



## Histopathology and microscopic morphology of protozoan and metazoan parasites of free ranging armadillos in Brazil<sup>1</sup>

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**ABSTRACT.**- Arenales A., Hoppe E.G.L., Gardiner C., Mol J.P.S., Werther K. & Santos R.L. 2021. **Histopathology and microscopic morphology of protozoan and metazoan parasites of free ranging armadillos in Brazil.** *Pesquisa Veterinária Brasileira* 41:e06868, 2021. Escola de Veterinária, Universidade Federal de Minas Gerais, Campus Pampulha, Av. Pres. Antônio Carlos 6627, São Luiz, Belo Horizonte, MG 31270-901, Brazil. E-mail: [rsantos@vet.ufmg.br](mailto:rsantos@vet.ufmg.br)

This study assessed microscopic morphology of protozoan and metazoan parasites, as well as parasite-associated histopathologic changes in five Brazilian free-ranging armadillos. Three armadillos had intra sarcolemmal cysts of *Sarcocystis* sp. in skeletal muscles without microscopic changes. One *Dasyopus novemcinctus* was found parasitized with a nematode morphologically compatible with an oxyurid in the small intestine. One *Dasyopus* sp. had neutrophilic enteritis associated with adult and larval stages of *Strongyloides* sp. and one *D. novemcinctus* had multiple embryonated eggs free in the lumen of the small intestine with mild neutrophilic enteritis. These findings represent a contribution for expanding our knowledge on parasitic diseases of armadillos.

Index term: Histopathology, microscopic morphology, protozoan, metazoan, parasites, armadillos, Brazil, *Dasyopus novemcinctus*, *Sarcocystis* sp., *Strongyloides* sp., parasitism.

**RESUMO.**- [Histopatologia e morfologia microscópica de parasitos protozoários e metazoários de tatus de vida livre no Brasil.] Este estudo avaliou a morfologia microscópica de parasitos protozoários e metazoários, bem como lesões associadas ao parasitismo em cinco tatus de vida livre no Brasil. Três tatus tinham cistos de *Sarcocystis* sp. Intra-sarcolemal em músculos esqueléticos sem alterações microscópicas. Um *Dasyopus novemcinctus* estava parasitado com um nematodo morfologicamente compatível com oxiurideo no intestino delgado. Um *Dasyopus* sp. apresentou enterite neutrofilica associada com estágios larvais de *Strongyloides* sp. e um *D. novemcinctus* apresentou múltiplos ovos embrionados livres no

lúmen do intestino delgado, associado a enterite neutrofilica discreta. Estes achados representam uma contribuição para a expansão do conhecimento sobre doenças parasitárias de tatus.

**TERMOS DE INDEXAÇÃO:** Histopatologia, morfologia microscópica, parasitos, protozoários, metazoários, tatus, Brasil, *Dasyopus novemcinctus*, *Sarcocystis* sp., *Strongyloides* sp., parasitismo.

### INTRODUCTION

Armadillos are mammals belonging to the Xenarthra superorder and the Cingulata order. These animals are naturally found only in the American continent, including 21 species of which 11 are registered in Brazil (Medri et al. 2011). Some of those species are a conservation concern, including the giant armadillo (*Priodontes maximus*) and the Southern long-nosed armadillo (*Dasyopus hybridus*), both having decreasing populations so they are considered vulnerable (Abba & Gonzalez 2014) or near threatened (Anacleto et al. 2014) according to the International Union for Conservation of Nature (IUCN) red list, respectively. Most of the animals included in this study are *Dasyopus novemcinctus* (nine-banded armadillo), which are considered of least concern according to the IUCN.

