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Gastrointestinal parasitism in nine-banded armadillos (*Dasypus novemcinctus*) under different hunting pressures in the Reserva Natural del Bosque Mbaracayú, Paraguay.

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The aim of this study was to characterize the gastrointestinal parasitic fauna of nine-banded armadillos (*Dasypus novemcinctus*) of the Reserva Natural del Bosque Mbaracayú (RNBM), Canindeyú, Paraguay, and to evaluate how human activities, particularly hunting, may influence parasitism patterns and zoonotic risk. We recovered 15 distinct parasites from fresh fecal samples of 73 individuals of *D. novemcinctus* including Protozoa*: Eimeria* sp., *Giardia* sp*.,* coccidia (Family Adeleidae), unidentified coccidia; Cestodes: *Hymenolepis* sp.*;* Nematodes: *Trichuris* sp., Trichostrongyloidea, *Cruzia* sp., *Ancylostoma* sp., *Uncinaria* sp., *Aspidodera* sp., *Strongyloides* sp. and Acanthocephala. We report high prevalence of potentially zoonotic parasites including *Ancylostoma, Trichuris, Uncinaria* and Coccidia. We also found an increase in parasite richness in areas where hunting was reported *vs.* areas without hunting and the same trend for Nematode probability of presence, especially during the cold dry season. These results suggest that human activities may be affecting patterns of parasitism in wild armadillos. In addition, traditional consumption of these animals in this region may present a route of human exposure to zoonotic parasites.

El objetivo de este estudio fue caracterizar la fauna parasitaria gastrointestinal de armadillos de nueve bandas (*Dasypus novemcinctus*) de la Reserva Natural del Bosque Mbaracayú (RNBM), Canindeyú, Paraguay y evaluar cómo las actividades humanas, particularmente la caza, pueden influir en los patrones de parasitismo y el riesgo de zoonosis. Recuperamos 15 parásitos distintos de muestras fecales frescas de 73 individuos de *D. novemcinctus*, incluidos protozoos: *Eimeria* sp., *Giardia* sp., coccidias (familia Adeleidae), coccidias no identificadas; Cestodos: *Hymenolepis* sp.; Nematodos: *Trichuris* sp., Trichostrongyloidea, *Cruzia* sp., *Ancylostoma* sp., *Uncinaria* sp., *Aspidodera* sp., *Strongyloides* sp. y Acanthocephala. Informamos una alta prevalencia de parásitos potencialmente zoonóticos, incluidos *Ancylostoma, Trichuris, Uncinaria* y Coccidia. También encontramos un aumento en la riqueza de parásitos en áreas donde se reportó caza *versus* áreas sin caza y la misma tendencia en la probabilidad de presencia de nematodos, especialmente durante la estación fría y seca. Estos resultados sugieren que las actividades humanas pueden estar afectando los patrones de parasitismo en los armadillos salvajes. Además, el consumo tradicional de estos animales en esta región puede presentar una vía de exposición humana a parásitos zoonóticos.

Keywords: Zoonoses, helminths, prevalence, human-wildlife interface

Introduction

Nine-banded armadillos (*Dasypus novemcinctus* Linnaeus, 1758) extend from Colombia and Venezuela to Argentina (MacDonald 1985; Redford and Eisenberg 1992; Loughry *et al.* 2015; Barthe *et al.* 2024). In Paraguay, they occur throughout the country, but are considerably rare in arid areas (Smith 2007; De la Sancha *et al.* 2017; Smith and Ríos 2018). They are nocturnal, generalist insectivores that spend their days in self-dug burrows, often shared by multiple individuals from the same family group (Taber 1945; Nowak 1991; Parera 2002; Smith 2007). In Paraguay, many human cultural groups use this species as a food resource including Aché, Mbyá, and Guaraní indigenous groups and farmers from rural settlements (McBee and Baker 1982; Esquivel 2001; Neris *et al.* 2002; Hill *et al.* 2003; Smith 2007; Morales 2009).

