PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 45:e07585, 2025
DOI: 10.1590/1678-5150-PVB-7585

Original Article
Wildlife Medicine
CC BY

Mortality by *Entamoeba invadens* in green anacondas (*Eunectes murinus*) from a zoological garden[1]

Bruno C. Souza[2], João Paulo S. Cavasani[2], Ícaro G. Santos[2], Larissa Sabino[2], Daniel G. Ubiali[3], Caroline A. Pescador[2], Edson M. Colodel[2], Fernando H. Furlan[2]*

ABSTRACT.- Souza BC, Cavasani JPS, Santos IG, Sabino L, Ubiali DG, Pescador CA, Colodel EM, Furlan FH. **Mortality by *Entamoeba invadens* in green anacondas (*Eunectes murinus*) from a zoological garden.** *Pesquisa Veterinária Brasileira 45:e07585, 2025.* Laboratório de Patologia Veterinária, Hospital Veterinário, Universidade Federal de Mato Grosso, Av. Fernando Corrêa da Costa 2367, Boa Esperança, Cuiabá, MT 78060-900, Brazil. E-mail: fernando.furlan@ufmt.br

Five green anacondas (*Eunectes murinus*) from the same enclosure died sequentially in the zoological garden of the Federal University of Mato Grosso, Midwest Brazil. The primary lesions in all snakes were severe necrotizing enterocolitis and hepatitis, associated with intralesional positive-PAS trophozoites morphologically compatible with *Entamoeba*. The protozoa were randomly distributed in the intestine and liver of the five anacondas and in the stomachs, kidneys, and lungs of three anacondas. The snake enclosure had contact with the room next door, which housed alligators and turtles. Based on clinical history, gross and histopathological findings, and immunofluorescence test, which positively marked the respective trophozoites, it was concluded that *Entamoeba invadens* infected the anacondas.

INDEX TERMS: Enterocolitis, amoebiasis, *Entamoeba*, snakes.

RESUMO.- [**Mortalidade por *Entamoeba invadens* em sucuris verdes (*Eunectes murinus*) de um zoológico.**] Cinco sucuris verdes (*Eunectes murinus*) do mesmo recinto morreram sequencialmente no jardim zoológico da Universidade Federal de Mato Grosso, em Cuiabá, Centro-Oeste do Brasil. As lesões primárias em todas as serpentes foram enterocolite e hepatite necrótica severa, associada a trofozoítos intralesionais PAS positivos, morfologicamente compatíveis com *Entamoeba*. Os protozoários estavam distribuídos aleatoriamente no intestino e fígado das cinco sucuris e nos estômagos, rins e pulmões de três sucuris. O recinto das serpentes tinha contato com o recinto vizinho que abrigava jacarés e tartarugas. Com base nos achados clínicos, macroscópicos, histopatológicos, e no teste de imunofluorescência, que marcou trofozoítos, concluiu-se que as sucuris estavam infectadas por *Entamoeba invadens*.

TERMOS DE INDEXAÇÃO: Enterocolite, amebíase, *Entamoeba*, serpentes.

INTRODUCTION

"Anaconda" or "sucuri" are aquatic top predator snakes with a wide distribution in South America. *Eunectes murinus*, *Eunectes akayima* and *Eunectes notaeus* are currently recognized species across nine countries (Rivas et al. 2024). Reptiles have numerous protozoaires that belong to the microbiota and usually are harmless to their host. Protozoa can become pathogenic when confined due to the high parasitic load provoked by continuous self-infection (Greiner & Mader 2006).

Infections due to *Entamoeba histolytica* in primates and *Entamoeba invadens* in reptiles are known to cause serious diseases (Richter et al. 2008). Amoebae in chelonians may cause gastrointestinal diseases associated with infection by *Entamoeba ranarum, Entamoeba insolita, Entamoeba testudines, Entamoeba barreti, Entamoeba terrapinae,* and *E. invadens* species (García et al. 2014). These species can cause high mortality due to mainly severe necrotizing enterocolitis and hepatitis (Donaldson et al. 1975, Jakob & Wesemeier 1995, Kojimoto et al. 2001, Richter et al. 2008, Baseler et al. 2014, McFarland et al. 2021). However, registering diseases caused by this agent in South America is rare. The first official morphologic description of *E. invadens* was made by Rodhain in 1938. However, there are signs that the Brazilians Cunha and Fonseca described the same species in 1917 under *Entamoeba serpentis* (Ghosh 1968).

Herein, we describe an amebiasis outbreak caused by *E. invadens* in green anacondas in captivity.

[1] Received on March 17, 2025.
Accepted for publication on May 19, 2025.

