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Article

Ticks on *Didelphis albiventris* from a Cerrado area in the Midwestern Brazil

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Abstract

This experiment studied tick fauna associated to *Didelphis albiventris* Lund 1840 from a Cerrado area (Mato Grosso do Sul State, non-adjoining to Pantanal) inserted in a pasture and agricultural activities matrix, with few natural preserved forest patches. Authors also summarized data on ticks parasitizing *Didelphis* spp. in Brazil, and discussed infestation patterns in different biomes and locations. Study took place in Cervinho Farm, Bandeirantes Municipality. For captures, Tomahawk-like traps were distributed along two forest patches (30 each) during five nights. Captures occurred monthly (July/2013 to September/2014), sampling both fragments on alternate months. Animals were sedated and ticks were collected and stored in vials containing ethanol (70%) for identification. 51 *D. albiventris* were captured in 15 campaigns. Tick prevalence was 100%, and 49.5% of the animals were co-infested by two or more tick species. Four parasitizing species were found: *Amblyomma sculptum* Berlese, 1888 (78 nymphs), *Ixodes loricatus* Neumann, 1899 (56 adults), *Amblyomma dubitatum* Neumann, 1899 (45 nymphs), *Amblyomma coelebs* Neumann, 1899 (32 nymphs) and *Amblyomma* sp. (123

larvae). *A. sculptum* was the most abundant tick, but most frequent species were *A. coelebs* and *A. dubitatum*, followed by *I. loricatus*. Co-occurrences of more than two species were observed among all tick pairs. *D. albiventris* usually presents high prevalence of tick infestation. However, this study presented 100% prevalence. Knowledge of host-parasite relation and interactions between different ticks that coexist on a same host are essential, since such interaction may favor pathogen dissemination. This is of special relevance regarding *D. albiventris*, known for participating in maintenance of ecological cycles of Spotted Fever Group rickettsiae.

Keywords: Opossum, Central West, Ticks, Co-infestation, Brazil

Introduction

The white-eared opossum, *Didelphis albiventris* Lund 1840, is a widespread didelphid marsupial in the Neotropical region that inhabits savannahs, gallery and humid forests (Emmons & Feer 1997). This species exhibits omnivorous and opportunistic feeding habits, presenting a great adaptability to disturbed landscapes (Cáceres & Monteiro-Filho, 2006), including to urban areas.

The synanthropic behavior of *D. albiventris* enables this species to participate, in some level, of the epidemiological cycle of human diseases (Muller *et al.* 2005). In Brazil, *Didelphis* spp. is the most important marsupial genus which harbors ectoparasites (Linardi 2006), mainly fleas and ticks. Ticks are among the most significant vectors for human diseases (Hoskins & Cupp 1988). Many of the tick-borne pathogens are also found in opossums (Melo *et al.* 2016), this animal can participate in the epidemiological cycle of *Rickettsia rickettsii* (Horta *et al.* 2009), the responsible agent for causing the Brazilian Spotted Fever (Parola *et al.* 2013).

Recently, Sponchiado *et al.* (2015) revealed, in five municipalities located in the Mato Grosso do Sul State, a great richness of tick species infesting this opossum in various fragments of Cerrado at the border of the Pantanal. The number of species was higher than the commonly observed on this host. It is reasonable to suppose that the ixodid fauna associated with this host in more preserved areas, as described by Sponchiado *et al.* (2015), could be more diverse than the ones from anthropogenic sites. To this respect, for detecting the existence of interaction patterns, or variations in these patterns, it is needed to characterize the tick fauna of this marsupial from areas under various disturb conditions and located in different biomes. In this sense, we presented data on the tick fauna associated to *D. albiventris* from a Cerrado area in the Mato Grosso do Sul State (non-adjoining to the Pantanal), inserted in a matrix of pastures and agricultural activities, and with few natural and preserved forest patches. In addition, we summarized the data from the literature on ticks parasitizing *Didelphis* spp. in Brazil, and provided a discussion regarding infestation patterns in different biomes and localities.

