**Joint Pathology Center**
**Veterinary Pathology Services**

# WEDNESDAY SLIDE CONFERENCE 2023-2024

## Conference #19 — 7 February 2024

**CASE I:**

**Signalment:**
Adult female corn snake (*Pantherophis guttatus*)

**History:**
An adult corn snake housed as part of a teaching colony presented for a two week history of regurgitation. During physical examination, a coelomic mass-effect was palpated as well as scale changes that prompted a scrape. Mites were identified and confirmed as *Ophyionissus natricis*. Supportive care was initiated. The snake was found dead in its enclosure several days after initial examination.

**Gross Pathology:**
The pleural surface is covered in a thick mat of fibrin. On cut section, there are multifocal 0.1-1.0 cm diameter abscesses containing caseonecrotic exudate.

**Laboratory Results:**
PCR on fresh gastrointestinal tissue was positive for *Salmonella* sp. and subsequent culture/genotyping identified *S. enterica ssp. enterica* serogroup C.

PCR on formalin-fixed paraffin embedded gastrointestinal tissue was positive for *Entamoeba invadens*.

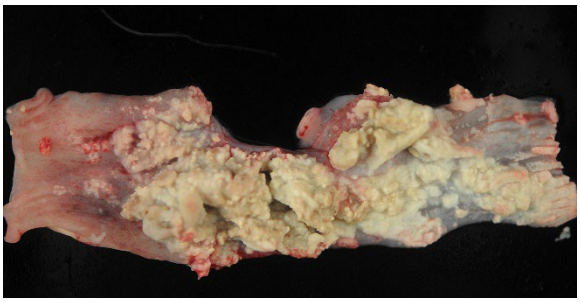

**Figure 1-1. Stomach and intestine, cornsnake. The mucosa is multifocally ulcerated with dense adherent fibrinonecrotic material extending deep into the tissue. (*Photo courtesy of:* Department of Population Health and Pathobiology, North Carolina State University College of Veterinary Medicine, Raleigh, NC. https://php.cvm.ncsu.edu/)**

**Microscopic Description:**
Stomach, ileum and colon: Multifocally the mucosa is eroded to ulcerated and overlain by thick mats of abundant fibrin mixed with cellular and karyorrhectic debris, numerous bacterial colonies, and marked histiocytic and heterophilic inflammation (diphtheritic membrane). In areas of ulceration where the membrane has been lifted, the exposed submucosa is often lined by numerous round protozoal organisms measuring 7-10 µm in diameter with a thin basophilic wall filled with fibrillar to granular basophilic cytoplasm and a single, often eccentrically placed, round basophilic nucleus 2-3 µm in diameter (ameboid trophozoites). Affected submucosa is sometimes expanded by granulation tissue and fibrin

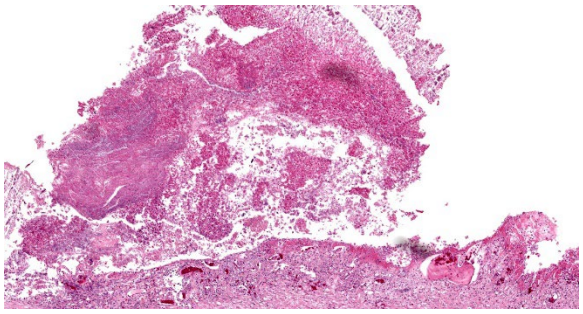

**Figure 1-2. Intestine, cornsnake. There is extensive necrosis of the mucosa with abundant adherent necrotic debris. (HE, 61X)**

Thrombi are frequently present within submucosal capillaries. Rarely, necrosis and inflammation extend into the tunica muscularis but does not breach the serosal membrane.

Special stains: Periodic acid-Schiff (PAS) stain applied to sections of gastrointestinal tract highlights the cell wall of amoebic trophozoites.

**Contributor's Morphologic Diagnosis:**
Gastrointestinal tract: Severe multifocal, segmentalfibrinonecrotic and histiocytic enteritis with intralesional amoebic trophozoites, diphtheritic membranes and fibrin thrombi.

**Contributor's Comment:**
Of amoebic infections in reptiles, *Entamoeba invadens* is one of the more significant pathogenic species.[4,7] Turtles and crocodiles are considered host reservoirs for the organism and transmission follows the fecal-oral route.[4,7] Infective cysts are shed in feces and can persist in the environment for prolonged periods.[4] Ingestion of cysts by susceptible species results in enteritis, as seen in this case, with some cases extending to the liver as well.[4,7] Rare systemic infections have been reported.[4] The pathogenesis of *Entamoeba invadens* is similar to *Entamoeba histolytica*, a pathogenic amoeba that causes dysentery in humans and nonhuman primates.[9,14] *E. invadens* is often used as a laboratory model for *E. histolytica* due to its ability to undergo encystation in vitro, which *E. histiolytica* lacks.[7,9]

The life stages of *Entamoeba* species include the transmissible cyst and the infective trophozoite.[10] Hosts ingest cysts from contaminated environments and the cyst hatches into a trophozoite via "excystation" within the intestinal lumen.[10] Trophozoites then attach to epithelial cells inducing cell injury and death through a variety of mechanisms.[2] Attachment of trophozoites to mucins coating the intestinal mucosa and enterocytes occurs via the amebic Gal/GalNAc lectin.[2,10] Trophozoites then induce cell death by releasing pore-forming polypeptides called amoebapores and by biting off portions of viable cells, a phenomenon referred to as trogocytosis.[2] Both result in increased intracellular calcium, culminating in cell death.

Amoeba-induced enterocyte death results in ulcerative lesions of the gastrointestinal tract and exposes the underlying stroma into which trophozoites can invade by releasing proteases that break down the extracellular matrix.[2] This invasion allows for passage of trophozoites into the blood vessels where they can colonize extraintestinal sites in an embolic fashion. In particular, the liver is thought to be infected through a hematogenous route from the portal vein, resulting in liver abscesses.[12] Some trophozoites transform back into cysts via "encystation" which are passed into the feces to perpetuate the life cycle.[10]

Severity of disease may be affected by the environment (namely temperature), host immune status, and protozoal virulence factors.[4,7] Additional host factors may include

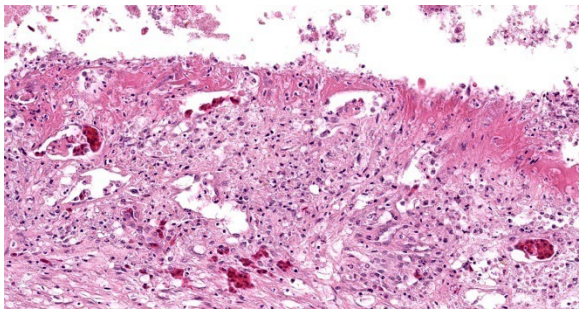

**Figure 1-3. Intestine, cornsnake. Higher magnification of the necrotic mucosa. There are numerous flagellates infiltrating the mucosa. (HE, 381X)**

diet. Typically, carnivorous lizards and snakes develop disease with *E. invadens* infection while herbivorous turtles infrequently develop lesions.[7] This may be due in part to the protective gut microenvironment that an herbivorous diet imparts. Virulence of *E. invadens,* namely encystation and invasion into tissues, decreases in environments rich in glucose and gram-negative bacteria.[7,9,14] When comparing the diets of studied reptiles and chelonids, the majority of animals on a carnivorous diet developed *E. invadens*-induced lesions while those on omnivorous and herbivorous diets had intermediate and low rates of lesion development.[7]

Aside from *E. invadens*, snakes can develop disease from *E. ranarum* infection.[8,11] Both can present as a necrotizing gastroenteritis, though extra-intestinal lesions have not been reported with *E. ranarum*.[4,8,11] For cases of *E. invadens* that only manifest as intestinal disease, as was seen in this case, ancillary testing is needed to definitively determine the species present. Moreover, it is difficult to distinguish between amoebic species on routine histologic examination and so molecular testing, namely polymerase chain reaction assays, have been developed to distinguish between species.[1,4] Special stains can be performed to highlight certain features of the organisms. PAS will stain the walls of cysts and trophozoites while trichrome stain can identify certain cellular features such as the dark pink chromatoid bodies.[4] The latter is best visualized in fecal preparations.[4]

Differential diagnoses for gastroenterocolitis in snakes include salmonellosis and coccidiosis.[4] While *Salmonella* spp. PCR and cultures were positive in this case, the yielded bacterium, *Salmonella enterica* ssp. *Enterica,* is considered a commensal in reptiles.[4] It's possible dysbiosis secondary to intestinal amebiasis led to overgrowth of *S. enterica* ssp. *enterica* and contributed to morbidity in this case. Coccidiosis was also considered based on routine histologic evaluation due to the relatively smaller size of the organisms than is previously reported in cases of *E. invadens*;[4] however, fecal analysis of cohabitants of this snake did not find coccidian organisms and PCR detected *E. invadens* in this case.