[2] Laboratório de Patologia Veterinária, Hospital Veterinário, Universidade Federal de Mato Grosso (UFMT), Av. Fernando Corrêa da Costa 2367, Boa Esperança, Cuiabá, MT 78060-900, Brazil. *Corresponding author: fernando.furlan@ufmt.br

[3] Setor de Anatomia Patológica (SAP), Departamento de Epidemiologia e Saúde Pública, Instituto de Veterinária (IV), Universidade Federal Rural do Rio de Janeiro (UFRRJ), Rodovia BR-465 Km 7, Seropédica, RJ 23890-000, Brazil.

OUTBREAK REPORT

Ethical approval. This study was conducted following the criteria established by the Ethics Committee for the Use of Animals (CEUA) of the "Universidade Federal de Mato Grosso" (UFMT) under protocol No. 23108.031812/2023-07.

Fig. 1. Infection by *Entamoeba invadens* in green anacondas (*Eunectes murinus*). Case 1 shows bloody feces in the cloaca.

From May to June 2007, five green anacondas (*Eunectes murinus*, Boidae) (Cases 1-5) from UFMT Zoo's collection died and were submitted to necropsy in the Veterinary Pathology Laboratory at UFMT. Samples of multiple organs were fixed in 10% formalin, routinely processed, embedded in paraffin, and stained with hematoxylin and eosin (HE). Selected tissues were stained by periodic acid-Shiff (PAS). All histological stains were performed as described by Prophet et al. (1992). The responsible reported a release of a free-ranging green anaconda in the same enclosure about two months before the mortality. In addition, the snake enclosure had contact with the room next door, which housed alligators and turtles.

The five snakes in the enclosure died within three days. Four affected snakes were found dead in the enclosure with no clinical signs (Cases 1-4). One green anaconda presented apathy, anorexia and bloody feces (Fig. 1) and died during the physical examination (Case 5). During necropsy, the primary lesions were similar in the five snakes and occurred in the intestines and livers. Fibrino-necro-hemorrhagic or fibrinous proliferative colitis (Fig. 2 and 3) was characterized grossly by the necropsies. The intestinal walls are thickened with an irregular mucosa covered with a yellow-brownish and hemorrhagic fibrin pseudomembrane. Two snakes presented colon intussusception (Fig. 3). The liver presented at capsular

