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Communication

[*Comunicação*]

Gastrointestinal parasites in marsupials from Atlantic Forest of Northeastern Brazil

[*Parasitos gastrointestinais em marsupiais da Mata Atlântica do nordeste do Brasil*]

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Marsupials have been recognized as animals of great ecological and sanitary relevance due to their role as disseminators of seeds and the involvement in the life cycle of several pathogens of zoonotic concern (Bezerra-Santos *et al*., 2021). Over the last decades, the contact between these animals and humans has been intensified because of the loss of natural habitat, which may imply in a negative impact for both animal and human species (Cooper *et al*., 2018). Currently, Brazil encompasses a high diversity of marsupials with at least 62 recognized species (Faria *et al*., 2019), being many of them directly affected by anthropic actions.

The synanthropic behavior presented by some marsupial species (e.g., *Didelphis* spp.) may facilitate the sharing of pathogens with domestic species (Roque *et al*., 2013). For instance, the black-eared opossum (*D. aurita*) captured in an urban area of Southeastern Brazil presented a high prevalence of *Ancylostoma caninum* (Bezerra-Santos *et al*., 2020), a common species to domestic dogs. Additionally, to helminths, gastrointestinal (*Eimeria* spp.) and blood (*Trypanosoma cruzi* and *Leishmania* sp.) protozoa are also reported, demonstrating that they can harbor a wide plethora of parasites of medical and veterinary concern (Teodoro *et al*., 2019).

Marsupials can thrive in different environments, benefiting from the resources (food and shelter) available in human-modified areas (Roque *et al*., 2013). The access of these animals to food available in the peri-urban areas is pivotal to their infection with zoonotic pathogens (Aragón-Pech *et al*., 2018). This synanthropic behavior is typical for species of generalist habitat; who have presented a good adaptation to situations of loss of natural habitat and fragmentation. Undoubtedly, this behavior acquired by some species is a consequence of anthropic actions, such as demographic pressures, agricultural exploitation, and disorderly occupation of the territory (Pinto *et al*., 2006).

Currently, the Atlantic Forest, an important natural habitat of several marsupial species, is restricted to only about 12.4% of its original area, and the area remains predominantly distributed in small forest fragments (SOS Mata Atlântica, 2021). Most of the remnants of the Atlantic Forest are very small and inserted in an anthropic matrix (Ribeiro *et al*., 2009). This scenario changes the natural structure of arboreal mammal populations, favoring the emergence of small and isolated populations that are more susceptible to parasitic infections (Fahrig, 2003). Therefore, the aim of this study was to report the occurrence of gastrointestinal parasites of marsupials captured in fragments of the Atlantic Forest in Northeastern Brazil.

The study was conducted in two remnants of the Atlantic Forest located in the state of Sergipe, Northeastern Brazil, the forest fragment of the

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Federal University of Sergipe – UFS – Campus, and the legal forest reserve of Santana farm – SF. The forest fragment of the UFS is in the São Cristóvão Campus (10º55'S, 37º04'W), peri-urban parkland surrounded by a 30ha forest remnant (Rocha *et al*., 2011). The legal forest reserve of SF (600ha; 10°32'07"S, 36°45'54"W) is a sugarcane plant located between the municipalities of Japoatã and Pacatuba (Pedroso *et al*., 2020). A predominant seasonal semi-deciduous vegetation features these areas, with annual temperature mean ranging from 24 to 30°C, and tropical climate (*As*) according to the Köppen's climate classification (Alvares *et al*., 2013).

From June 2017 to January 2018 marsupials were captured using live traps (Tomahawk 40cm x 12cm x 12cm; Zootech®), which were armed on the ground along six transects of 15 points and checked daily, totaling a sampling effort of 90 trap-nights. The traps were baited with a mixture of corn meal, sardine, peanut butter, banana, oatmeal, bacon, and vanilla essence to attract species with different types of diet (Astúa *et al*., 2006). The animals were marked with numbered aluminum earrings ("Ear Tags") and released at the same point of capture. Fresh fecal samples were obtained after spontaneous defecation. All material was kept in isothermal boxes at 4°C until laboratory processing.

The captured animals were identified by external morphometry according to Faria *et al*. (2019). All animals were handled according to the American Society of Mammalogists (Sikes and Gannon, 2011). The research was authorized by the ICMBio permit (SISBio 11283-2).