**Contributing Institution:**
Department of Population Health
and Pathobiology
North Carolina State University
College of Veterinary Medicine
https://php.cvm.ncsu.edu/

**JPC Diagnoses:**

1. Gastrointestinal tract: Gastroenterocolitis, necrotizing, multifocal, moderate, with numerous flagellates and superficial bacteria.
2. Kidney: Urate stasis with rare gouty tophi.
3. Presumed ovary: Granulomas, multiple.

**JPC Comment:**

The contributor provides an excellent overview of enteric disease caused by *Entamoeba invadens* and the virulence factors that make *Entamoeba* spp. in general so damaging. As

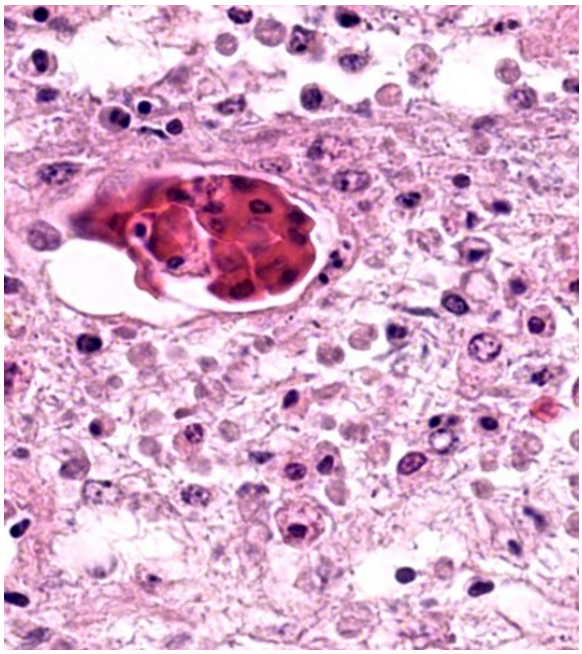

**Figure 1-4. Intestine, cornsnake. Numerous flagellates infiltrate the necrotic mucosa and submucosa. (HE, 892X)**

the contributor notes, the organisms in this case are smaller (approximately 4 µm in diameter) than reported for *E. invadens*, which drew additional, careful scrutiny during conference discussion.

In additional to their small size, the examined protozoa are also irregularly shaped and have eccentric, small, darkly basophilic, nuclei measuring approximately 1 µm in diameter. The cytoplasm is occasionally tapered at one end into a thin filament, which is interpreted as a flagellum. The protozoa are often present in large clusters of dozens of organisms and are overlying ulcerated epithelium.

These characteristics are most consistent with infection with flagellates, which commonly infect the intestines of snakes and are associated with ulceration and secondary bacterial infections.[13] Flagellates infect similar anatomic regions as *Entamoeba* sp., which can make differentiation of these protozoa challenging. *Entamoeba invadens* are larger (10-15 µm in diameter) than flagellates and have discrete round nuclei *Entamoeba invadens* typically occur individually (rather than in clusters of dozens of organisms) and are more invasive with more necrosis, hemorrhage, and inflammation than the flagellates seen in this case.[13]

Interestingly, a previous publication investigated 51 snakes with histologically suspected *Entamoeba invadens* infection. The authors used immunohistochemistry to confirm or rule out *Entamoeba* infection. Only 22 cases were confirmed as *Entamoeba invadens*; all other cases were intestinal flagellate infections with histological feature similar to the present case. Histologic lesions secondary to flagellate infection included inflammation and ulceration of the intestines with a fibrinonecrotizing membrane, and flagellates were reported on the surface of ulcerated mucosa, as seen in this case.[5] This work underscores the histologic similarity between *Entamoeba invadens* and flagellate infections in snakes, and the present case serves as an excellent example of the difficulty in differentiating these infections.

Flagellates that cause enteritis in snakes are not well characterized. Most cases are assumed to represent *Monocercomonas* spp. based on the few reports of this flagellate in snake enteritis lesions; however, confirmation of the genus of infective flagellates is rarely performed in cases of snake enteritis.[15] Flagellates can infect other organs in reptiles. Recent reptile work has described renal flagellates, suspected of finding their way to the kidney via reflux into the urinary system from the cloaca; flagellate-associated gastroenteritis was also commonly reported in that case series.[6] Conference participants did find rare

amoebas of the appropriate size within the luminal contents, consistent with the contributor's PCR results; however, snakes can be asymptomatic but PCR positive for *Entamoeba invadens*, which is suspected in this case.[3]

This week's conference was moderated by Dr. David Needle, Associate Professor at the University of New Hampshire and Senior Veterinary Pathologist and Pathology Section Chief at the New Hampshire Veterinary Diagnostic Laboratory. Dr. Needle led participant discussion of this description-rich slide, which, after much discussion of the etiologic agent as previously detailed, moved to more ancillary lesions, including the gouty tophus within the kidney. Participants noted the abundant pigment within the renal tubules, which is a normal finding in reptiles and is of unknown origin. Participants also noted the unidentified tissue surrounded by macrophages with intracytoplasmic yellow pigment, which participants thought might represent degenerative changes within aged ovaries, though mycobacterial granulomas could not be ruled out in the absence of acid-fast staining.

Consideration of the morphologic diagnosis recapitulated much of the previous conference discussion, with participants convinced that the gastrointerstinal pathology was caused by the flagellates aggregated within areas of tissue necrosis rather than by the occasional amoeba found within the lumen of the gastrointestinal tract.

**References:**


1. Bradford CM, Denver M, Cranfield MR. Development of a polymerase chain reaction test for *Entamoeba invadens*. *J Zoo Wildl Med*. 2008;39(2):201-207.
2. Espinosa-Cantellano M, Martínez-Palomo A. Pathogenesis of intestinal amebiasis: from molecules to disease. *Clin Microbiol Rev*. 2000;13(2):318-31.
3. Heng Y, Hsu CD, Mathew A, Oh PY, Li WT, Xie S. Management of *Entamoeba invadens* in the herpetological collection at the Singapore zoo. *J Wildl Med*. 2023 Jul;54(2):272-281.
4. Jacobson ER, Garner MM. *Infectious Diseases and Pathology of Reptiles: Color Atlas and Text*. 2nd ed. CRC/Press; 2021:860-862, 904-905.
5. Jakob W, Wesemeier HH. Intestinal inflammation associated with flagellates in snakes. *J Comp Pathol*. 1995;112 (4):417-21.
6. Juan-Sallés C, Garner MM, Nordhausen RW, Valls X, Gallego M, Soto S. Renal flagellate infections in reptiles: 29 cases. *J Zoo Wildl Med*. 2014 Mar;45(1):100-9.
7. McFarland A, Conley KJ, Seimon TA, Sykes JM. A retrospective analysis of amoebiasis in reptiles in a zoological institution. *J Zoo Wildl Med*. 2021;52(1): 232-240.
8. Michaely LM, von Dornberg K, Molnar V. Entamoeba ranarum infection in a ball python (*Python regius*). *J Comp Path*. 2020;179:74-78.
9. Mi-Ichi F, Yoshida H, Hamano S. Entamoeba encystation: new targets to prevent the transmission of amebiasis. *PLoS Pathog*. 2016;12(10):e1005845.
10. 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;508(7497): 526-530.
11. Richter B., Kübber-Heiss A., Weissenböck H. Diphtheroid colitis in a Boa

constrictor infected with amphibian Entamoeba sp. *Vet Parasitol*. 2008;153(1-2):164-167.
12. Usuda D, Tsuge S, Sakurai R, et al. Amebic liver abscess by Entamoeba histolytica. *World J Clin Cases*. 2022;10(36): 13157-13166.
13. Walden HDS, Greiner EC, Jacobson ER. Parasites and parasitic diseases of reptiles. In: Jacobson ER, Garner MM, eds. *Infectious Diseases and Pathology of Reptiles*. 2nd ed. CRC Press;2020.
14. Watanabe K, Petri WA. Learning from the research on amebiasis and gut microbiome: is stimulation by gut flora essential for effective neutrophil mediated protection from external pathogens? *Gut Microbes*. 2019;10(1):100-104.
15. Zwart, Peernel and SFM, Teunis. Monocercomoniasis in reptiles. September 1982. Conference: First International colloquiem on pathology of reptiles and amphibians. At: Angers (France). 73-76.

**CASE II**:

**Signalment:**
Adult male white-eared opossum (*Didelphis albiventris*)

**History:**
The animal was found dead in an urban park in the city of Belo Horizonte, Brazil, and was immediately submitted to necropsy.