Material and methods

The field study was carried out in the Cervinho Farm (19° 55’ 04” S; 54° 21’ 50” W, 560m a.s.l.), Bandeirantes Municipality, Mato Grosso do Sul State. This farm is within the Cerrado biome, in the Midwestern Brazil, and encompasses an area that presents a few preserved patches mixed with agricultural and cattle ranching activities. Sixty Tomahawk-like traps (45x21x21cm) were distributed along two forest patches (30 in each), during five nights. The traps were baited with two mixtures; banana, pineapple essence and corn flour, and canned sardine with corn flour. The captures occurred monthly from July 2013 to September 2014, and the two forest fragments were sampled on alternate months, resulting in 2,250 night-traps. In each campaign, all working traps were daily checked and the bait was replaced. Initially, the captured animals were mechanically restrained for

body weight measuring (electronic balance SF-40). Afterward, they were anesthetized with Tiletamin-Zolazepam (Zoletil®) for determination of age, sex, application of numerical ear tags and collection of ticks. The animals were released in its capture area after the fully recover from the anesthesia. Ticks were maintained in ethanol (70%) until taxonomic identification according to dichotomous keys and descriptions (Onofrio *et al.* 2006; Onofrio *et al.* 2009; Martins *et al.* 2010a; Martins *et al.* 2016; Nava *et al.* 2014) and comparison with specimens from reference collection. The larvae were retained at the genus level. Mean infestation intensity, mean abundance and prevalence, according to Bush *et al.* (1997), were used to describe ticks infestation on hosts. Literature data were summarized for describing tick species parasitizing *Didelphis* spp. in Brazil. For this purpose, only studies presenting clear association between host and tick species and taxonomic identification at species level for ticks were considered.

Results

Field data: In our campaigns, we inspected a total of 51 specimens of *D. albiventris*, which were captured in all the 15 campaigns. The prevalence of ticks was 100%, and 49.5% of the animals were co-infested by two or more tick species. Four tick species were found parasitizing this host; *Amblyomma sculptum* (n = 78 nymphs), *Ixodes loricatus* (n = 56 adults), *Amblyomma dubitatum* (n = 45 nymphs), *Amblyomma coelebs* (n = 32 nymphs), and larvae of *Amblyomma* sp. (n = 123). *A. sculptum* was the most abundant tick, but the most frequent ticks were *A. coelebs* and *A. dubitatum*, followed by *I. loricatus* (Table 1). The higher co-infestation rate was verified for three hosts, parasitized by the four tick species (Table 2). Co-occurrences were observed for all pairs of tick species; *A. sculptum* plus *A. dubitatum* (occurring in 10 hosts), *A. sculptum* + *A. coelebs* (n = 4), *A. sculptum* + *I. loricatus* (n = 5), *A. dubitatum* + *A. coelebs* (n = 8), *A. dubitatum* + *I. loricatus* (n = 10), and *A. coelebs* + *I. loricatus* (n = 10). The most frequent unique interactions were *A. sculptum* + *A. dubitatum* (n = 6) and *A. dubitatum* + *A. coelebs* + *I. loricatus* (n = 5). Larvae of *Amblyomma* sp. peaked in June and July 2014, and *A. sculptum* nymphs in September 2013. The other tick species were collected in low numbers, but in general, they were concentrated in the dry season (Figure 1).

Literature data: Twenty-eight studies on tick infestations on *Didelphis* spp. in Brazil were selected from literature (Table 3). Most of them present results for *D. albiventris*, but some works also include *Didelphis aurita* and *Didelphis marsupialis.* The six Brazilian biomes just were investigated, but the majority (n = 15 studies) was carried out in Atlantic Forest areas, followed by Cerrado areas (n = 11). Sixteen tick species are reported on *Didelphis* spp.: *Amblyomma aureolatum*, *Amblyomma auricularium*, *Amblyomma brasiliense*, *A. coelebs*, *A. dubitatum*, *Amblyomma fuscum*, *Amblyomma geayi*, *Amblyomma humerale*, *Amblyomma ovale*, *Amblyomma parkeri*, *Amblyomma parvum*, *A. sculptum*, *Amblyomma yucumense*, *I. loricatus*, *Ixodes schulzei*, and *Ornithodoros mimon*. The most frequent tick species was *I. loricatus*, observed in 14 works, followed by *A. sculptum*, found in nine studies. The former is reported in eight studies conducted within Areas of Conservation (AC's) (05 on *D.. aurita* in Atlantic Forest and 03 on *D. albiventris* in Cerrado), one on non-informed area (on *D. aurita* in Cerrado), and in five outside AC's (02 on *D. albiventris* and 01 on *D. aurita* in Atlantic Forest and 02 on *D. albiventris* in Cerrado). The later occurred outside AC's; two in Atlantic Forest, on *D. aurita* and *D. albiventris*, and five in Cerrado, one on *D. aurita* and four on *D. albiventris* and two studies conducted within Areas of Conservation (AC's) (01 on *D. aurita* in Atlantic Forest and 01 on *D. albiventris* in Cerrado).