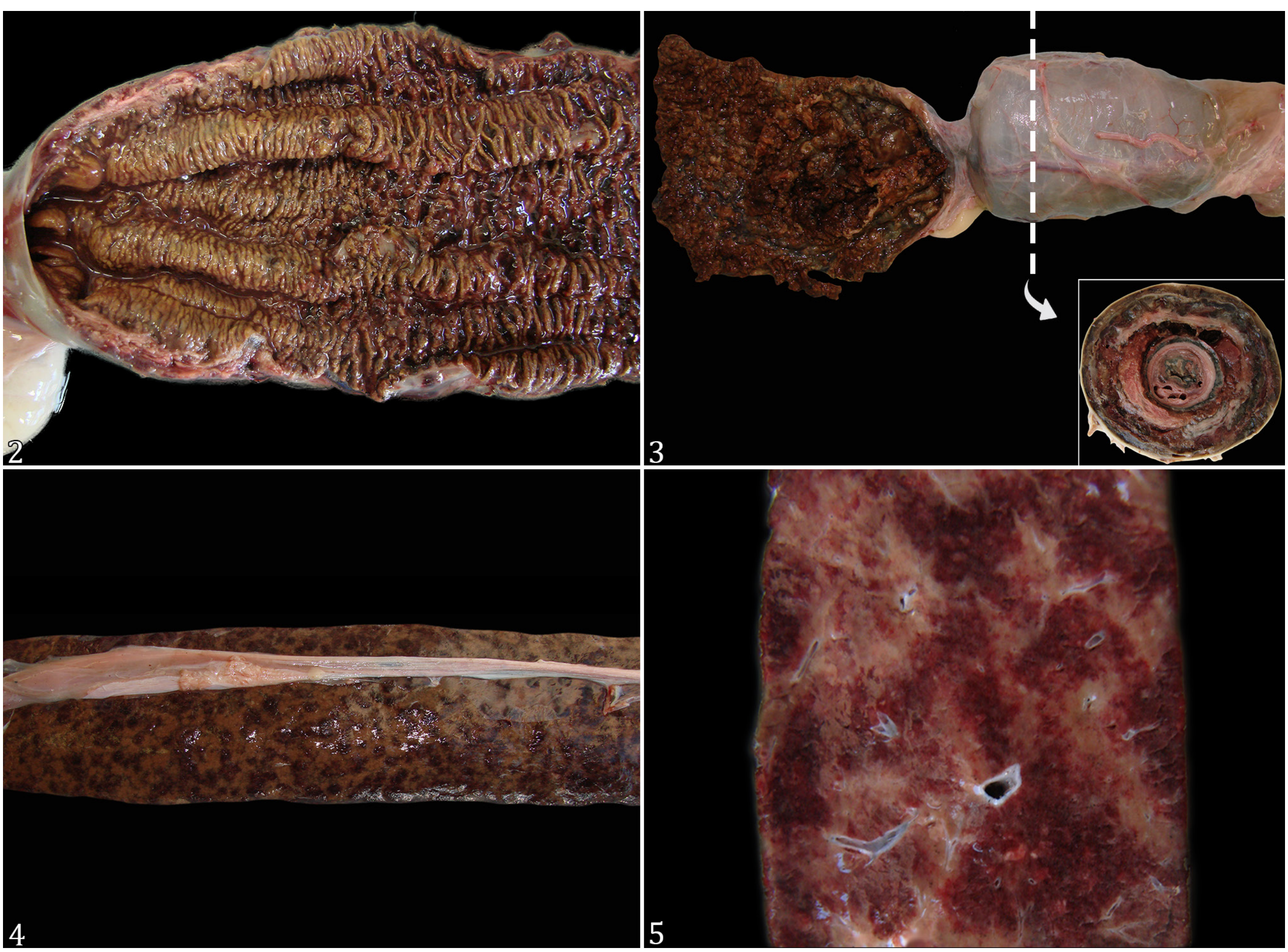

Fig. 2-5. Infection by *Entamoeba invadens* in green anacondas (*Eunectes murinus*). (**2**) Case 1. Note the necrohemorrhagic colitis with a brown fibrinonecrotic membrane and thickened and irregular mucosa. (**3**) Case 5 showing necrohemorrhagic colitis and colon intussusception. Inset: The intestinal transverse cut surface shows a congested segment of the intestinal loop inside another. (**4** and **5**) Multifocal to coalescent necrotizing hepatitis presenting mottled, well-defined dark red areas on the capsular (Case 3) cut surface (Case 2).

and cut surfaces about 40% multifocal-to-coalescing necrotic foci characterized by firm, dark-red areas about 0.5 to 1 cm of diameter (Fig. 4 and 5). Cases 3-5 presented gastric multifocal ulcers ranging from 0.1 cm to 1.5 cm (Fig. 6). Macroscopic lesions are summarized in Table 1 and were classified as absent, mild, moderate and severe according to severity.

Histologically, the primary lesions occurred in the intestine and liver. The enteric lesions were characterized by necrotizing-fibrinoid diffuse pronounced enteritis associated with amoebic trophozoites. The mucosa and submucosa were replaced by severe diffuse necrosis and hemorrhage. It was noted epithelial necrosis with villi fusion, and high fibrin deposition intermixed with multifocal bacterial coccobacillary basophilic aggregates. Transmurally, expanding from the lamina propria to the serosa, there was a mixed infiltrate made of heterophils, macrophages and lymphocytes (Fig. 7). In the intestine's muscular layers, the muscle fibers and the conjunctive tissue of the serosa and mesentery were multifocally expanded by edema, hemorrhage and heterophils, macrophages, and lymphocytes infiltrate. Multifocally, the larger diameter vessels presented vacuolization in muscular tunicae, sometimes with a heterophilic perivascular intramural infiltrate containing occasional intraluminal bacterial coccobacillary basophilic aggregates. The liver presented necrotizing hepatitis, evidenced by the multifocal-to-coalescing extensive necrotic areas (Fig. 8) associated with moderated infiltration of heterophils, macrophages, and lymphocytes. Additionally, there were gastric and renal lesions in anacondas from Cases 3-5. In the stomach sections, ulcerative gastritis was evidenced by epithelium discontinued areas and multifocal necrosis in the mucosa, which was associated with fibrin deposition. The mucosa and submucosa were expanded by an intense infiltration of heterophils, which was related to moderate hemorrhage and edema. There was lymphohistiocytic infiltration at the border and the walls of the vessels. Mild to moderate multifocal membranous degeneration of glomeruli and interstitial nephritis was noted in the kidneys, characterized by diffuse vacuolization of the tubular epithelium and multifocal inflammatory infiltrate associated with vasculitis in the interstitium. Case 3 presented in the lungs intrafoveolar edema containing moderate lymphocytes, macrophages and heterophils infiltration. The walls of the larger diameter vessels presented severe vasculitis. Amoebic trophozoites were identified in the lesion areas of the gastrointestinal tract, liver, kidneys and lungs (Fig. 9). The trophozoites were PAS-stained (Fig. 10), with an oval cytoplasm of 10 to 25 μm presenting a thin cellular wall and a broad eosinophilic cytoplasm and a round to oval pale basophilic nucleus measuring 3-5 μm (Fig. 8). The trophozoites were also found within renal tubules and blood vessels. Additionally, bacterial coccobacillary basophilic aggregates were observed multifocally in vessels from the stomach, liver, lungs, and kidneys from Cases 3-5.