**Gross Pathology:**
There was an extensive area of skin laceration with exposure of bone and muscle in the dorsal proximal portion of the tail. Lungs were pale pink with diffuse decreased crepitation. The myocardium had multifocal to coalescing 0.2 to 0.4 cm white areas. The liver was diffusely yellow and friable. The spleen was markedly enlarged with multifocal to coalescent white-red areas. The kidneys were diffusely yellow with pinpoint multifocal white areas. The pelvis of the right kidney was moderately dilated, as was the right ureter, which was partially obstructed by a white friable plug. The gastric and duodenal lumens had multiple cylindrical white worms which were 3 cm long and 0.3 cm in diameter (morphologically compatible with nematodes).

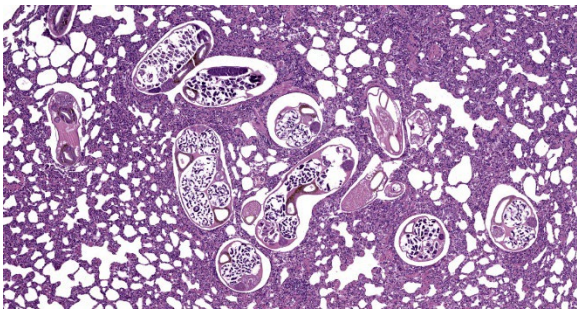

**Figure 2-2. Lung, opossum. Bronchioles contain multiple cross and tangential sections of adult male and female metastrongylid nematodes. (HE, 35X)**

**Laboratory Results:**
Samples of liver, spleen and kidney were submitted for bacterial culture. *Streptococcus didelphis* was isolated from all samples.

The nematodes found in the stomach and duodenum were identified as *Turgida turgida*.

**Microscopic Description:**
Lung: Bronchi and bronchioles are expanded by moderate amount of mucus, intact and degenerate neutrophils, and some macrophages. Interspersed with the mucus, there was a moderate number of nematode larvae approximately 50 µm in length x 7 µm in diameter. Alveolar septa were diffusely and moderately thick and filled by neutrophils and histiocytes. Multifocally, in the alveolar lumena and terminal bronchioles there are multiple adult

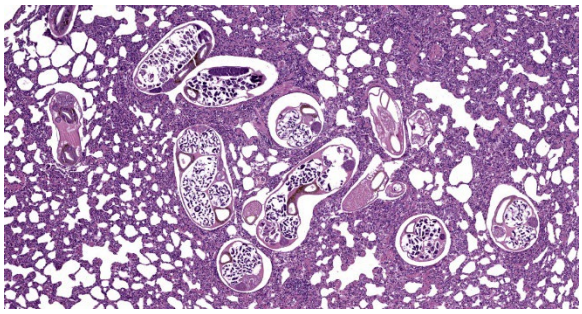

**Figure 2-2. Lung, opossum. Bronchioles contain multiple cross and tangential sections of adult male and female metastrongylid nematodes. (HE, 35X)**

metastrongyles approximately 260 µm in diameter. The parasites have a small and ornamented cuticle, a pseudocoelom, an intestine with cuboidal cells full of brown intracytoplasmic granules, a uterus with numerous larvae in females and testes with globose spermatozoa in males. There are mild multifocal areas of rupture and coalescence of alveolar septa (emphysema). Additionally, multifocally there are foamy macrophages in the alveolar septa and lumena adjacent to the adult parasites. Blood vessels are filled with leukocytes.

**Contributor's Morphologic Diagnoses:**

1. Lung: Bronchitis and bronchiolitis, catarrhal, neutrophilic and histiocytic, multifocal, moderate, with intraluminal nematode larvae.
2. Lung: Interstitial pneumonia, neutrophilic and histiocytic, diffuse, moderate, with nematodes morphologically compatible with *Didelphostrongylus hayesi* in the lumen of alveoli and terminal bronchioles, and mild multifocal emphysema.

**Contributor's Comment:**

White-eared opossums (*Didelphis albiventris*) are marsupial mammals that inhabit forests and peri-urban areas in Brazil, Bolivia, Argentina, Paraguay, and Uruguay, and, according to the IUCN red list, the species is considered of Least Concern for the risk of extinction.[3] Parasitic infections are frequently reported in these opossums, often by parasites that also can affect domestic animals and humans, such as ectoparasites, protozoans (*Leishmania* spp., *Toxoplasma gondii*, and *Trypanosoma cruzi*), and helminths (*Toxocara* sp., *Ancylostoma caninum*, and *Schistosoma* spp.).[1]

*Didelphostrongylus hayesi* is a nematode of the Order *Strongylida* and superfamily *Metastrongyloidea* that infects the lungs of opossums. Infection occurs by ingestion of snails (*Mesodon perigraptus* and *Triodopsis albolabris*) which are the intermediate hosts.[9] The infection can be mild to severe, and the animals may be asymptomatic or symptomatic, with dyspnea, fever and apathy. Radiographically, the lungs may have a bronchial pattern and fecal examination may support the *in vivo* diagnosis by visualizing larval stages.[7] At necropsy, the lungs may be hyperemic with a cranioventral pattern. Lungs do not collapse when the thoracic cavity is opened, and they are firm, with disseminated micronodules and adult parasites of approximately 1.5 to 2.0 mm may be seen in the bronchi. In cases of mild to moderate infection, as in this case, gross changes may be mild or absent. Histologically, there are larvae and adult parasites in the lumen of bronchi, bronchioles and alveoli, with mucus and an inflammatory infiltrate that can be neutrophilic, eosinophilic, or histiocytic. The infiltrate associated with the adult parasites is mild or absent, whereas larvae and eggs induce a more exacerbated inflammatory response, with granulomatous or pyogranulomatous inflammation.[7,8] There is also hyperplasia of the smooth muscle of the bronchi and bronchioles, hyperplasia of the respiratory epithelium, and emphysema.[4, 8]

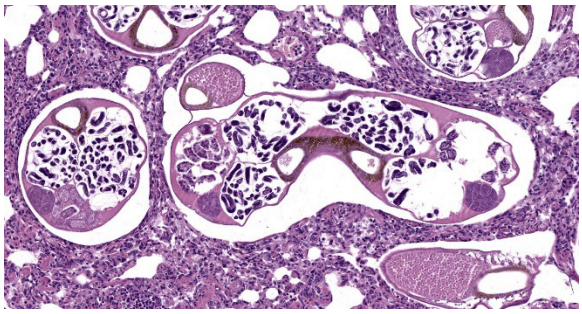

**Figure 2-3. Lung, opossum. Higher magnification of adult nematodes. The larger female has multiple cross sections of a uterus containing larvated eggs and an intestine lined by few epithelial cells which contains hemosiderin. The males (arrows) have a smaller diameter and a single cross section of a testis. (HE, 164X)**

Worm infections of the lower airways (trachea, bronchi, bronchioles, and alveoli) are important causes of morbidity and mortality in domestic and wild animals. Histological lung lesions by helminthic infections can range from mild, such as bronchitis and bronchiolitis in cases of mild infections by *Dictyocaulus viviparus,* to granulomatous, necrotizing, hyperplastic and emphysematous lesions. The intensity of the lesions will depend on the immune status of the host, as well as the parasite load and reproductive stages of the parasites.[2]

Traumatic injuries frequently occur in opossums due to their synanthropic habits, whether due to car accidents, or attacks by dogs or people. In this case, the animal presented with areas of skin laceration and systemic involvement by *Streptococcus didelphis*. Neutrophilic and histiocytic interstitial pneumonia, in addition to being associated with the parasites, may result from the systemic bacterial process. *Streptococcus didelphis* is a poorly known bacterium that is associated with cutaneous lesions in opossums.[10] A study identified the bacteria in nine opossums that had skin lesions. It is believed that skin lesions may have been the entry point for this agent in this case and the cause of the subsequent development of systemic lesions.[10]

**Contributing Institution:**
Departamento de Clínica e Cirurgia Veterinária
Escola de Veterinária
Universidade Federal de Minas Gerais
Av. Presidente Antônio Carlos,
Belo Horizonte, MG, Brazil.
www.vet.ufmg.br

**JPC Diagnoses:**
1. Lung: Bronchopneumonia, catarrhal and lymphohistiocytic, multifocal, mild to moderate, with metastrongyle adults, larvae, and eggs.
2. Lung: Pneumonia, interstitial, lymphohistiocytic and neutrophilic, diffuse, moderate.