Although the parasites of *D. novemcinctus* are well characterized at the northern extent of their range (i.e.: Chandler 1946; Radomski *et al.* 1991; Varela-Stokes et al. 2008; Jimenez-Ruiz et al. 2013), previous research in the southern extent of their range is more limited. For example, Fujita *et al.* (1995) reported some nematodes recovered from the gastrointestinal tract of just two specimens (Dept. of San Pedro, Central Paraguay): *Ancylostoma* sp., *Aspidodera esperanzae* (possibly *A. binansata* -Jimenez-Ruiz *et al.* 2006), *Moeniggia complexus, M. pintoi* and an unidentified species of Heterakinae. Navone (1990) recorded the nematode *Aspidodera vazi* (Aspidoderidae) and the cestode *Mathevotaenia surinamensis* (Anoplocephalidae) in the Paranaense and Chaco regions of Argentina, with *Aspidodera fasciata* (Aspidoderidae) additionally recorded in the Chaco region. In Santa Cruz, Bolivia Jimenez-Ruiz *et al.* (2003) reported Aspidoderid nematodes *Aspidodera binansata* (n=173) and *Lauroia bolivari* sp. nov (n=22) from the gastrointestinal tract of one individual. Hoppe and do Nascimento (2007) reported at least 13 species of nematodes recovered from the gastrointestinal tract of nine individuals in Pantanal (Mato Grosso, Brazil). Also, in Bolivia, Deem *et al.* (2009) reported the following parasites from 22 individuals: Heterakidae, *Aspidodera* sp., *Trichuris* sp., Adelaidae (protozoan), and *Strongylus* sp.

The species can coexist with humans in rural areas because of its largely nocturnal habits and large litter size but has declined over much of its range in eastern Paraguay because of destruction of optimal habitats and hunting pressure (Smith 2007). Armadillos of the studied species may present zoonotic pathogens thus hunting and human consumption of these species or sharing edge habitats may have a significant public health impact (Stallknecht *et al.* 1987; Yaeger *et al.* 1988; Truman 2005; Deem *et al.* 2009).

The aim of the current study was twofold: 1) to begin the characterization of the gastrointestinal parasitic fauna of nine-banded armadillos (*Dasypus novemcinctus*) of the Reserva Natural del Bosque Mbaracayú (RNBM), Canindeyú, Paraguay; and 2) to explore differences in parasite diversity and prevalence between areas exposed to human activities (i.e.: traditional and illegal hunting) and more remote, undisturbed areas within the reserve. In addition to determining the parasites present and their prevalence in *Dasypus novemcinctus* within the RNBM, we also aimed to determine if the parasite communities and/or the prevalence of specific taxa differed in armadillos from hunted areas compared to those from non-hunted areas. By comparing infection patterns across zones with contrasting hunting pressure, we also aimed to evaluate the influence of these human practices on parasite in armadillos, as well as the potential zoonotic risks for individuals involved in their hunting and consumption (Medkour *et al.* 2020; Inada 2023; Mozer and Prost 2023; Salvarani *et al.* 2025).

Methods

Study site. Our study was conducted as part of a larger research program to investigate infectious diseases dynamics in the protected area Mbaracayú Forest Nature Reserve (RNBM) and its surroundings in Paraguay. Specifically, this study was done at the Reserva Natural del Bosque Mbaracayú (RNBM), (24° 00' - 24° 15' S, 55° 00' - 55° 32' W), Depto. Canindeyú, Paraguay. The RNBM represents a c. 640 km^2 protected area composed mostly of Upper Parana Atlantic Forest. Mbaracayú was established in 1991 and is privately managed by a Paraguayan non-governmental organization (Fundación Moises Bertoni). The study area is also within a UNESCO biosphere reserve, which includes the protected area Mbaracayú Forest Nature Reserve (RNBM), as a core preserved area.

The climate is warm, humid, and temperate, with an average annual temperature of 21–22 °C and annual rainfall ranging from 1,650 to 1,800 mm, peaking between October and March. Based on the classification by Owen *et al.* (2010), the year is divided into three seasons: transitional (T: February–May), dry (D: June–September), and wet (W: October–January). Frosts can occasionally occur from June to October. The landscape consists of a mosaic of natural forest and savanna ecosystems, including High Forest, Medium and Low Forests with bamboo, wetlands, cerrado, and native grasslands. Forests range from mature stands with open understories to dense, bamboo-dominated areas (*Chusquea ramosissima*, *Guadua* spp.), while savannas include Cerrado formations and grassy riparian zones, contributing to the area's ecological complexity (FMB/BM 2005).

Sampling was conducted in two types of areas within the RNBM: (1) areas under direct human influence, such as zones where hunting occurs, and (2) remote, minimally disturbed forested areas where no hunting occurs (Figure 1). One of the human-impacted sites was located near Arroyo Bandera, the closest community to the reserve. In this region, indigenous communities have legal authorization to practice traditional hunting, while illegal hunting by non-Indigenous individuals has also been reported both along the edges and within the interior of the reserve. These designations were based on reports from rangers responsible for protection and monitoring of the RNBM. In the hunting zones, the most predominant domestic species observed was the dog (*Canis lupus familiaris*), which is commonly used by hunters as a companion and a hunting aid during their excursions into the forest (Kowalewski *et al.* 2019).

