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Nellore cattle (*Bos indicus*) and ticks within the Brazilian Pantanal: ecological relationships

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Abstract Pantanal is a huge floodplain mostly in Brazil, and its main economic activity is extensive cattle raising, in farms characterized by an extremely wildlife-rich environment. We herein describe tick infestations of cattle and of the natural environment in Pantanal of Nhecolândia in Brazil, at areas with and without cattle during both dry and wet seasons. Environmental sampling resulted in three tick species: *Amblyomma sculptum* (423 nymphs and 518 adults), *Amblyomma parvum* (7 nymphs and 129 adults), *Amblyomma ovale* (3 adults) as well as three clusters and two individuals of *Amblyomma* sp. larvae. A significantly higher number of adult *A. sculptum* ticks was found in areas with cattle in the wet season. From 106 examinations of bovines 1710 ticks from three species were collected: *Rhipicephalus microplus* (55.7 % of the total), *A. sculptum* (38 %) and *A. parvum* (4.1 %), as well as 32 *Amblyomma* sp. larvae. A significant similarity was found between *Amblyomma* tick fauna from environment and on cattle during both seasons. All *A. sculptum* females on bovines were flat whereas many of *A. parvum* females and *A. sculptum* nymphs were engorging. Although *R. microplus* was the most abundant tick species on cattle, overall highest tick prevalence on bovines in the dry season was of *A. sculptum* nymphs. Lack of *R. microplus* in environmental sampling, relationship between cattle and increase in adult *A.*

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sculptum numbers in the environment as well as suitability of bovine for the various tick species are discussed.

Keywords *Amblyomma sculptum* · *Rhipicephalus microplus* · *Amblyomma parvum* · Bovines · Brazil · Ecology

Introduction

Brazil is a huge country with a rich biodiversity distributed over several biomes. This biodiversity includes approximately 67 tick species mostly from *Amblyomma* genus (Krawczak et al. 2015). Tick species occurrence vary according to biomes and ecosystems, with a few species prevailing within rainforests as is the case of *Amblyomma incisum* and *Amblyomma aureolatum* (Szabó et al. 2009; Pinter et al. 2004) and others in the Brazilian savannah, as is the case of *Amblyomma sculptum* Berlese (from *Amblyomma cajennense* complex) (Szabó et al. 2007a; Veronez et al. 2010). Several exogenous tick species established in the country with colonization as well, and are mostly associated with domestic animals and anthropogenic sites as is the case of *Rhipicephalus sanguineus* on dogs and *Rhipicephalus microplus* on cattle (Szabó et al. 2001; Fonseca et al. 2005).

Brazilian commercial bovine herd is the largest in the world and cattle breeding vary within the country according to the several biomes, climates and social conditions (ABIEC 2015). Nevertheless, *R. microplus* ticks are considered a major pest for cattle across the country and are responsible for huge economic losses (Grisi et al. 2002). In a very few instances, however, ticks are not a major concern. This is the case of some farms within Brazilian Pantanal where infestation control is not justified because tick incidence is considered low (Barros and Pellegrin 2002).

The Pantanal is the world's largest freshwater wetland with an estimated area of 138.183 km^2 (larger than Portugal or Bulgaria) mostly in Brazil but extending into Paraguay and Bolivia (Alho 2005). It is a seasonally flooded plain; from October to April, rains cause the Paraguay River and its tributaries to overflow and over 80 % of the Pantanal plains are submerged. It has a mosaic of phytophysiognomies mostly from the Cerrado biome (Alho et al. 1988; Silva et al. 2000; Abreu et al. 2010) such as savannah, open fields, 'dirty' fields (with many small trees and shrubs), swamps with hydrophilic vegetation, forest corridors ("cordilheiras"), forest patches ("capões") among others. At the same time, Pantanal has a rich wildlife with several ticks with at least eight different species described from wild animals (Campos Pereira et al. 2000).