**JPC Comment:**
*Didelphostrongylus hayesi* is an opossum lungworm with a wide distribution and high prevalence, above 70% in some studies, in opossums in the Americas.[8] Oppossums become infected after ingesting intermediate hosts which, as the contributor notes, are terrestrial snails. The migratory path of the nematode larvae from the gut to the lung is unknown, but once in the lung, third-stage larvae mature into adults in the airways, particularly the intrapulmonary bronchi.[8] Unlike other lungworms, which produce eggs that are deposited in host tissues or feces for futher development, *D. hayesi* produces eggs which hatch within their uteri.[6] The hatched stage one larvae migrate up the trachea, are coughed up, swallowed, and then passed in the feces where they contaminate the environment and are ingested by their intermediate gastropod hosts.[6]

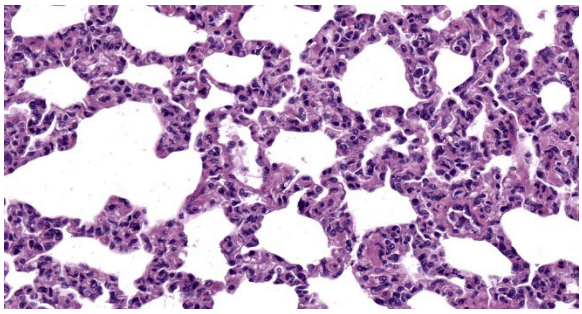

**Figure 2-4. Lung, opossum. Alveolar septa are hypercellular with hyperplasia of intraseptal macrophages, lymphocytes, and neutrophils. (HE, 380X)**

Typical gross lesions include lungs that do not collapse, focal to coalescing areas of emphysema, white to tan raised nodules on the pleural surface that roughly track the branching of intrapulmonary airways, and visible adult forms of *D. hayesi* on the visceral pleura.[8] Histologically, *D. hayesi* shares many common characteristics with its metastrongyle brothers and sisters, to include a smooth cuticle, a pseudocoelom, an intestine composed of few multinucleated cells with an indistinct brush border, and coelomyarian musculature. In the United States, opossum lungs are frequently co-infected with *D. hayesi* and the capillarid nematode *Eucoleus aerophilus*.[8]

Discussion of this case centered initially on the histologic appearance of the lungworm itself, with some participants noting an apparent eosinophilic fluid within the pseudocoelom, typically a feature of spirurids. The moderator noted that this is commonly seen in wildlife nematode infections and speculated that this could be a degenerative change. Other participants noted that spirurids typically have, among other distinguishing features, prominent lateral cords, which are not present in the examined section.

Participants noted the rather dramatic smooth muscle hyperplasia within vascular walls throughought the section, the extent of which seems inconsistent with the small number of organisms in section. Other participants noted that a prominent smooth muscle layer is normal in the vasculature of some species, such as the cat, and that opossum control tissue would be helpful in determining if the vascular "lesions" are, in fact, pathologic.

Finally, participants discussed the thickened alveolar septa and prominent diffuse interstitial pneumonia, lesions which are not typically associated with lungworm infection. Participants reviewed the clinical history and speculated that the interstitial pneumonia was likely due to bacterial sepsis. This separate pathogenesis warranted two separate differential diagnosis, with the bronchopneumonia attributed to the metastrongyles and the interstitial pneumonia attributed to sepsis.

**References:**


1. Bezerra-Santos MA, Ramos RAN, Campos AK, Dantas-Torres F, Otranto D. *Didelphis* spp. opossums and their parasites in the Americas: A One Health perspective. *Parasitol Res*. 2021; 120:4091-4111.
2. Caswell JL, Willians K. Respiratory system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 6th ed. Vol 2. 2016;536-590.
3. Costa LP, Astúa D, Brito D, Soriano P, Lew D. *Didelphis albiventris* (amended version of 2015 assessment). The IUCN Red List of Threatened Species 2021.
4. Duncan RBJ, Reinemeyer CR, Funk RS. Fatal lungworm infection in a opossum. *J Wildl Dis*. 1989;25(2):266-269.
5. Gardiner CH, Poynton SL. *An Atlas of Metazoan Parasites in Animal Tissues*. American Registry of Pathology. Washington, DC;1999:27-29.

6. Jones KD. Oppossum nematodiasis: diagnosis and treatment of stomach, intestine, and lung nematodes in the Virginia opossum (*Didelphis virginiana*). *J Exot Pet Med*. 2013;22(4):375-382.
7. Lamberski N, Reader JR, Cook LF, Johnson EM, Baker DG, Lowenstine LJ. A retrospective study of 11 cases of lungworm (*Didelphostrongylus hayesi*) infection in opossums (*Didelphis virginiana*). *J Zoo Wildl Med*. 2002;33(2):151-156.
8. López-Crespo RA, López-Mayagoitia A, Ramírez-Romero R, Martinez-Burnes J, Prado-Rebolledo OF, Garcia-Marquez LJ. Pulmonary lesions caused by the lungworm (*Didelphostrongylus hayesi*) in the opossum (*Didelphis virginiana*) in Colima, Mexico. *J Zoo Wildl Med.* 2017;48(2):404-412.
9. Prestwood AK. *Didelphostrongylus hayesi* gen. et sp. n. (*Metastrongyloidea*: *filaroididae*) from the opossum, *Didelphis marsupialis*. *J Parasitol*. 1976;62 (2):272-275.
10. Rurangirwa FR, Teitzel CA, Cui J, et al. *Streptococcus didelphis* sp. nov., a *streptococcus* with marked catalase activity isolated from opossums (*Didelphis virginiana*) with suppurative dermatitis and liver fibrosis. *Int J Syst Evol Microbiol*. 2000;50:759-765.

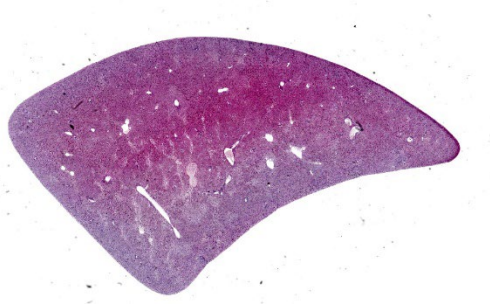

**Figure 3-1. Liver, porcupine. The submitted section of liver does not show any lesion (except subjective rounding of edges) at subgross magnification. (HE, 5X)**

**CASE III**:

**Signalment:**
5-year-old male porcupine (*Erethizon dorsatum*)

**History:**
This porcupine was born in captivity and transferred to another zoo one year later. The animal showed the following clinical signs: apathy, shortness of breath, and ataxia. The

clinical examination revealed a body temperature of 33.2°C. The animal died shortly after the onset of symptoms.

**Gross Pathology:**
Liver: On gross examination, the liver was predominantly wine-red, with some black-red areas. The liver margins were partly blunt-edged. The cut surface was demarcated, wine-red and firm. Furthermore, there were several to abundant beige-coloured, soft, sharply demarcated foci with a diameter of 1 to 5 mm.

**Microscopic Description:**
Liver: There are multifocal, randomly distributed hypercellular foci affecting 30% of the liver parenchyma. The foci consist of many

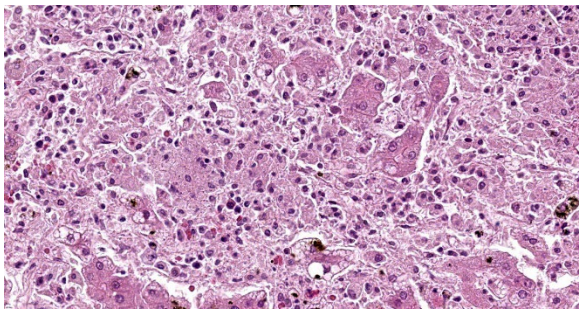

**Figure 3-2. Liver, porcupine. There are areas of hepatocellular necrosis and loss with infiltration of macrophages and neutrophils randomly scattered throughout the section. (HE, 480X)**

macrophages, lymphocytes, and few neutrophils. Scattered hepatocytes display cytoplasmic swelling, pallor, membranous rupture, nuclear pyknosis and karyorrhexis (lytic necrosis). Within the inflammatory foci there are rare intrahepatocytic, intracytoplasmic, 15-20 µm in diameter, oval apicomplexan cysts with a thin capsule and more than 20, 1-2 µm, basophilic, banana-shaped bradyzoites, and rare extracellular, 1-2 µm, basophilic, oval tachyzoites. Portal areas are expanded by 2-5 layers of lymphocytes, macrophages, and plasma cells. There is minor and variably distributed bile duct hyperplasia and portal fibrosis.

Inflammatory changes with variable severity were found in the heart, lungs, intestines, spleen, urinary bladder, and brain (not on submitted slide).

**Contributor's Morphologic Diagnosis:**
Liver: Hepatitis, lymphohistioplasmacytic and necrotizing, multifocal, random, subacute, moderate with intralesional apicomplexan cysts.

