Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 23

22 April 2020

CASE I: 69887 (JPC 4117530).

Signalment: Neutered male Domestic Short Hair cat of unknown age, *Felis catus*

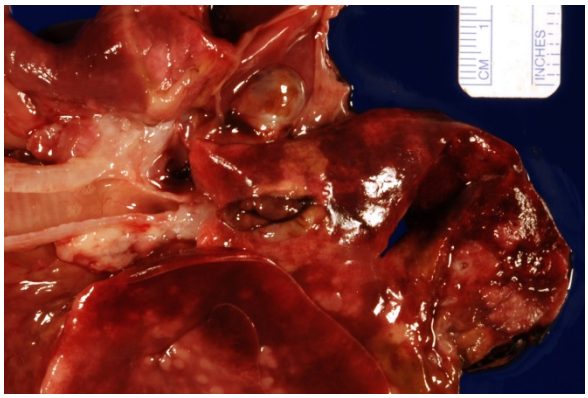
History: The animal was a rescue shelter cat current on all vaccinations. The animal was presented with dyspnea, hemoptysis and subsequent cardiac arrest.

Gross Pathology: The most notable postmortem findings were restricted to the lungs, which were uncollapsed, moist, firm, meaty and diffusely mottled dark red to pink with multifocal to coalescing 2-4mm white/tan raised round nodules. Impression smear of these nodules showed numerous degenerate neutrophils and macrophages. There were also multifocal, dome shaped, raised, 1-2 cm nodules with central cavitations containing few ovoid, reddish-brown ~5 x 10 mm adult trematodes.

Laboratory results: NA.

Microscopic Description: Lung: Pyogranulomatous inflammation effaces and replaces up to 40% of the lung parenchyma and occludes bronchi and bronchioles. This inflammation is composed of numerous degenerate and viable neutrophils and macrophages with fewer multinucleated giant cells, lymphocytes and plasma cells. Associated with inflammation, there are

Lungs, cat. The lung lobes are uncollapsed, moist, firm, meaty, and diffusely mottled dark red to pink with multifocal to coalescing, 2-4 mm white to tan/grey, raised, round nodules. (Photo courtesy of: Johns Hopkins University School of Medicine, Department of Molecular and Comparative Pathobiology, http://www.hopkinsmedicine.org/mcp)



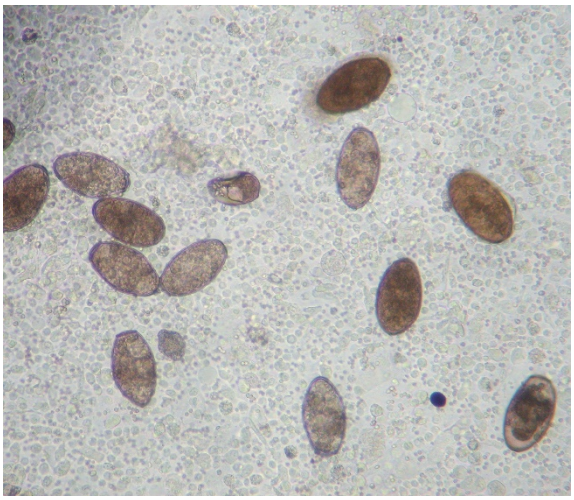
Lung, cat. There are multifocal, dome-shaped, raised 1-2 cm nodules with central cavitations containing few ovoid, reddish-brown, approximately 5x10 mm adult trematodes. (Photo courtesy of: Johns Hopkins University School of Medicine, Department of Molecular and Comparative Pathobiology, http://www.hopkinsmedicine.org/mcp)

multiple adult trematodes, parasitic eggs and multifocal bacterial colonies in the alveolar, bronchiolar and bronchial lumena. Adult trematodes are ~ 6mm X 4mm with a 40um thick spiny tegument and a spongy parenchyma that contains numerous subtegumental vitellaria with eosinophilic globular yolk material, centrally located uterus with numerous egg, few testis containing sperms, and intestinal caeca. The parasite eggs in the adult trematode and airways are 50um X 120um and embryonated with curved 110um X 30um larva, and have 1-3um thick, gold-brown, operculate, ansiotropic shell. Bacterial colonies are composed of numerous 1-2um cocci enmeshed in a brightly eosinophilic protein matrix (Splendore-Hoeppli material). Bronchi and bronchioles are partially filled with sloughed epithelial cells and low numbers of macrophages, neutrophils, lymphocytes and plasma cells and surround by moderate numbers of the same inflammatory cells, hyperplastic peribronchial mucous glands and mild fibrosis. Many bronchi containing adult trematodes are lined by lined by squamous epithelium (squamous metaplasia). Less affected areas have pigment-laden macrophages, eosinophilic proteinaceous material (edema) and hemorrhage. Multifocally, small and medium caliber blood vessels have tunica media and intima thickened by hypertrophic and vacuolated smooth muscle cells and endothelial cells, respectively, and multifocally surrounded by paler connective tissue separated by clear space (edema). There is multifocal bronchiolar and alveolar smooth muscle hypertrophy. The pleura is thickened up to 50um by inflammatory infiltrate and increased fibrosis and lined by plump and cuboidal mesothelial cells (reactive).