TABLE 1. Infestation parameters of ticks collected on *Didelphis albiventris* from forest fragments in Cerrado areas, Mato Grosso do Sul, Brazil, between July 2013 and September 2014.

Tick species	Infested hosts (n)	Ticks (n)	P (%)	MI	MA
Amblyomma sculptum	11	78N	21.6	7.1	1.5
Amblyomma coelebs	20	32N	39.2	1.6	0.6
Amblyomma dubitatum	20	45N	39.2	2.3	0.9
Amblyomma sp.	11	123L	21.6	11.2	2.4
Ixodes loricatus	18	56A (28M, 28F)	35.3	3.1	1.1

n: total number; P: prevalence; MI: mean intensity; MA: mean abundance; L: larvae; N: nymph; A: adult; M: males; F: females.

TABLE 2. Number of *Didelphis albiventris* infested by two or more tick species, in Cerrado areas, Mato Grosso do Sul, Brazil, between July 2013 and September 2014.

Number of hosts with co-infestation	*Amblyomma sculptum*	*Amblyomma coelebs*	*Amblyomma dubitatum*	*Ixodes loricatus*
6	x		x	
1	x		x	x
1	x			x
3	x	x	x	x
5		x	x	x
2		x		x
1	x	x		
1		x		
2			x	
1			x	x

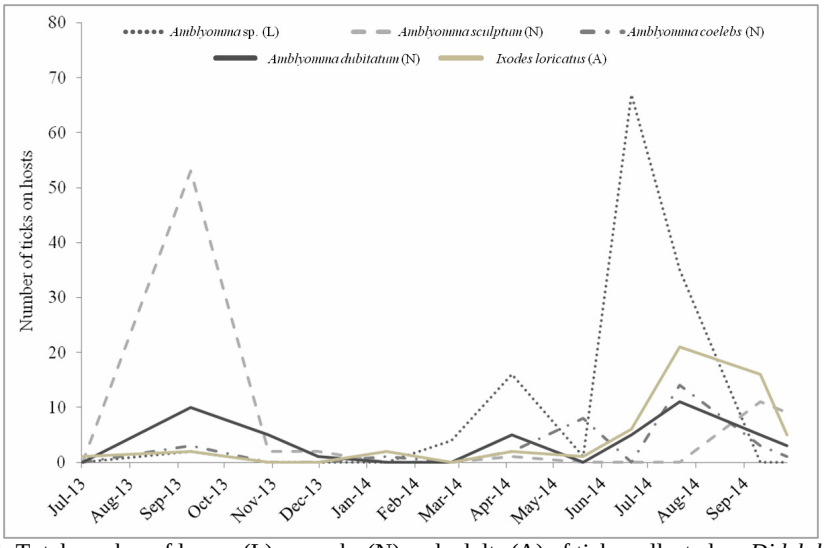

FIGURE 1. Total number of larvae (L), nymphs (N) and adults (A) of ticks collected on *Didelphis albiventris* in a Cerrado area, Mato Grosso do Sul, Brazil, between July 2013 and September 2014.

TABLE 3. Reports on the composition of tick species infesting *Didelphis* spp. in Brazil.