**Contributor's Comment:**
Due to the typical histomorphological findings, an infection with *Toxoplasma gondii (T. gondii)* was suspected as the cause of the lesions in the present case. Immunohistochemistry was performed and *T. gondii* was verified as the causative agent.

Toxoplasmosis is one of the most common and widespread diseases in humans and warm-blooded animals worldwide.[10] In 1908, the parasitic pathogen was first isolated from a gundi (*Ctenodactylus gundi*) in Tunis and a rabbit from South America.[1] The genus toxoplasma contains *T. gondii* as the only species, which can be divided into 189 different genotypes.[1,3] The epidemiological and pathogenetic significance depends on the respective genotypes. In the northern hemisphere, especially in Europe, the most common genotypes are 1 and 3. Genotypes 2 and 5 are mainly isolated in North America and genotypes 2 and 3 in Africa. In China, genotypes 9 and 10 represent the majority of isolates.[1] *T. gondii* has a very broad host spectrum; thus, numerous warm-blooded animals as well as humans are susceptible as intermediate host. Cats and wild felids serve as final hosts.

*Epidemiology*: Approximately one third of the world's human population is infected with *T. gondii*, although prevalence varies from region to region. In the USA and the United Kingdom, about 16 to 40% of people are infected.[7,9] In contrast, the infection rate in Central and South Africa and continental Europe is 50 to 80%.[9] Evidence of infection was found in 31 of the world's 39 felid species.[10] Prevalence in wildlife animals depends on various physical, biological, and ecological factors as well as climatic conditions and the susceptibility of host species.[10] The prevalence in marine mammals (e.g., seals, sea otters and dolphins) is particularly interesting, as it ranges from 47 to 100%. There have been some reports of toxoplasmosis in New World porcupines empha

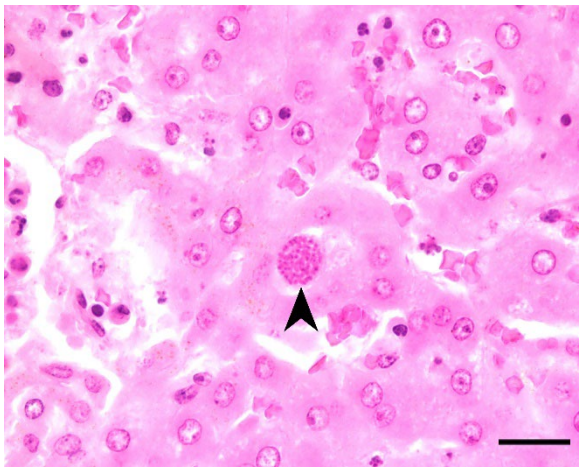

**Figure 3-3. Liver, porcupine. Cytoplasmic cyst of *Toxoplasma gondii* within a hepatocyte. (*Photo courtesy of:* Institute of Veterinary Pathology, Faculty of Veterinary Medicine, Leipzig University; An den Tierkliniken 33, 04103 Leipzig, Germany; https://www.vetmed.uni-leipzig.de/institut-fuer-veterinaer-pathologie) (HE, 400X)**

sizing this species as susceptible to the disease. Numerous comparable cases of toxoplasmosis have been described in various animals in zoologic gardens, including New World primates, Australian marsupials, and Pallas' cats.[2] Toxoplasmosis is an important cause of sporadic and epizootic mortality in zoo populations. In most cases, the affected animals are over 12 months old. Old world monkeys, rats, cattle, and horses seem highly resistant to infection.[1,2]

*Pathogenesis/development cycle*: *T. gondii* exhibits facultative heteroxenic development.[3,12] Depending on the position in the developmental cycle, different morphological stages of *T. gondii* occur.[8] Tachyzoites represent slightly curved, crescent-shaped cells with an apical complex at the anterior pole and a nucleus in the posterior half of the cell. The cell contains one chromosomal and one mitochondrial genome as well as a genome of circular DNA. Outside the host cell, the tachyzoites are only viable for a short period of time and are destroyed during gastric passage. During endodyogeny, bradyzoites are formed within the cyst lumina. The cysts, which are up to 150 µm in size, develop intracellularly in various tissues and, due to their resistant wall, have a relatively long life span in the host. A cyst can carry up to several thousand bradyzoites. The oocyst contains two sporocysts with four sporozoites each. Overall, development in the final and intermediate host proceeds along different paths, whereby only nucleated cells are infected. Both intermediate and definitive hosts can become infected under natural conditions mainly via three infectious routes: oral ingestion of oocysts from the environment (horizontal); oral ingestion of cysts within the tissues of intermediate hosts (horizontal); and diaplacental or galactogenic transmission of tachyzoites (vertical).

A particular key role in transmission or spread is played by cats, which are the only domestic animals that serve as a final host. In cats that are primarily infected by cysts, a massive excretion of oocysts, which can last up to 20 days, occurs after a prepatency period of 3 to 10 days. The majority of bradyzoites released from ingested cysts usually remain in the small intestine. They initiate merogony and gametogeny in the intestinal epithelium, giving rise to the unsporulated oocysts. The remaining bradyzoites immediately penetrate the intestinal wall and reach the other internal organs via the lymphatic and blood pathways, resulting in extraintestinal development of the parasite. In contrast, when the cat is infected with oocysts, asexual reproduction must immediately take place in the extraintestinal organs. Some of the tachyzoites subsequently migrate into the intestinal wall and initiate development in epithelial cells until oocysts are formed. After a prepatency of 18 to 36 days,

about 50 % of the cats excrete oocysts with the faeces.

The oocysts excreted by the cat are initially unsporulated and non-infectious. The generation of the infectious sporulated oocysts takes 1 to 5 days under conditions of sufficient oxygen supply, moisture, and temperature. Due to their very high resistance to environmental destruction, the sporulated oocysts pose a high risk of infection for humans and other animals. Once the intermediate host is infected, the bradyzoites or sporozoites immediately penetrate the intestinal wall after oral ingestion of the oocysts or cysts. This is followed by lymphohaematogenous spread into various organ systems, including mesenteric lymph nodes, liver, lungs and striated muscles. Two asexual multiplication phases are carried out in the organs. Intestinal development is absent.

*Macroscopic and microscopic findings*: Depending on the localization of the parasitic structures, macroscopic and histologic lesions can be found in several organs. The lungs, brain, and liver are most commonly affected by the changes.[4] The lungs show interstitial pneumonia, type II pneumocyte hyperplasia, and a necrotizing component associated with edema and hemorrhage. The liver shows mild hepatomegaly and clearly visible grey, white, or yellow foci. In histopathology of the liver, as in the other affected organs, necrosis is dominant. In the brain, *T. gondii* is found in most cases as a tachyzoite form in macrophages, glial cells, or neurons. Furthermore, it is possible to detect tissue cysts. In all affected organs, macrophages, lymphocytes and plasma cells are the dominant cell population of the inflammatory component. The formation of granulomas can occur. There may be a minor and variable neutrophilic component.

In a previously published case report of toxoplasmosis in a porcupine, microscopic changes were found in the liver, lungs, heart, and spleen, in agreement with the present case.[6] Reported histopathologic findings in porcupines include necrotizing hepatitis, lymphohistiocytic and necrotizing myocarditis, lymphohistiocytic encephalitis, and lymphohistiocytic interstitial nephritis.[6] Thin-walled tissue cysts with numerous bradyzoites, as well as tachyzoites, have been described within alveolar macrophages, free in the alveoli, between myocardial fibers, and within glomeruli and tubular epithelial cells.[6]

*Diagnosis*: *T. gondii* infection can be diagnosed by many different methods. For the selection of the most suitable diagnostic procedure, the immune status of the patient, the clinical signs, and the severity of the symptoms should be considered. The detection can be done indirectly using serology or by direct detection of the parasite antigen or DNA.[10] The following techniques can be used: polymerase chain reaction (PCR; parasite detection), enzyme-linked immunosorbent assays (ELISAs; IgG/IgM/IgA detection; serology), indirect fluorescence antibody tests (IFATs; serology), comparative Western blotting (serology), Sabin-Feldman dye test (serology), and mouse bioassay. In veterinary medicine, histopathology and immunohistochemistry are of primary diagnostic importance. Furthermore, the diagnosis can be confirmed by electron microscopy.