At present, extensive beef cattle production with specialization in calf and heifer production and a herd estimated at four million head, is the main economic activity in Pantanal. In fact, over 90 % of the region is in private hands, of which 80 % has been used for Nellore cattle (*Bos indicus* L.) production for more than 250 years (Abreu et al. 2010). In contrast, Pantanal is considered the most conserved ecosystem in Brazil, with over 85 % of its area still dominated by native vegetation (reviewed by Abreu et al. 2010). Most cattle production in the Pantanal is therefore extensive calf rearing on native pastures (Bliska and Gonçalves 1998), on large properties with management directed by the flood regime (Abreu et al. 2010). At the same time, Pantanal has a rich wildlife fauna and bovines share environment with wild animals on both pastures as well as forestall formations used by cattle to rest.

We herein describe and compare tick infestations of cattle and of the natural environment in Brazilian Pantanal in wet and dry seasons. In addition, we evaluate the variations in questing ticks between areas with and without cattle and between phytophysiognomies (forest versus grasslands), characterize tick distribution along the cattle body, and analyze the similarity between free-living ticks and cattle infestation.

Materials and methods

Study site

The study was carried out in the Nhumirim farm, in Nhecolândia, a sub-region of Pantanal, Mato Grosso do Sul State, Brazil (18°59′15″S; 56°37′03″W), at 89 m asl. There are basically two seasons in this region; one that is hot and rainy from October to March (monthly rainfall means from 150 to 300 mm) and another drier with milder temperatures from April to September (monthly rainfall means bellow 100 mm). The temperature amplitude is overall high with minimums close to zero to as high as 40 °C (Tarifa 1986).

Free-living ticks were collected in two different areas (a fenced area with 681.4 ha, without cattle since 1987, and a cattle raising area with 288.5 ha) distant 02 km each other. In each area (with and without cattle), surveys for ticks were carried out along grasslands and forests. In cattle raising area, both phytophysiognomies are commonly used by bovines.

Free-living ticks

Host-seeking ticks from the environment were collected by three methods: visual search, cloth dragging (Terassini et al. 2010) and CO_2 (dry ice) traps (Oliveira et al. 2000). Sampling by visual search and dragging was performed together and five times in the dry (September 2010, June and September of 2011 and 2012) and twice in the wet season (December 2011 and 2012). In each field trip transects of 40 m were sampled in the following areas; forest with cattle (n = 4 transects), forest without cattle (n = 4), grassland with cattle (n = 4) and grassland without cattle (n = 4), totalizing 16 transects in each campaign. Within sampling areas, grassland transects were no <100 m from nearest forest and replicates of transects in the same phytophysiognomy were 150–300 m far from each other. Search for ticks in transects was initially visual followed by cloth dragging. Dry ice traps were used separately in the wet season of 2012 and 2013 (February) to increase sampling of adult tick species. Five CO_2 traps at least 5 m apart were mounted in each forest fragment in both reserve (n = 4) and cattle raising areas (n = 4), and inspected for ticks 1.5–2.0 h later. Furthermore, five traps CO_2 were placed in open vegetation adjacent to two forest fragments in each area.

Ticks on cattle

At each campaign, concurrently with collection of free-living ticks, fifteen (sixteen on one occasion—July 2011) Nellore cows (*B. indicus*) maintained in extensive cattle raising system (a mean number of 64.11 cows/288.5 ha) at Nhumirim Farm were examined for ticks. To this end, animals (approximately, 18 months old) were restrained in a cattle crush and one side of the body inspected. For an accurate sampling of immatures, time-

independent collection was performed at a demarcated area of 100 cm^2 in center of the dewlap. Afterwards ticks were collected for 1 min at each of the following sites: head, perineum and ventral region.

Molting success of nymphs engorging on Nelore cattle from Pantanal

Several engorging nymphs were found on Nelore cattle in the Nhumirim farm throughout the study. Therefore molting success of a sample of 28 engorged nymphs collected from these bovines (n = 18), was taken and held in laboratory at 80 % of humidity and 27 °C to determine molting rate.

Tick identification

Ticks were identified according to morphologic features (Onofrio et al. 2006; Martins et al. 2010; Nava et al. 2014) and comparisons to reference collection of the Tick Museum of Federal University of Uberlândia.