Study subjects. Sample collection occurred from June 2012 to August 2013, and from September 2016 to June 2017. For subsequent analyses, samples were classified according to the three seasonal periods previously defined (transitional, dry and wet). Fresh fecal samples were collected from *D. novemcinctus*, primarily from individuals captured by hand by Aché technicians with expertise in traditional hunting practices. Additional samples were obtained opportunistically from fresh feces found in the field, attributed to non-captured individuals. Once captured, each armadillo was placed in an individual breathable bag and transported to our nearby base camp where our veterinarian performed a health assessment and obtained a fecal sample from each individual. All armadillos were weighed while in the bag and then immobilized

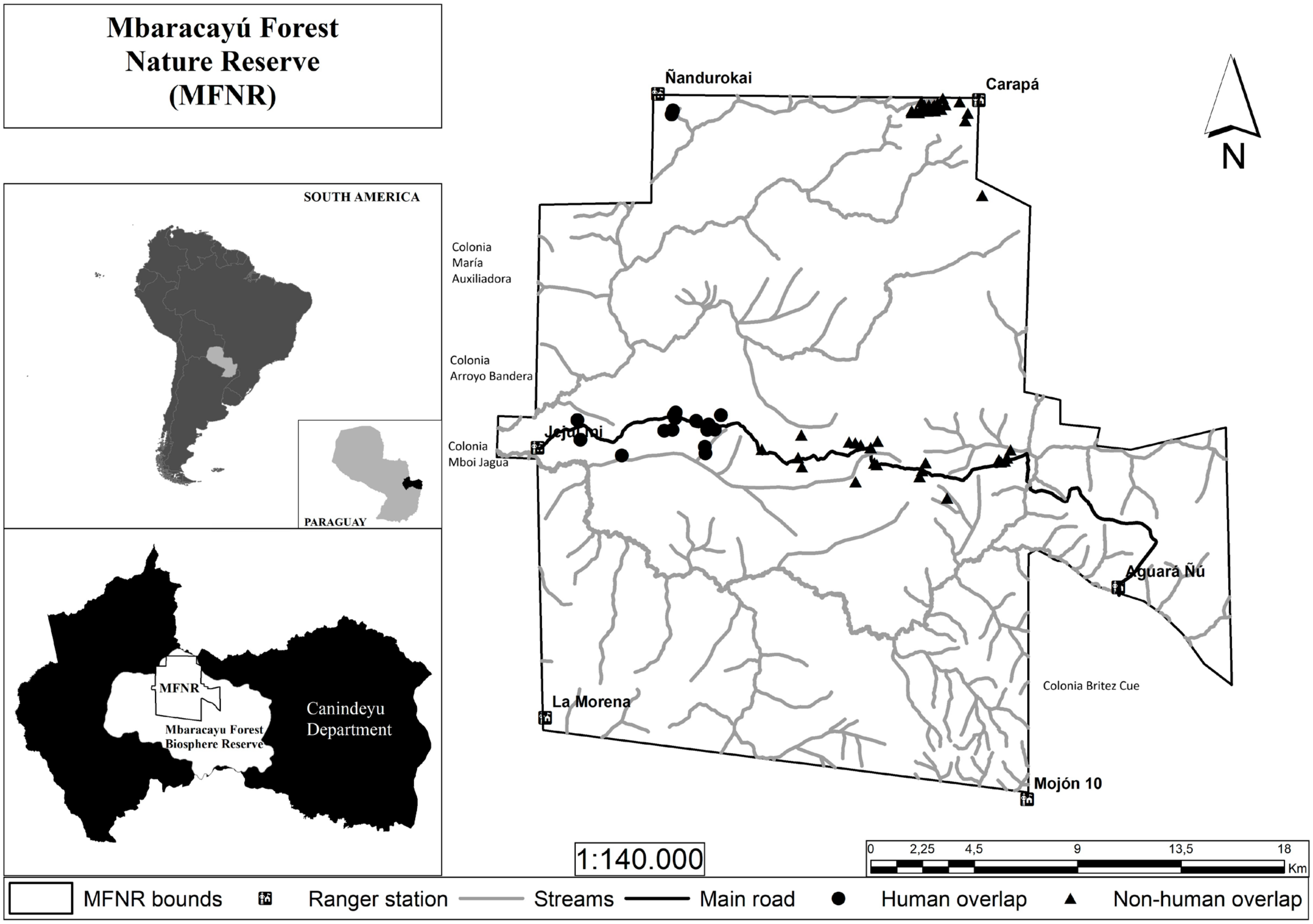


Figure 1. Location of *Dasypus novemcinctus* sampling sites with and without hunting within the Reserva Natural del Bosque Mbaracayú, Paraguay.

using ketamine-zolazepam for physical examination and collection of biologic samples. After sampling, armadillos were released at the site of capture. All fecal samples were preserved in 10% buffered formalin in 20 ml plastic tubes and labeled. We did not repeat capture sites, and armadillos were marked previous release to avoid recapture.