A better understanding of host-pathogen interactions is a key element in wildlife conservation (Worbesen 2005). In spite

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of recent studies on diseases of other Xenarthra species such as anteaters (Arenales et al. 2020a) and sloths (Arenales et al. 2020b), there are a few studies focused on pathologic changes associated with protozoan and metazoan parasites and parasitic diseases of armadillos. Previous reports described *Leishmania* (Lainson & Shaw 1989), *Sarcocystis neurona* (Cheadle et al. 2001, Tanhauser et al. 2001), *Mathevotaenia* (Gomes et al. 2012, Ríos et al. 2016) and *Angiostrongylus cantonensis* (Dalton et al. 2017) affecting armadillos. In addition, there is a survey in Texas (Chandler 1946), and a few reports on helminths in Brazilian mammals that include parasites from armadillos (Vicente et al. 1997, Lux Hoppe & Nascimento 2007, Lux Hoppe et al. 2009). However, histopathological findings are usually absent in those previous reports. Therefore, the focus of this study was to describe histopathological changes associated with protozoan and metazoan parasites in five free ranging armadillos from Brazil.

## MATERIALS AND METHODS

Five free ranging armadillos were referred to the wildlife pathology service at the "Faculdade de Ciências Agrárias e Veterinárias", "Universidade Estadual Paulista 'Júlio de Mesquita Filho'" (Unesp, Jaboticabal/SP, Brazil) for necropsy, from 1994 to 2017. Several tissue samples from internal organs were sampled, including lungs (5/5), liver (5/5), skeletal muscle (4/5), kidney (4/5), spleen (3/5), heart (3/5), stomach (3/5),

small intestine (3/5), trachea (3/5), esophagus (3/5), tongue (2/5), testes (2/5), brain (2/5), cerebellum (2/5), urinary bladder (2/5), ovary (1/5), skin (1/5), thymus (1/5), lymph node (1/5), pancreas (1/5), and adrenal (1/5). Samples were fixed in 10% buffered formalin, processed for paraffin embedding, sectioned at 4- $\mu$ m, and stained with hematoxylin and eosin (HE). Tissues samples with morphologically detectable *Sarcocystis* sp. were processed for DNA extraction from paraffin embedded tissue samples and subjected to PCR amplification of rRNA 18s sequences as previously described by Harrus et al. (2011).

## RESULTS

Table 1 summarizes the findings observed in the armadillos included in this study. Animals 2, 4, and 5 had occasional intrasarcolemmal cysts morphologically compatible with *Sarcocystis* sp. in skeletal muscles, including the tongue and esophagus. These cysts were not associated with any microscopic change. Importantly, cysts of *Sarcocystis* sp. had two distinct microscopic morphologic patterns: cysts observed in the tongue of Animal 2 had a thick capsule with a brush-like outer layer, containing large bradyzoites with lower densities and an abundant matrix (Fig.1). In contrast, cysts of *Sarcocystis* sp. observed in Animals 4 and 5 had a thinner and smoother capsule, without the brush-like outer layer, smaller bradyzoites, representing a denser population within scarce matrix (Fig.2 and 3). The attempt

**Table 1. Characterization of armadillos included in this study and microscopic changes associated with protozoan and metazoans**

Animal	Host species	Sex	Age	Histopathology
1	<i>Dasyopus novemcinctus</i>	M	Adult	Small intestine: luminal embryonated eggs; no lesions associated
2	<i>Dasyopus novemcinctus</i>	F	Adult	Tongue: intrasarcolemmal <i>Sarcocystis</i> sp. cysts (brush-like outer capsule); no lesions associated
3	<i>Dasyopus</i> sp.*	F	Young	Small intestine: neutrophilic diffuse and mild enteritis with intralesional <i>Strongyloides</i> sp. (adults and larvae stages)
4	<i>Dasyopus</i> sp.*	F	Adult	Esophagus and skeletal muscle: intrasarcolemmal <i>Sarcocystis</i> sp. cysts; no lesions associated
5	<i>Dasyopus novemcinctus</i>	M	Adult	Duodenum: luminal oxyurid nematode, tongue: intrasarcolemmal <i>Sarcocystis</i> sp. cysts; no lesions associated

\* Species not identified; NR = not reported.