Analyses

Free-living ticks: Total tick numbers of each ticks species are presented descriptively. Statistical analysis (comparison between phytophysiognomies, areas with and without cattle, and between dry and wet season) was restricted to *A. sculptum*, the only species captured in numbers high enough. Tick infestations between physiognomies (forestall and open field) and areas (with and without cattle) were compared separately for nymphs and adults. Data of ticks collected by visual search and dragging were pooled, transformed (log x + 1) and analyzed by two-way Anova. The latter analysis was used to test whether the both factors, phytophysiognomy and presence/lack of cattle, exerted isolated or combined effect upon free living tick occurrence. Ticks collected using dry ice traps were much less numerous and thus data was analyzed separately with a non-parametric test (Mann–Whitney).

Cattle infestation: Infestation of each host was estimated by doubling the number ticks from found on one side of the body. Infestation of cattle is expressed by mean abundance, prevalence (Bush et al. 1979) and also by median and interquartile interval (1st–3rd Quartiles) for each tick species as well as to all ticks together. Anatomical sites of tick parasitism on the host were compared in the wet and dry seasons by Kruskal–Wallis followed by Dunn's test. Similarity between host infestation (considering all *Amblyomma* species together and without *R. microplus,* not found in the environment) and free-living ticks (collected by visual search and dragging) was analyzed by the Morisita-Horn index (CM_H) according to Magurran (1988) and all other tests followed Zar (1989) and Rózsa et al. (2000).

Results

Free-living ticks

Dragging and visual search

Tick numbers by dragging and visual search resulted in three tick species: *A. sculptum* (423 nymphs and 241 adults), *Amblyomma parvum* Aragão (seven nymphs and 39 adults) and

Amblyomma ovale Koch (three adults). Additionally, three clusters and two individuals of *Amblyomma* sp. larvae were collected as well. Total tick numbers found according to location and season are presented in Table 1. A detailed description of the prevailing free-living tick species, *A. sculptum*, is shown in Table 2. Partial results of environmental infestations but with a different analysis were already presented elsewhere (Ramos et al. 2014a).

During the dry season, tick occurrence was not influenced by combined effect of physiognomy (forestall and grassland) and area (with or without cattle) for any developmental stage (Two-way Anova, $F_{nymphs} = 0.233$; $F_{adults} = 0.008$; g.l. = 1.0 and $p > 0.05$ for all cases). Forests presented a higher tick number than grassland vegetaion ($F_{nymphs} = 5.302$; $F_{adults} = 12.191$; g.l. = 1.0 and $p < 0.05$ for all cases) (Fig. 1a, b) but collections in the both areas (with and without cattle) resulted in comparable number of ticks ($F_{nymphs} = 0.037$; $F_{adults} = 0.028$; g.l. = 1.0 and $p > 0.05$ for all cases) (Fig. 1c, d). On the other hand, an interaction ($r^2 = 0.40$) between physiognomy and presence/lack of cattle was observed; forest areas with cattle had significantly more adult ticks than the reserve forest ($F = 5.075$; g.l. = 1.0; $p < 0.05$; Fig. 1e, f).

Dry ice

Dry ice traps set up solely in the wet season attracted overall 366 adult ticks (*A. sculptum* and *A. parvum*) in the forests of areas with cattle and reserve, and only one *A. sculptum* in open vegetation (in area with cattle). From these a total of 43 *A. sculptum* (mean ± standard deviation = 14.33 ± 2.08 ticks by fragment) and four *A. parvum* (1.33 ± 1.15) were captured in the reserve against 233 *A. sculptum* (77.67 ± 40.86) and 86 *A. parvum* (28.67 ± 15.01) in areas with cattle. Forests with cattle were significantly more infested by

Table 1 Total number of free-living *Amblyomma* ticks collected by visual search and dragging in the Brazilian Pantanal (Nhecolândia sub-region, Nhumirim farm, Corumbá, MS) between 2010 and 2012

