Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 25

4 May 2022

CASE I: Z215/20 (JPC 4167239)

Signalment:
Adult (>3 year-old), male, red-chested mustached tamarin, *Saguinus labiatus*.

History:
Bauru Zoo has a New World primate (NWP) population ranging from 30 to 55 individuals over the past eleven years (2010-2020), with 16 different species of genre *Alouatta*, *Ateles*, *Callicebus*, *Lagothrix*, *Leontopithecus* and *Saguinus*. Since 2010, there were 27 deaths associated with *Prosthenorchis* sp. parasitism, representing 67.5% of total deaths of tamarins and lion tamarins (27/40) and 49.1% of deaths in all NWP (27/55). Deaths associated to parasitism were reported in seven years (2010, 2012, 2013, 2015, 2016, 2019 and 2020) with an average of one case per year over the first nine years (2010-2018), and a marked increase in the number of deaths over the last two years (2019-2020) with an average of nine cases per year. All deaths associated to *Prosthenorchis* sp. parasitism affected tamarins and lion tamarins, including 18 (66.7%) belonging to the genus *Saguinus* (15 *S. bicolor*, two *S. labiatus* and one *S. niger*) and nine (33.3%) belonging to the genre *Leontopithecus* (four *L. rosalia*, four *L. chrysomela* and one *L. chrysopygus*).

Gross Pathology:
Grossly, there were multiple yellowish to gray soft nodules disseminated on the serosa of the ileum, cecum and colon, with 0.2 a 0.5 cm in diameter. In the intestinal lumen, there were dozens of cylindrical adult parasites deeply attached to the mucosa, morphologically compatible with acantho-cephalans, and the intestine wall was diffusely thick and edematous. Additionally, the animal had severe cachexia.

Laboratory Results:
None.

Microscopic Description:
Small intestine. Focally extensive area of ulceration extending into the mucosa, submucosa and muscular layers with a

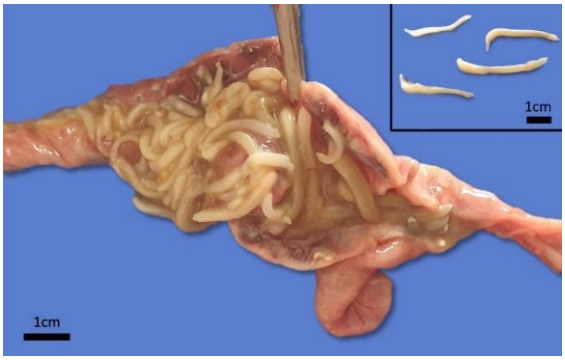

Figure 1-1. Ileum, tamarin. Numerous cylindrical adult acanthocephalans are embedded within the thickened, edematous mucosa. (Photo courtesy of: Departamento de Clínica e Cirurgia Veterinária, Escola de Veterinária, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos, 6627 – CEP 30161-970, Belo Horizonte, MG, Brazil.)

moderate and diffuse lymphohistio-plasmacytic and neutrophilic infiltrate, marked fibroplasia, and an intralesional acanthocephalan parasite. There is a longitudinal section of an adult parasite with a delicate cuticle, a thick hypodermis, a thin muscular layer, and a pseudocoelomatic cavity filled with a uterus with multiple eggs, which is morphologic compatible with an adult female acanthocephalan parasite. The acanthocephalan is deeply attached to the intestine wall by its hooks, which are inserted deeply at the external intestinal muscular layer. Both transversal and longitudinal intestinal muscular layers are completely lost. There are myriad of bacteria within the path of parasite migration within the intestine wall. There are many acanthocephalan eggs in the intestinal lumen. The serosa is diffusely thick, edematous, with ectasia of lymphatic vessels.

Contributor's Morphologic Diagnoses:
Small intestine: Ulcerative and necrotizing enteritis, transmural, chronic, with intra-lesional acanthocephalan parasite.

Contributor's Comment:
Acanthocephalan parasitism in NWP is known to be an important cause of malnutrition and death in captivity. Once established in a colony it is extremely difficult to control or eliminate from the environment.[8,12,17] *Prosthenorchis* sp. are acanthocephalan parasites found in the small and large intestines (ileum, cecum and colon) of different mammalian species,[2,5] and have been reported in captive and free-ranging NWP, with two species recognized to infect this group of animals: *P. elegans* and *P. spirula*.[2]

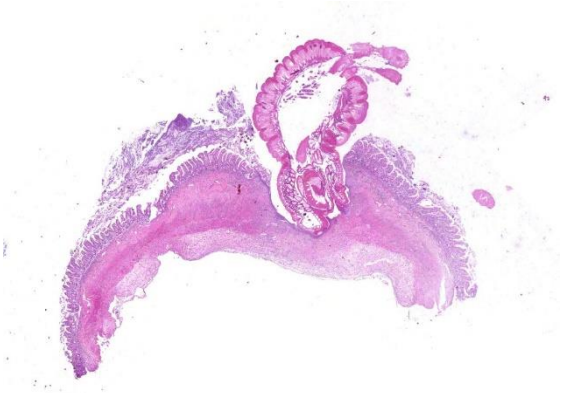

Figure 1-2. Ileum, tamarin. Cross-section of a single acanthocephalan embedded within the muscularis. There is thickening of muscularis and underlying serosa. (HE, 6X)

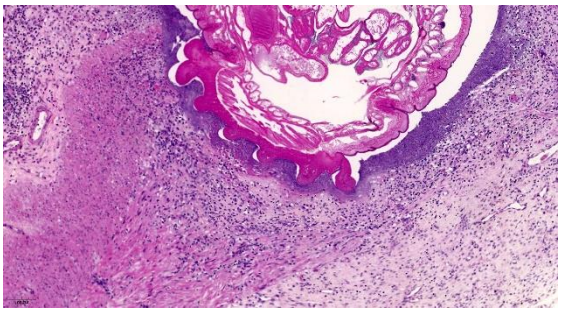

Figure 1-3. Ileum, tamarin. The acanthocephalan is attached by hooks deep in the muscularis. The attachment site is surrounded by necrotic debris, numerous bacilli, and a thick layer of inflamed fibrous connective tissue effacing normal architecture. (HE, 400X) (Photo courtesy of: Departamento de Clínica e Cirurgia Veterinária, Escola de Veterinária, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos, 6627 – CEP 30161-970, Belo Horizonte, MG, Brazil.)

NWP are the definitive hosts of this parasite, and the infection occurs by ingesting intermediate hosts, including cockroaches and beetles, carrying the infective larvae. Usually, captive primates are infected by ingestion of intermediate hosts that may be offered as environmental enrichment or found inside the enclosures as environmental pests.[17] A feature of acanthocephalans, including *Prosthenorchis* sp., is a well-developed proboscis that attaches deep in the intestinal mucosa.[1,9-12] It feeds on the intestinal content by osmosis, reducing the absorption of nutrients by the host, which in chronic infections results in malnutrition and cachexia.[8,9,11,12] In some cases, this parasite can perforate the intestine wall and be found free in the abdominal cavity, causing a severe and acute peritonitis, which leads to a quick

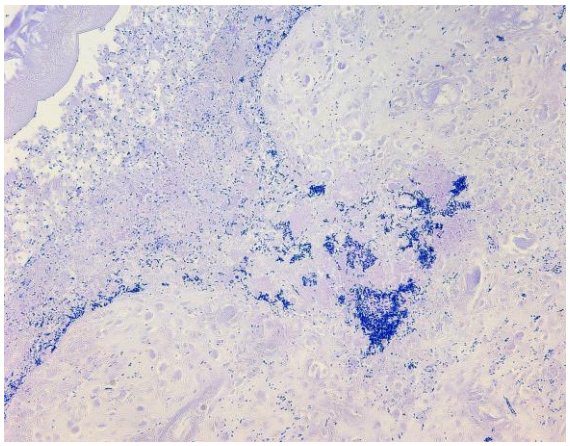

Figure 1-4. Ileum, tamarin. Numerous bacilli are present at the attachment site, as demonstrated by Gram staining. (GoodPasture, 100X). (Photo courtesy of: Departamento de Clínica e Cirurgia Veterinária, Escola de Veterinária, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos, 6627 – CEP 30161-970, Belo Horizonte, MG, Brazil.)

death.[1,11,12] In addition, acanthocephalan parasitism favors bacterial translocation and secondary bacteremia, which usually contributes to clinical impairment of the animal.[8]

There is a wide variety of feeding habits among NWP, ranging from folivorous to omnivorous.[17] Therefore, although acanthocephalan parasitism may affect any genus of NWP, some species are more predisposed to be parasitized by *Prosthenorchis* sp. due to their feeding habits. For instance, *Saguinus* and *Leontopithecus* have approximately 80% of their diet composed of fruits, and the remaining of the diet includes insects, small vertebrates, nuts, and nectar, according to availability.[3,9,13] These species also have dentition more adapted to predate small vertebrates and insects,[17] justifying the high prevalence of this parasitism in these genera. It is known that wild tamarin groups that have more contact with anthropic environment tend to have a higher prevalence of *P. elegans*,[18] although the parasite seems to be less pathogenic for free-ranging animals.[15] Indeed, acanthocephalan parasitism has being often reported affecting free-ranging NWP in the Brazilian Atlantic Forest, particularly in peri-urban areas.[1,10,16] Tamarins and lion tamarins are found in all Latin America and some of them, such as *S. bicolor*, are critically endangered.[6,14] Therefore, it is essential for a successful conservation strategy to better understand the threats for the survival of these species, both in captivity and in wildlife, including acanthocephalan parasites.