*Differential diagnosis*: The genera *Hammondia*, *Neospora* and *Besnoitia* should be considered as etiologic differential diagnoses.[3] Like the genus *Toxoplasma*, these belong to the family Toxoplasmatidae. A reliable differentiation of the oocysts of the

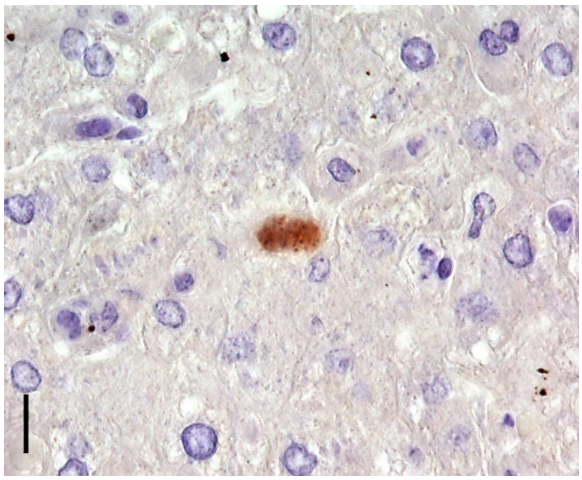

**Figure 3-4. Liver, porcupine. Apicomplexan cysts are strongly immuopositive for antibodies against *Toxoplasm gondii*. (*Photo courtesy of:* Institute of Veterinary Pathology, Faculty of Veterinary Medicine, Leipzig University; An den Tierkliniken 33, 04103 Leipzig, Germany) (anti-*T. gondii*, 400X)**

named genera is not possible by means of histological examination alone.

**Contributing Institution:**
Institute of Veterinary Pathology
Faculty of Veterinary Medicine
Leipzig University
Leipzig, Germany
https://www.vetmed.uni-leipzig.de/institut-fuer-veterinaer-patholgie/

**JPC Diagnosis:**
Liver: Hepatitis, necrotizing, subacute, multifocal, random, mild to moderate, with intracytoplasmic apicomplexan zoites.

**JPC Comment:**
The contributor provides an excellent, thorough overview of toxoplasmosis, and rightly notes that *Toxoplasma gondii* infection has been documented in a wide range of mammalian hosts. Prevalence rates vary, but are throught to surpass 50% in dogs, rabbits, and sea otters; 60% in rats, mice, and birds; and 70% in humans, cats, bears, and deer.[13]

*T. gondii* has been extraordinarily successful by protozoal standards, and its successful transmission to many species worldwide is due, in part, to its ability to modify its hosts' behavior for its own benefit.[13] This "manipulation hypothesis" explains how parasites that are immature in the intermediate host ensure that they are eaten by the appropriate definitive host so the parasite can mature and complete its life cycle.[13] The classic toxoplasmosis pairing is the cat, which serves as the definitive host in which gamteogeny occurs, and the mouse, in which shizogeny occurs, and this classic pair provides an illustrative example of the manipulation hypothesis. Infective oocysts, which develop in the feline host, are ingested by the wild rodent, where the parasites undergo asexual reproduction followed by encystation in multiple tissues, including the brain.[13] Because sexual maturation can be accomplished only in felines, there is strong evolutionary pressure for the parasite to develop mechanisms to ensure transmission from the mouse to the cat through predation.[13]

Researchers investigating the behaviors of *T. gondii*-infected mice found that the infected mice showed decreased learning capacity and memory compared to their uninfected counterparts. Because cats are more attracted to moving, exposed prey, investigators conducted a series of studies to determine if *T. gondii* infection increased activity levels. Researchers determined that infected mice were more active than their uninfected counterparts and spent more time in exposed or novel environments than control mice.[13]

Subsequent mouse and rat studies evaluated whether *T. gondii* infection affected rodents'

perception of cat predation risk as measured by their response to cat odor.[13] Cat odor is well-known to elicit aversion behavior in laboratory rodents, even after hundreds of generations have been raised in the laboratory environment with no lived experience with feline predation.[13] Results show that, while uninfected rodents show the expected aversive behaviors toward cat-treated areas, infected rodents exhibited not only a reduction in this aversive behavior, but also exhibited a significant, potentially suicidal preference for cat-treated areas.[13]

Convinced that *T. gondii* has behavior-modifying effects, researchers investigated whether treatment with antipsychotic drugs, many of which were known to inhibit *T. gondii* replication in cell cultures, could ameliorate the behavioral effects of *T. gondii* in rodents.[13] Infected but untreated rodents demonstrated the same attraction to feline odors as seen previously; however, following treatment with schizophrenia drugs such as haloperiodol and valproic acid, such cat-seeking behaviors, as well as the numbers of neurons and glial cells immunohistochemically positive for *T. gondii*, were significantly reduced.[13]

The mechanisms by which *T. gondii* modifies rodent behavior are currently unknown, though neuromodulation has been suggested as the dominant mechanism. Studies attempting to determine the neurologic basis of anxiety often use the reaction of rodents to cats as a research model, and such studies have found that blocking certain receptors in the amygdala or providing serotonin antagonists causes rodents to display the same lack of aversion to cats that *T. gondii*-infected rodents display.[13] Additional research has shown substantial differences in certain neurotransmitter levels in infected versus uninfected rodents, suggesting additional mechanisms by which *T. gondii* may be achieving its behavior modulatory effects.[13] Additional research is needed to further elucidate the protozoal magic that causes normally avoidant rodents to run headlong into the jaws of a primary predator. Though interesting in its own right, understanding these mechanisms could have profound implications for human behavior given the high levels of suspected *T. gondii* infection in the human population.

The moderator emphasized the need for molecular diagnostics when attempting to differentiate among the various apicomplexans, many of which look identical on H&E examination. Participants appreciated this straightforward example of hepatic toxoplasmosis, which sparked a discussion of how an organism such as *T. gondii*, which has no known virulence factors, can cause such significant necrosis.

**References:**


1. Aguirre AA, Longcore T, Barbieri M, et al. The One Health approach to toxoplasmosis: epidemiology, control, and prevention strategies. *EcoHealth*. 2019;16(2):378-90.
2. Denk D, Neck S de, Khaliq S, Stidworthy MF. Toxoplasmosis in zoo animals: A retrospective pathology review of 126 cases. *Animals*. 2022;12(5).
3. Deplazes P, Eckert J, Samson-Himmelstjerna G von, Zahner H. *Lehrbuch der Parasitologie für die Tiermedizin.* 3. Auflage. Stuttgart: Enke Verlag; 2013; 87-94.
4. Dubey JP, Carpenter JL. Histologically confirmed clinical toxoplasmosis in cats: 100 cases (1952-1990). *J Am Vet Med Assoc.* 1993;203(11):1556-1566.
5. Control of intestinal protozoa in dogs and cats. ESCCAP Recommendation No. 6.

https://www.esccap.de/empfehlung/protozoen/. Updated May 30, 2022. Accessed May 30, 2022.
6. Fayyad A, Kummerfeld M, Davina I, et al. Fatal systemic Toxoplasma gondii infection in a red squirrel (Sciurus vulgaris), a swinhoe's striped squirrel (Tamiops swinhoei) and a New World porcupine (Erethizontidae sp.). *J Comp Pathol.* 2016;154 (2-3):263-267.
7. Fuglewicz AJ, Piotrowski P, Stodolak A. Relationship between toxoplasmosis and schizophrenia: A review. *Adv Clin Exp Med.* 2017;26(6):1031-1036.
8. Halonen SK, Weiss LM. Toxoplasmosis. *Handb Clin Neurol.* 2013;114:125-145.
9. Hill D, Dubey JP. Toxoplasma gondii: transmission, diagnosis and prevention. *Clin Microbiol Infect.* 2002;8(10):634-40.
10. Robert-Gangneux F, Dardé M-L. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin Microbiol Rev.* 2012; 25(2):264-296.
11. Schnieder T, Boch J, eds. *Veterinärmedizinische Parasitologie.* 6. Aufl. Stuttgart: Parey; 2006; 144-148, 298, 366-367, 428-432.
12. Tenter AM, Heckeroth AR, Weiss LM. Toxoplasma gondii: from animals to humans. *Int J Parasitol.* 2000;30(12-13): 1217-1258.
13. Webster JP. The effect of *Toxoplasma gondii* on animal behavior; playing cat and mouse. *Schizophr Bull*. 2007;33(3):752-756.