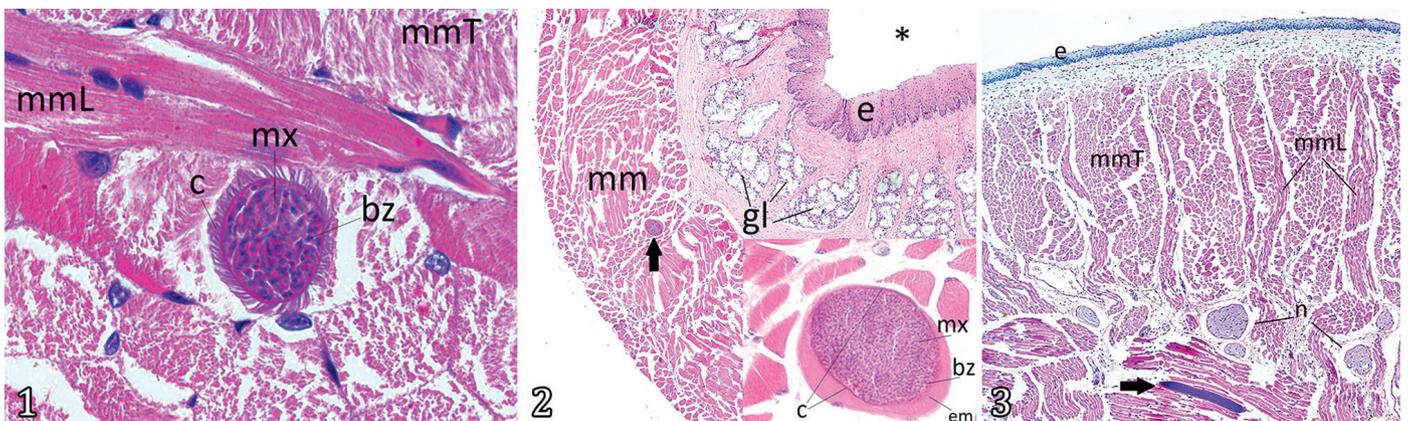


Fig. 1-3. Armadillos with intrasarcolemmal *Sarcocystis* sp. cysts with two distinct morphologic appearance. (1) *Dasyopus novemcinctus*, Animal 2. Tongue, skeletal muscle cells in transversal (mmT) and longitudinal (mmL) sections. Sarcolemma containing a *Sarcocystis* sp. cyst with thick and brush-like outer capsule (c) with large and low density bradyzoites (bz), and abundant matrix (mx). HE, obj.100x. (2) *Dasyopus* sp., Animal 4. Esophagus; muscular esophageal layer (mm) with a cyst of *Sarcocystis* sp. (arrow); esophageal lumen (\*), epithelium (e) and esophageal glands (gl). HE, obj.5x. Inset: higher magnification of a *Sarcocystis* sp. cyst. Notice a different morphological aspect when compared to Figure 1: thinner and smoother capsule (c), without brush appearance, smaller and higher density bradyzoites (bz) and fewer matrix (mx) amounts. HE, obj.40x. (3) *Dasyopus novemcinctus*, Animal 5. Tongue. Epithelium (e) skeletal muscle cells in transversal (mmT) and longitudinal (mmL) sections and nerves (n); *Sarcocystis* sp. cyst (arrow) in the sarcolemma, with morphological features similar to Figure 2. HE, obj.5x.

for amplification of rRNA 18s sequences by PCR (Harrus et al. 2011) for a phylogenetic identification of these parasites was not successful in any of the samples.

Animal 5 (*Dasypus novemcinctus*) had sections of a nematode within the duodenal lumen. These parasites measured approximately 200µm in diameter, with a thick cuticle, thin hypodermis, large lateral alae, platymyarian musculature, and intestine lined by columnar and uninuclear cells with a prominent brush border on the apical side (Fig.4-5). These parasites were surrounded by abundant cellular debris with minimal to mild neutrophilic infiltrate. The parasite was morphologically identified as an oxyurid nematode.