Contributing Institution:
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JPC Diagnosis:
Ileum: Enteritis, necrotizing and pyo-granulomatous, transmural, chronic, severe, with acanthocephalid adult and eggs.

JPC Comment:
The contributor provides a concise review of host factors and the pathogenesis associated with acanthocephalan parasitism in NWMs. The phylum Acanthocephala is a diverse group of over 1,100 species of pseudo-coelomates colloquially known as “thorny-headed worms” due to their characteristic proboscis with hook-like projections used to anchor the parasite to the host’s

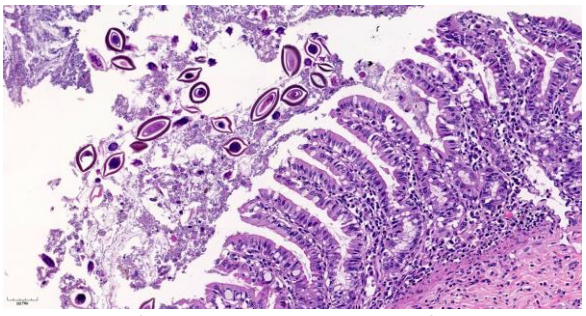

Figure 1-5. Ileum, tamarin. Numerous characteristic acanthocephalan eggs are present within the intestinal lumen. (HE, 400X) (Photo courtesy of: Departamento de Clínica e Cirurgia Veterinária, Escola de Veterinária, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos, 6627 – CEP 30161-970, Belo Horizonte, MG, Brazil.)

gastrointestinal tract.[4,7] As noted by the contributor, these parasites have a complex life cycle with intermediate and definitive hosts. Arthropods such as crustaceans and insects serve as intermediate hosts, which are in turn ingested by definitive and/or paratenic vertebrate hosts such as reptiles, amphibians, fish, birds, marine and terrestrial mammals, and humans.[7,19]

Acanthocephalans are readily identifiable from other metazoan parasites in histologic sections by the combined features of a pseudocoelom, an anterior end armed with a proboscis, and the absence of a digestive tract. Additional characteristic features include a thin peripheral cuticle, thick hypodermis composed of a subcuticular felted layer and thicker inner layer of cross fibers occasionally interrupted by lacunar channels, and two layers of muscle (circular and longitudinal) bordering the pseudocoelom. Acanthocephalans are dioecious, with females containing both immature ova also known as "egg balls" and embryonated eggs within the pseudocoelom whereas males contained paired testes. Finally, acanthocephalans possess a unique structure known as lemniscus that plays a role in the eversion and retraction of the proboscis.[4] In the case of *Prosthenorchis* spp., the worms are most commonly found attached to the luminal aspect of the terminal ileum, cecum, and colon, typically within a nodule.[1]

Acanthocephalan eggs have been discovered in copralites from prehistoric humans, indicating acanthocephaliasis may be an ancient disease of humans. Humans continue to be infected during the modern era, most commonly by *Macracanthorhynchus hirudinaceus*, *M. ingens*, and *Moniliformis moniliformis*, which parasitize pigs, raccoons, and rodents as their primary definitive hosts, respectively. The majority of cases involve children, likely as the result of putting objects such as insects, in their mouths. Insects and arthropods used for medicinal purposes have also been linked to human infections. Finally, humans may also become infected by consuming paratenic hosts, as is suspected with *Bolbosoma* spp. infection. Cetaceans are considered definitive hosts of this genus, while marine planktonic crustaceans and fish likely serve as intermediate and paratenic hosts, respectively.[7]

Conference participants reviewed the previously discussed features of acanthocephalans in addition to those of other metazoan parasites while discussing this case. Unfortunately, the highly characteristic feature of an armed proboscis with hook-like projections was not in the plane of section. Nevertheless, the presence of a pseudocoelom and absence of a gastrointestinal tract is diagnostic for an acanthocephalan. Based on the patient's signalment and clinical history, the moderator agreed the acanthocephalan in this case is most likely *Prosthenorchis elegans*. However, the moderator cautioned against attempts to definitively identify the species, and in many cases the genus, of metazoan parasites based solely on histologic sections.

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intermediate hosts in the acanthocephalan Polymorphus minutus sensu lato (Polymorphidae). *Parasitology*. 2018;145(11):1421-1429.

CASE II: 174994-20 (JPC 4167688)

Signalment:
2-year-2-month-old, male castrated, Coon-hound mix, *Canis familiaris*, dog.

History:
Over one year history of chronic diarrhea, anemia, and iron deficiency, treated for suspected inflammatory bowel disease with prednisolone. Near the time of biopsy, he developed gastrointestinal bleeding and suspected steroid-induced hepatopathy. The dog was off prednisolone for 2 weeks prior to the biopsy.

Gross Pathology:
The liver was diffusely dark brown to almost black. The small and large intestines were thickened with a mottled pink-white serosal surface. The mesenteric lymph nodes were enlarged.

Laboratory Results:
None.

Microscopic Description:
Liver: Examined is a single section of a liver wedge biopsy, with minimal crush artifact. Throughout the section, portal tracts and random areas of the parenchyma are moderate to severely expanded by multiple nodular aggregates of macrophages, multi-nucleated giant cells, lymphocytes, plasma cells, and eosinophils, often centering on up to 80 µm diameter, round to irregular to folded trematode eggs (granulomas). The eggs are characterized by a yellow-clear, up to 2 µm thick, hyalinized wall, with multiple, approximately 5 µm diameter, round and larger, irregular basophilic structures

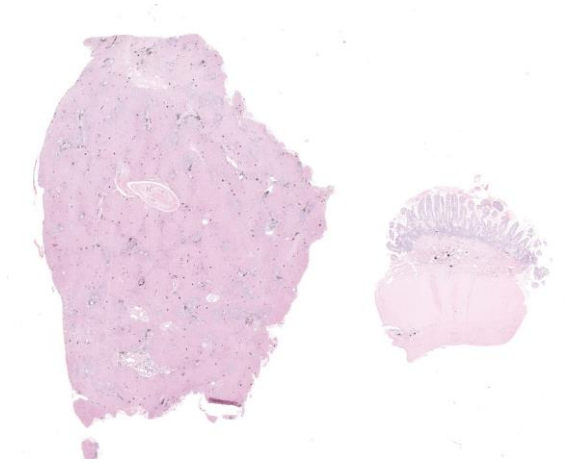

Figure 2-1. Liver, intestine, dog. One section of liver and a section of intestine are submitted for examination. At subgross magnification, there is scattered pigment within the section of liver. (HE, 6X)

(miracidium). Occasionally, the eggs are replaced by irregular, clumped, deeply basophilic concretions (mineralization). A single portal vein is severely dilated and contains an approximately 200 µm wide male trematode. The trematode is characterized by a pale eosinophilic tegument with unevenly spaced, 4 µm wide and up to 18 µm long, hyalinized eosinophilic spines that are most concentrated in the anterior segment, spongy parenchyma, intestine filed with coarsely granular, golden-brown pigment (hemo-siderin) and neutrophils, testes, and vitellaria. The infiltrating macrophages are often laden with coarsely granular, black-brown cyto-plasmic pigment (parasitic exhaust/"fluke pigment"). The nodular inflammatory aggregates are associated with streaming bundles of collagen (fibrosis) with increased numbers of irregular biliary profiles that occasionally lack a central lumen (ductular reaction). Diffusely, hepatic lobules are slightly small, and hepatocytes are similarly small and binucleated. Hepatocytes often contain moderate amounts of finely granular, brown-yellow cytoplasmic pigment (lipo-fuscin). Additionally, midzonal hepatocytes are minimally distended with variably sized, poorly demarcated cytoplasmic clear spaces separated by thin eosinophilic wisps

