Histopathology and microscopic morphology of protozoan and metazoan parasites of free ranging armadillos in Brazil

Alexandre Arenales1, Estevam G.L. Hoppe2, Chris Gardiner3, Juliana P.S. Mol2, Karin Werther4 and Renato L. Santos2*

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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 Dasypus novemcinctus was found parasitized with a nematode morphologically compatible with an oxyurid in the small intestine. One Dasypus 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, Dasypus novemcinctus, Sarcocystis sp., Strongyloides sp., parasitism.

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-nose armadillo (Dasypus 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 Dasypus 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

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 Dasypus novemcinctus estava parasitado com um nematode morfologicamente compatível com oxiurídeo no intestino delgado. Um Dasypus sp. apresentou entere neutrofílica associada com estágios larvais de Strongyloides sp. e um D. novemcinctus apresentou múltiplos ovos embrionados livres no lúmen do intestino delgado, associado a entere neutrofílica discreta. Estes achados representam uma contribuição para a expansão do conhecimento sobre doenças parasitárias de tatus.

TERMOS DE INDICAÇÃO: Histopatologia, morfologia microscópica, parasitos, protozoários, metazoários, tatus, Brasil, Dasypus novemcinctus, Sarcocystis sp., Strongyloides sp., parasitismo.

Histopatologia e morfologia microscópica de parasitos protozoários e metazoários de tatus de vida livre no Brasil1

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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), Sarcozystis 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-µm, and stained with hematoxylin and eosin (HE). Tissues samples with morphologically detectable Sarcozystis 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 Sarcozystis sp. in skeletal muscles, including the tongue and esophagus. These cysts were not associated with any microscopic change. Importantly, cysts of Sarcozystis 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 Sarcozystis 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      | Dasypus novemcinctus | M   | Adult | Small intestine: lumenal embryonated eggs; no lesions associated |
| 2      | Dasypus novemcinctus | F   | Adult | Tongue: intrasarcolemmal Sarcozystis sp. cysts (brush-like outer capsule); no lesions associated |
| 3      | Dasypus sp.* | F   | Young | Small intestine: neutrophilic diffuse and mild enteritis with intralesional Strongyloides sp. (adults and larvae stages) |
| 4      | Dasypus sp.* | F   | Adult | Esophagus and skeletal muscle: intrasarcolemmal Sarcozystis sp. cysts; no lesions associated |
| 5      | Dasypus novemcinctus | M   | Adult | Duodenum: lumenal oxyurid nematode, tongue: intrasarcolemmal Sarcozystis sp. cysts; no lesions associated |

* Species not identified; NR = not reported.

Fig. 1-3. Armadillos with intrasarcolemmal Sarcozystis sp. cysts with two distinct morphologic appearance. (1) Dasypus novemcinctus, Animal 2. Tongue, skeletal muscle cells in transversal (mmT) and longitudinal (mmL) sections. Sarcolemma containing a Sarcozystis 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) Dasypus sp., Animal 4. Esophagus; muscular esophageal layer (mm) with a cyst of Sarcozystis sp. (arrow); esophageal lumen (*), epithelium (e) and esophageal glands (gl). HE, obj.5x. Inset: higher magnification of a Sarcozystis 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) Dasypus novemcinctus, Animal 5. Tongue. Epithelium (e) skeletal muscle cells in transversal (mmT) and longitudinal (mmL) sections and nerves (n); Sarcozystis 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.
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 is 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 subject 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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