In the small intestine of one armadillo (Animal 3) there were larval and adult stages of *Strongyloides* sp. Adult

parasites measured approximately 30µm in diameter, and had two cross sections of the genital tract and a distinct intestine (Fig.6). Additionally, in *Strongyloides* sp. infections only females parasitized hosts. Adult parasites were coiled and located within the superficial epithelium associated with minimal to mild neutrophilic inflammatory infiltrate in the adjacent lamina propria, characterizing mild acute enteritis (Fig.7). Early stage developing eggs were also observed on the superficial mucosa among cellular debris (Fig.8). In addition, multiple 10 to 15µm in diameter metazoan embryonated eggs (Fig.9) were free in the small intestine lumen of Animal 1, with a mild neutrophilic enteritis.

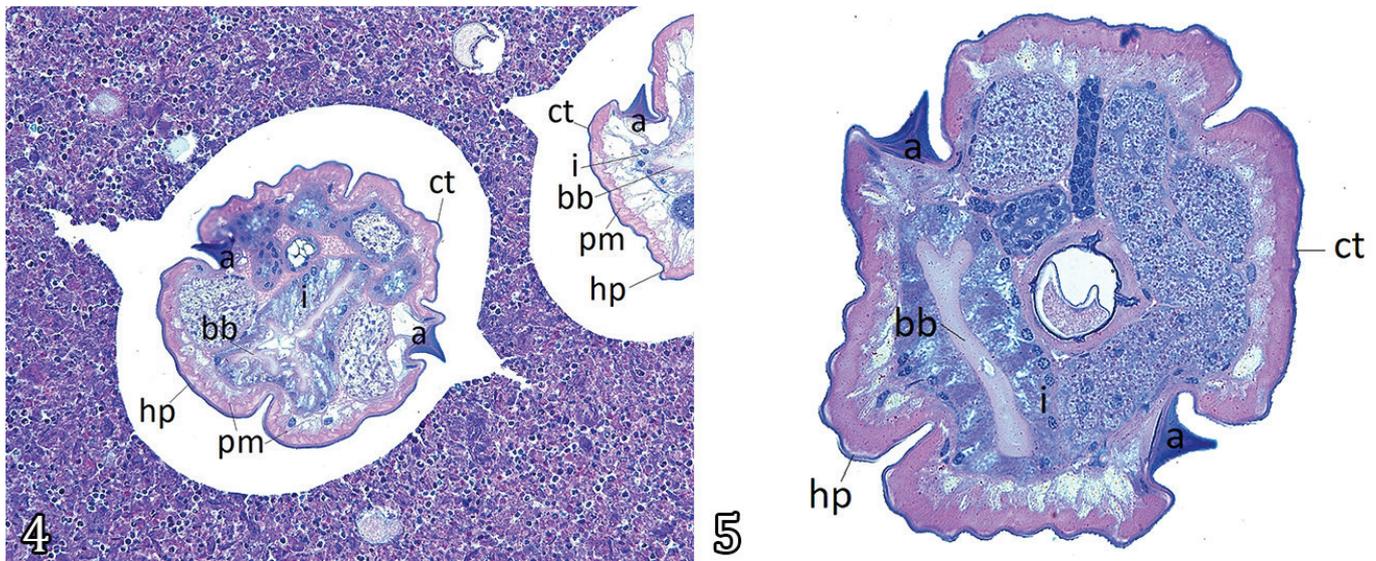


Fig.4-5. *Dasypus novemcinctus*, Animal 5. Cross sections of an oxyurid nematode in the intestinal lumen, surrounded by debris and bacteria, measuring approximately 200µm in diameter, with a thick cuticle (ct), thin hypodermis (hp), lateral alae (a), platymyarian musculature (pm), characterized by large and separated muscle fibers, parallel to the cuticle; and an intestine (i) lined by columnar and uninuclear cells with prominent brush border (bb). (4) Two transversal sections of a nematode surrounded by abundant cellular debris with scarce neutrophils. HE, obj.10x. (5) Transversal cut section of nematode in a different portion. HE, obj.20x.