Season, tick species and stage	Forest with cattle (n = 4)	Forest without cattle (n = 4)	Grassland with cattle (n = 4)	Grassland without cattle (n = 4)
Dry season				
Amblyomma sculptum				
Adults	57	85	0	0
Nymphs	230	155	10	27
Amblyomma parvum				
Adults	07	20	01	02
Nymphs	05	0	01	01
Amblyomma ovale				
Adults	01	02	0	0
Wet season				
Amblyomma sculptum				
Adults	106	13	0	0
Nymphs	01	0	0	0
Amblyomma parvum				
Adults	08	01	0	0

Table 2 Free-living *Amblyomma sculptum* ticks collected in transects of 40 m^2 each by visual search and dragging in the Brazilian Pantanal (Nhecolândia sub-region, Nhumirim farm, Corumbá, MS) between 2010 and 2012

Season, physiognomy and area	Minimum–maximum		Median (1st–3rd quartiles)	
	Nymphs	Adults	Nymphs	Adults
Dry				
Forest without cattle	0.00–22.00	0.00–25.00	6.00 (2.75–10.75)	0.00 (0.00–1.25)
Grassland without cattle	0.00–14.00	–	0.00 (0.00–1.00)	–
Forest with cattle	0.00–43.00	0.00–12.00	5.5.00 (1.75–19.00)	0.00 (0.00–3.00)
Grassland with cattle	0.00–04.00	–	0.00 (0.00–0.25)	–
Wet				
Forest without cattle	–	0.00–5.00	–	1.00 (1.00–2.00)
Grassland without cattle	–	–	–	–
Forest with cattle	–	0.00–51.00	–	10.50 (0.75–16.25)
Grassland with cattle	–	–	–	–

the both adult tick species in the wet season (U = 9.0, d.f. = 1.0, $p < 0.05$). None of the techniques for environmental sampling captured *R. microplus*.

Natural infestation on Nellore cattle

Overall tick infestation prevalence of 106 examined bovines was of 90.79 % in the dry season and 66.67 % in the wet season (Table 3). From 106 examinations of bovines 1710 ticks from three species were collected: *R. microplus* (n = 952; 55.67 % of the total), *A. sculptum* (n = 656; 38 %) and *A. parvum* (n = 70; 4.1 %), as well as 32 *Amblyomma* sp. larvae (Table 3). *R. microplus* infestation prevalence was of 61 % in the dry season, 47 % in the wet season with a mean infestation intensity of, respectively, 27.6 and 10.4 ticks. *A. sculptum* nymphs were more numerous than adults during the dry season (nymph infestation prevalence of 87 % and mean infestation intensity of 7.44 against 44 % and 2.32 of adults, respectively), and from these, 70.9 % were engorged or engorging. At the same time, solely adults were observed in the wet season, albeit in low numbers (infestation prevalence of 20 % and mean infestation intensity of 2.34 ticks). Most of *A. parvum* ticks were female (n = 42 against 28 males) and six nymphs of this species were found in the dry season (not shown in Table 3).

Both *R. microplus* and *A. sculptum* were found on all examined anatomical sites whereas *A. parvum* was found solely on the head. During the dry season *R. microplus* was found in similar numbers on all anatomical sites (area of 100 cm^2 in center of the dewlap, perineum and ventral region) except for lesser numbers on the head (K = 16.89, $p < 0.005$, d.f. = 3.0). During the rainy season ventral region was more infested with this *R. microplus* than all other sites (K = 9.35, $p < 0.05$, d.f. = 3.0). Ventral region and head