Fig. 6. Infection by *Entamoeba invadens* in green anacondas (*Eunectes murinus*). Case 5 with multifocal disseminated ulcerative gastritis.

Formalin-fixed paraffin-embedded intestine and liver sections from five green anacondas were submitted to the Amoeba Laboratory from the Center for Disease Control and Prevention (CDC), Waterborne Disease Prevention Branch, to immunofluorescence testing (DIF) with fluorescein isothiocyanate-labeled antibodies targeting *Acanthamoeba* spp, *Balamuthia mandrillaris, Hartmannella vermiformis*, *Naegleria fowleri, Entamoeba histolytica*, and *E. invadens*. Tissue sections were tested for the presence of amoebas by applying conjugated antiamoebic sera for 30 min at a 25% dilution, washed in PBS, and then mounted in glycerol (Visvesvara 1987). Amoebae in the colon and liver only reacted with an antibody specific to *E. invadens*, resulting in strongly positive trophozoites (Fig. 11).

DISCUSSION

The present outbreak in green anacondas causing peracute death or acute apathy, anorexia and bloody feces was associated mainly with enterocolitis and necrotizing hepatitis. The diagnosis was based on intralesional positive PAS trophozoites morphologically compatible with *Entamoeba invadens* confirmed by immunofluorescence assay.

Entamoeba is a genus of protozoa that has adapted to the digestive tract of many vertebrate and invertebrate hosts. Most *Entamoeba* species are non-pathogenic. However, some species can cause severe, including fatal, diseases invading the

Table 1. Distribution and gradation of macroscopic lesions in the infection by *Entamoeba invadens* in green anacondas (*Eunectes murinus*)

Case	Proliferative colitis			Multifocal ulcerative gastritis	Multifocal necrotic hepatitis
	Fibrinous	Fibrino-necro-hemorrhagic	Intussusception		
1	-	+++	-	-	+++
2	-	+++	-	-	+++
3	+++	-	Yes	++	++
4	-	+++	-	++	++
5	-	+++	Yes	+++	++

- Absent, ++ moderate, +++ severe.

colon and hematogenously spreading to the liver and other organs. *Entamoeba histolytica* and *E. invadens* are important pathogens in this genus. While *E. histolytica* causes human amoebiasis, *E. invadens* causes amoebiasis in snakes, which is a highly contagious and potentially fatal disease (Hooshyar et al. 2015). Detecting histologically *Entamoeba* species is not challenging due to their characteristic morphology and staining with PAS or trichrome stains. However, it is impossible to identify species based on morphology (Fotedar et al. 2007, Saidin et al. 2019). Although some authors recommend the need for molecular tests to differentiate *E. invadens* from other amoebas from the *Entamoeba* genus (Michaely et al. 2020), antibody-based antigen detection is quite specific (Fotedar et al. 2007, Baseler et al. 2014, Saidin et al. 2019), since antibodies from other *Entamoeba* (e.g., *E. histolytica*) do not react with *E. invadens*, and vice versa (Brewer et al. 2008, Baseler et al. 2014), as we observed in this study. Other techniques used to establish definite diagnosis include polymerase chain reaction (PCR) (Bradford et al. 2008, McFarland et al. 2021), immunohistochemistry (Jakob & Wesemeier 1995) and *in-situ* hybridization (Richter et al. 2008). The main free-living amoebae potentially pathogenic to humans and animals belong to the *Entamoeba*, *Acanthamoeba*, *Balamuthia*, *Naegleria* and *Sappinia* genera (Visvesvara et al. 2007, García et al. 2014).

The intestinal lesions in the green anacondas of this outbreak are similar to those described in other enterocolitis related to *E. invadens* infection in multiple snake species (Donaldson et al. 1975, Jakob & Wesemeier 1995, Kojimoto et al. 2001, Richter et al. 2008, Baseler et al. 2014). The inflammation pattern of enteric necrosis and hepatic, renal, and vascular lesions is often described in amebiasis by *E. invadens* cases in snakes. Amoebic trophozoites usually spread in a hematogenous way throughout the hepatic or renal portal system (Kojimoto et al. 2001, Baseler et al. 2014, Park et al. 2019). The pathogenesis of *Entamoeba* spp. infection starts with fecal-oral transmission via contaminated food or water