Contributor's Morphologic Diagnosis:

Lung, pneumonia pyogranulomatous and lymphoplasmacytic, multifocal to

Paragonimus kellicotti eggs. (Photo courtesy of: Johns Hopkins University School of Medicine, Department of Molecular and Comparative Pathobiology, http://www.hopkinsmedicine.org/mcp)



Paragonimus kellicotti eggs. (Photo courtesy of: Johns Hopkins University School of Medicine, Department of Molecular and Comparative Pathobiology, http://www.hopkinsmedicine.org/mcp)

coalescing, chronic active, severe with pleuritis and trematode adults and eggs and bacterial cocci

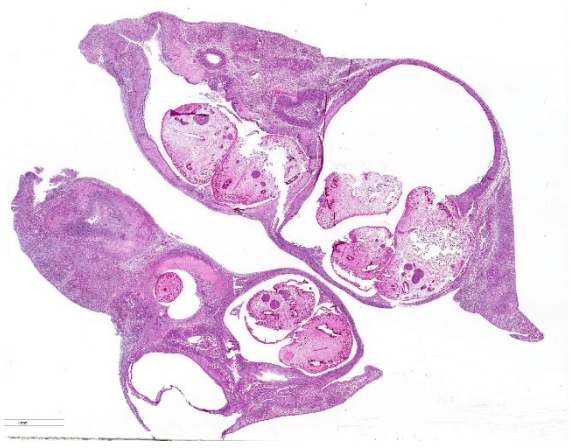
Etiologic diagnosis: Pulmonary paragonimiasis

Cause: *Paragonimus kellicotti*

Contributor's Comment: Pulmonary paragonimiasis is a parasitic disease caused by trematodes of the genus *Paragonimus* and the family *Troglotrematidae*. It is an important food-borne zoonotic disease affecting crayfish eating mammals and human worldwide, but it is most common in China, southeast Asia, and North America.[1] At least 28 species of *Paragonimus* have been discovered.[1] *P. westermani* (China and southeast Asia) and *P. kellicotti* (North America) are the two most common species.[2]

Adult trematodes are usually found in the lung of definitive hosts (human and wild and domestic animals, including dogs and cats).[1] Trematodes are also rarely found in other viscera and brain (extrapulmonary paragonimosis).[1] The first and second intermediate hosts are small aquatic snails (cercariae stage) and crayfish or crabs (metacercariae stage), respectively.[2,4] Once the second intermediate hosts are ingested by definitive hosts such as dogs and cats, the metacercariae are liberated into the intestine and subsequently migrate across the peritoneal and pleural cavities into the lung where they mature, form cysts and cause pyogranulomatous inflammation and fibrosis.[2,4]. The mature adults lay eggs into the bronchioles or bronchi and the eggs are coughed up the tracheobronchial tree, swallowed and passed in feces or excreted in the sputum.[2,4] In the external environment, the eggs hatch and release ciliated miracidia, which infect the first intermediate hosts such as aquatic snails.[2]

Clinical signs include intermittent cough, weakness and lethargy (4). Pathologic lesions are principally due to the presence



Lung, cat. Two sections of lung are presented, with cross sections of cysts containing paired adult trematodes. The remainder of each section is atelectatic with patch areas of inflammation. (HE, 7X)

Lung, cat. Higher magnification of the adult trematode, demonstrating a spiked tegument, spongy body cavity, numerous vitellarian glands (black arrows), cross section of cecum (green arrow) and testes (yellow arrows). (HE, 67X)

and migration of adult trematodes and eggs and metabolites produced by trematodes (1). Common pulmonary lesions include pyogranulomatous pneumonia, catarrhal and eosinophilic bronchitis and pleuritis (4). In addition, pneumothorax also can happen rarely due to rupture of parasitic cysts (4). Ectopic extrapulmonary paragonimosis occur more often than in other mammalian species (1). Common sites extrapulmonary Paragonimosis are the brain, spinal cord, abdominal cavity and subcutis (1).

Contributing Institution:

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Department of Molecular and Comparative Pathobiology
http://www.hopkinsmedicine.org/mcp

JPC Diagnosis: Pneumonia, pyogranulomatous, multifocal, mild with encysted adult trematode and eggs.

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JPC Comment: Paragonimiasis is an uncommon acquired infection of crustacean-eating animals. Of the approximately 30 named species of *Paragonimus*, ten are considered pathogenic, but the overwhelming number of cases occur, as mentioned above, as a result of *P. westermani* infection in Asia, and *P. kellicotti* in the United States.