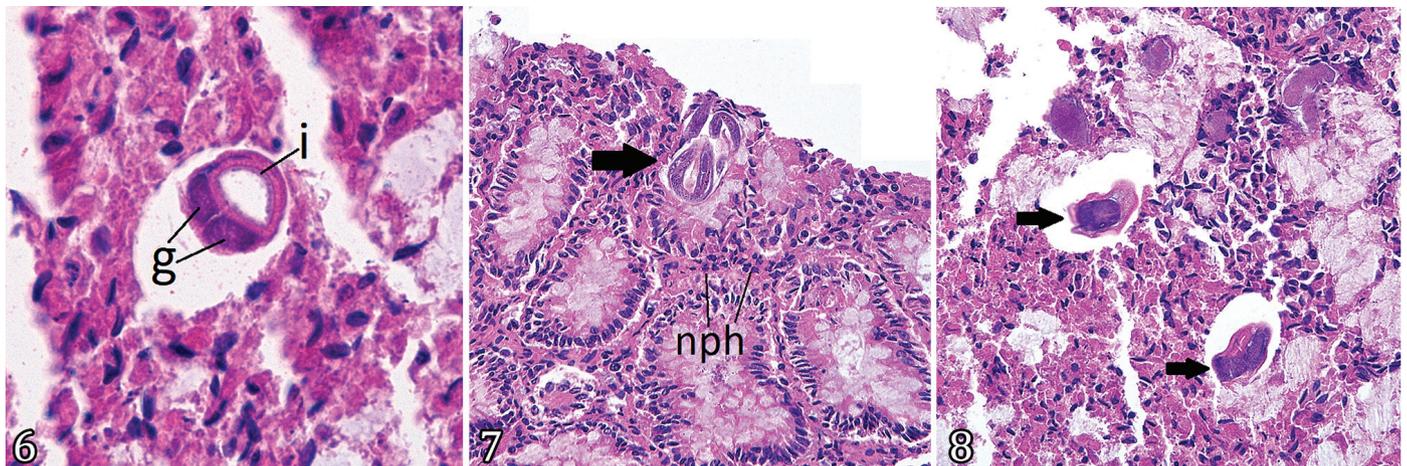


Fig.6-8. *Dasypus* sp., Animal 3. Small intestine, adult female nematodes morphologically compatible with *Strongyloides* sp., measuring approximately 30µm in diameter: (6) Transversal section; intestine (i) and genital tracts (g) characterizing a female. HE, obj.40x. (7) Longitudinal section; nematode coiled (arrow) within the superficial mucosa with minimal neutrophilic (nph) inflammatory infiltrate in the lamina propria, characterizing a mild acute enteritis. HE, obj.20x. (8) Early stage eggs (arrows) on the superficial mucosa among cellular debris. HE, obj.10x.