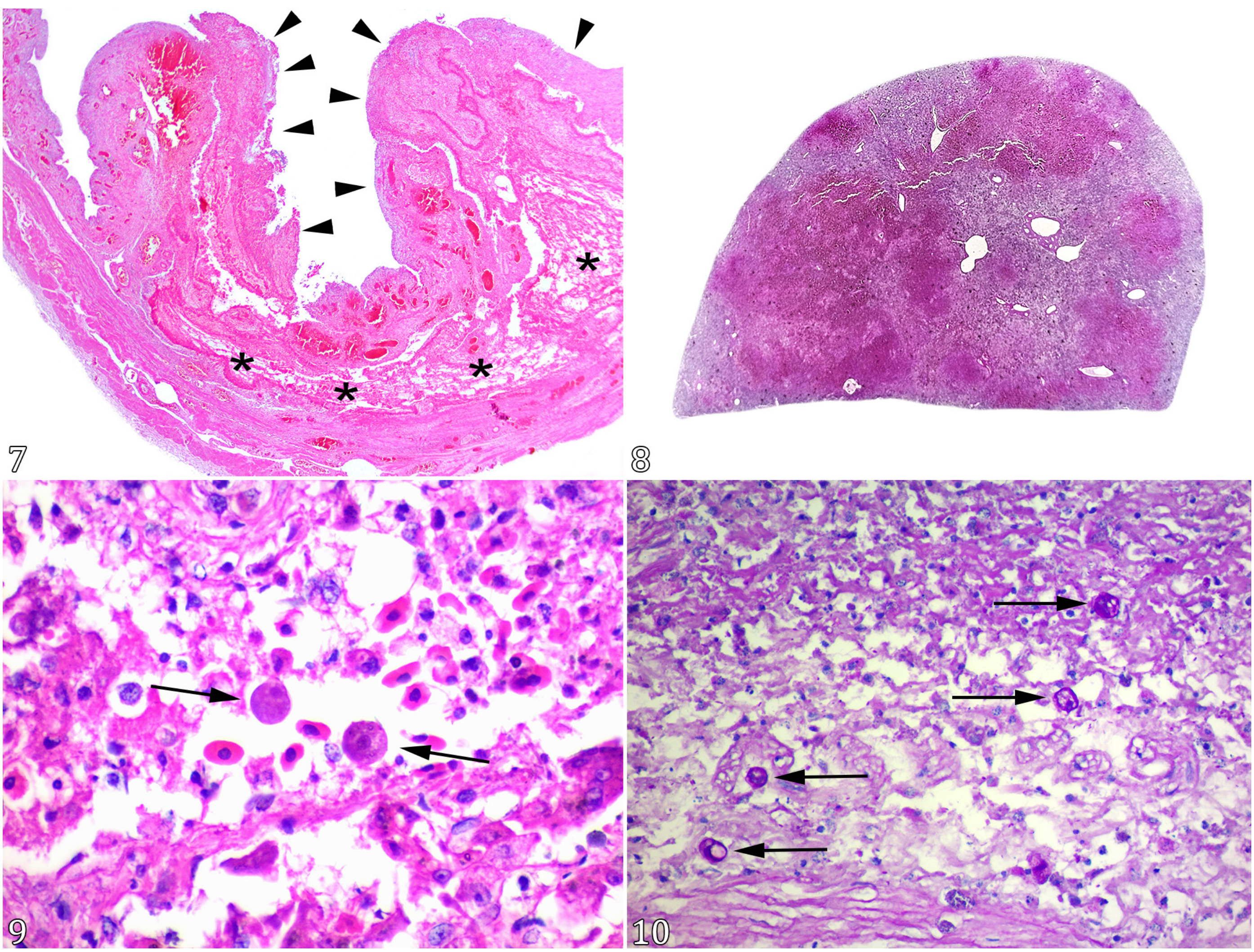

Fig. 7-10. Infection by *Entamoeba invadens* in green anacondas (*Eunectes murinus*). (**7**) Case 4. Colon wall thickening, inflammatory infiltration and hemorrhage. Note the submucosa expansion (asterisks) and the fibrinonecrotic membrane over the thickened and irregular mucosa (arrowheads). HE, submacroscopic. (**8**) Case 1 shows multifocal to coalescent necrotizing hepatitis and hemorrhage. HE, submacroscopic. (**9**) Liver from Case 2 with hepatocyte disorganization, hepatocellular vacuolization and necrosis and free amoebic trophozoites (arrow). HE, obj. 63x. (**10**) Colon from Case 3 showing *E. invadens* trophozoite demonstrating cytoplasm with glycogen in the submucosa. PAS, obj. 40x.

by ingestion of infective cysts, which develop into motile trophozoites in the intestine, followed by binary fission of trophozoites that invade mucosa or are transformed into cysts and pass in the feces. The trophozoites attach to the gastrointestinal epithelium, causing epithelial cell death, and may invade the lamina propria, crypts of colonic glands and submucosa. The liver lesions result from trophozoites circulating through the portal vein (Ralston et al. 2014).