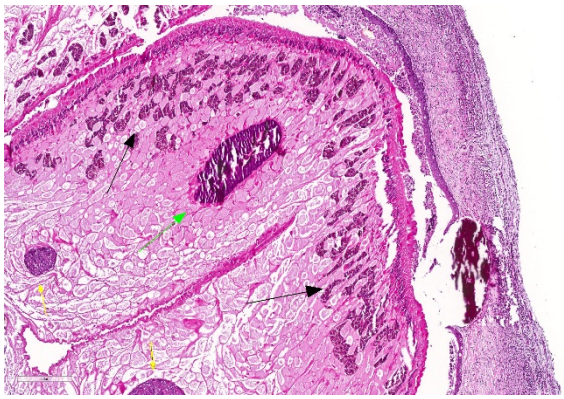
P. rudis was the first lung fluke to be described by Natere in 1828, *P. westermani* was first described by Conrad Kerbert in 1878 in a Bengal tiger at the Amsterdam Zoo and named for the zoo's curator, C.F Westerman. The next year, B.S. Ringer identified the first case of human paragonimiasis in the lung, and the following year, Patrick Manson and Erwin von Baetz independently diagnosed cases by viewing fluke eggs in human sputum for the first time. The first case of *P. kellicotti* in the U.S. was identified in a dog in Ohio by Kellicott in 1894, and in a cat by Ward, and the first human case of *P. kellicotti* was identified by Abend in 1910.

Human paragonimiasis is far more common in Asia, where it is considered endeminc in some areas of China and Southeast Asia in humans who eat raw, undercooked, or alcohol-pickled freshwater crayfish. In the U.S., cases of non-native paragonimiasis (*P. westermani* infection from eating imported Asian crabs) still outweigh cases of native paragonimiasis (*P. kellicotti* infection from consuming raw or undercooked crayfish).

Published risk factors for cases of native paragonimiasis include young males (males outweigh females 15:1), alcohol consumption, and paddling, boating, or camping along the upper Mississippi river valley, with a particular concentration in the state of Missouri. Many cases of nonnative paragonimiasis arise as a result of eating poorly cooked imported crabs, often in sushi restaurants. In parts of Asia where "drunken crabs" (ethanol-picked crabs) are a delicacy, up to 30% of the local population have antibodies to *P. westermani*. A resurgence in consumption of wild boar meat in Japan has resulted in a number of cases of infection with *P. miyazakii.*

The contributor has described the complex life cycle of lung flukes with multiple intermediate hosts. Young pathologists are often intrigued by the very characteristic presence of two hermaphroditic flukes in each cyst (as illustrated in this case). *Paragonimus* flukes are indeed hermaphroditic, with presence of both testicular and ovarian tissue within an individual. While under certain circumstances they can self-fertilized, cross-fertilization is generally the rule (hence the presence of two flukes) and the cysts are known as "mating cysts".)

In humans, the prepatent period for both nonnative and native paragonimasis is 2 to 16 weeks with an average of 10 weeks. The initial clinical signs occur days following ingestion with abdominal cramps, diarrhea and fever (likely the period of migration of metacercaria out of the intestine.). After 2-16 weeks, a constellation of clinical signs occur, including fever, "rusty" hemoptysis



Lung cat. Cross section of encysted adult trematode demonstrating spiny tegument and spongy parenchyma, and cross sections of ceca (black arrows), vitellarian glands (green arrows), testes (blue arrows) and uterus with eggs (red arrows). (HE, 40X).

(due to the presence of red blood cells from rusty mating cysts.), and peripheral eosinophilia. Respiratory infections are the rule, although aberrant migration may result in cutaneous infections (*trematoda larval migrans)* or cerebral infections. Complications of respiratory infections in humans include pneumothorax, constrictive pleuritis and pleural effusions. Following diagnosis, treatment with praziquantel generally effects a cure.

References:

1. Madarame H, Suzuki H, Saitoh Y, Tachibana M, Habe S, Uchida A and Sugiyama H. Ectopic (subcutaneous) *Paragonimus miyazakii* infection in a dog. Vet Pathol. 2009 Sep;46(5):945-8.
2. Blair D. (2014) Paragonimiasis. In: Toledo R., Fried B. (eds) Digenetic Trematodes. Advances in Experimental Medicine and Biology, vol 766. Springer, New York, NY.
3. Gardiner CH, Poyton SL. An Atlas of Metazoan Parasites in Animal Tissue. Washington, DC: Armed Forces Institute of Pathology; 1990:46-48.

4. Caswell JL, Williams KJ. Respiratory System. In: Maxie ME, ed. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. 6th ed. Vol 2, Philadelphia, PA: Elsevier; 2016:591.

CASE II: PV300 (JPC 4119013).