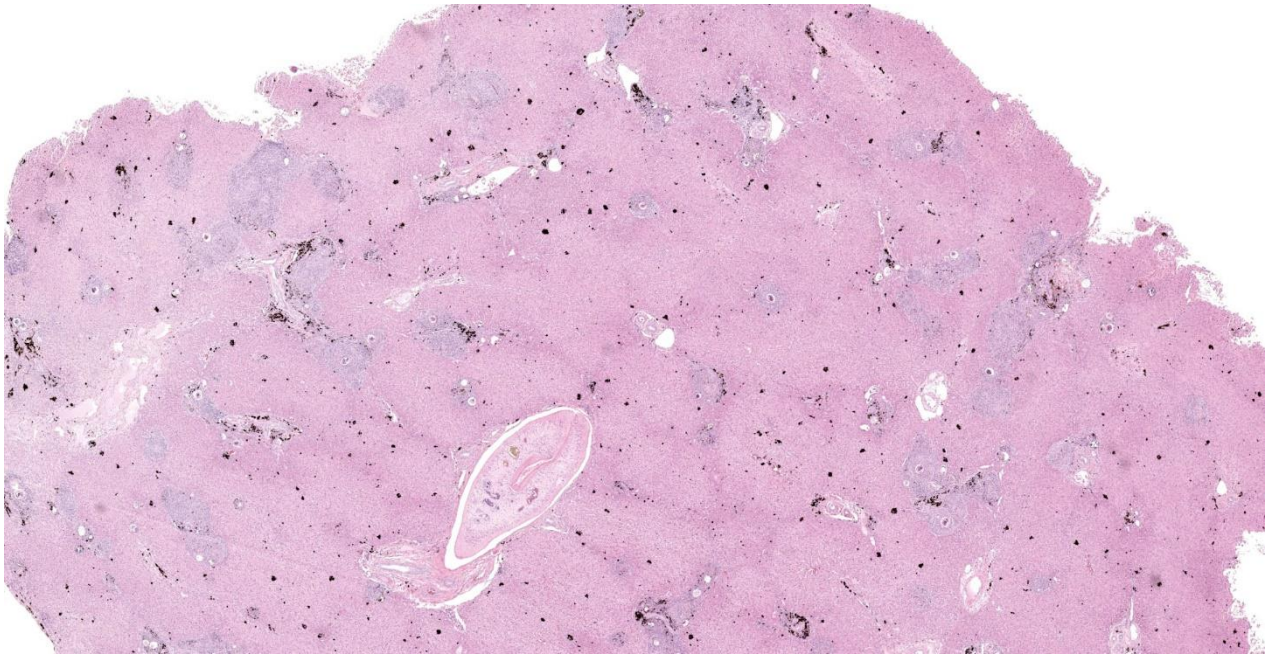

Figure 2-2. Liver, dog. Portal areas are markedly expanded by an inflammatory exudate and pigment, and an adult schistosome is present within a hepatic vein. (HE, 18X)

extending from the cell membrane to the nucleus (glycogen).

Ileum: Examined is one ileal full-thickness specimen with minimal crush artifact. Within the lamina propria, submucosa, and muscularis are dozens of intact and mineralized trematode eggs, as described previously. The eggs are often surrounded by small numbers of macrophages, multinucleated giant cells, lymphocytes, and plasma cells, where multinucleated giant cells occasionally contain the trematode eggs (phagocytosis). These inflammatory cells together with small numbers of neutrophils and eosinophils, moderately infiltrate the lamina propria of the villus tips and deep mucosa. The connective tissue of the mid-mucosa is expanded by wispy, pale eosinophilic material and clear spaces (edema). The crypts are often elongated and slightly tortuous with increased numbers of mitotic figures, stacking of nuclei, and decreased numbers of goblet cells (crypt hyperplasia). The villus to crypt ratio is within normal limits at approximately 2:1.

Contributor's Morphologic Diagnoses:
Liver: Moderate, portal and random, chronic granulomatous and eosinophilic hepatitis with intralesional trematode eggs and adult trematode, portal fibrosis, ductular reaction, and fluke pigment deposition, consistent with schistosomiasis

Ileum: Moderate, generalized, chronic granulomatous enteritis and myositis with intralesional trematode eggs, mid-mucosal edema, and crypt hyperplasia, consistent with schistosomiasis.

Contributor's Comment:
Canine schistosomiasis is caused by *Heterobilharzia americana*, a digenean trematode in the family Schistosomatidae. These trematodes are unique in that they are not hermaphroditic and have separate sexes, the eggs are non-operculated, and the metacercariae are not encysted. The males have a gynecophoral canal that holds the females. Historically, *H. americana* was thought to be endemic to the South Atlantic

and Gulf Coast regions of the United States, but recent reports indicate that the distribution of this parasite and natural occurrence in the domestic dog is broader than previously known, especially in the Midwestern United States including Kansas and Indiana.[6,7,12] The present case is also a dog from Illinois, without known travel history, further supporting the recent spread of distribution.

The life cycle of *Heterobilarzia* is complex, where asexual reproduction occurs in a lymnaeid snail and sexual reproduction in a mammalian host. Raccoons (*Procyon lotor*) are considered the most important natural definitive host,[1] although a variety of other species such as wild and domestic canids, nutria (*Myocastor coypus*), and bobcats (*Lynx rufus*) may also be infected.[10] In species of veterinary importance, infection in horses occur at a relative regularity resulting in hepatic, intestinal serosal, and mesenteric granulomas.[4] Infection is rare in other equids and domestic species with single case reports in a Grant's zebra (*Equus burchelli boehmi*)[13] and llama (*Lama glama*),[3] respectively.

Dogs are exposed to infection when swimming or wading in freshwater infested with lymnaeid snails, where cercariae emerging from the snails penetrate the dog's skin by proteolytic enzymes secreted by the acetabulum glands. Subsequently, the cercariae will detach their tail and become schistosomules that spread to the lungs and then to the liver hematogenously, where sexual maturation occurs, and the adult trematodes will migrate to the mesenteric vein through the portal system for sexual reproduction. Schistosomiasis is mainly due to egg-induced tissue reaction (mainly TH_2 response), while the adults elicit minimal host response. Occasionally, adult trematodes induce eosinophilic endophle-bitis, intimal proliferation, and thrombosis in mesenteric and portal veins.[11] In the presented case, in addition to the liver and ileum, similar granulomatous lesions associated with eggs were found in the concurrently submitted duodenum, jejunum,

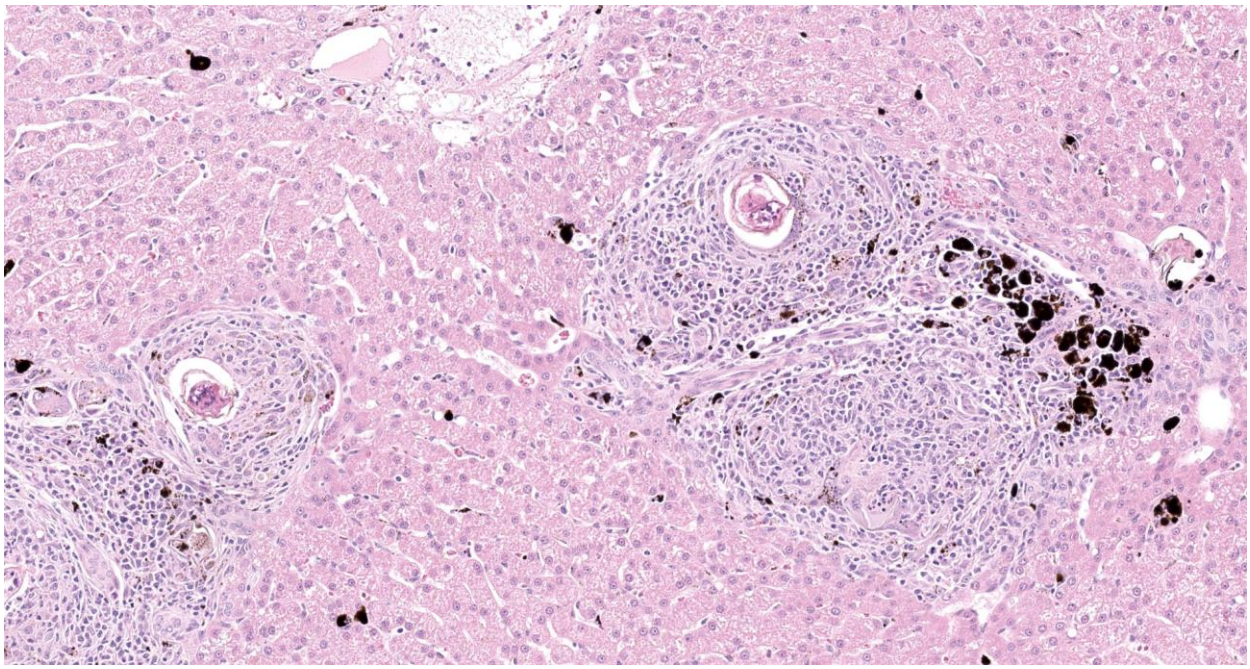

Figure 2-3. Liver, dog. Portal areas are expanded by chronic granulomatous inflammation, extending into the surrounding parenchyma and centered on trematode eggs. Hematin pigment is present within macrophages in the expanded portal areas and concentrated in Kupffer cells and clusters of macrophages within the adjacent hepatic parenchyma. (HE, 133X)