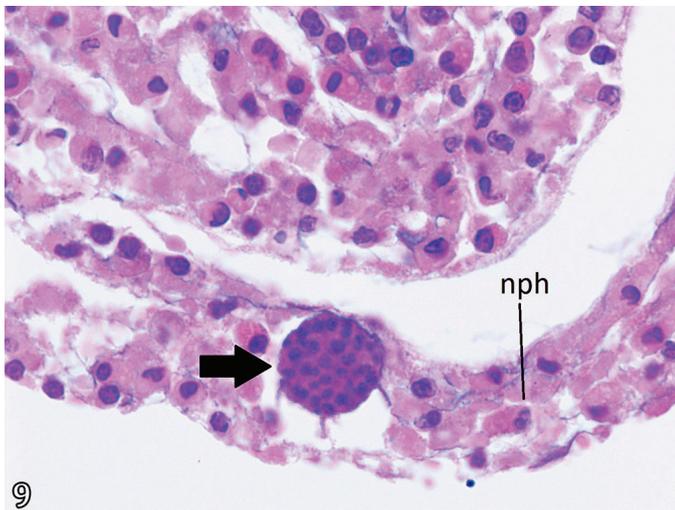


Fig.9. *Dasypos novemcinctus*, Animal 1. Small intestine, lumen containing free embryonated eggs (arrow), which are multilobulated and contains multicellular structures, measuring approximately 20x10 $\mu$ m with scant neutrophils (nph) in the laminae propria. HE, obj.100x.

## DISCUSSION

This study described microscopic changes associated with protozoan and metazoan parasites in free-ranging armadillos. Parasite specimens from Animal 5 were not available for parasitological identification and the parasites could not be identified morphologically based on histological sections. Oxyurid nematodes are common in many species of both invertebrate and vertebrate animals (Hugot et al. 1996, Carreno 2014), although it has not been previously reported in armadillos. However, identification of these parasites in this study was based solely on histologic features, which did not allow for a conclusive parasitological diagnosis. Finding of neutrophils and debris associated with this parasite suggests a parasite-elicited enteritis, which could be associated with clinical disease. However, oxyurid nematodes do not usually elicit a pathological response in their hosts (Roberts & Janovy 2009).

*Strongyloides* helminths were observed in Animal 3 (*Dasypos* sp.), associated with a neutrophilic enteritis. To the best of our knowledge this is the first report of a microscopic diagnosis of parasitic enteritis in an armadillo. Two Species of *Strongyloides* parasites have been described in *Dasypos novemcinctus*, i.e., *Strongyloides ratti* and *Strongyloides dasypodis* (Lux Hoppe & Nascimento 2007, Lux Hoppe et al. 2009) but specific identification of the parasite based on histological morphology was not possible in this case. Additionally, *Strongyloides* were the probable source of the embryonated eggs observed in Animal 1 (Gardiner & Poynton 1999).

In regard to *Sarcocystis* sp., as described in this study, an early report described two species based on histological morphology: *Sarcocystis dasypi* (small and more density bradyzoites with thinner capsule) and *Sarcocystis diminuta* (thicker capsule and large and fewer bradyzoites) (Lindsay et al. 1996). Although this morphologic distinction is interesting under an anatomic pathology point of view, it is not possible to identify *Sarcocystis* species based on morphology in histological sections (Dubey et al. 2016). *Sarcocystis* sp. are found in the skeletal muscle of intermediate hosts as a terminal asexual

stage (Dubey et al. 2016). It has been described in armadillos, with 75% of *D. novemcinctus* (nine-band armadillo) in Brazil infected with *Sarcocystis* sp. (Antunes et al. 2012). In fact, more recent studies with molecular techniques demonstrated that nine-band armadillos are intermediate hosts for *Sarcocystis neurona* in the United States (Cheadle et al. 2001, Tanhauser et al. 2001). Therefore, considering the occurrence of equine protozoal myeloencephalitis in Brazil (Masri et al. 1992, Paixão et al. 2007, Henker et al. 2020), it reasonable to hypothesize that Brazilian armadillos may act as intermediate hosts for *S. neurona*. Thus, detection of *S. neurona* in armadillos in Brazil would be an interesting topic of future investigations. Unfortunately PCR amplification of rRNA 18s failed in these samples, which may have been influenced by over-fixation since these were archive samples subjected to variable (often prolonged) fixation periods.

## CONCLUSION

This study demonstrated parasites in free-ranging armadillos in Brazil, as well as parasite-associated histopathologic changes, which is a relevant contribution for expanding our knowledge on parasitic diseases of armadillos.

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**Conflict of interest statement.**- The authors declare no conflict of interest.

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