Fecal samples were processed individually and analyzed through FLOTAC technique (Cringoli *et al*., 2010). The FLOTAC was performed with two flotation solutions (sodium chloride, specific gravity, s.g. = 1.200 and zinc sulfate, s.g. = 1.350). All parasitic stages observed were morphologically identified based on previous taxonomic keys (Taylor *et al*., 2017).

Initially, relative and absolute frequencies were calculated through descriptive statistics. Subsequently, the Lilliefors test was used to verify the normality of the data. The Chi-square (χ^2) test was used to compare the overall positivity for different marsupial species. Additionally, the Kruskal-Wallis test was used to compare different helminths/protozoa (genus/family) according to each marsupial species. For all tests, a significance level of 5% was considered, and the BioEstat software version 5.3 was used (Ayres *et al*., 2007).

A total of 88 animals (37 *Marmosops incanus*, 30 *Marmosa demerarae*, 20 *Didelphis albiventris*, and 01 *Marmosa murina*) were captured. The most parasitized species was *D. albiventris* (45.95%) followed by *M. incanus* (43.24%) and *M. demerarae* (23.3%), without significant difference ($\chi 2$ = 3.579; p = 0.1671). *M. murina* was not parasitized.

Overall, eggs of helminths were detected in 47.72% (42/88) of samples, whereas oocysts of protozoa in 32.95% (29/88). In particular 12 different types of parasites were diagnosed, but those of the Family Ancylostomatidae predominated over the others (Table 1).

Interestingly, *M. incanus* and *M. demerarae* hosted six different gastrointestinal parasites (H = 14.4282; p = 0.0007). Although frequent, *Ancylostoma* sp. eggs did not differ statistically from other helminths (H= 5.9910; p = 0.1120). It is important to highlight that *D. albiventris* was the most parasitized species, hosting 83.33% (10/12) of the gastrointestinal parasites herein diagnosed, with predominance of *Ascaris* sp. eggs (H = 6.6093; p = 0.3585). Coccidea predominated over *Giardia* sp. and *Entamoeba* sp. (H = 14.7016; p = 0.0006). The detailed information about co-infections is summarized in Table 2.

This study reports the parasitism by different gastrointestinal parasites in marsupials captured from the Atlantic Forest of Northeastern Brazil. Although only half of the animals were parasitized, those positive samples represent three species of wild mammals captured. This finding demonstrates the susceptibility of several species of marsupials to gastrointestinal parasites, which may be related also to the generalist feeding behavior presented by these animals (Jiménez *et al*., 2011). It is known that the diet changes according to the environment where they are inserted, so it is possible to have availability of food from wild, rural, and urban environments (Bezerra-Santos *et al*., 2021). It is important to note that *D. albiventris* was the species more parasitized. This animal has been considered a generalist mammal with high prolificity and adaptability to the anthropic environments. Therefore, its interaction with other synanthropic animals, domestic animals and humans may contribute for the sharing of pathogens among all species involved (Janes *et al*., 2017).

Table 1. Absolute and relative frequencies for each gastrointestinal parasite found in marsupials

Host	*M. incanus*			*M. demerarae*			*D. albiventris*			Total		
	N	AF	RF%	N	AF	RF%	N	AF	RF%	N	AF	RF%
Helminths												
Ancylostoma sp.	37	10	27.03	30	5	16.67	20	3	15.00	88	18	20.45
Ascaris sp.	37	0	0.00	30	0	0.00	20	4	20.00	88	4	4.55
Aspiculuris sp.	37	0	0.00	30	0	0.00	20	2	10.00	88	2	2.27
Capillaria sp.	37	1	2.70	30	2	6.67	20	1	5.00	88	4	4.55
Hymenolepis sp.	37	0	0.00	30	0	0.00	20	3	15.00	88	3	3.41
Physaloptera sp.	37	0	0.00	30	1	3.33	20	0	0.00	88	1	1.14
Strongyloides sp.	37	0	0.00	30	1	3.33	20	0	0.00	88	1	1.14
Toxocara sp.	37	0	0.00	30	0	0.00	20	1	5.00	88	1	1.14
Trichuris sp.	37	3	8.11	30	3	10.00	20	2	10.00	88	8	9.09
Protozoa												
Coccidiida oocyst	37	9	24.32	30	2	6.67	20	11	55.00	88	22	25.00
Entamoeba sp.	37	1	2.70	30	0	0.00	20	4	20.00	88	5	5.68
Giardia sp.	37	1	2.70	30	0	0.00	20	1	5.00	88	2	2.27

N: number of specimens evaluated; AF: absolute frequency; RF: relative frequency.