In this study, it was not possible to identify the source of infection. The introduction of a sick snake would be a possibility, but identifying the individual anaconda released in the enclosure two months before the deaths was impossible. Consequently, we always recommend quarantine before releasing animals from free-ranging habitats in enclosures to avoid unknown or subclinical infections. The contamination could also be associated with the flow of people between enclosures because to access the anaconda enclosure, treaters had to pass through an enclosure that housed yacarés (*Caiman yacare*) and red-footed tortoises (*Chelonoidis carbonaria*).

Chelonians and crocodilians are *E. invadens* hosts, and this amoeba species seems less pathogenic (Meerovitch 1958). The definitive host remains asymptomatic while the amoeba is in its cyst stage. If the trophozoites stay on the mucosa, they can make cysts released in the lumen. The symptomatic stage occurs when trophozoites destroy the mucosa through protease activity and hit the lamina propria, activating pro-inflammatory responses (Watanabe & Petri 2015). The trophozoite-releasing process depends on the proper conditions of the intestinal lumen. In this way, tissue invasion occurs due to the erratic cycle stimulated by the intestines of snakes, as they are accidental hosts. The same does not frequently occur in turtles, which, being phylogenetically more primitive, evolved along amoebae for a longer time. This offers the necessary conditions in the intestinal lumen for their encystation and life cycle conclusion without lesions (Meerovitch 1958). Environmental factors, such as food or temperature change, are considered triggers for symptoms and consecutive death of hosts (Jacobson et al. 1983). This might be the reason for these snakes having died acutely or peracutely.

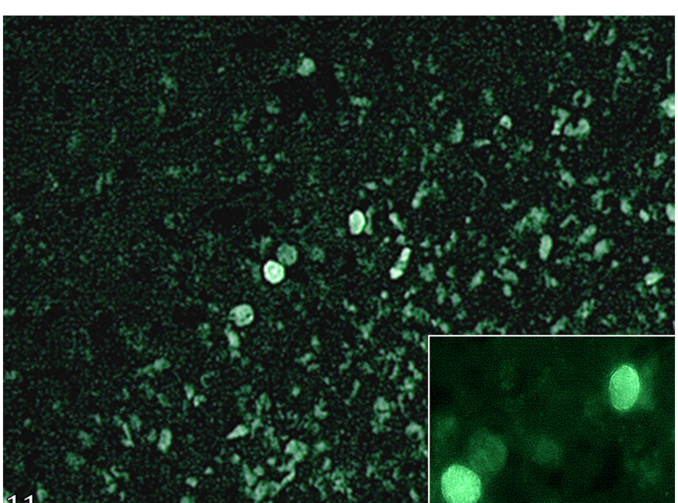

Fig. 11. Infection by *Entamoeba invadens* in green anacondas (*Eunectes murinus*). Immunofluorescence from Case 3 showing amoebic trophozoites tested to anti-*E. invadens* antibodies strongly positive marked. DIF, obj. 20x. Inset: detail showing the amoebic trophozoites strongly positive marked DIF, obj. 100x.

Harmless commensals to highly pathogenic, numerous protozoa have been reported in reptiles. Confinement in enclosures, continuous autoinfection and host immune status determine protozoa's pathogenicity. Beyond amoebas, *Cryptosporidium* spp. is the leading cause of gastrointestinal diseases (Brower & Cranfield 2001, Richter et al. 2008). Snakes with *Cryptosporidium* sp. infection have either chronic gastritis or no clinical signs. Fifteen juvenile rough green snakes (*Opheodrys aestivus*) died without previous clinical signs due to *Cryptosporidium* sp. infection, causing severe heterophilic and lymphocytic enteritis with epithelial necrosis (Richter et al. 2008). *Monocercomonas* sp. is a harmless flagellate protozoa in the snake gastrointestinal tract, but it may lead to enteritis (Zwart et al. 1984, Jakob & Wesemeier 1995). *Entamoeba ranarum* has typically been considered a disease of amphibians that act as replicators or reservoirs, with rare findings of disease in snakes, causing a state of severe enterocolitis (Shilton et al. 2019, Michaely et al. 2020, McFarland et al. 2021).