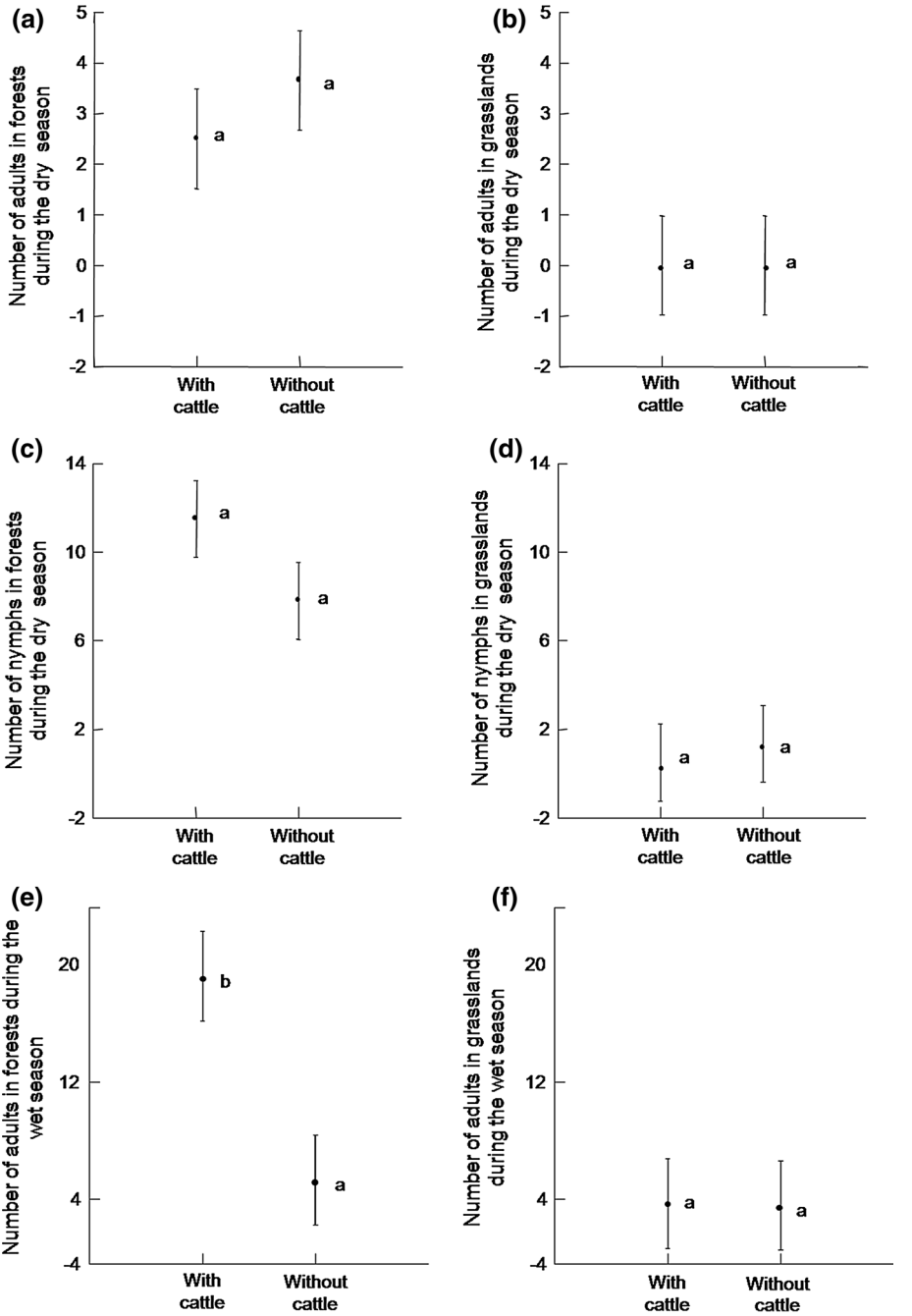

Fig. 1 Least square means of free-living *Amblyomma sculptum* ticks collected by transect (40 m^2) in forests and grasslands with and without cattle during dry (**a**–**d**) and wet (**e**, **f**) seasons, between 2010 September and 2012 December, at Nhumirim Farm, Nhecolândia Pantanal, Brazil. *Lowercase letters* indicate significant difference ($p < 0.05$) between areas

Table 3 Natural tick infestation of Nellore cattle (*Bos indicus*) during the dry (2010 to 2012, n = 76) and wet (2011/2012 and 2012/2013, n = 30) seasons in the Brazilian Pantanal, Nhecolândia sub-region (Nhumirim Farm, Corumbá, MS)