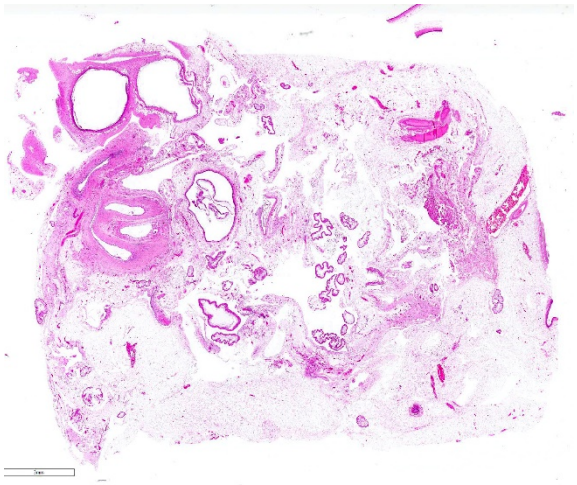
Signalment: A 6.9 years old, female spayed, mixed breed (*canis familiaris*).

History: Ascites. Ultrasound examination revealed many cysts in the abdominal cavity. The cysts were sampled on exploratory laparotomy.

Gross Pathology: Open and friable cyst approximately 7 cm in diameter. On section many spaces, approximately 2 cm in diameter that contain clear fluid.

Laboratory results: NA.

Microscopic Description: The tissue sections consist of mesenteric/peritoneal fat with peritoneal connective tissue with minimal fibrosis and mononuclear cell infiltration. There is formation of many cystic spaces composed of a wall of mature fibrous tissue with an inner layer of palisading macrophages surrounding degenerate cestode larvae with a thick, smooth capsule, a subjacent layer of somatic cells and a loose body cavity with numerous calcareous corpuscles. No scolices are observed.

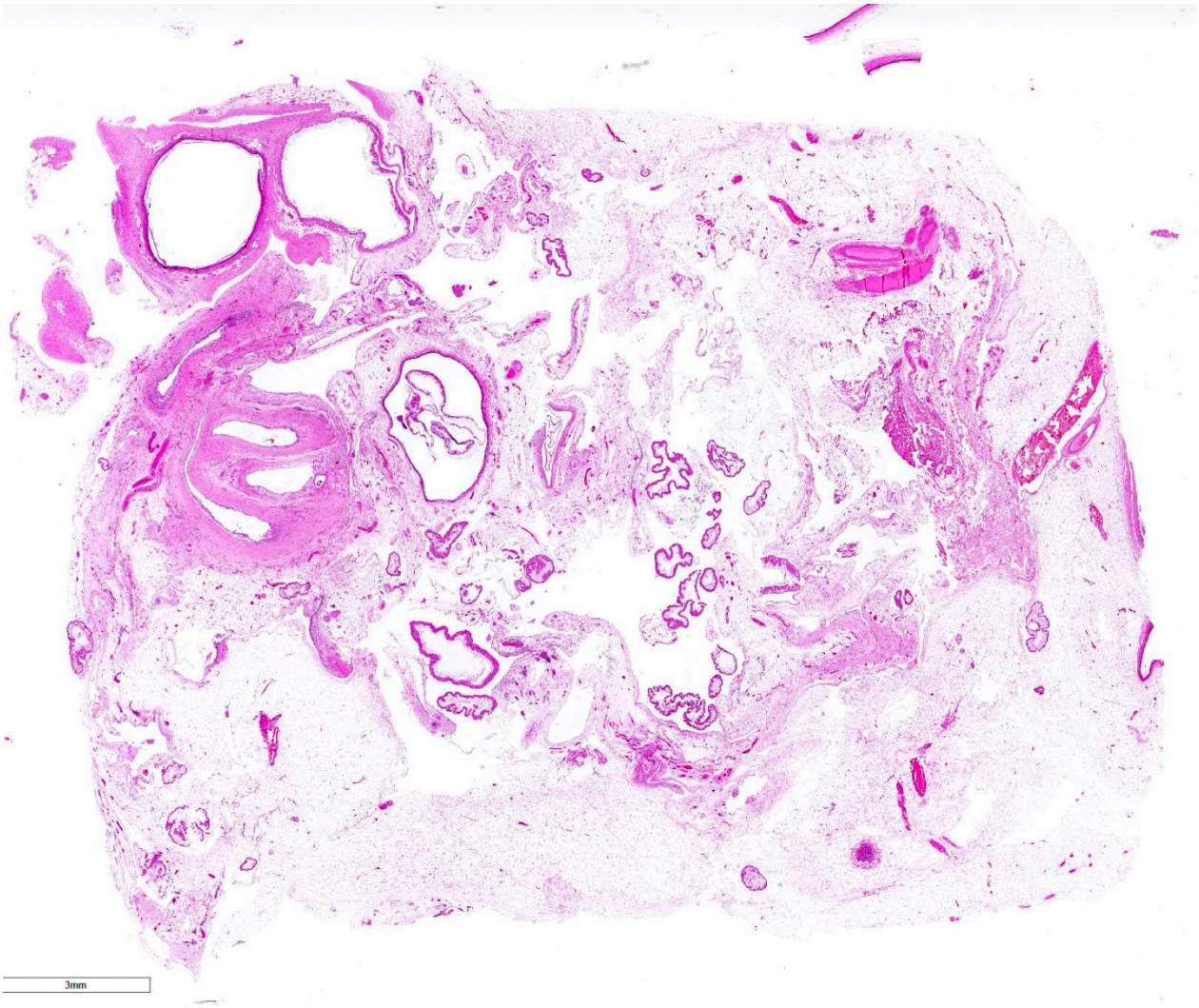


Mesentery, dog. Subgross examination of a section of mesenteric fibroadipose tissue contains cross sections of numerous metacestodes which are both encysted and free in the tissues. (HE, 7X)