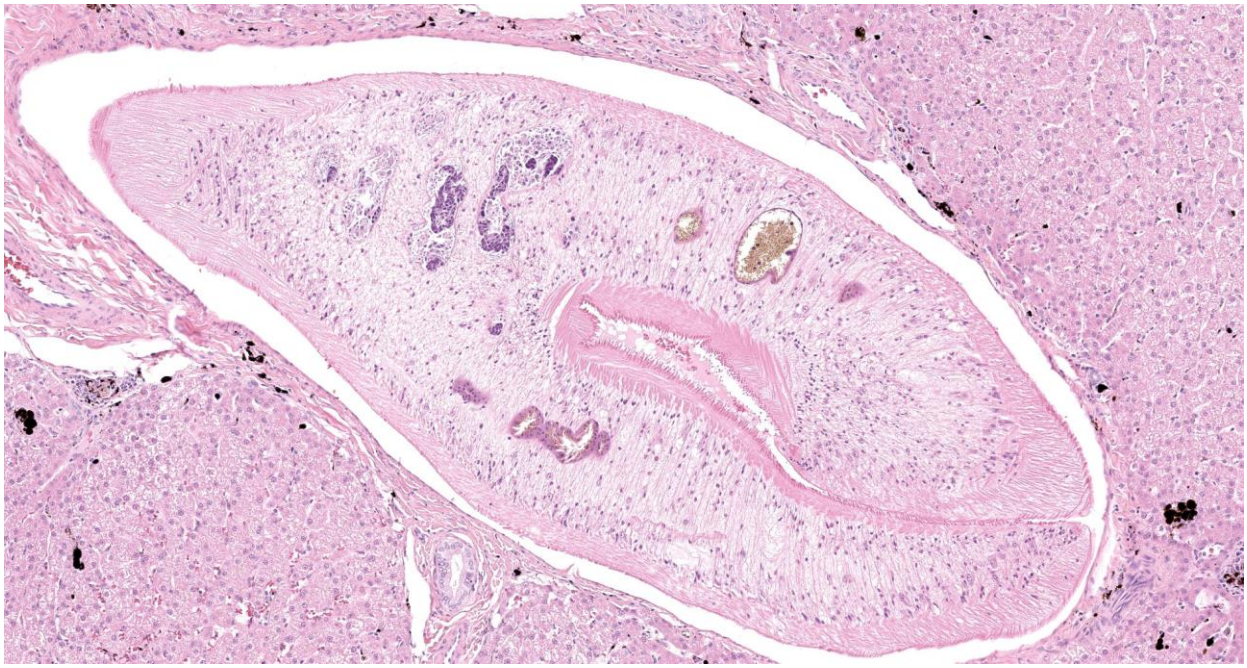

Figure 2-4. Liver, dog. Within a hepatic vein, there is a cross section of an adult male trematode with a central gynecophoric canal, a ridged cuticle, a spongy body cavity, and cross sections of a testis and hemosiderin-filled ceca. (HE, 107XX)

and mesenteric lymph node, but not in the stomach.

Clinical signs in infected dogs are usually nonspecific, and may include diarrhea, vomiting, weight loss, and anorexia.[5] Clinicopathologic findings are also usually nonspecific, including hyperglobulinemia, increased liver enzyme activities, and eosinophilia.[5] The prognosis for treated dogs is generally positive, while the disease is often fatal in severely infected dogs despite aggressive treatment. The outcome of the presented case is unknown.

Dogs in other parts of the world may get infected with other members of Schistosomatidae, such as *Schistosoma japonicum* and *S. mansoni*. Other schistosomes of veterinary importance include *Orientobilharzia* spp., affecting ruminants, and some species of avian schistosomes such as *Trichobilharzia* spp.

Contributing Institution:
Cornell University College of Veterinary Medicine,
Department of Biomedical Sciences,
Section of Anatomic Pathology,
New York State Animal Health Diagnostic Center
https://www.vet.cornell.edu/animal-health-diagnostic-center

JPC Diagnosis:

1. Liver: Hepatitis, portal, granulomatous, chronic, diffuse, marked with numerous trematode eggs, fluke pigment, and an intravascular adult schistosome.

2. Small intestine: Enteritis, granulomatous, multifocal, moderate with numerous mucosal and submucosal trematode eggs.

JPC Comment:
The contributor provides an excellent review of the host range, life cycle, pathogenesis, clinical signs, and histomorphologic features of the digenean trematode *Heterobilharzia americana*.

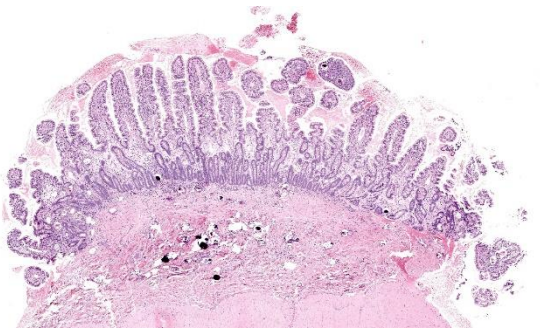

Figure 2-5. Intestine, dog. Mineralize trematode eggs are present within the lamina propria and submucosa. (HE, 32X)

H. americana is endemic in the southeastern United States with northward extension along the Atlantic coast to the Carolinas. This distribution closely corresponds to the geographic range of the only species snail historically confirmed to be susceptible to infection by *H. americana*, the semi-tropical *Galba cubensis*. However, a second amphibious snail species, *G. humilis*, was recently discovered to also be susceptible to both experimental and natural *H. americana* infection. Unlike *G. cubensis*, *G. humilis* is widespread throughout North America. Furthermore, *G. humilis* was recently implicated in the westernmost *H. americana* outbreak to date, with infections reported in 12 dogs living near a man-made pond in Moab, Utah. Although *G. cubensis* has been reported as far north as Oklahoma, it is possible other species, such as *G. humilis*, are associated the sporadic infections in northern states such as Indiana and Illinois. *G. humilis* also serves as one of several intermediate host's for *Fasciola hepatica*, a trematode that inhabits the bile ducts of sheep and cattle, causing cholangiohepatitis.[8]

G. cubensis and *G. humilis* are closely related to at least 40 subspecies and species of "fossarine" lymnaeids, which are relatively small snails with shell heights of less than 15mm and typically live very close to or above the waterline but may also be completely submerged. Collectively, fossarine lymnaeids are distributed throughout much of North America, the Caribbean, and regions of Central and South America. Given their close genetic relationship, multiple *Galba* spp., in addition to those previously identified, may potentially serve as intermediate hosts for *H. americana*, though additional research is needed to verify this hypothesis.[8]

Interestingly, infective cercariae are released from snails under nocturnal conditions. This is an indication of a host-adaptation of *H. americana* for the common raccoon (*Procyon lotor*), which typically forages in and around aquatic habitats during the night. Furthermore, the westward expansion of *H. americana* may be partially due to an increase in the raccoon's range itself, as this opportunist was not commonly found in the American west until the 20th century as the result of urbanization, decreased predation, climate change, and expanding agriculture. Although *H. americana*'s maintenance and gradual expansion is facilitated by the raccoon, infected dogs imported from endemic regions also may play a major role in its introduction to distant new habitats.[8]

Humans are also affected by *H. americana*, which causes severe cercarial dermatitis, also known as "swimmer's itch", but does not result in patent infection.[8] However, humans are very commonly parasitized by other schistosomes, making schistosomiasis the second most common parasitic disease of humans worldwide, following malaria. The most common species known to infect humans are *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*.

Schistosomiasis has plagued mankind for millennia, as symptoms consistent with *S. haematobium* were described on ancient Egyptian papyrus and calcified *S. haematobium* eggs were recovered from

3,200 year old Egyptian mummies.[9] Over two millennia later, Napoleon's troops referred to Egypt as the "land of menstruating males" as hematuria from schistosomal cystitis was so common that it was interpreted as an indication of puberty.[2] The parasite was first described in humans by Theodore Bilharz in 1851. As a result, the condition is also commonly referred to as "bilharzia" or "bilharziasis".[9]

Schistosomiasis is estimated to cause approximately 280,000-500,000 deaths per year. In addition, schistosomiasis is associated with a tremendous morbidity rate, with over 250 million infections, resulting in an estimated loss of 3.3 million life-years per year according the DALYs index ("Disability-Adjusted Life Years"). Unfortunately, human schistosomiasis is grouped in a category of diseases known as "Neglected Tropical Diseases", which also includes several helminth, protozoal, viral, and bacterial diseases. These diseases predominately affect underserved populations living in the poorest conditions and contribute toward the maintenance of social inequality, inherently creating significant barriers for the development of the most severely affected countries.[9]

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CASE III: N19-705A (JPC 4152931)

Signalment:
Adult, 18 kg, intact male North American opossum (*Didelphis virginiana*), marsupial.

History:
The opossum was submitted from the wildlife center. The patient presented for emaciation (body condition score 2/9) and multiple abrasions in the skin of the right aspect of the chin, tail base and bilaterally on the carpal paw pad, as well as a deep puncture wound in the right chin that extended into the oral cavity. The opossum declined clinically and displayed marked respiratory distress; euthanasia was performed due to poor prognosis.