Parasitological Analyses. Fecal samples were examined using direct microscopic examination. Techniques to recover eggs included Willis, Sheather and zinc sulphate methods (MAAF 1986; Thienpont *et al.* 1986; Foreyt 1989) at the Parasitological Laboratory of the Centro para el Desarrollo de la Investigación Científica (CEDIC, Asunción, Paraguay). Slides (18 mm x 18 mm) prepared by each method were observed under a light microscope (LEICA DM500) after the addition of a drop of Lugol's iodine and photographed with a Leica camera (ICC50W). Parasites were identified based on egg, larvae, or cyst coloration, shape, contents, and size following Anderson *et al.* (1974), Khalil *et al.* (1994), Lainson and Shaw (1982), Mehlhorn *et al.* (1992), and Navone (1986; 1987; 1988, 1990). Each slide was scanned thoroughly under both 10x and 40x objective lenses to confirm presence or absence of helminth eggs and larvae and protozoan cysts. Representatives of each parasite species recovered per sample were measured at 400x to the nearest 0.1 µm with the Leica Application Suite software (2016) and representative parasite species were photographed. Morphological examination of eggs and oocysts allowed diagnosis to the genus level or higher levels for some parasites. Thus, the taxonomic level achieved through morphological identification of parasite eggs or oocysts depended on the parasite, as some genera had distinctive eggs enabling genus-level diagnosis, while others could only be identified at higher taxonomic levels, such as the superfamily Trichostrongyloidea

Statistical analyses. We estimated the prevalence (as the proportion of individuals positive for a given parasite divided by the total number of armadillos examined) and richness (as the number of the parasite species/group present per individual). Using Generalized Linear Models (GLM), we analyzed the effects of environmental, hunting and sex of the individual on richness and parasite occurrence (presence/absence of parasites) (Table 1). For these analyses we divided data into broad categories, presence of nematodes, presence of cestodes and presence of protozoan parasites (Model 2.a; 2.b; 2.c respectively). Full models were then restricted by removing non-significant terms in a stepwise

Table 1. Variables summary for GLM model.

Model	Variable *Dependent variables*	Definition
1	Richness	Count (min= 1; max=9)
2. a	Presence nematodes	Binary response (absent=0; presence=1)
2. b	Presence cestodes	Binary response (absent=0; presence=1)
2. c	Presence protozoa	Binary response (absent=0; presence=1)
	Independent Variables	
	Hunting	Categorical variable, 2 levels (yes, no)
	Sex	Categorical variable, 2 levels (male, female)
Environmental	*Season	Categorical variable, 3 levels (T= transitional; D= dry; W= wet)
	Monthly total precipitation	Continuous variable (min=29; max=408, mean=166.8)

backward elimination manner, using likelihood ratio tests, until we obtained a final model that contained only significant variables. GLM were fit by maximum likelihood (Laplace Approximation), using the function "glm" from the MASS package (Venables and Ripley 2002). Poisson (log) distribution was used for the richness model and Binomial (logit) distribution for the prevalence set of models. For the first model we verified Poisson distribution of dependent variable "richness" graphically (plot distribution of cumulative frequencies and qq-plot). The assumptions of normally distributed and homogeneous residuals (Pearson residuals) were checked by visually inspecting qq-plots and the residuals plotted against the fitted values. We used R version 3.2.1 (The R Project for Statistical computing, www.r-project.org) for the analyses, and statistical significance was set at a p-value of 0.05. for all interpretations.

Compliance with ethical standards. We have followed ARRIVE guidelines and all procedures complied with relevant legislation, including the U.K. Animals (Scientific Procedures) Act (1986), EU Directive 2010/63/EU, and the NIH Guide for the Care and Use of Laboratory Animals. We used the minimum number of animals required for statistically valid results. The study adhered to Paraguayan law (N° 96/92 on wildlife), with permits for scientific collection (N°06/2012 and 270/2016) granted by Ministry of the Environment and Sustainable Development (MADES-Paraguay in Spanish).

Results

We collected a total of 78 fresh fecal samples. Of these, 66 correspond to *D. novemcinctus* captured by hand and the other 12 samples correspond to fresh fecal samples of non-captured armadillos. Of the 66 weighed armadillos, 32 were females and 34 males. There were no differences in weight between females (4.6 ±0.8 kg) and males (4 ± 1.2 kg) (p>0.05). All captured individuals showed good health status based on physical examination.