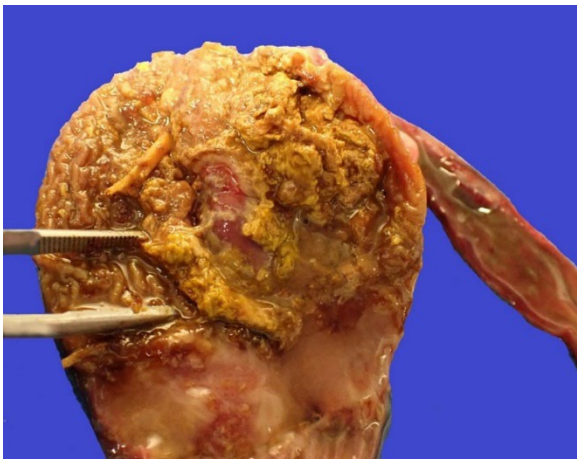

**Figure 4-1. Ventriculus, hornbill. There is a thick fibrinonecrotic membrane adherent to the mucosa of the ventriculus. (*Photo courtesy of:* International Zoo Veterinary Group Pathology, https://www.izvg.co.uk/pathology/about.htm)**

**CASE IV**:

**Signalment**:
1-year-old male Visayan hornbill (*Penelopides panini*)

**History**:
The animal was found dead from suspected hypothermia following an enclosure heating malfunction. The animal was submitted for necropsy. No other birds were affected.

**Gross Pathology**:
The bird weighed 0.5 kg (slightly underweight) and was in lean body condition with ample skeletal muscle bulk and minimal body fat. The ventriculus contained a moderate amount of yellow, soft, diphtheritic material replacing the koilin layer centered on a central, ovoid, 14 x 18 mm mucosal ulcer. The adjacent duodenum was diffusely and transmurally reddened and contained moderate volumes of red-tinged mucoid fluid overlying eroded intestinal mucosa. Concurrent macroscopic findings included moderate diffuse

splenomegaly and marked diffuse pulmonary edema.

**Laboratory Results:**
Microbiology: Anaerobic cultures of the ventriculus and small intestine isolated a moderate growth of *Clostridium perfringens*.

Aerobic cultures produced mixed scant growth of *Proteus* spp., *Escherichia coli*, and *Enterococcus* spp. Selective cultures for *Salmonella, Campylobacter, Shigella,* and *Yersinia* were sterile.

PCR: The bacterial isolate was identified as *Clostridium perfringens* by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) analysis.

A PCR toxin profile identified alpha toxin; beta, epsilon, iota, beta-2, and enterotoxin were all absent.

**Microscopic Description**:
Ventriculus-duodenal junction: Overall affecting approximately 70-80% of the examined ventriculus, there is widespread multifocal-to-coalescing erosion and degeneration of the koilin layer, characterized by superficial erosive scalloping and variable separation of the superficial horizontal koilin matrix layers due to fibrillary to finely globular protein degeneration with numerous intralesional small interspersing colonies of gram-positive rod-like bacteria that range in size from 1-2 x 3-6 µm. Multifocal areas of more extensive koilin necrosis are seen, characterised by complete loss of horizontal koilin matrix architecture and replacement by hypereosinophilic amorphous koilin protein debris. Where extensive, koilin necrosis is confluent with regions of ventricular mucosal ulceration, with tissue replaced by hypereosinophilic lytic cytonuclear debris admixed with abundant fibrin, moderate heterophilic and histiocytic inflammation, and numerous small (approx. 50-micron diameter) to medium-sized (approx. 400-micron diameter) colonies of dense rod-like bacteria, together forming a fibrinonecrotising (diphtheritic) membrane. Remnant epithelial mucosa exhibits variable features of crypt dilatation (containing either fine fibrillary hypoeosinophilic proteinaceous content or hypereosinophilic koilin matrix) and epithelial cell degeneration (including cell swelling, loss of apical cilia, loss of cytoplasmic detail, karyorrhexis and nuclear pyknosis), the latter multifocally leading to lytic necrosis (cytoplasmic rupture, exudation of degenerate cytoplasm and karyolitic nuclei). Mild to moderate mixed inflammatory infiltrates (mostly degenerate heterophils, lymphocytes, and plasma cells with occasional macrophages) are seen throughout the lamina propria. Ventricular smooth muscle layers are multifocally disrupted by infiltrating heterophils with variable degrees of myofibrillar swelling, fiber splitting, striation loss, and nuclear pyknosis and karyorrhexis. Mild heterophilic infiltrates occasionally infiltrate through the subserosal layers, and are mostly associated with variably degenerate adipose tissue. Myenteric plexi are unaffected. Ventricular blood vessels exhibit relative increases in circulating heterophils (granulocytosis). Small sections of duodenum are also present in the examined section, which exhibit mild to moderate superficial autolytic mucosal exfoliation. Where better preserved, mild multifocal areas of lytic mucosal necrosis are observed with associated loss of villi and crypts, plus blunting and fusion of remaining adjacent villi. Prominent lymphoplasmacytic infiltrates are seen within the lamina propria, with occasional heterophils scattered throughout. The

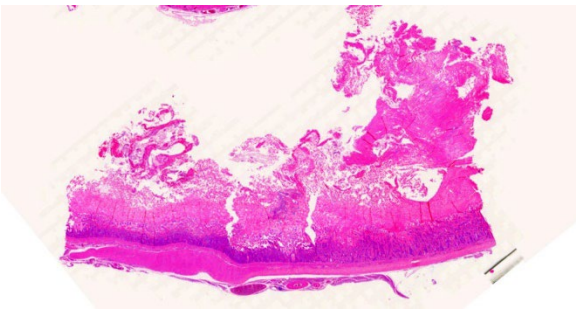

**Figure 4-2. Ventriculus, hornbill. There is extensive loss of koilin and partial-thickness mucosal necrosis. A large fibrinonecrotic membrane extends into the lumen. (HE, 9X)**

submucosa, muscle layers, and serosa appear generally unremarkable. Gram stain confirms the prescence of numerous gram-positive, rod-like bacteria, consistent with *Clostridium perfringens*. Ziehl-Neelsen stain for acid-fast organisms is negative. PAS stains were negative for fungal organisms.

Pancreas: Histologically unremarkable.

**Contributor's Morphologic Diagnosis**:
Severe, subacute, multifocal-to-coalescing, fibrinonecrotising and ulcerative ventriculitis with koilin necrosis and intralesional gram-positive rod-like bacteria consistent with *Clostridium perfringens*, gizzard.

**Contributor's Comment**:
The gram-positive anaerobic spore-forming bacteria *Clostridium perfringens* is a major enteric pathogen in animals, represented by 7 distinct toxinotypes (A to G) based on the detection of 6 toxin genes: alpha (cpa gene), beta (cpb), epsilon (etx), iota (itx), enterotoxin (cpe), and necrotic enteric B-like toxin (netb).[1,2] Whilst typically found in intestinal flora of healthy birds, physiologic stress may promote *C. perfringens*-associated necrotic enteritis (NE), a significant disease caused by types A (cpa only), C (cpa and cpb), and G (cpa and netb-toxin).[3,5] Necropsy findings in this case include distended and friable small intestines with multifocal-to-coalescing fibrinonecrotising mucosal ulcers covered by "Turkish-towel"-like diphtheritic pseudomembranes.[2] *C. perfringens* may subsequently invade the portal system and biliary tract, causing cholangiohepatitis.[2] Histologically, gram-positive rod-like bacteria may be seen initially adhered to villous tips and later embedded within foci of mucosal to transmural intestinal necrosis.[2]

Outbreaks in avian collections occur infrequently, with clinical infection characterized by sudden onset of high mortality and small intestinal mucosal necrosis. NE typically affects domestic poultry but has also been described in more wild and exotic avian species such as lorikeets, macaws, toucans, and crows.[1-3,5,9] *C. perfringens* can be ubiquitous in the environment, particularly if soil-based substrate is used in the enclosure, therefore review of enclosure hygiene protocols is usually recommended in zoological collections when cases occur.

This case was particularly unusual in that the ventriculus was the primary organ affected, rather than the small intestine, which is more typical in other avian species. The most immediate cause of death was attributed to bacteraemic sepsis, based on the observation of heterophilic infiltrates within other viscera (i.e., liver, lung, and spleen) plus necrotizing hepatitis associated with similar intralesional gram-positive rod-like bacteria. To the contributor's knowledge, this is the first reported case of *C. perfringens*-associated ventriculitis in a hornbill, and in the Order Bucerotiformes.

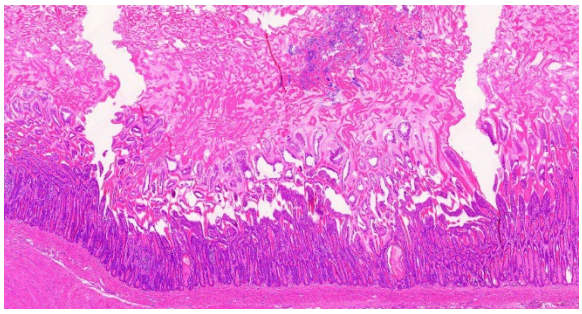

**Figure 4-3. Ventriculus, hornbill. Higher magnification of the mucosal necrosis with numerous bacterial colonies. (HE, 48X)**

**Contributing Institution:**
International Zoo Veterinary Group
Station House, Parkwood Street
Keighley, West Yorkshire
United Kingdom
http://www.izvg.co.uk/pathology/pabout.htm

**JPC Diagnoses:**

1. Ventriculus: Koilin erosion and ulceration, multifocal, subacute, moderate, with glandular hyperplasia and numerous extracellular bacteria.
2. Ventriculus: Vasculitis, fibrinoid and heterophilic, multifocal, moderate.
3. Adipose: Atrophy, diffuse, moderate.