Contributor's Morphologic Diagnosis:

Mild chronic peritonitis with cestode larvae - most compatible with canine peritoneal larval cestodiasis

Contributor's Comment: Most parasites found in the peritoneal cavity occur during their normal migration to another site. Only a few larval and adult helminths use the abdominal cavity as their normal habitat. Cysticerci may be found on the peritoneum in rabbits and ruminants during their normal development; they are nonpathogenic and incite no tissue response beyond a thin bland fibrous capsule. Rarely, cysticerci are reported in the abdomen of carnivores, which are abnormal hosts.[10]



Mesentery, dog. Higher magnification of degenerating metacestodes within fibrous cysts (black arrows) and viable asexually replicating metacestodes free in the tissue. (HE 38X)

The etiology in this case is most compatible with infection with the tapeworm *Mesocestoides* spp. The larvae of *Mesocestoides* can proliferate extensively in the abdominal cavity of carnivores and cause a pyogranulomatous and proliferative peritonitis known as parasitic ascites or canine peritoneal larval cestodiasis (CPLC).[1,9,10]The diagnosis in this case was confirmed by PCR. The differential diagnosis is infection with *Spirometra* spp. which may also encyst in the peritoneal cavity of carnivores.

Canine peritoneal larval cestodiasis (CPLC) is an unusual parasitic disease in dogs that is caused by asexual proliferation of larval *Mesocestoides*. *Mesocestoides* spp. are tapeworm parasites. Adult worms reside in the intestinal tracts of their final hosts, which include carnivores, birds and occasionally humans. Infection with adult worms is usually asymptomatic. In contrast, the third stage larvae, called tetrathyridium, live in the serosal cavities of second intermediate hosts, which include amphibians, reptiles, birds, and rodents.

Dogs and cats can also harbor tetrathyridia in their peritoneal cavities.[6,9] Proglottids from adult parasites are not directly infectious to definitive or secondary intermediate host species.[6,9]

Diagnosis of infection by *Mesocestoides* spp. can be confirmed by morphologic identification of tetrathyridia or by PCR.

Recommended treatment involves long term therapy with praziquantel.[8] Praziquantel has been reported effective to eliminate peritoneal tetrathyridia definitely. Early recognition of CPLC improves the prognosis, as it may be an incidental, but severe finding.[8]

Contributing Institution:

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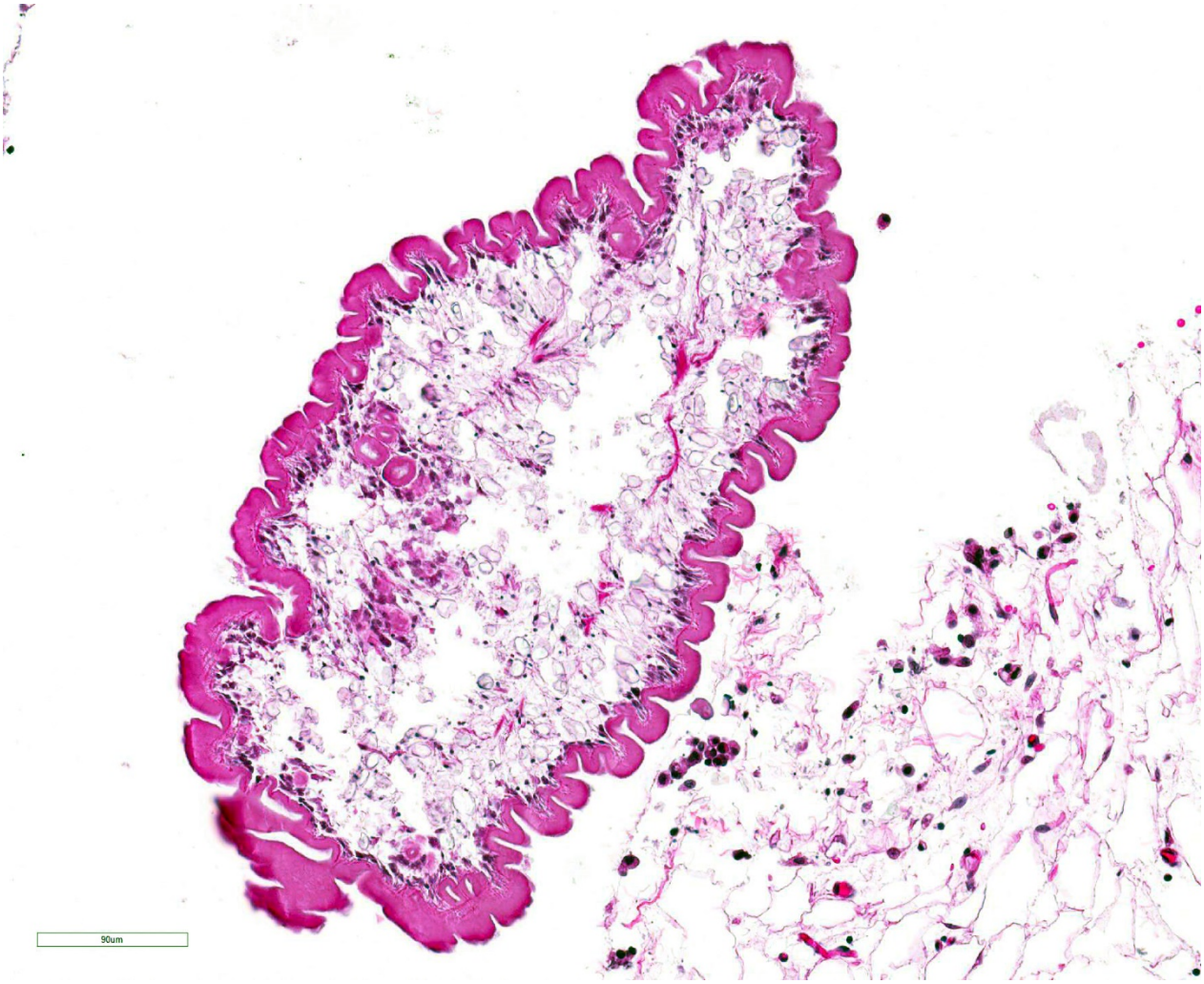
JPC Diagnosis: Fibroadipose tissue: Encysted and free tetrathyridia multiple with mild granulomatous steatitis.



Mesentery, dog. Cross-section of a mesocestode (tetrathyridium) demonstrating a thick eosinophilic tegument, subtegumental somatic cell nuclei, a spongy parenchymatous body and numerous oval clear calcareous corpuscles. (HE 246X)

JPC Comment: The uniqueness of the cyclophyllidean cestodes belonging to the Family Mesocestoidae is matched only by the continued mystery of its life cycle. First identified in 1863, published life cycles remain presumptive 150 years later. Gravid proglottids are shed in the feces of a carnivore definitive host (which is rarely man).[2,7] Eggs are contained within the parauterine organ, a structure unique to *Meoscestoides* and a diagnostic feature when examining proglottids.[7] Eggs lack a shell, and possess an emybryophore enveloping the naked oncosphere.[7] The eggs are ingested by the first intermediate host (presumptively an arthropod) where it develops into a second, as yet undescribed larval (or metacestode) stage. The metacestode possesses all of the somatic structures of the eventual adult cestode with the exception of reproductive organs. [7] The arthropod host is then consumed by a vertebrate secondary host, usually an amphibian or reptile (but rodents and birds may also serve as intermediate hosts), where upon the larva passes through a series of post-larval forms, ultimately becoming a tetrathyridia, the infective stage for the final carnivore host.[2] Terrestrial carnivores including dogs, cats, procyonids, mustelids, and opossums, are most commonly identified as definitive hosts.[2] While in the secondary intermediate host or the definitive host, tetrathyridia possess the ability to divide asexually, sometimes achieving large numbers and infections similar to that illustrated in this case. Only in the definitive carnivore host does the tetrathyridium evaginate, attach to the intestinal mucosa, and mature into an adult cestode.[2]

Human infections with metacestodes are quite rare, and only two genera *(M. lineatum* in Asia, Europe and Africa and *M. variablilis* in North America) have been reported in these cases.[4] Most cases involve the consumption of uncooked viscera or blood containing metacestodes (tetrathyridia).[4]

In the dog, the disease is referred to as canine peritoneal larval cestodiasis, and this type of infection is less commonly seen in cats.[1,3] Asexual proliferation of tetrathyridia results in variable degrees of granulomatous peritonitis with occasional extension into abdominal organs and the thoracic cavity. Clinical signs are generally vague, and include lethargy, weight loss, vomiting, and ascites. Some cases are identified during routine OHE and castration (including WSC 2014, Conference 3 Case 3.)[1,3]

In 2014, a similar asexually proliferative metacestode infection was first identified in a captive juvenile Borneo orangutan which we had been American-born and currently resided in a zoo in Michigan. Cestode parasites of the genus *Versteria* are most commonly seen in small mammals including mustelids and weasels. Atypical infection of this non-human primate manifested as cysts within the liver, lung, and spleen containing numerous metacestodes which were identified as unique members of the genus *Versteria* with 12% difference from the DNA of the closest species (*V. mustelae).*[5]