We recovered 15 distinct parasite taxa from 73 (out of 78) individuals including Protozoa: *Eimeria* sp., *Giardia* sp., coccidia (Family Adeleidae), unidentified coccidia; Cestodes: *Hymenolepis* sp.; Nematodes: *Trichuris* sp., Trichostrongyloidea, *Cruzia* sp., *Ancylostoma* sp., *Uncinaria* sp. *Aspidodera* sp., *Strongyloides* sp. and Acanthocephala (possible *Travassosia* sp.) (Table 2, Figure 2). The total

Table 2. Prevalence of parasites recovered in fresh fecal samples from 73 individual *Dasypus novemcinctus* (out of 78 individuals examined) at Reserva Natural del Bosque de Mbaracayú, Paraguay. Twenty individuals are from hunting areas and 53 individuals are from areas without hunting.

Parasite/group	Prevalence (%)		
Protozoa	Total	Hunting	Without Hunting
Giardia sp.*	6.9	21.4	3.4
Eimeria sp. (Coccidia)	5.5	14.3	3.4
Unidentified Coccidia	49.3	71.4	44.1
Adeleidae (Coccidia)	16.4	42.9	10.2
Cestoda			
Unknown Cestode 1	5.5	14.3	3.4
Hymenolepis sp.*	4.1	7.2	3.4
Nematoda			
Strongyloides sp.	6.9	7.2	6.8
Trichostrongyloidea	8.2	21.4	5.1
Trichuris sp.	90.4	100	88.1
Uncinaria sp.*	20.6	42.9	15.3
Ancylostoma sp.	67.1	57.1	69.5
Cruzia sp.	4.1	7.1	3.4
Aspidodera sp.	15.0	21.4	13.6
Unknown Nematode 1	1.4	7.1	0
Acanthocephala (possibly *Oligacanthorhynchus*)	1.4	7.1	0

(*) indicate new records for the species.

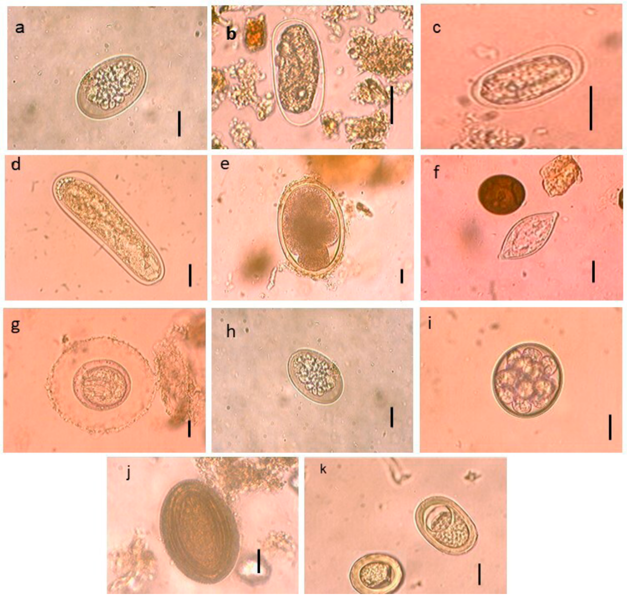
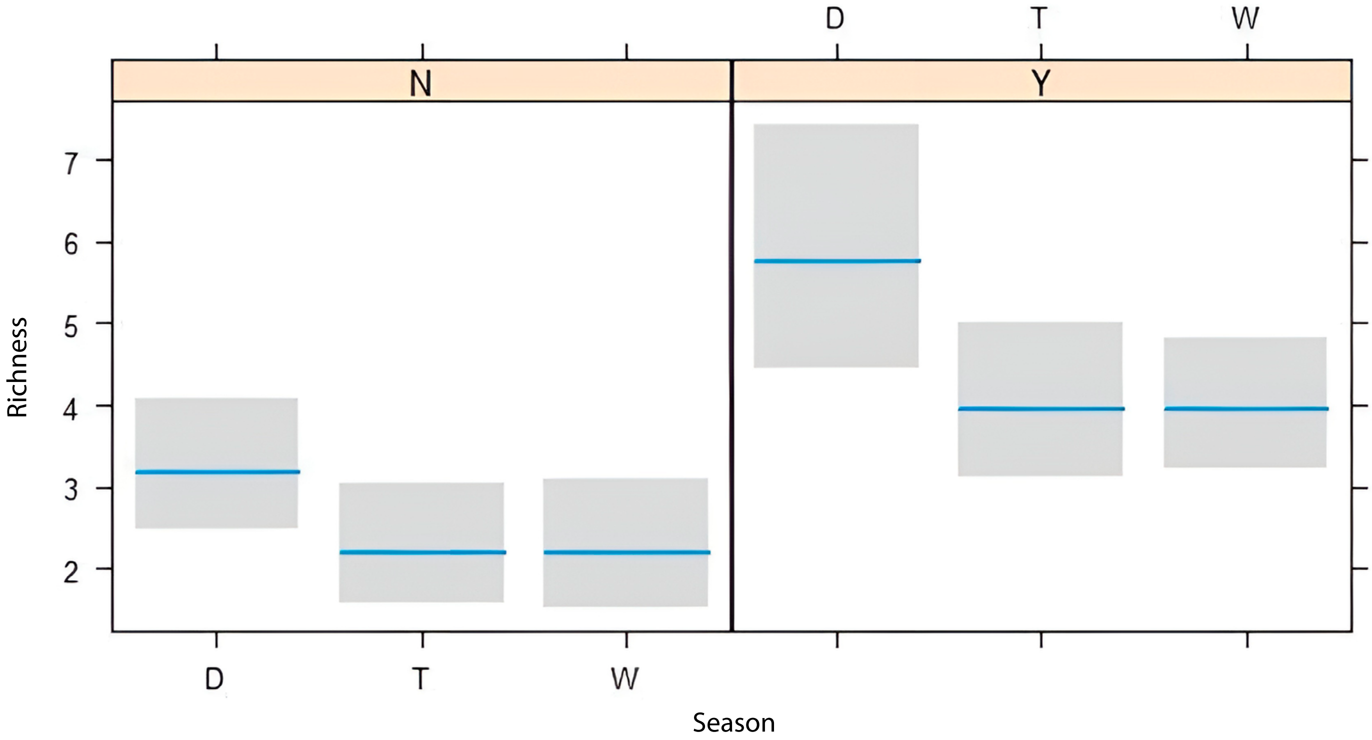