Locality—Biome (Conservation status of the area)	Host species	Ticks
1—Paraná - Atlantic Forest (AC)	*D. albiventris, D. marsupialis*	*Ixodes loricatus*
2—São Paulo - Atlantic Forest (AC)	*D. aurita*	*I. loricatus*
3—Rio de Janeiro - Atlantic Forest (AC)	*D. aurita*	*Ixodes didelphidis***
4—Rio Grande do Sul - Atlantic Forest, Pampa (OAC)	*D. albiventris*	*Amblyomma aureolatum, I. loricatus*
5—Santa Catarina - Atlantic Forest (AC)	*D. aurita*	*A. aureolatum, Amblyomma cajennense*, I. loricatus*
6—São Paulo - Atlantic Forest (OAC)	*D. albiventris*	*A. cajennense*, Amblyomma dubitatum*
7—Mato Grosso do Sul - Cerrado (AC)	*D. albiventris*	*I. loricatus*
8—Goiás - Cerrado (AC)	*D. albiventris*	*Amblyomma coelebs*
9—Minas Gerais - Atlantic Forest[a,b], Cerrado[c] (OAC)	*D. aurita*	*A. cajennense*, I. loricatus, A. coelebs*
10—Pernambuco - Atlantic Forest (AC)	*D. albiventris, D. aurita*	*Amblyomma fuscum, I. loricatus*
11—Distrito Federal - Cerrado (AC)	*D. albiventris*	*I. loricatus, Ixodes schulzei*
12—Rio Grande do Norte - Atlantic Forest (AC)	*D. albiventris*	*Ornithodoros mimon*
13—São Paulo - Cerrado (OAC)	*D. albiventris*	*A. cajennense*, A. coelebs*
14—Mato Grosso do Sul - Cerrado bordering the Pantanal (OAC)	*D. albiventris*	*A. sculptum, A. dubitatum, A. coelebs, Amblyomma ovale, Amblyomma parkeri, Amblyomma parvum, O. mimon*
15—Minas Gerais - Cerrado (OAC)	*D. albiventris*	*A. sculptum, A. dubitatum, I. loricatus*
16—Minas Gerais - Cerrado (OAC)	*D. albiventris*	*A. dubitatum, I. loricatus*
17—Rio Grande do Sul - Atlantic Forest (OAC)	*D. albiventris*	*A. ovale, I. loricatus*
18—Mato Grosso - Pantanal (OAC)	*D. albiventris*	*A. sculptum*
19—Mato Grosso - Cerrado[a], Amazon Forest[b] (NI)	*D. albiventris[ab], D. marsupialis[b]*	*A. coelebs[ab], A. coelebs, Amblyomma humerale, A. parkeri[b]*
20—Espírito Santo - Atlantic Forest (OAC)	*D. aurita*	*A. coelebs, A. dubitatum*
21—Santa Catarina - Atlantic Forest (OAC)	*D. albiventris*	*A. ovale*
22—Pernambuco - Caatinga (OAC)	*D. albiventris*	*Amblyomma auricularium, A. dubitatum*
23—São Paulo - Cerrado (NI)	*D. aurita*	*I. loricatus*
24—Pará - Amazon Forest (OAC)	*D. marsupialis*	*Amblyomma geayi, A. humerale*
25—Rio Grande do Sul - Atlantic Forest (AC)	*D. aurita*	*Amblyomma yucumense*
26—Mato Grosso do Sul - Cerrado (AC)	*D. albiventris*	*A. cajennense**
27—Pernambuco - Atlantic Forest (AC)	*D. albiventris*	*A. fuscum*
28—São Paulo - Atlantic Forest (AC)	*D. aurita*	*A. cajennense*, Amblyomma brasiliense, A. fuscum, A. ovale, I. loricatus*