As additional differential diagnoses, we must include secondary bacterial infections often associated with Gram-negative bacilli. They are bacteria present in snake microbiota, but due to parasite or viral primary infections (Jacobson et al. 1992) could cause immunosuppression and become pathogenic, resulting in enterocolitis. The main bacterial genera that may cause enteritis and hepatitis are *Citrobacter* and *Salmonella* (Onderka & Finlayson 1985, Jacobson et al. 1992), *Providencia, Pseudomonas*, and *Aeromonas* (Kolesnikovas et al. 2001, Park et al. 2019). *Aeromonas hydrophila* is an opportunist bacterium frequently isolated in snakes. It may remain asymptomatic or cause gastritis and enteritis that evolve into septicemia and affect multiple organs (Kolesnikovas et al. 2001). Septicemia was the second cause of death in a series of cases in the reptiles from Testudines order in the Midwest of Brazil (Santos et al. 2022). In the present outbreak, we found bacterial colonies in the intestines and inside vessels from the intestines, liver, lungs, and kidney of three anacondas, which fits the probable diagnosis of septicemia in those snakes. An essential characteristic of amoebas is that they carry pathogenic microorganisms, such as bacteria, characterizing an association known as endosymbiosis (Lovieno et al. 2010).

CONCLUSIONS

Infection by *Entamoeba invadens* may cause systemic infection in green anacondas (*Eunectes murinus*) kept in captivity, resulting in a systemic disease characterized mainly by enterocolitis, gastritis and necrotic hepatitis.

The definitive diagnosis should demonstrate the etiologic agent associated with gross and histologic lesions.

In institutions where snakes, chelonians and crocodilians are kept, planning the enclosures and adapting management to isolate the snake environments is necessary.

Acknowledgments.- We thank the staff of the Division of Foodborne, Waterborne and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases (CDC) for their technical support with the immunofluorescence stain and for their assistance in preparing the images. The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The authors are grateful to the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq), “Fundação de Amparo à Pesquisa do Estado de Mato Grosso” (FAPEMAT) and “Pró Reitoria de Pesquisa da Universidade Federal de Mato Grosso” (PROPeq).

Conflict of interest statement.- The author declares that there are no conflicts of interest.

Credit author statement.- Souza BC, Cavasani JPS, Santos IG, Sabino L – Case analysis and article writing; Ubiali DG, Pescador CA, Colodel EM, Furlan FH – Case diagnosis and article writing.

Data availability statement.- The datasets used and analyzed during the current study are available from the corresponding author upon request.

REFERENCES

Baseler LJ, Visvesvara GS, Ramos-Vara JA. Pathology in practice. *E invadens* infection in a ball python. J Am Vet Med Assoc 2014; https://doi.org/10.2460/javma.245.5.501

Bradford CM, Denver MC, Cranfield MR. Development of a polymerase chain reaction test for *Entamoeba invadens*. J Zoo Wildl Med 2008; https://doi.org/10.1638/2007-0145.1

Brewer LA, Denver MC, Whitney M, Eichinger DJ. Analysis of commercial *Entamoeba histolytica* ELISA kits for the detection of *Entamoeba invadens* in reptiles. J Zoo Wildl Med 2008; https://doi.org/10.1638/2007-0182.1

Brower AI, Cranfield MR. *Cryptosporidium* sp.-associated enteritis without gastritis in rough green snakes (*Opheodrys aestivus*) and a common garter snake (*Thamnophis sirtalis*). J Zoo Wildl Med 2001; https://doi.org/10.1638/1042-7260(2001)032[0101:CSAEWG]2.0.CO;2

Donaldson M, Heyneman D, Dempster R, Garcia L. Epizootic of fatal amebiasis among exhibited snakes: Epidemiologic, pathologic, and chemotherapeutic considerations. Am J Vet Res 1975; https://doi.org/10.2460/ajvr.1975.36.06.807

Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J. Laboratory diagnostic techniques for *Entamoeba* species. Clin Microbiol Rev 2007; https://doi.org/10.1128/cmr.00004-07

García G, Ramos F, Pérez RG, Yañez J, Estrada MS, Mendoza LH, Martinez-Hernandez F, Gaytán P. Molecular epidemiology and genetic diversity of *Entamoeba* species in a Chelonian collection. J Med Microbiol 2014; https://doi.org/10.1099/jmm.0.061820-0

Ghosh TN. Observations on the type specimen of *Entamoeba serpentis* Cunha and Fonseca, 1917. J Protozool 1968; https://doi.org/10.1111/j.1550-7408.1968.tb02104.x

Greiner EC, Mader DR. Parasitology. In: Mader D. Reptile Medicine and Surgery. 2006; https://doi.org/10.1016/B0-72-169327-X/50025-0

Hooshyar H, Rostamkhani P, Rezaeian M. An annotated checklist of the human and animal *Entamoeba* (Amoebida: Endamoebidae) species – a review article. Iran J Parasitol 2015;10(2):146-156. PMid:26246811

Jacobson E, Clubb S, Greiner E. Amebiasis in red-footed tortoises. J Am Vet Med Assoc 1983; https://doi.org/10.2460/javma.1983.183.11.1192

Jacobson ER, Gaskin JM, Wells S, Bowler K, Schumacher J. Epizootic of ophidian paramyxovirus in a zoological collection: pathological, microbiological, and serological findings. J Zoo Wildl Med 1992;23(3):318-327.