**JPC Comment:**
*Clostridium perfringens* and its various subtypes and toxins are well-known to veterinary students, residents, and practitioners, who have developed all manner of mnemonics dedicated to classifying *C. perfringens* types based on toxin production. This pathogen is so well-known because it is so ubiquitous in the environment and is the cause of many distinct enteric diseases in humans and in a wide variety of animal species, particularly among herbivores.[8]

The currently classification scheme classifies *C. perfringens* types based on the production of particular "typing toxins," but all isolates produce numerous other toxins and hydrolytic enzymes that contribute to virulence.[8] In addition, genes encoding beta, epsilon, and iota toxins are encoded on plasmids which also contain conjugation loci, and transfer of epsilon toxin plasmids from type D strains to type A strains, with subsequent production of epsilon toxin, which effectively turns type A into type D, has been observed, meaning that the beloved classification scheme is essentially based on the plasmid complement carried by an individual organism.[8]

The only clostridial toxin isolated in this case, alpha toxin (CPA), is a chromosomally-encoded toxin that is produced by all strains of *C. perfringens*.[8,10] CPA is a phospholipase that compromises cellular function by degrading phosphatidylcholine and sphingomyelin, both of which are components of eukaryotic cell membranes.[10] Release of CPA causes damage to erythrocyte and other cell membranes, resulting in cell lysis via phospholipid degradation and the activation of other cellular mechanisms that culminate in lysis. CPA also activates the arachidonic acid metabolic pathways, resulting in the production of thromboxanes, leukotrienes, and prostaglandins, resulting in inflammation and vasoconstriction.[10] In small amounts, CPA impaires leukocyte migration and causes their intravascular aggregation at the periphery of lesions, leading to a reduction in blood flow, subsequent hypoxia and necrosis, and the creation of an anaerobic niche for the proliferation of *C. perfrigens*.[10]

The role of CPA in mammalian intestinal disease is controversial. *C. perfringens* type A causes yellow lamb disease, a rare form of enterotoxemia in lambs that is characterized by depression, anemia, icterus and hemoglobinuria.[10] It is generally assumed that these clinical

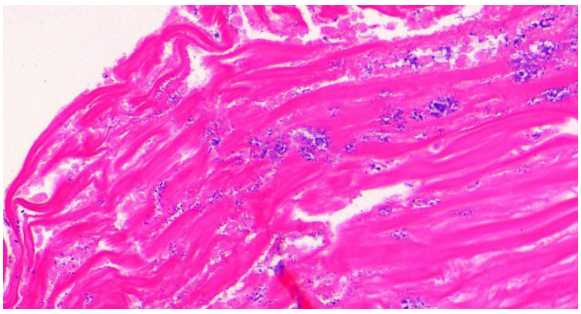

**Figure 4-4. Ventriculus, hornbill. There are numerous bacterial colonies within the degenerate koilin in the ventricular lumen. (HE, 48X)**

signs and the associated gross and microscopic lesions are caused by the action of CPA, but no definitive proof is available.[10] Similarly, *C. perfrigens* type A is blamed for enteritis, abomasitis, and enterotoxemia in cattle, horses, goats, and pigs, though no definitive evidence for CPA's role in the pathogenesis of these diseases has been discovered.[10] The picture is further muddied by the detection of CPA in the intestinal contents of clinically healthy animals, making the presence of CPA diagnostically irrelevant for type A diseases in many species.[10] *C. perfringens* type A is known to produce gas gangrene (also known as malignant edema) in many domestic animals and in humans and CPA has long been assumed to be the causative agent; however, in a recurring theme, there is no concrete evidence that the disease produced by the bacterium is the result of CPA.[10]

The controversy surrounding CPA extends to avian diseases. While *Clostridium perfrigens* type A is frequently found as the sole agent in a variety of enteric avian diseases, it is unclear if CPA is responsible for the observed lesions.[3,5,8] In fact, CPA was long considered the key virulence factor for necrotizing enteritis in broiler chickens until recent research demonstrated that NetB, not CPA, is the main lesion-causing virulence factor produced by the newly-described *C. perfringens* type G strain.[5,7]

This case prompted robust discussion from conference participants, who noted, as did the contributor, how the ventriculus is an unusual and surprising location for *C. perfringens*-associated disease. Participants were surprised at the extent of the koilin destruction and ulceration, which would be an unusual manifestation of clostridial disease and is more intense than would be expected from stress induced from a relatively short period of cold exposure.

In general, koilin erosion and ulceration in the ventriculus of birds has many associated conditions, and is likely a multifactorial disease. In chickens, koilin erosion and ulceration is considered a syndrome and termed "gizzard erosion and ulceration," or GEU. Numerous causes for GEU have been proposed, including starvation, genetic factors, inappropriate feed, toxins, nutritional deficiencies, and infections. *C. perfringens* is one infection agent that has been putatively associated with this condition and may have caused the koilin erosion and ulceration in this case.[4]

Also of particular interest was the multifocal heterophilic vasculitis present through the ventriculus. The cause of this vasculitis is not apparent in the examined section and vasculitis is not a lesion commonly associated with clostridial infection in birds, leading participants to believe that this likely represents a separate pathologic process. As heterophilic vasculitis and koilin ulceration can be caused by fungal infections in birds,[6] participants scrutinized the section for fungal hyphae, but no fungal elements were observed. Other possible causes of vasculitis include bacterial sep-

sis, viral disease, and immune-mediated vasculitis. In this case, the cause of the vasculitis is ultimately unknown.

Participants also noted fat atrophy in the examined section, corresponding to the clinical history that the bird was underweight. The fat atrophy, vasculitis, and severe koilin layer ulceration and erosion comprise an unusual constellation of lesions which prompted an array of hypothetical pathogeneses, none of which could be definitely confirmed by the examined section alone. In the end, participants chose to separate each lesion into separate morphs due to the uncertain relationships between them.

**References**:


1. Asaoka Y, Yanai T, Hirayama H, et al. Fatal necrotic enteritis associated with *Clostridium perfringens* in wild crows (*Corvus macrorhynchos*). *Avian Pathol*. 2004;33 (1):19-24.
2. Boulianne M. Clostridial Diseases. In: Swayne DE, Boulianne M, Logue CM, et al., eds. *Diseases of Poultry*. 14th ed. Wiley-Blackwell; 2020.
3. De Santi M, Schocken-Iturrino RP, Casagrande MF, et al. Necrotic enteritis caused by *Clostridium perfringens* in blue and gold macaws (*Ara ararauna*). *J Avian Med Surg*. 2020;34(1):65-69.
4. Gjerve AG, Kaldhusdal M, Eriksen GS. Gizzard erosion and ulceration syndrome in chickens and turkeys: a review of causal or predisposing factors. *Avian Pathol*. 2013;42(4):297-303.
5. Grau-Roma L, Navarro M, Blatter S, et al. *Clostridium perfringens*-associated necrotic enteritis-like disease in coconut lorikeets (*Trichoglossus haematodus*). *Vet Pathol*. 2021;58(2):423-427.
6. Jeffrey JS, Chin RP, Shivaprasad HL, Meteyer CU, Droual R. Proventriculitis and ventriculitis associated with zygomycosis in ostrich chicks. *Avian Dis*. 1994;38(3):630-4.
7. Lee KW, Lillehoj HS. Role of *Clostridium perfringens* necrotic enteritis B-like toxin in disease pathogenesis. *Vaccines (Basel)*. 2022;10(1):61.
8. Rood JI, Adams V, Lacey J, et al. Expansion of *Clostridium perfringens* toxin-based typic scheme. *Anaerobe*. 2018;53:5-10.
9. Trupkiewicz J, Garner MM, Juan-Salles C. Passeriformes, Caprimulgiformes, Coraciiformes, Picipformes, Bucerotiformes and Podiformes. In: Terio K, Mcaloose D, St. Leger J, eds. *Pathology of Wildlife and Zoo Animals*, 1st ed. Academic Press;2018.
10. Uzal FA, Vidal JE, McClane BA, Gurjar AA. *Clostridium perfringens* toxins involved in mammalian veterinary disease. *Open Toxinology J*. 2010;2:24-42.