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Diagnostic Exercise
From the Latin Comparative Pathology Group and the Davis-Thompson Foundation

Necro-hemorrhagic enteritis and necrotizing hepatitis in green anaconda (*Eunectes marinus*)

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History:

An adult, male, green anaconda (*Eunectes marinus*) from "Universidade Federal de Mato Grosso" (UFMT) Zoo's collection showed apathy, anorexia and bloody feces. This snake died during clinical evaluation and was necropsied.

Necropsy findings:

The autopsy revealed a colon intussusception. The intestinal wall was thickened in this area with an irregular mucosa covered with a yellow-brownish and hemorrhagic fibrin pseudomembrane (Figure 1A). The hepatic lesion affected about 40% of the capsular and cut surfaces and was characterized by multifocal-to-coalescing firm and dark-red areas about 0.5 to 1cm in diameter (Figure 1B). Samples from all the organs and various muscle fragments were collected and fixed in 10% neutral-buffered formalin for morphologic studies.

Follow-up questions

- *Morphologic diagnosis:*
- *Etiology:*
- *Differential diagnosis:*

**The Diagnosti Exercises are an initiative of the Latin Comparative Pathology Group (LCPG), the Latin American subdivision of The Davis-Thompson Foundation (DTF). These exercises are contributed by members and non-members from any country of residence. Consider submitting an exercise! A final document containing this material with answers and a brief discussion will be posted on the DTF website: https://davisthompsonfoundation.org/diagnostic-exercise/*

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ANSWERS

Histological description: Histopathology:

The enteric lesions comprised mucosa and submucosa replacement by severe diffuse necrosis and hemorrhage. There is epithelium necrosis with fusion of villi and high fibrin deposition. Transmurally, expanding from the lamina propria to the serosa, there was an infiltrate of heterophils, macrophages and lymphocytes. The intestinal muscular layers, the serosa's conjunctive tissue, and mesentery were multifocally expanded by edema, hemorrhage and heterophils, macrophages, and lymphocytes infiltrate (Figure 2A). In the liver, we observed multifocal-to-coalescent coagulation necrosis associated with mixed inflammation similar to that presented in the intestine, characterizing necrotizing hepatitis. Amoebic trophozoites were identified in the lesion areas of the intestinal tract and liver. The trophozoites were PAS-stained, with an oval cytoplasm of 5 to 25µm presenting a thin cellular wall, a broad eosinophilic cytoplasm and a round to oval pale basophilic nucleus measuring 3-5µm (Figure 2B). Formalin-fixed paraffin-embedded intestine sections were submitted to the Amoeba Laboratory from the Center for Disease Control and Prevention (CDC), Waterborne Disease Prevention Branch, for immunofluorescence testing with anti-*Entamoeba invadens* (Amoebid: Entamoebidae) antibody, which resulted in strongly positive trophozoites (Figure 2B).

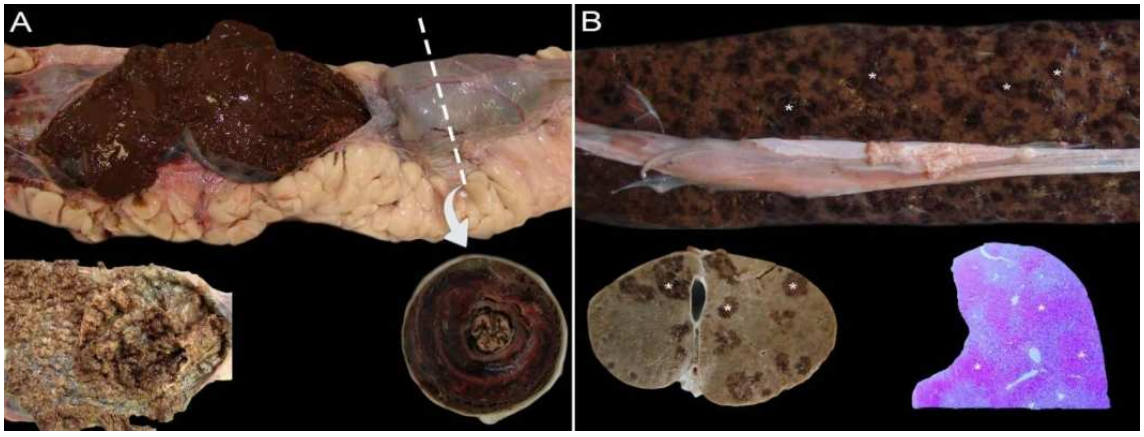


Figure 1. A – Large intestine showing a colon intussusception (upper right) and the cut surface of that region (Arrow). The intestinal mucosa is irregular and covered with brown feces (upper left). After washing, the mucosa revealed a yellow-brownish and hemorrhagic fibrin pseudomembrane (down left). B – Liver: non-formalin-fixed capsular surface (upper), formalin-fixed cut surface (down left), showing multifocal-to-coalescing dark-red areas about 0.5 to 1cm of diameter (asterisks) corresponding to liver necrosis in the sub-macroscopic view of a H&E-stained photomicrograph of liver section (below, right)