Gross Pathology:
Diffusely, all the lung lobes were mottled pale pink, light yellow and white with myriad irregular, indistinct and coalescing nodules forming consolidated and semi-firm parenchyma and numerous tiny black and white, threadlike, curvilinear to serpentine foci randomly scattered throughout. The bronchi and bronchioles were filled with abundant yellow, thick, mucoid material. At the tip of the left cranial lung lobe is a small bulla.

Laboratory Results:
None.

Microscopic Description:
Lung: Nearly all the bronchi and bronchioles are distended (ectasia) and occluded by numerous larvae and a few adult nematodes intermixed with large numbers of degenerate and non-degenerate neutrophils, macrophages, lymphocytes, plasma cells, fewer eosinophils, sloughed epithelial cells, abundant cellular and pyknotic debris and mucus. The adult nematodes have a smooth cuticle with underlying hypodermis, lateral cords, meromyarian-platymyarian musculature. The pseudocoelom contains a digestive tract composed of large epithelial cells with a brush border and numerous intracytoplasmic brown granules, ovaries/ testis and uterus harboring numerous embryonated ova and larvae. Larvae are approximately 90 um in length and 10 um in width with linear arrangment of nuclei. The bronchial and bronchiolar epithelium is extensively eroded, attenuated or hyperplastic. The submucosa is infiltrated by large numbers of lymphocytes, plasma cells, and macrophages, which also extend into the peribronchial tissues. In addition, bronchial smooth muscles are thickened (hypertrophy) and the peribronchial mucous glands are enlarged with abundant mucus production (hyperplasia). More than 60% of the alveoli, particularly adjacent to the airways, are filled and expanded by numerous adult nematodes and larvae as described above. The alveoli are also extensively effaced by multifocal to coalescing granulomas in which large numbers of macrophages, multinucleated giant cells and few other inflammatory cells

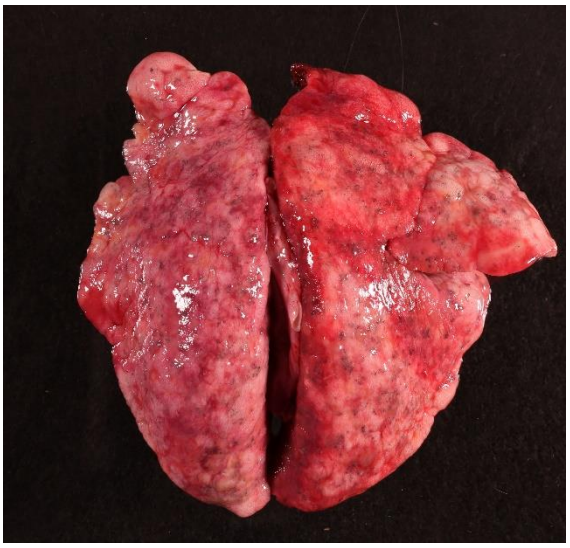

Figure 3-1. Lung, opossum. There are numerous red to black nodules scattered throughout all lung lobes. (Photo courtesy of: Department of Biomedical Sciences, Cummings School of Veterinary Medicine, Tufts University, 200 Westboro Rd, N Grafton, MA 01536, Website: https://vetmed.tufts.edu/pathology-service/services/anatomic/)

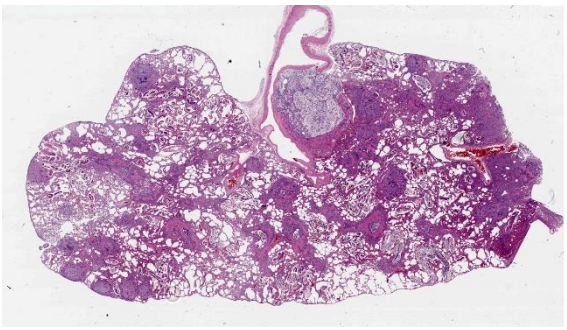

Figure 3-2. Lung, opossum. There are multifocal to coalescing areas of inflammation and consolidation which are centered on bronchioles. (HE, 6X)

are centered on nematode larvae. The granulomatous inflammation is vaguely intermixed and encircled by activated fibroblasts and collagen fibers that extend into the septa of adjacent alveoli. The alveoli subjacent to the granuloma are compressed and collapsed (atelectasis). Some of the remaining alveoli are distended and fused (emphysema). The pleura is intermittently lined by cuboid (reactive) mesothelial cells.

Contributor's Morphologic Diagnoses:
Lung: Pneumonia, granulomatous, multifocal to coalescing, chronic, severe with adult and larval metastrongyle sp., bronchiolar ectasia and occlusion, smooth muscle hypertrophy, pulmonary interstitial fibrosis, atelectasis, and emphysema, North American opossum (*Didelphis virginiana*), marsupial.

Contributor's Comment:
The Virginia opossum (*Didelphis virginiana*), commonly known as the North American opossum, is the only marsupial found north of Mexico. As a successful opportunist, they frequently inhabit urban areas due to the associated proximity to food sources. The opossum is a hardy creature that seems to adapt to heavy parasitic infections quite well.[1,4] Interestingly, wild opossums are seldom reported to have rabies and to serve as a poor-quality host for ticks and pathogens (dilution hosts), diverting tick blood meals away from competent hosts.[7] In addition, they are short-lived animals; few live longer than 2 years. Whether the short life span of the opossum correlates with parasitism is unknown. In this case, the opossum presented with severe emaciation, respiratory distress, and multiple traumatic injuries. At necropsy, poor body condition and heavy internal and external parasitism were identified. The stomach and duodenum are impacted by nematodes (*Physaloptera turgida*), which is very common in the opossum population as described[1,6,7] and the small intestine was heavily parasitized by *Mesoceastoides* spp.[1] However, comprehensive microscopic examination of multiple skeletal muscles and visceral organs from this opossum did not capture *Besnoitia darlingi*, which is commonly noted in opossum. *Didelphostrongylus hayesi* belongs to the order Strongylida, superfamily Metastrongyloidea. With 48 to 79% of the prevalence rate, *D. hayesi* is one of the most common pulmonary nematodes in the opossum.[1,7] *D. hayesi* has an indirect life cycle requiring terrestrial snails (*Mesodon perigraptus* or *Triodopsis albolabris*) as an intermediate host. After intermediate hosts are ingested, the larvae migrate from the alimentary tract to the lung, likely through the blood and lymphatic circulation and direct penetration. Subsequently, the third-stage larvae mature into the adult stage in the

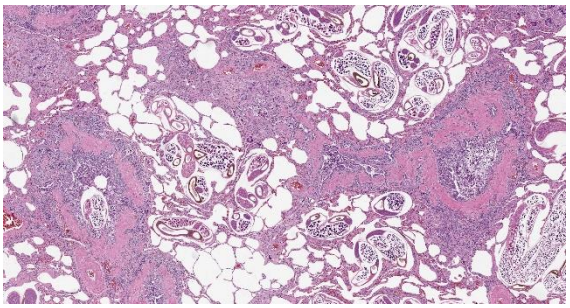

Figure 3-3. Lung, opossum. Numerous cross and tangential sections of metastrongyle adults are present within airways and adjacent alveoli. (HE, 30X)

airways, particularly intrapulmonary bronchi. As presented in this case, numerous nematodes admixed with mucus and inflammatory cells occlude the bronchiolar lumina and alveolar spaces. Unlike other lungworms, *D. hayesi* are distinguished by ovoviviparity, in which the eggs are hatched within the uteri. The newly hatched larvae (L1) migrate up the trachea, are swallowed and are then shed in the feces.[4] The pathology caused by *D. hayesi* can be mild to severe. In a recent study, 20 of 44 opossums trapped in the state of Colima, Mexico, carried *D. hayesi* in their airways, but none of them showed overt emaciation, suggesting that *D. hayesi* has little or no effect on the general health status.[8] However, prominent gross pulmonary lesions were observed in 5 of 11 opossums with *D. hayesi* infection in another retrospective study.[6] In this case, severe respiratory distress occurred as the result of airway obstruction, granulomatous pneumonia, and extensive fibrosis. The clinical deterioration was enhanced by comorbidities, including trauma-associated stress, malnutrition and gastric impaction due to heavy parasitism.

At necropsy, the gross changes in the lungs, including the diffuse consolidation of parenchyma with numerous granulomatous nodules, terminal emphysema, are impressive. Under the microscope, the consolidation of the lungs corresponds with bronchiolar and alveolar spaces filled by nematodes, inflammatory cells, and debris, in combination with granulomatous inflamemation typically centered on larvae; adult worms evoked a relatively mild inflamematory response.[2,6,8] Tiny black foci randomly scattered throughout the lungs, as grossly observed, correspond to the parasitic brown pigments within the epithelium of conspicuous digestive tracts of the nematodes.[2] The inflammation is mainly composed of macrophages, multinucleated giant cells and lymphocytes, plasma cells.