Figure 2. Parasites recovered from fecal samples of *Dasypus novemcinctus* within the Reserva Natural del Bosque Mbaracayú, Paraguay. (a) *Ancylostoma* sp. 1 (b) *Uncinaria* sp. (c) *Strongyloides* sp. (d) Trichostrongyloidea (e) *Cruzia* sp. (f) *Trichuris* sp. (g) *Hymenolepis* sp. (h) *Aspidodera* sp. (i) Coccidio, Adeleidae Family (j) Acanthocephala (possible *Travassosia* sp.) (k) Cestode 1 Scale bar is 20 µm.



N = Sites without hunting; Y = Sites with hunting.

Figure 3. Richness GLM results. Blue line represents expected richness and gray boxes are standard deviations according to GLM. Seasons are T, transitional (February–May); D, dry (June–September); W, wet (October–January) following Owen *et al.* (2010).

prevalence of gastrointestinal parasites was 93.6%. Both *Ancylostoma* sp. and *Trichuris* sp. presented the highest prevalence: 67.1% and 90.4% respectively. When coccidia parasites were pooled, the general prevalence reached 71.2% (Table 2). The number of distinct parasites per individual ranged from 1-9.

Parasite richness was higher in sites with hunting and during the dry season compared to sites without hunting and the wet season, respectively (Figure 3). Neither sex nor precipitation were significant contributors to the final model (Table 3). Presence / absence models showed a trend toward hunting pressure being associated with a higher probability of the presence of nematodes (Table 4). Null models were selected in protozoan and cestode models.

Table 3. GLM results, independent variables estimate of the model selected for richness.

Model	Estimate	Std. Error	z value	Pr(>|z|)
(intercept)	1.16	0.12	9.27	0.001***
hunting (Yes)	0.59	0.15	3.81	0.001***
season (T)	-0.37	0.16	-2.33	0.01*
season (W)	-0.37	0.16	-2.32	0.02*

Table 4. GLM results, independent variables estimate of the model selected for presence / absence of nematode.

Model	Estimate	Std. Error	z value	Pr(>|z|)
(intercept)	1.7	0.54	3.14	0.01**
hunting (Yes)	18.8	2614.2	0.007	0.99

Discussion

In this study we recovered 15 distinct parasites from 73 wild *D. novemcinctus* in Reserva Natural del Bosque Mbaracayú (RNBM) including taxa known to be prevalent in species of the Order Xenarthra, including coccidia of the Families Adeleidae and Eimeriidae (Lange and Wittenstein 2001; Ezquiaga *et al.* 2014), nematodes of the genera *Aspidodera* (Vicente 1966; Vicente *et al.* 1997; Hoppe and Nascimento 2007) and *Cruzia* (Fujita *et al.* 1995; Vicente *et al.* 1997; Hoppe and Nascimento 2007), and an acanthocephala, presumed to be *Oligacanthorhynchus* (syn, *Travassosia*) sp., (Travassos 1917; Lent and Freitas 1938; Smales 2007; Gomez-Puerta 2012). Notably, *Giardia* spp., *Hymenolepis* spp., and *Uncinaria* spp. were recorded for the first time in this host species, expanding the known parasitic diversity of *D. novemcinctus*.