	Amblyomma sp. Larvae	*Amblyomma sculptum*				*Amblyomma parvum* Total	Total of *Amblyomma* ticks	*Rhipicephalus microplus*				
		Adults	Nymphs		Total			Larvae	Adults	Nymphs		Total
			Total	Engorged						Total	Engorged	
Dry season												
Median	0.00	0.00	6.00	3.00	6.00	0.00	6.00	0.00	2.00	0.00	0.00	6.00
1st Quartile	0.00	0.00	2.00	1.50	2.00	0.00	4.00	0.00	0.00	0.00	0.00	0.00
3rd Quartile	0.00	0.50	10.60	8.00	10.60	0.00	12.50	0.00	6.00	8.00	8.00	28.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	4.00	4.00	42.00	40.00	44.00	4.00	52.00	4.00	36.00	46.00	44.00	78.00
Total	32.00	44.00	598.00	424.00	642.00	36.00	710.00	36.00	356.00	412.00	390.00	804.00
Prevalence	10.52	25.00	85.53	75.00	89.47	21.05	90.79	14.47	52.63	48.68	48.68	53.95
Mean abundance	0.42	0.58	7.87	5.58	8.45	0.47	9.34	0.47	4.68	5.42	5.13	10.58
Wet season												
Median	–	0.00	–	–	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1st Quartile	–	0.00	–	–	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3rd Quartile	–	0.00	–	–	0.00	2.00	2.00	0.00	4.00	2.00	2.00	10.00
Minimum	–	0.00	–	–	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	–	4.00	–	–	4.00	6.00	8.00	2.00	20.00	14.00	14.00	20.00
Total	–	14.00	–	–	14.00	34.00	48.00	2.00	94.00	52.00	52.00	148.00
Prevalence	–	20.00	–	–	20.00	26.67	33.33	3.33	36.67	33.33	33.33	46.67
Mean abundance	–	0.47	–	–	0.47	1.13	1.60	0.07	3.13	1.73	5.20	10.57

were more infested with *A. sculptum* then other sites in the dry season ($K = 59.20$, $p < 0.001$, d.f. $= 3.0$), and no difference in *A. sculptum* numbers was found among anatomical sites in the rainy season.

Morisita-Horn index (CmH), calculated only for *Amblyomma*, indicated similarity between tick fauna from environment and on cattle during both seasons ($CmH_{DRY} = 0.999$, $CmH_{WET} = 0.998$). Considering tick stage separately (either nymphs or adults), infestation similarity was higher in the dry season ($CmH_{DRY} = 0.998$, $CmH_{WET} = 0.735$).

Molting success of nymphs engorging on Nelore cattle from Pantanal

All 28 engorged *Amblyomma* nymphs collected from naturally infested cattle molted to *A. sculptum* adults.

Discussion

Three tick species were recovered from environment in Pantanal. From these *A. ovale* was the less numerous, with only three adults sampled. Adults of this tick species parasitizes carnivores (Labruna et al. 2005), and birds and rodents are probable hosts for larvae and nymphs (Ogrzewalska et al. 2009; Guglielmone and Nava 2011; Szabó et al. 2013) and there is only one record on cattle (Hoffman 1962). The other two tick species, *A. sculptum* and *A. parvum*, were found in much higher numbers and seem to be the most prevalent species in this region of Pantanal not only in the environment but also on wild animals (Campos-Pereira et al. 2000; Cançado et al. 2013).

Amblyomma parvum ticks have broad host range and were described previously from several domestic and wild animals within the Neotropical region (Nava et al. 2008). It is also a tick species aggressive to humans (Szabó et al. 2007a; Guglielmone et al. 2014; Ramos et al. 2014b) with potential to transmit disease agents. In fact, a *Rickettsia* species of unknown pathogenicity and the Q fever agent, *Coxiella burnetii*, were described from *A. parvum* (Pacheco et al. 2007, 2013).