**A. cajennense = A. sculptum; **I. didelphidis = I. loricatus*
AC = Areas of Conservation, OAC = outside Areas of Conservation, NI = non informed
References (coordinates) = 1—Barros and Baggio, 1992 (25º14'S, 50º00"W), 2—Bossi *et al*., 2002 (24º32'S, 47º15'W), 3—Bittencourt and Rocha, 2003 (23°11'S, 44°12'W), 4—Muller *et al*., 2005(31º46'S, 52º20"W), 5—Salvador *et al*., 2007 (27°17'S, 48°21'W; 27°28'S, 48°33'W; 27°43'S, 48°31''W), 6—Perez *et al*., 2008 (22°42'S, 47°38'W), 7—Miziara *et al*., 2008 (20º26'S, 54º38'W), 8—Martins *et al*., 2011 (18°06'S, 52°55'W), 9—Saraiva *et al*., 2012 (a—19º03'S, 42º55'W, b—20º36'S, 42º08'W, c—23º08'S, 43°30˙W), 10—Dantas—Torres *et al*., 2012 (7º47'S, 34º43'W), 11— Bomfim, 2013 (15°56'S, 47°56'W), 12—Labruna *et al*., 2014 (05º47'S, 35º15'W), 13—Silveira *et al*., 2015 (21º19'S, 51º49'W), 14—Sponchiado *et al*., 2015 (20º17'—21º15'S, 54º53'—56º31'W), 15—Tolesano—Pascoli, 2014 (18°55'S, 48°17'W), 16—Coelho *et al*., 2016 (18°54˙S, 48°15'W), 17—Krawczak *et al*., 2016 (28º08'S, 54º44''W), 18—Wolf *et al*., 2016 (16°15'S, 56°37"W), 19—Witter *et al*., 2016 (a—15°27'S, 55°44'W; b—09°54'S, 55°54'W), 20—Acosta *et al*., 2016 (18°24'S, 40°13'W; 19º56'S, 40º36'W), 21—Blanco *et al*., 2017 (26º46'S, 48º38'W), 22—Fontalvo *et al*., 2017 (09°19'S, 40°33'W; 08º40'S, 40º08'W), 23—Martins *et al*., 2017 (coordinates not informed), 24—Soares *et al*., 2015 (02º24'S; 54º42'W; 04º05'S; 54º54'W), 25—Krawczak *et al*., 2015 (27°14'26" S, 53°58'19" W and 27°13'51" S, 53° 51'04" W), 26—Costa *et al*., 2002 (coordinates not informed), 27—Martins *et al*., 2010b (08º00'S,35º01'W; 07º53'S,35º10'W), 28— Szabó *et al*., 2013b (24°25'23·6"S; 47°03'06·6"W

Discussion

In our study, *I. loricatus* and *A. sculptum* were not the most prevalent on hosts, but they were the most abundant tick species collected on *D. albiventris*. *Amblyomma sculptum* is a generalist tick and

commonly found on this host species, mainly in immature stages (Oliveira, 2004; Estrada-Pena *et al.* 2004). *Ixodes loricatus* is also associated to marsupials, but especially the adult ticks (Barros-Battesti *et al.* 2006).

Both host-parasite interactions between stages and species were reported on our study. Otherwise, the most frequent species were *A. coelebs* and *A. dubitatum*. Nymphs of *A. coelebs* seem to infest a broad host range, and they have been previously reported on *D. albiventris* (Labruna *et al.* 2002a; Labruna *et al.* 2005; Martins *et al.* 2011; Saraiva *et al.* 2012; Sponchiato *et al.* 2015). Tapirs (*Tapirus terrestris*) are the primary hosts for this tick species (Labruna & Guglielmone 2009), and are commonly reported in this region (Cáceres *et al.* 2008). *Amblyomma dubitatum* is a tick that depends on humid areas and parasitizes mainly capybaras, *Hydrochoerus hydrochaeris* (Aragão, 1936; Nava *et al.* 2010; Szabó *et al.* 2007; Queirogas *et al.* 2012). In this sense, the occurrence of this tick species is essentially associated with the presence of its primary host and adequate environment.

Co-infestation was verified in almost 50% of the hosts in this study. Co-occurrences of more than two species were common, and it was observed among all tick species pairs. Despite the importance of the interactions of different tick species on individual hosts in mechanisms of pathogens transmission among vectors (Randolph *et al.* 1996; Szabó *et al.* 2013a), few studies have mentioned these association patterns.

Didelphis albiventris usually presents high prevalence by ticks. Herein, we described 100% of prevalence. In the same region of Mato Grosso do Sul, Sponchiado et al. (2015) found 84.38% of this host species parasitized by ticks. Studies in different regions of the Brazil have reported prevalence ranging from 40 to 100%, using distinct capture efforts (Coelho *et al.* 2016; Saraiva *et al.* 2012; Muller *et al.* 2005; Dantas-Torres *et al.* 2012; Sponchiado *et al.* 2015). In many of these previous reports, *D. albiventris* also exhibits a high intensity of tick infestation. Intensity rates might oscillate due to collection accuracy and duration, behavior and age of hosts, as well as environmental factors, that obviously varies in different studies.