The richness found in our research is similar to that recorded by Hoppe and do Nascimento (2007) in Brazil, and higher than that recorded by Fujita *et al.* (1995) in Paraguay, Navone (1990) in Argentina and Deem *et al.* (2009) in Bolivia. However, methodological differences and ecological contexts across these studies must be considered. For instance, Fujita *et al.* (1995) examined only two museum specimens, with no specific environmental information available. Navone (1990) analyzed 17 gastrointestinal tracts from individuals collected in temperate grasslands and subtropical regions of Buenos Aires and Corrientes provinces. Hoppe and do Nascimento (2007) also relied on gastrointestinal tract analysis, although detailed ecological context was not provided. In contrast, Deem et al. (2009) analyzed fecal samples from 22 individuals collected within the Gran Chaco region of Bolivia, a dry, thorny forest and savanna ecosystem. These variations among samples (feces vs. gastrointestinal tracts), sample size, and habitat likely influence parasite detection and diversity, and should be carefully considered when comparing across studies. Nevertheless, a summary of parasite records across different countries is presented in Table 5, to contextualize our findings and highlight general patterns and knowledge gaps.

Table 5. Reported parasites of *Dasypus novemcinctus* across its distribution range.

Parasite	**Sample**	**Location**	**Reference**
Brachylaemus virginianus, Oncicola canis, Hamanniella sp., *Aspidodera fasciata, Physocephalus* sp., *Ascarops* sp.	GI tracts (N= 8)	USA	Chandler 1946
Aspidodera vazi (Aspidoderidae) and the cestode *Mathevotaenia surinamensis* (Anoplocephalidae), *Aspidodera fasciata (Aspidoderidae)*	GI tracts (N=17)	Argentina	Navone 1990
Macracanthorhynchus ingens, Centrorhynchus sp., *Physaloptera* sp.	GI tract (N=117)	USA	Radomski *et al.* 1991
Ancylostoma sp., *Aspidodera esperanzae* (possibly *A. binansata* -Jimenez-Ruiz *et al.* 2006), *Moeniggia complexus, M. pintoi*, unidentified species of Heterakinae.	GI tract (N= 2)	Paraguay	Fujita *et al.* 1995
Aspidodera binansata, Lauroia bolivari sp. nov	GI tract (N=1)	Bolivia	Jimenez-Ruiz *et al.* 2003
Aspidodera sogandaresi, Aspidodera vazi, Aspidodera binansata, Lauroia trinidadensis, *L. trinidadensis,*	GI tract (N= 13)	Mexico Panama USA	Jimenez-Ruiz *et al.* 2006
Macielia macieli, M. flagellata, Moennigia moennigi, M. complexus, M. pintoi, M. littlei, Delicata variabilis, Hadrostrongylus speciosum, Strongyloides ratti, Aspidodera fasciata, A. binansata, A. vazi, Cruzia spp.	GI tract (N= 9)	Brazil	Hoppe and do Nascimento 2007
Aspidodera cf. soganderesi, Macracanthorhynchus ingens	GI tract (N=?)	USA	Varela-Stokes *et al.* 2008
Heterakidae, *Aspidodera* sp., *Trichuris* sp., Adelaidae, *Strongylus* sp. eggs	Fecal samples (N= 22)	Bolivia	Deem *et al.* 2009
Mathevotaenia sp., *Centrorhynchus* sp.	GI tract (N= 30)	Brazil	Gomes *et al.* 2011.
Cestoda, Trematode eggs, Trichostrongylidae and Ascarididae eggs, *Capillaria* sp.	Fecal samples (N= 4)	Brazil	Santos *et al.* 2024

GI= gastrointestinal, N= number of samples collected.