Amblyomma sculptum (Berlese 1888) is a tick species recently resurrected (Nava et al. 2014) within the *A. cajennense* (Fabricius 1787) complex (Beati et al. 2013). It is associated with the Cerrado Biome encompassing southeast and central Brazil (Beati et al. 2013; Nava et al. 2014). Thus, previous reports of *A. cajennense* from these regions of Brazil should be regarded as *A. sculptum* as is the case of Pantanal. *A. sculptum* populations are usually the most prevalent tick species within its range in the Cerrado Biome (Campos-Pereira et al. 2000; Szabó et al. 2007a; Veronez et al. 2010). This tick species depends on a few key hosts for adult ticks such as ungulates (horses and tapirs) and capybaras to maintain high levels of environmental infestation (Castagnolli et al. 2003; Labruna et al. 2001; Heijden et al. 2005). Recently it was shown that feral pigs, abundant animals in the Pantanal, are suitable hosts for *A. sculptum* as well (Ramos et al. 2014c). Notably, *A. sculptum* is, among those from *A. cajennense* complex in Brazil, that is associated with the transmission of the highly lethal *Rickettsia rickettsii*, agent of the Brazilian Spotted-Fever (Labruna 2009).

Both *A. sculptum* and *A. parvum* were found overwhelmingly within forestall formations. This phytophysiognomy provides hosts for ticks; it is a refuge for wild fauna (Oliveira-Filho and Teixeira 1992; Mamede and Alho 2006; Desbiez et al. 2009), and an area with shadow and resting place for bovines. It also provides suitable microhabitats,

such as the litter layer, for ticks that need humidity, and shelter from sun. In fact, *A. sculptum* at anthropogenic sites in southeast Brazil seems to be associated with shadow, bushes and shrubs in pastures (Labruna et al. 2001) and gallery forest fragments (Souza et al. 2006).

In all of the sampled years, nymphs and adult ticks were found in the dry season and only adults in the wet season. It is important to highlight that comparisons between dry and wet seasons may be biased due to differing sampling efforts. Such uneven sampling occurred because flooding of grasslands during the wet season precludes environmental sampling of ticks and moving within the Pantanal is hampered. On the other hand, these observations match the seasonal pattern in Brazil of the major tick species found, *A. sculptum* (Serra-Freire 1982a; Labruna et al. 2002) as well as of many other *Amblyomma* of the country (Szabó et al. 2007b; Labruna et al. 2009). At the same time, larvae were found in much lesser numbers. Reason for that is not clear but, it might have occurred due to temporal or spatial bias of sampling and/or due to the patchy distribution of larva batches (Table 3).

A noteworthy observation was the significantly higher number of adult ticks in areas with cattle in the wet season, without a correspondent higher number of nymphs or larvae in the dry season in the same location. In this regard, we speculate that bovines, a huge biomass within Pantanal, feed nymphs of *A. sculptum* originating more adults. At the same time, lack of suitable hosts for adults in the same proportion lowers tick numbers again. This hypothesis is based on the tick infestations of bovines observed in this study (discussed below) and should be tested by additional field observations as well as experimental infestations of bovines.

Three tick species were found on bovines in our study; *A. sculptum*, *A. parvum* and *R. microplus*. Among these, the latter was the most numerous tick species on hosts. In fact, *R. microplus* is the main cattle tick in Brazil, causes huge economic losses and, in many herds, routine acaricide treatments of bovines are mandatory (Grisi et al. 2002). However, *R. microplus* tick numbers found on bovines in our study should be considered a glimpse at a highly kinetic life cycle precluding trustful analysis. This tick species may have several generations per year (undetermined in Pantanal) and our observations may have occurred along any moment of any of the tick generations. On the other hand, lack of *R. microplus* in samples from the environment is at first sight an interesting observation. Several aspects of this tick's biology and of the environment, apart from the aforementioned generation issue, may explain this apparent discrepancy. First of all, *R. microplus* is a one-host tick species and neither nymphs nor adults quest for host in the environment. At the same time larvae of this tick species exhibit a highly aggregated pattern in the environment (Zimmerman and Harris 1987) decreasing the chances of sampling by dragging. Furthermore, this tick species is typically from open fields on pastures (Furlong et al. 2002). In Pantanal total or partial flooding of pastures may eliminate ticks from the vegetation. Extensive cattle ranching with decreased animal density and Nellore breed, considered more resistant to *R. microplus* than *Bos taurus* L. breeds (da Silva et al. 2013) may also lessen tick populations from pastures. In spite of all, *R. microplus* was still the most numerous tick on bovines demonstrating the survival of a population under apparently harsh conditions. This indicates that any environmental and\or bovine management alteration in Pantanal that accidentally favors this tick species could result in unbearably increased infestation levels as occurs elsewhere.