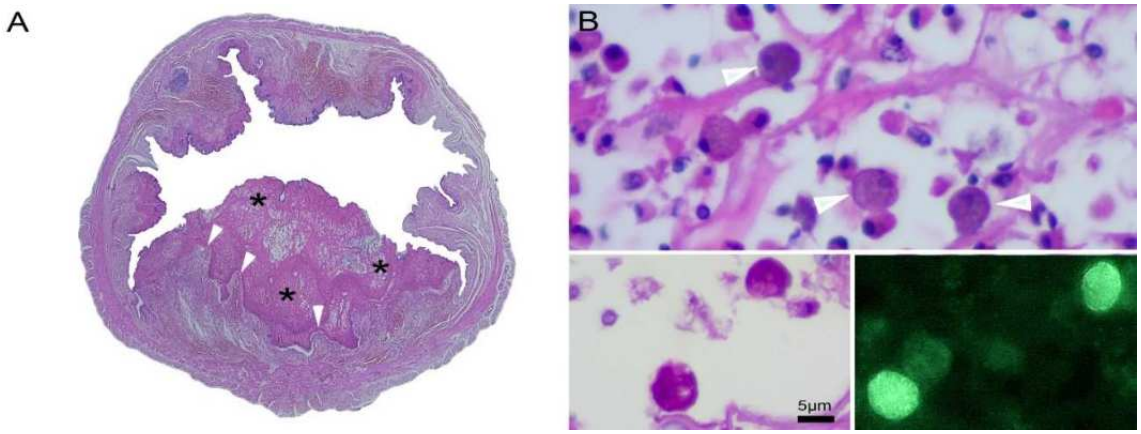


Figure 2. Photomicrograph of section of the colon of the snake described in Figure 1. A is a sub-macroscopic view of a transversal section showing epithelium necrosis with a fusion of villi and high fibrin deposition (Asterisks). The intestinal wall is thickened due to a transmural mixed inflammatory infiltrate. B - H&E-stained transversal section of the colon showing (upper), in the lamina propria, amoebic trophozoites with an oval cytoplasm presenting a thin cellular wall and a broad eosinophilic cytoplasm and a round to oval pale basophilic nucleus (Arrowhead). The trophozoites were PAS-stained (down left), and the immunofluorescence test with anti-*Entamoeba invadens* antibody resulted in a strongly positive reaction (down right), Bar=5 µm.

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Morphologic diagnosis:

1. Large intestine, Colon: severe diffuse acute fibrinonecrotic enteritis with intralesional extracellular amoebic trophozoites.
2. Liver: severe multifocal-to-coalescent acute diffuse necrotizing hepatitis with intralesional extracellular amoebic trophozoites.

Etiology:

Entamoeba spp.

Differential diagnosis:

Entamoeba invadens, E. ranarum, Salmonella sp, *Cryptosporidium* sp. *Monocercomonas* sp.

Comments:

E. invadens is an amoeba commensal to crocodilians and turtles and rarely causes disease in these animals. In snakes, it is known to cause acute gastric lesions, such as necrotic enteritis and necrotic hepatitis, which were observed in this specimen. Infection by *E. invadens* occurs after ingesting cysts in environments contaminated with feces from reservoir hosts or other infected animals. (1) These cysts contain trophozoites that invade enterocytes after hatching in the intestinal lumen and subsequently cause cell death. (2)

The pathogenic Entamoeba species in snakes is widely regarded to be *E. invadens*, which can cause severe amoebiasis with up to 100% mortality, as described in this case. Although *E. ranarum* was described causing a similar disease in snakes and the differential between these species require molecular genetic tools (3)

Others differential diagnosis includes bacterial infection by gram-negative bacilli, mainly from *Salmonella* sp.; however, no isolation or molecular detection of these bacteria was found. Coccidia of the genus *Cryptosporidium* sp. are known to cause gastrointestinal diseases in snakes. However, clinical signs are chronic or absent, unlike what was usually observed in this case. (4) *Monocercomonas* sp. is a flagellate protozoan commensal to snakes that can cause necrotic enteritis in snakes, but it has relatively more minor trophozoites. [5]

The source of contamination of this snake is uncertain; however, after an investigation of the shelters, it was noted that the route taken by the handlers to feed the snakes included passage through enclosures of alligators (*Caiman yacare*) and red-footed tortoises (*Chelonoids carbonara*), which is a plausible hypothesis for the origin of the contamination of this animal.

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Disclosures:

The authors have nothing to disclose.

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