References:

1. Boyce W et al. Survival analysis of dogs diagnosed with canine peritoneal larval cestodiasis (Mesocestoides spp.). *Vet Parasitol* 2011. 180:256– 261
2. Centers for Diseases Control: DPDX – Identifcation of Parasites of Public Health Concern. https://www.cdc.gov/dpdx/mesocestoidiasis/index.html
3. Crosbie PR, Boyce WM, Platzer EG. Diagnostic procedures and treatment of eleven dogs with peritoneal infections caused by Mesocestoides spp. *J Am Vet Med Assoc* 1998; 213:1578-1583.
4. Fuenza MV, Galan-puchdes MT, Maline JB. A new case report of human *Mesocestoides* infection in the United States. *Am J Trop Med Hyg* 2003; 68(5)566-567.
5. Goldberg TL, Gendron-Fitzpatrick A, Deering KM, Wallace RS, Clyde VL, Lauck M, Rosen GE, Bennett AJ, Greiner EC, O'Connor DH. Fatal metacestode infction in Bornean orangutan caused by unknown Versteria species. *Emerg Inf Dis* 2014; 20(1):109-113.
6. Kashiide T et al. Case report: First confirmed case of canine peritoneal larval cestodiasis caused by Mesocestoides vogae (syn. M. corti) in Japan. *Vet Parasitol* 2014. 20:154–157.
7. McAlister CT, Tkach VV, Conn DB. Morphological ad molecular characterization of post-larval pretetrathrydia of Mesocestoides sp. (Cestoda Cyclophyllida) from Ground Skink from Southeastern Oklahoma. *J Parasitol* 2018; 104(1):246-253.
8. Papini R et al. Effectiveness of praziquantel for treatment of peritoneal larval cestodiasis in dogs: A case report. *Vet Parasitol* 2010. 170:158–16,
9. Uzal FA et al. Alimentary system. In: Jubb, Kennedy and Palmer's *Pathology of Domestic Animals*. Ed: M Grant Maxie. 6th ed. vol. 2, Elsevier, 2016:223.
10. Uzal FA et al. Alimentary system. In: Jubb, Kennedy and Palmer's *Pathology of Domestic Animals*. Ed: M Grant Maxie. 6th ed. vol. 2, Elsevier, 2016:255.
11. Wirtherle N et al. First case of canine peritoneal larval cestodosis causedby Mesocestoides lineatus in Germany. *Parasitology International*. 2007. 56:317–320

CASE III: 61290 (JPC 4117380).

Signalment: 7.5 year old, female Malayan Snail-eating turtle, *Malayemys subtrijuga*

History: This captive-hatched animal was found dead with no history of clinical signs.

Gross Pathology: An approximately 2.0 cm long by 0.3 cm diameter off-white to yellow,

Lung, turtle. Numerous adult pentastomes are present within the lungs and one is contained within on of the mainstem bronchi. (Photo courtesy of: Disease Investigations, Institute for Conservation Research, San Diego Zoo Global, PO Box 120551, San Diego, CA 92112, http://institute.sandiegozoo.org/disease-investigations)

Pentastome. Adult pentastomes are pseudosegmented and had a flattened anterior end with four hooklets. (Photo courtesy of: Disease Investigations, Institute for Conservation Research, San Diego Zoo Global, PO Box 120551, San Diego, CA 92112, http://institute.sandiegozoo.org/disease-investigations)

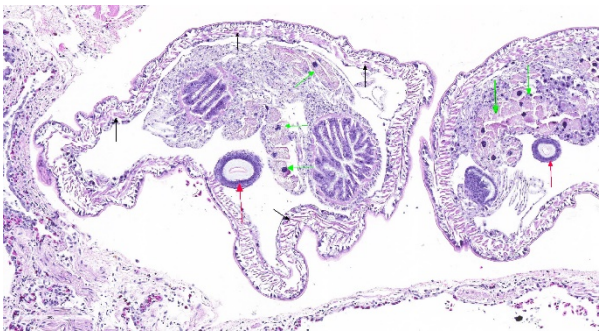
round parasite (identified as a pentastome) was within the caudal oral cavity near the base of the tongue. Approximately 5-10 similar parasites were throughout the parenchyma of each lung, and a single parasite was folded within the lumen of the right bronchus. Under a dissecting scope, the parasites had a slightly expanded and flattened anterior end with four hooks on the flattened surface.There was very subtle banding or pseudosegmentation of the parasite body, and most of the body cavity was filled with a thick, highly convoluted white to off-white tubular structure (presumed uterus). The turtle's lungs were diffusely pink-red and wet, yet floated when placed in 10% neutral buffered formalin.