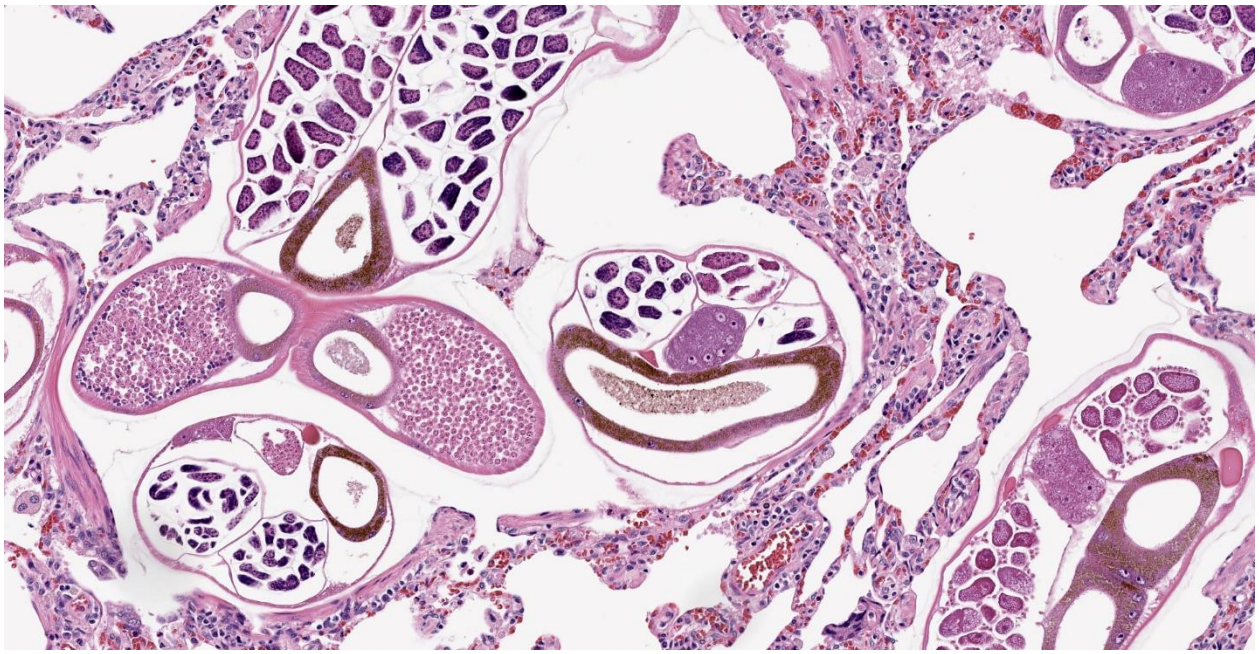

Figure 3-4. Lung, opossum. Higher magnification of male and female metastrongyle adults with thin cuticles, platymarian musculature, intestinal epithelium with cytoplasmic hemoglobin pigments, and gonads with embryonated eggs and testes with spermatocytes. (HE, 144X)

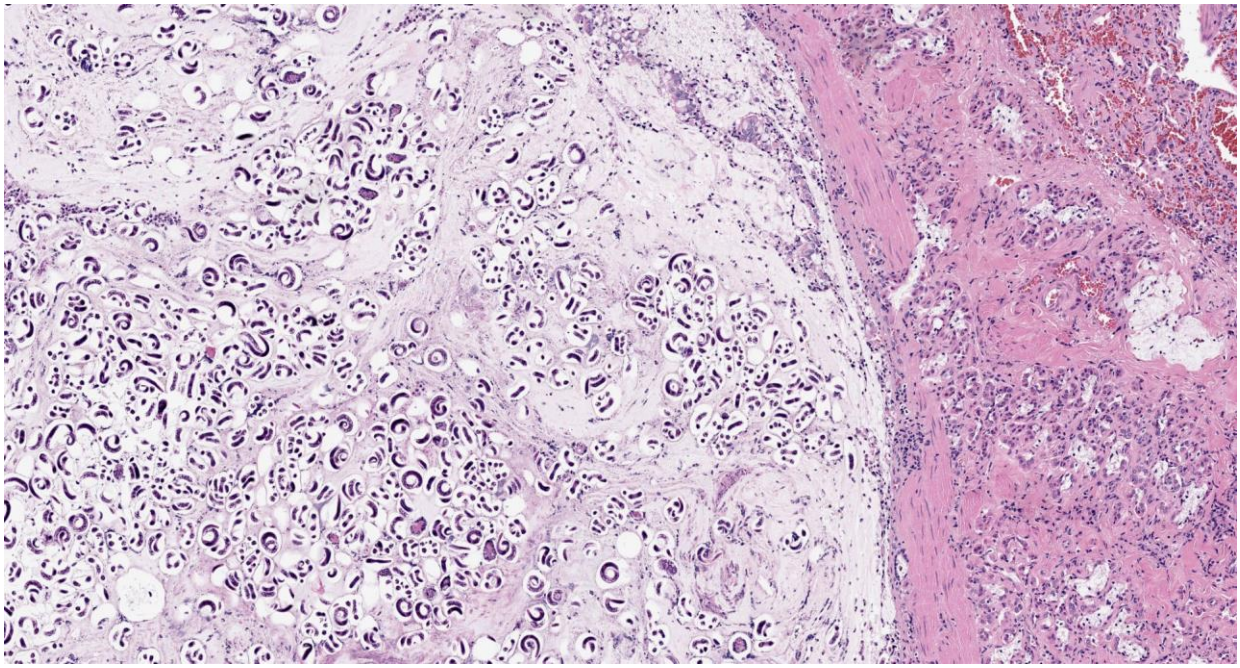

Figure 3-5. Lung, opossum. Bronchi are ectatic and filled with abundant mucin, metastrongyle larvae and eggs, and there is marked hyperplasia of submucosal glands. (HE, 82X)

The eosinophilic component is minor, suggesting chronicity. In addition, the lungs failed to collapse due to the thickening of alveolar septa arising from interstitial fibrosis and infiltration of inflammatory cells. The emphysema and atelectasis are the consequence of verminous airway obstructions. Bronchiolar and alveolar ductular smooth muscle hyperplasia, recognized as a feature of *D. hayesi* in the lungs[6], is noted in this case.

The morphological changes seen in this case are compatible with a late-stage lungworm infection in the opossum or other species, such as *Aelurostrongylus abstrusus* in cats.[2,6,8] It is deemed that these parasites induce pulmonary injuries via direct mechanical irritation and/or their secretory products, which is exacerbated by enzyme and free radical released by inflammatory cells. Typical features include type II pneumocyte hyperplasia, alveolar bronchiolization, as well as hyperplasia and metaplasia of goblet cells in the airway, which are evidence of a response to the stimulation and damage of pneumocytes. Surfactant overproduction can also result in the accumulation of alveolar macrophages.[8] In this case, there is severe granulomatous inflammation, fibrosis and smooth muscle hypertrophy that occasionally obscures these features. This suggests a relatively increased severity and chronicity. No evidence of concurrent bacterial coinfection or other parasite infection, such as *Eucoleus aerophilus* (formerly *Capillaria aerophilus*) or *Besnoitia darlingi*, was identified in this case.

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200 Westboro Rd, N Grafton, MA 01536
Website: https://vetmed.tufts.edu/pathology-service/services/anatomic/

JPC Diagnosis:

Lung: Bronchopneumonia**,** lymphoplasmacytic, diffuse, severe, with multifocal granulomas, marked smooth muscle

hyperplasia, and metastrongyle adults, larvae, and eggs.

JPC Comment:
The contributor provides a concise summary *Didelphostrongylus hayesi*'s lifecycle, prevalence, histologic features, and associated pulmonary lesions described in infected Virginia opossums (*Didelphis virginiana*).

D. hayesi is similar to many other metastrongyle lungworms in that adults are most commonly found in the bronchioles and bronchi, which may result in goblet cell metaplasia within the bronchiolar epitheium, a location not typically inhabited by goblet cells. As a result, chronic bronchiolitis may induce excessive mucous production, which in turn may form mucous plugs and obstruct lower airways. In addition, parasitized opposums may also exhibit hyperplasic bronchial glands, resulting in a high gland to wall thickness ratio, otherwise known as the Reid index. An increased Reid index is an indication of prolonged mucosal irritation and may be increased as the result of multiple conditions, such as allergies, inflammation, and parasitic bronchitis.[8]

Rarely, *D. hayesi* infection in opossums is associated with a lesion known as alveolar bronchiolarization. Also known as peri-bronchiolar metaplasia or "lambertosis" in human pathology, this phenomenon occurs as the result of an erratic remodeling process in which bronchiolar epithelial cells migrate to and repopulate alveoli following injury and fibrosis. Lambertosis is an eye-catching lesion given that it is composed of clusters of bronchiolar cells lining the alveolar basement membrane and is easily mistaken for pulmonary adenoma, as evidenced reports of "extensive adenomatoid proliferation of alveolar epithelium" in early reports describing opossums with verminous pneumonia.[8]