Jakob W, Wesemeier HH. Intestinal inflammation associated with flagellates in snakes. J Comp Pathol 1995; https://doi.org/10.1016/S0021-9975(05)80022-2

Kojimoto A, Uchida K, Horii Y, Okumura S, Yamaguch R, Tateyama S. Amebiasis in four ball pythons, *Python reginus*. J Vet Med Sci 2001; https://doi.org/10.1292/jvms.63.1365

Kolesnikovas CKM, Ramos MCC, Catão Dias JL. Microbiological findings in the Brazilian rattlesnake (*Crotalus durissus*). Bol Asoc Herpetol Esp 2001;12(1):25-28.

Lovieno A, Ledee DR, Miller D, Alfonso EC. Detection of bacterial endosymbionts in clinical *Acanthamoeba* isolates. Ophthalmology 2010; https://doi.org/10.1016/j.ophtha.2009.08.033

McFarland A, Conley KJ, Seimon TA, Sykes JM. A retrospective analysis of amoebiasis in reptiles in a zoological institution. J Zoo Wildl Med 2021; https://doi.org/10.1638/2020-0148

Meerovitch E. A new host of *Entamoeba invadens* rodhain. Can J Zool 1958; https://doi.org/10.1139/z58-036

Michaely LM, Von Dörnberg K, Molnár V, Tappe D, Tannich E, Hewicker-Trautwein M, Wohlsein P. *Entamoeba ranarum* infection in a ball python (*Python regius*). J Comp Pathol 2020; https://doi.org/10.1016/j.jcpa.2020.07.008

Onderka DK, Finlayson MC. Salmonellae and salmonellosis in captive reptiles. Can J Comp Med 1985;49(3):268-270. PMid:4041972

Park CH, Han JB, Park SI. Dual infection with *Entamoeba invadens* and *Aeromonas hydrophila* in a captive anaconda (*Eunectes murinus*) leading to necrotising gastroenteritis and hepatocyte death. Veterinárni Medicína 2019; https://doi.org/10.17221/140/2018-VETMED

Prophet EB, Mills B, Arrington JB, Sobin LH. AFIP Laboratory Methods in Histotechnology. Washington: American Registry of Pathology; 1992.

Ralston KS, Solga MD, Mackey-Lawrence NM, Somlata, Bhattacharya A, Petri Jr WA. Trogocytosis by *Entamoeba histolytica* contributes to cell killing and tissue invasion. Nature 2014; https://doi.org/10.1038/nature13242

Richter B, Kübber-Heiss A, Weissenböck H, Schmidt P. Detection of *Cryptosporidium* spp., *Entamoeba* spp. and *Monocercomonas* spp. in the gastrointestinal tract of snakes by *in-situ* hybridization. J Comp Pathol 2008; https://doi.org/10.1016/j.jcpa.2007.11.001

Rivas JA, Quintana PDL, Mancuso M, Pacheco LF, Rivas GA, Mariotto S, Salazar-Valenzuela D, Baihua MT, Baihua P, Burghardt GM, Vonk FJ, Hernandez E, García-Pérez JE, Fry BG, Corey-Rivas S. Disentangling the anacondas: Revealing a new green species and rethinking yellows. Diversity 2024; https://doi.org/10.3390/d16020127

Saidin S, Othman N, Noordin R. Update on laboratory diagnosis of amoebiasis. Eur J Clin Microbiol Infect Dis 2019; https://doi.org/10.1007/s10096-018-3379-3

Santos UG, Queiroz CRR, Hirano LQL, Santos MVB, Cavalcante AKS, Macêdo JTSA, Pedroso PMO. Anatomopathological findings of necropsied Testudines in the Distrito Federal, Brazil. Pesq Vet Bras 2022; https://doi.org/10.1590/1678-5150-PVB-6953

Shilton CM, Slapeta J, Shine R, Brown GP. Pathology associated with an outbreak of entamoebiasis in wild cane toads (*Rhinella marina*) in tropical Australia. Vet Pathol 2019; https://doi.org/10.1177/0300985819868729

Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. FEMS Immunol Med Microbiol 2007; https://doi.org/10.1111/j.1574-695X.2007.00232.x

Visvesvara GS. Laboratory diagnosis. In: Rondanelli EG. Amphizoic Amoeba: human pathology. Padova: Piccin Nuova Libraria; 1987.

Watanabe K, Petri JR. Molecular biology research to benefit patients with *Entamoeba histolytica* infection. Mol Microbiol 2015; https://doi.org/10.1111/mmi.13131

Zwart P, Teunis SFM, Cornelissen JMM. Monocercomoniasis in reptiles. J Zoo Anim Med 1984; https://doi.org/10.2307/20094704