Overall parasite richness was higher in areas with hunting, with the strongest effect occurring during the dry season (from June to September). We found a trend toward the same pattern when comparing the probability of parasite presence / absence (i.e., higher probability of presence in areas with hunting and during the dry season) for nematodes, but not cestodes or protozoa. These results suggest that human activities within the reserve may alter patterns of parasitism in wild armadillos. One possible explanation for our results is that in the areas where illegal hunting is known to occur, poachers establish camps and use their dogs for hunting (Ramirez Pinto, pers. comm.). Both, dogs and humans, defecate on the ground and could contribute to a high rate of soil and grass contamination with infective parasitic stages. Studies in nearby communities have reported a high diversity of gastrointestinal parasites in domestic dogs, including *Uncinaria stenocephala*, *Strongyloides stercoralis*, *Ancylostoma caninum*, *Toxocara canis*, *Cystoisospora* sp., *Trichuris vulpis*, *Giardia duodenalis*, and *Taenia* sp. (Martínez 2019). Notably, some of these parasites—such as *Giardia*, *Trichuris*, and *Uncinaria*—were also detected in armadillos from hunting areas, suggesting potential overlap. Given their feeding habits, armadillos may be especially exposed to fecal-oral transmission routes and could act as accidental or paratenic hosts for these parasites. Furthermore, the higher parasite richness observed during the colder winter months (June to August) in comparison with the rest of the year, may be related to lower temperatures and food availability, which could compromise immune function and increase host susceptibility to infection. Additionally, some nematodes, such as *Trichuris,* produce highly resistant eggs that can remain viable in the environment under these conditions (Traversa *et al.* 2011). These survival traits, combined with seasonal nutritional stress and immune compromise, may contribute to sustained environmental infective pressure and help explain the observed increase in parasite richness during the dry season. Concerning cestodes and protozoa, there was not a significant association or trend between these groups of parasites and the independent variables. In general, cestodes have indirect cycles and use insects as intermediate hosts. Although we did not collect specific data on the diet of the captured armadillos, field observations confirm the presence of termite mounds in several areas where individuals were captured. As insectivorous mammals, armadillos are known to dig into the soil and termite nests in search of food, which could provide them access not only to termites and ants but also to insect larvae developing underground. In the case of protozoa is probably that the presence of some parasites (e.g., Adeleidae) is associates to the mechanical passage of oocysts from infected prey through the gastrointestinal tract of the nine - banded armadillos, rather than reflecting true parasitism (Lange and Wittenstein 2001; Ezquiaga *et al.* 2009).

Although we recovered some taxa with zoonotic potential, such as nematodes of the genera *Ancylostoma* and *Trichuris* (at high prevalence), *Uncinaria* (at moderate prevalence), and protozoa such as *Giardia* to a lesser extent (Table 2; Figure 2), it is important to note that the presence of these genera does not necessarily imply a direct zoonotic risk. Most species within these groups can infect both animals and humans; however, not all species possess the ability to infect humans or cause disease. Future molecular analyses are essential to identify the specific species involved and to accurately assess their zoonotic potential.

Understanding these risks is particularly important given that the traditionally consumption of these animals by legal and illegal hunters may represent a potential route of human exposure to zoonotic parasites- either through ingestion of contaminated meat or through exposure to body fluids and feces during hunting and preparation. Moreover, Ache hunters eat part of the game during their hunting expeditions (Ramirez Pinto, pers. comm.), which increases the risk of transmission when meat is consumed raw or undercook, or when handling and transportation practices do not follow food safety practices (Thompson 2013; Van Vliet *et al.* 2017).

Based on these practices, we suggest that exposure to parasites may occur not only through ingestion of infected tissue, but also via handling of raw meat or cross-contamination during food preparation. The risk may arise from indirect contact with fecal material present in the environment during traditional hunting practices. In such contexts, hunters—often accompanied by dogs—frequently dig into burrows to manually extract the animals, increasing the likelihood of exposure to soil and fecal contamination (Natalini pers. comm.) This, combined with the consumption practices described above, creates multiple potential pathways for zoonotic transmission.

Our study represents a descriptive approximation, and we do not aim to explore transmission dynamics in depth. While we acknowledge that environmental conditions such as soil moisture can influence the transmission of hookworms and other parasites, our current data are insufficient to make conclusive statements in this regard. Nonetheless, this is indeed an important aspect that warrants further investigation through targeted ecological and longitudinal studies.

Considering these findings, which highlight the possibility of zoonotic transmission, future studies employing molecular analyses and opportunistic necropsies are needed to improve our classification of the gastrointestinal parasites of armadillos. Mechanisms to reduce zoonotic risks identified may promote human health and local support for conservation.

Conclusions

We characterized the gastrointestinal parasitic fauna of nine-banded armadillos (*Dasypus novemcinctus*) recovering 15 distinct parasites from fresh fecal samples of 73 individuals at the Reserva Natural del Bosque Mbaracayú (RNBM), Canindeyú, Paraguay. We also report an increase in parasite richness in areas where hunting was reported in contrast to areas without hunting and the same trend for Nematode probability of presence. These findings suggest that human activities may be affecting patterns of parasitism in wild armadillos.

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