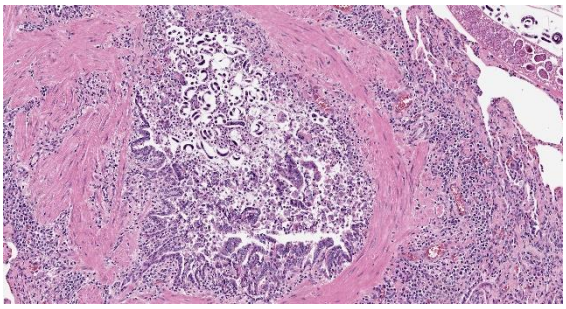

Figure 3-6. Lung, opossum. Bronchioles are filled with mucin and metastrongyle larva. There is airway, smooth muscle, and BALT hyperplasia. (HE, 112X)

As noted by the contributor, the capillarid nematode *Eucoleus aerophilus* is also associated with verminous pneumonia in opossums, with dual infections commonly reported in the United States. Both *D. hayesi* and *E. aerophilus* are associated with granulomatous reactions, however, severe reactions are predominantly associated with the latter.[8]

Both adult *D. hayesi* and *E. aerophilus* nematodes may both be found in the bronchi. However, the former is also found in the bronchioles while the latter is also inhabits in the trachea of canids, felids, and some omnivorous animals such as opossums. Despite its wide host-range and zoonotic potential, little is known about *E. aerophilus*' life cycle although it is hypothesized animals become infected following the ingestion of an earthworm intermediate host. *E. aerophilus* infection is particularly common in wild foxes, with prevalence rates as high as 88% in Norway and 84% in the Pannonian and Fruska Gora Mountain regions of Serbia. In addition *E. aerophilus* is a parasite of economic importance as it is considered to be an agent of massive mortality in farmed silver foxes.[5]

E. aerophilus belongs to a group of nematodes known as aphasmids, which differ from other nematodes by lacking a pair of sensory papilla on the caudal aspect, known

as phasmids. A characteristic feature of aphasmids in histologic sections is a structure composed of a row of esophageal gland cells (stichocytes), known as the stichosome, which surrounds the esophagus. In addition, aphasmids produce either embryonated or unembryonated eggs with bipolar plugs.[3]

During the conference, the moderator discussed a unique feature of *D. hayesi* amongst metastrongyles: its meromyarian-platymyarian musculature. This feature is in contrast to other metastrongyles, which have coelomyarian-polymyarian musculature. The reason for this variance is unclear, although the moderator mused this feature may represent a retained feature from an evolutionary divergence from nematodes with similar musculature, such as strongyles or trichostrongyles.

As noted in the contributor's description, the section includes multifocal areas of atelectasis and emphysema. Participants discussed these features and postulated the atelectasis likely occurred as the result of both bronchiolar obstruction with collapse of the downstream airways in addition to compression from adjacent granulomas. Furthermore, many remaining alveoli are emphysematous, which is consistent with the clinical history of dyspnea and likely increased inspiratory effort.

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CASE IV: S884/20 (JPC 4166939)

Signalment:
Adult, male raccoon (*Procyon lotor*)

History:
The animal was shot due to central nervous signs and submitted for necropsy to clarify the cause of disease.

Gross Pathology:
At necropsy, the animal was in a moderate nutritional status. In addition to a hemaskos and generalized swollen lymph nodes, the lungs exhibited multifocal greyish nodular lesions up to 3 mm in diameter that are slightly raised above the pulmonary surface. The liver had slightly rounded edges and a firm consistency. The spleen was slightly swollen. The stomach was empty.

Laboratory Results:
Fungal structures were identified as *Emmonsia crescens* using qPCR (target ITS-2/28s). Using Immunofluorescence, canine distemper virus antigen was found in the lung, urinary bladder, spleen and brain. Immunohistochemistry of the lung revealed low to moderate numbers of CDV antigen-containing bronchial and bronchiolar epithelial cells, peribronchial glandular epithelial cells, interstitial cells, and typ II-pneumocytes. Using antibodies against CD3 and CD20, low numbers of T- and B-lymphocytes were seen in the pulmonary interstitium. In addition, CD204-expressing macrophages were found immunohisto-chemically in low numbers in the pulmonary interstitium and in moderate numbers in fungal-associated granulomas. Moreover, *Staphylococcus spp*. was isolated via bacterial culture of lung tissue.

Microscopic Description:
In the lung, affecting about 60% of the tissue, there is a multifocal loss of parenchymatous architecture by concentrically arranged granulomas. Granulomas measure up to 500 µm in diameter and often show a central, fungal adiaspore. These fungal structures have a diameter of up to 150 µm and a thick (up to 8 µm), round, eosinophilic to amphophilic, non-birefringent cell wall and contain finely stippled, amphophilic material. The adiaspore cell wall stains purple with the Periodic Acid-Schiff (PAS) reaction and black with Grocott´s methenamine silver method. The fungal elements are embedded into low to moderate amounts of cellular debris that is surrounded by several layers of epitheloid macrophages and eosinophils as well as low numbers of lymphocytes and plasma cells. Occasionally, multinucleated giant cells of foreign body type are present in the granulomatous wall.

Additionally, the remaining alveolar septa are diffusely, mildly thickened by an infiltration of mostly macrophages and lymphocytes. Multifocally, there is a mild hyperplasia of type II pneumocytes. Furthermore, bronchial and bronchiolar epithelial cells are slightly hypertrophic and infrequently contain small, cytoplasmic, eosinophilic inclusion bodies.

Contributor's Morphologic Diagnoses:
Lung, pneumonia, granulomatous and eosinophilic, severe, chronic, multifocal, with intralesional fungal conidia (adiaspores) and pneumonia, broncho-interstitial, lymph-ohistiocytic, mild, subacute, diffuse with eosinophilic, cytoplasmic inclusion bodies in bronchial epithelial cells.

Figure 4-1. Lung, raccoon: Numerous slightly raised white nodules ranging up to 3mm are distributed throughout all lobes. (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 17, D-30559 Hannover, Germany http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

Contributor's Comment:

Fungal infections are most commonly seen in animals that are immunocompromised due to infections, malnutrition, stress or various other reasons. Therefore, the respiratory tract is predisposed due to the direct contact with the environment. In the presented case, the morphological changes in the lung with granuloma formation together with the described intralesional fungal elements match the histological characteristics of an adiaspiromycosis. This fungal infection is reported from different continents (e.g. Europe, Northern America and Africa) and occurs in various mammals, including humans, otters, beavers, deer, rabbits and rodents. Inhalation of conidia leads to granulomatous or pyogranulomatous pneumonia with or without inflammatory involvement of the local lymph nodes.[1,7,10,12] Inhaled conidia enlarge and form nonreplicating adiaspores.[1] The underlying dimorphic fungus *Emmonsia* spp. is closely related to *Blastomyces spp.* and has two important pathogenic subspecies. In Europe, *E. crescens* characterized by adiaspores measuring up to 500 µm in diameter is more frequently found than *E. parva*, which forms smaller seized spores of up to 40 µm in diameter.[3, 5] The latter is more commonly seen in xerothermic regions like Africa, Asia and partially America.[3,5] Pathological findings are granulomatous to pyo-granulomatous pneumonia, as the infection is restricted to the lower respiratory tract.[3,7,10]

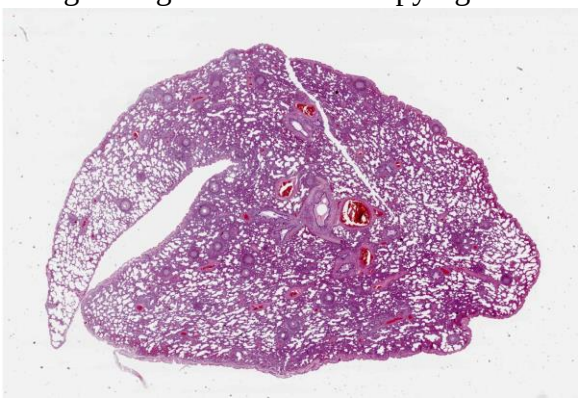

Figure 4-2. Lung, raccoon. There is diffuse mild atelectasis and granulomas are scattered throughout the lung. (HE, 6X)

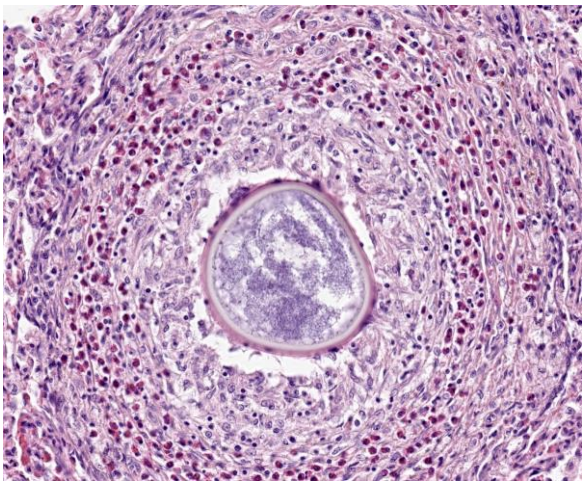

Figure 4-3. Lung, raccoon. Granuloma are centered on 150 µm diameter adiaspores with a thick trilaminar wall and vacuolated cytoplasm. (HE, 266X)