Table 2. Frequency of gastrointestinal co-infections

Marmosops incanus	AF	RF%
Ancylostoma sp. + Coccidiida oocyst	5	13.51
Ancylostoma sp. + *Trichuris* sp.	2	5.41
Ancylostoma sp. + *Capillaria* sp. + *Trichuris* sp.	1	2.70
Marmosa demerarae	AF	RF%
Ancylostoma sp. + *Trichuris* sp.	2	6.67
Ancylostoma sp. + Coccidiida oocyst + *Strongyloides* sp.	1	3.33
Ancylostoma sp. + *Capillaria* sp. + *Physaloptera* sp. + *Trichuris* sp.	1	3.33
Didelphis albiventris	AF	RF%
Aspiculuris sp. + *Hymenolepis* sp.	1	2.70
Coccidiida oocyst + *Hymenolepis* sp.	1	2.70
Ancylostoma sp. + *Ascaris* sp. + Coccidiida oocyst	1	2.70
Ancylostoma sp. + *Ascaris* sp. + Coccidiida oocyst +*Toxocara* sp.	1	2.70
Ascaris sp. + *Aspiculuris* sp. + *Capillaria* sp. + *Entamoeba* sp.	1	2.70
Ascaris sp. + Coccidiida oocyst + *Entamoeba* sp. + *Giardia* sp. + *Hymenolepis* sp. + *Trichuris* sp.	1	2.70

AF: absolute frequency; RF: relative frequency.

Eggs belonging to *Ancylostoma* sp. were those more frequently detected in this study. This retrieval has been a common finding in these animals, especially in *D. albiventris* where a positivity of up to 100% has been observed (Teodoro *et al*., 2019). Unfortunately, in this study the identification at level species of parasites was not achieved. However, recently the retrieval of *A. caninum* in *D. albiventris* sounded as an alert, indicating the strict sharing of this nematode species between wild and domestic animals (Bezerra-Santos *et al*., 2020).

Co-infections with other gastrointestinal parasites were a common finding. This is a consequence of the lifestyle of these animals, as well as the availability of food in some areas that exposes them to different species (Jimenez *et al*., 2011). Some of the parasites detected here may be related to zoonotic infections (e.g., *Giardia* and *Entamoeba*), raising concern about the spill-over of pathogens from humans and domestic animals into wildlife (Vermeulen *et al*., 2015). Among all marsupial species assessed, the role of *D. albiventris* as synanthropic species is notable, for this reason these animals have been extensively studied over the last years (Bezerra-Santos *et al*., 2021).

Overall, this study brings important data about the gastrointestinal fauna of marsupials demonstrating the wide variety of parasites in which these animals are exposed. It is important to adopt measures of conservation of natural habitat of these animals to avoid its interaction with domestic animals and human species. Although, at the moment of the sampling no animal presented clinical signs suggestive of the infection by gastrointestinal parasites, the impact of this kind of parasitism needs to be better assessed to clarify its role in the conservation of these marsupials' species.

Keywords: parasitological diagnosis, mammals, FLOTAC, endoparasites

RESUMO

Os marsupiais estão envolvidos no ciclo de vida de vários patógenos de interesse médico e veterinário. O objetivo deste estudo foi relatar a ocorrência de parasitos gastrointestinais em marsupiais capturados em fragmentos da Mata Atlântica, estado de Sergipe, nordeste do Brasil. De junho de 2017 a janeiro de 2018, marsupiais foram capturados usando-se armadilhas, e foram obtidas amostras fecais frescas após defecação espontânea. Os animais foram identificados morfometricamente e as fezes analisadas pela técnica FLOTAC. Foram capturados 88 animais, sendo 37 Marmosops incanus, *30* Marmosa demerarae, *2*0 Didelphis albiventris e 01 *Marmosa murina*. A espécie mais parasitada foi D. albiventris (45,95%) *seguida de* M. incanus (43,24%) e M. demerarae (23,3%). *No geral, ovos de helmintos foram detectados em 47,72% (42/88) das amostras, enquanto oocistos de protozoários em 32,95% (29/88). Ovos de* Ancylostoma *sp. predominaram sobre outros parasitos. Este estudo aponta para a ocorrência de parasitos gastrointestinais e contribui para um melhor entendimento do parasitismo em marsupiais que vivem em fragmentos florestais da Mata Atlântica.*

Palavras-chave: diagnóstico parasitológico, mamíferos, FLOTAC, endoparasitos

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