Overall highest tick prevalence on bovines was of *A. sculptum* nymphs in the dry season. Such prevalence is linked to high environmental infestation whereas the lower mean infestation intensity *A. sculptum* nymphs in relation to *R. microplus* is the

consequence of host infestation, not of larvae clusters as is the case of *R. microplus*, but rather individuals scattered in the environment. Same relationship between bovine and environmental infestations was not found in the case of adults of *A. sculptum.* During the wet season, when adults of *A. sculptum* prevail on vegetation in forest patches, only few adults were observed on cattle. Lower numbers of *A. sculptum* adults may be explained by as lower bovine suitability as hosts. This last affirmation is underscored by lack of engorging *A. sculptum* adults, as opposed to nymphs, on bovines in Pantanal. However, suitability of bovines to each *A. sculptum* instar should be addressed further by experimental studies.

Amblyomma parvum ticks were found in much lesser numbers in both environment and hosts to allow for a trustful analysis. Nonetheless, it is worthwhile mentioning that, in spite of prevailing of *A. sculptum* adults over *A. parvum* adults in the environment, adult *A. parvum* tick numbers on cattle were similar to that *A. sculptum* adults in the dry season and twice as high in the wet season, moreover all female ticks were engorging. In this regard, Olegário et al. (2011) demonstrated experimentally that *A. parvum* can attach and successfully feed on cattle. These observations indicate the higher suitability of Nellore cattle to *A. parvum* adults. It was also interesting to note that *A. parvum* adults were found solely on the head of hosts. Similar infestation pattern was observed by Nava et al. (2009) in Argentina but the reason for parasitism restriction to such anatomical site is unknown and should be addressed by additional studies.

Species of the *Amblyomma* genus are rarely mentioned on cattle infestations in Brazil (Serra-Freire 1982b; Borges and da Silva 1994). One reason for that are the many times overwhelming infestations with *R. microplus* ticks. Another reason may be the broadly used cattle tick infestation evaluation pattern by Wharton et al. (1973) and based on engorging *R. microplus* females over 4.5 mm of diameter precluding detection of immatures or adults of small tick species, such as *A. parvum*. Nonetheless, observations of bovine tick infestations similar to the ones we detected in Pantanal were described in Northwest Argentina (Guglielmone and Hadani 1980, 1982; Guglielmone et al. 1990; Mangold et al. 1994). These authors observed regular infestation of cattle by *R. microplus*, *A. cajennense* sensu lato and *A. parvum* and in the same prevalence order. Although these authors reported *Amblyomma neumanni* Ribaga on hosts as well, a tick species non-described in Brazil.

It is worthwhile mentioning that Mangold et al. (1994) observed *A. cajennense* and *A. parvum* only on cattle maintained in native pastures in forested habitats. At deforested habitats, cattle were parasitized only by *R. microplus* and *A. neumanni*. These reports in Argentina reinforce our observations that infestation by *A. parvum* and *A. sculptum* on livestock in the Pantanal is associated to natural forested areas. Coincidentally, Brazil is undergoing an increase in the awareness for habitat preservation and reconstitution of several natural areas. Under this scenario, it is possible to suppose a shift in the tick infestation of cattle in a few areas and infestations by both *A. parvum* and *A. sculptum* may increase. Thus, problems related to infestations by these tick species such as transmission of tick-borne diseases to bovines should be monitored.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (Animal Experimentation Ethics Committee of the Federal University of Uberlândia). Permits and Approvals (no 116/10) are on file in the office of M.P.J.S.

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