As a differential diagnosis, coccidioidomycosis due to infection with *Coccidioides* spp. is also characterized by (pyo-) granulomas up to 200 µm in diameter and fungal spherules, which contain numerous, 2-5 µm seized endospores.[5] Moreover, the observed bronchointerstitial pneumonia in association with the presence of eosinophilic, cytoplasmic inclusion bodies suggests an infection with canine distemper virus (CDV). This viral infection was confirmed by both, immunohistochemistry and immune-fluorescence. CDV-antigen was observed in various cell types in the lung as well as in the brain, spleen and urinary bladder of the raccoon. CDV, a pantropic morbillivirus of the family Paramyxoviridae, can infect a wide range of terrestrial carnivores and marine mammals and leads to various forms of disease.[2] Pathological findings include bronchointerstitial pneumonia, gastro-enteritis, demyelinating encephalomyelitis, chorioretinitis, hyperkeratosis (hard pad disease), metaphyseal bone lesions and enamel hypoplasia.[6] Furthermore, CDV compromises the immune response of the affected host by infecting lymphocytes

leading to severe lymphocytolysis.[4,13] The concomitant immunosuppression represents a predisposing factor for various secondary infections including viral, bacterial, fungal or parasitic infections. In the present case, the CDV infection is considered more likely as a secondary event superimposed on the adiaspiromycosis, because the fully developed fungal granulomas represent more chronic lesions. However, the CDV infection represents the most probable cause of the central nervous signs of the animal.

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JPC Diagnosis:

1. Lung: Pneumonia, eosinophilic and granulomatous, multifocal, severe, with numerous adiaspores.
2. Lung: Pneumonia, interstitial, histiocytic, diffuse, moderate, with rare cytoplasmic viral inclusions.

JPC Comment:
The contributor provides a succinct and insightful summary of *Emmonsia parva* and *E. crescens*. These dimorphic fungi cause adiaspiromycosis, a disease that primarily affects rodents but is also rarely reported in other species and humans. In addition, the contributor provides a concise review of canine distemper virus (CDV), a morbillivirus capable of infecting multiple domestic and wildlife species, including a recent report[14] describing CDV infections in multiple 2-toed sloths (*Choloepus didactylus*). As noted by the contributor, CDV infection is commonly associated with clinical signs involving the respiratory, gastrointestinal, integumentary, and central nervous systems. Furthermore, this entity was previously discussed at length during 21-22 WSC 5, Case 2.

Emmonsia spp. bear the name of Dr. Chester Wilson Emmons, an internationally recognized 'founding father' of medical mycology in the United States. After receiving his Ph.D from Columbia University in 1931, Dr. Emmons began his long and storied career by collecting, observing, and classifying dermatophytes based on their spores and accessory organs, resulting in redefining genera *Trichophyton*, *Microsporum* and *Epidermophyton* based on mycological terms and the elimination chaotic pre-existing taxonomic nomenclature. Dr. Emmons later colla-borated with Dr. Carrion at the School of Tropical Medicine at the University of Puerto Rico and unequivocally confirmed *Actino-myces bovis* is a normal commensal of the human mouth in 1935. He was recruited the following year as the first medical mycologist at the National Institutes of

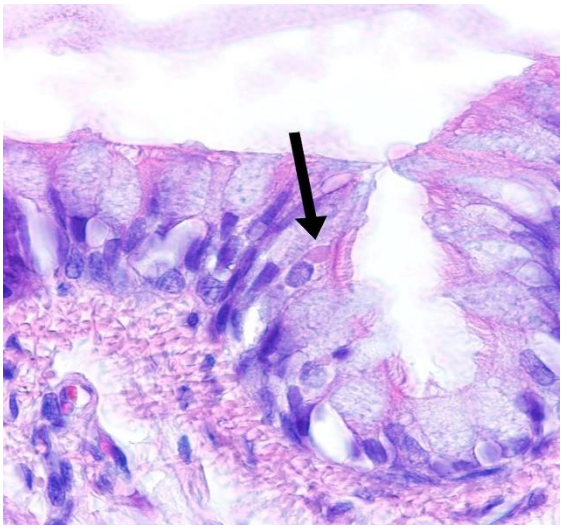

Figure 4-4. Lung, raccoon. Rare airway epithelial cells contain cytoplasmic viral inclusions. (HE, 400X) (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 17, D-30559 Hannover, Germany http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

Health (NIH) in an attempt by the institution to expand its investigative and surveillance capabilities in regard to infectious diseases in the United States. During his tenure, Dr. Emmons devoted his professional career toward the development of the medical community's recognition of saprophytic organisms as being significant causes of disease, an underappreciated concept at the time. Examples of these efforts include overseeing the discovery of coccidiomycosis in rodents in the desert southwest of the United States, original research in establishing the importance of histoplasmosis, the initial isolations of *Histoplasma capsulatum* and *Cryptococcus neoformans* from their natural habits, the isolation and identification of new agents of fungal diseases (such as *Emmonsia* spp.), and assisting in the development of the antifungal medication amphotericin B. Dr. Emmons retired from the National Institutes of Allergy and Infectious Diseases (NIAID), NIH as the head of the medical mycology division three decades later in 1966. Retirement did not suite Dr. Emmons, as he was later appointed as a faculty member of Arizona State University from 1973-1977, where he lectured, continued writing, and searched for *C. immitis* in the soil, amongst other academic endeavors.[11]

Emmons and Jellison first described *Emmonsia crescens* in a 1960 report in which they reclassified *Haplosporangium parvum* to *Emmsonsia parvum*; *E. crescens* was then segregated on the basis of its greater size in the adiaspore form. At the time, *Emmonsia parva*'s known range was the arid southwestern United States, whereas *E. crescens* had been identified throughout North, Central, and South America, Europe, and Asia. Interestingly, the etiology previously known as *H. parvum* had not identified until 1942 to the puzzlement of Emmons and Jellison, who commented how it seemed "improbable that a fungus that so frequently invades the lungs of rodents to have escaped the notice of mammalogists and mycologists until 1942." The same report describes Jellison identifying the fungus in preserved lung tissue from a rodent (*Microtus agrestis*) trapped in Sweden in 1845.[8]

Since Emmons and Jellison's 1960 report, adiaspiromycosis has been identified in over 118 species of mammals with a global distribution and is common in both rodents and small terrestrial mammals. For example, a 2009 report found nearly a third of native British mammals to be infected with *E. crescens*. Humans may also be infected, with the first human case of adiaspiromycosis reported in 1964 and subsequent cases reported worldwide. Both *E. crescens* and *E. parava* may result in human infections, with the former being more commonly implicated.[15]

The term "adiaspiromycosis" itself is derived from the characteristically large adiaspores that arise following marked enlargement of inhaled infective conidia. The term "adiaspore" is in reference to the fact these structures neither replicate nor disseminate

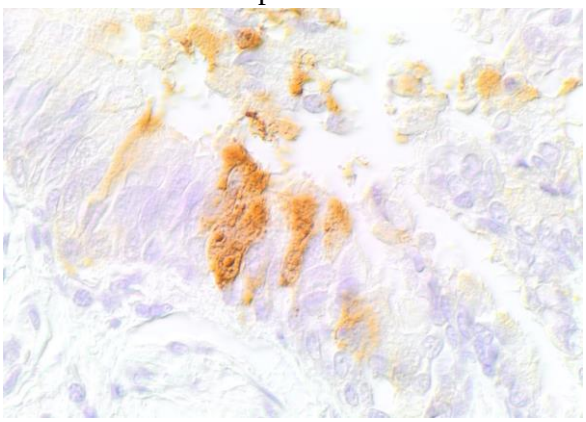

Figure 4-5. Lung, raccoon. Airway epithelium contains abundant CDV antigen. (anti-CDV, 600X). (Photo courtesy of: Department of Pathology, University of Veterinary Medicine Hannover, Buenteweg 17, D-30559 Hannover, Germany http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

from their original site of implantation, for which Emmons and Jellison coined the term from Greek α– (not, without), –δια– (by, through), and –σπορα (seed, sowing).[8,15] Thus, the severity of disease is determined by the inoculum size and the host's response, which may range from subclinical pneumonia to diffuse pulmonary disease and death.[15]

As noted by the contributor, *Emmonsia*'s large size and thick PAS-positive wall histologically resemble *Coccidioides* spp., as well as the mesomycetozoan parasite *Rhinosporidium seeberi*. However, in contrast the latter two organisms, endosporulation is not a feature of *Emmonsia* spp.[5]

The moderator emphasized the diffuse atelectasis in this case, noting the dilated patent alveolar ducts on a background of collapsed alveoli.

Conference participants noted considerable slide variability in regard to intracytoplasmic and intranuclear viral inclusions which made the diagnosis of canine distemper a challenging feature in this case.

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