The richness and the composition of species are diversity components that depend on characteristics of each area (biome, preservation condition, phytophysiognomy, matrix) and especially for this parasite, on the presence of primary hosts for each tick species. In disturbed areas of Cerrado, *D. albiventris* is mainly infested by *A. sculptum*, *A. dubitatum*, and *I. loricatus* (Coelho *et al.* 2016). Conversely, in areas inserted into most natural matrices, other tick species are described on this host. We observed four tick species infesting *D. albiventris* in a single area, including the three species commonly found in degraded areas as mentioned before. Sponchiado *et al.* (2015) reported seven tick species in collections performed in a greater number of fragments distributed along five municipalities bordering the Pantanal.

In general, seasonal pattern of ticks is measured by sampling of free-living ticks and collection on hosts during a minimal period of two consecutive years. In this study, occurrences on hosts showed a concentration of larvae and nymphs in drier months, and for the most abundant tick, *A. sculptum*, the nymphs peaked in September of the first year. As explained above, the assessment of the infestation on hosts is variable and depends on many factors. Nevertheless, on-host ticks represent, to some extent, the environmental infestation. Thus, is supposed to expect that, herein, *Amblyomma* tick species present a seasonal pattern as demonstrated for other areas in Brazil (Labruna *et al.* 2002b).

Coincident with the broad distribution of *D. albiventris*, which occurs in the six Brazilian continental biomes (Pantanal, Pampa, Caatinga, Amazon, Atlantic Forest and Cerrado) (Roma, 2007), the parasitism by ticks is described in all these regions. Despite the different number of studies conducted in each biome, the reports are concentrated in the last two eco-regions, and the most frequent ticks were, respectively, *I. loricatus* and *A. sculptum.*

Particularly on *D. albiventris*, *I. loricatus* was reported in five studies carried out in the Atlantic Forest and in three studies in Cerrado areas, whereas *A. sculptum* was observed on this host from two areas of Atlantic Forest and from five savannic areas (four from Cerrado). Both ticks occurred inside and outside AC's, in Cerrado and Atlantic Forest. These findings were expected, because these two tick species seem to be associated with microclimatic and vegetation attributes, and not primarily with the biome or conservational status of each area. *Amblyomma sculptum* is the most collected free-living tick from forest formations in savannic areas (Ramos *et al.* 2016; Veronez *et al.* 2010; Souza *et al.* 2006), and in Atlantic Forest, it is observed in deforested areas, with savannic characteristics. *Ixodes* spp. are associated to humid forests, and they are frequently found on small mammals from Atlantic Forest and Amazon (Barros-Battesti *et al.* 2000; Díaz *et al.* 2009; Arzua *et al.* 2003; Luz *et al.* 2013; Martins *et al.* 2014). In the Cerrado, *I. loricatus* was reported on hosts from forest phytophysiognomies (Coelho *et al.* 2016).

It is necessary to mention that some studies from literature are anecdotal notes on infestations, whereas others are systematized works. Therefore, the analyses of infestation parameters have to be careful, as discussed before. Despite this restriction, all of them contribute to the knowledge on richness of tick species on *D. albiventris*. Additionally, the condensed information provides a large-scale vision of the association between tick species and this host from diverse vegetal formations in Brazil. Furthermore, it is relevant to connect the comprehension on the host-tick interactions to serological data and future studies on the infection rates by rickettsiae in these ticks.

In this sense, Binder *et al.* (2017) suggested that *D. albiventris* participate in the maintenance of the ecological cycle of Spotted Fever Group rickettsiae in Mato Grosso do Sul. Thus, similar studies have to be encouraged in areas with great abundance of this marsupial, mainly in patches of urban or peri-urban forests, where *D. albiventris* is especially important because it can be involved with the eco-epidemiological chain of the Brazilian Spotted Fever (Horta *et al.* 2007; Horta et al. 2009).

Aknowlegdments

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Conflict of interests

The authors declare to have no conflict of interest.

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