Two smaller grey-tan, coiled, round pentastome nymphs measuring approximately 1.0 cm long by 0.1 cm diameter were in the adventitia of the mid esophagus, adjacent to the medial aspect of the left lung. No adipose stores were present, and there was some watery coelomic fluid.

Laboratory results: Parasite Identification: Whole parasites were submitted to the Zoological Medicine and Wildlife Disease Laboratory at the University of Florida for speciation using a pentastome PCR assay, which resulted in a sequence that identified the parasites as *Sebekia mississippiensis*.

Microscopic Description: Lung (sections from 3 blocks): There is some variability between slides. The interstitium of faveolar septa is diffusely expanded by edema and variable numbers of inflammatory cells with occasional foci of hemorrhage. The inflammatory infiltrate consists of predominately granulocytes, lymphocytes and plasma cells with fewer histiocytes and

Pentastome. Much of the body of the female contains a convoluted egg-filled uterus. (Photo courtesy of: Disease Investigations, Institute for Conservation Research, San Diego Zoo Global, PO Box 120551, San Diego, CA 92112, http://institute.sandiegozoo.org/disease-investigations)



Lung, turtle. Cross sections of the anterior end of the pentastomes demonstrates a thin cuticle, coelom, skeletal muscle (black arrows), chitinous hooklets (red arrows) and cross sections of an intestinal tract bordered by large eosinophilic glandular cells (green arrows). (HE ,140X)

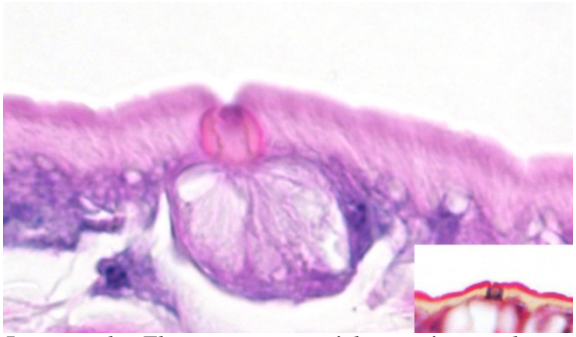
melanomacrophages. A few 1-2 mm diameter metazoan parasites expand and compress faveoli, fill a bronchial lumen, or are embedded in pleural connective tissue, depending on the section. These parasites have features consistent with pentastomes, including a thin, undulating to pseudosegmented eosinophilic cuticle which contains occasional openings lined by refractile, eosinophilic material (sclerotized pores). Underlying the cuticle, striated skeletal muscle fascicles with subcuticular glands make up the body wall, which encloses a body cavity containing a multicellular digestive tract lined by eosinophilic glands and a reproductive tract. In sections of mature parasites, the uterus is filled with developing eggs. Chitinous hooks are present in some sections. Also present in faveolar lumina are variable amounts of sloughed respiratory epithelium mixed with granulocytes, macrophages, red blood cells, debris and bacteria. The faveolar epithelium is frequently absent, hypertrophied or hyperplastic. There are 1-2 discrete heterophilic granulomas within the interstitium in some sections. These occasionally contain folded, hyalinized, eosinophilic membranous material (presumed pentastome cuticle).

Contributor's Morphologic Diagnosis:

Lungs: Moderate, diffuse, granulocytic and lymphoplasmacytic interstitial pneumonia with epithelial erosion and hyperplasia and adult and nymph pentastomes

Contributor's Comment: Pentastomiasis is a disease caused by a group of blood-sucking endoparasites with a world-wide distribution.[5] Variably considered a separate phylum or a subclass of phylum Arthropoda, Pentastomida is an interesting group of obligate parasites most closely related to branchiurans, or fish lice, a type of crustacean arthropod.[5,7] The distribution of definitive hosts and fossil records show pentastomids first appeared when reptiles flourished during the Mesozoic era.[7,9] Approximately 90% of pentastomes use reptiles as definitive hosts, and the life cycle is indirect.[7,9]

Adult pentastomes typically reside in the lower respiratory tract and produce larvated

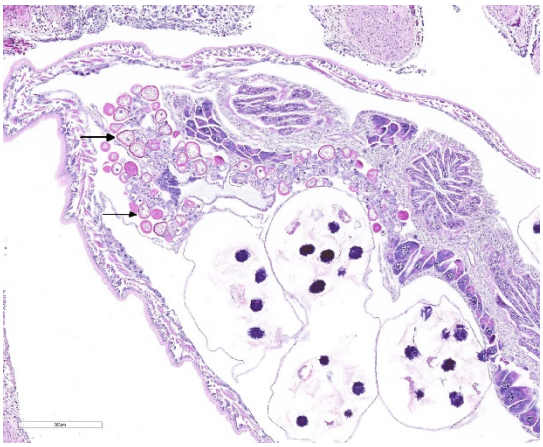


Lung, turtle. The pentastome cuticle contains regular spaced sclerotzedc openings, which stain black with a Movat's pentachrome (inset) (HE and Movat's pentachrome, 400X) (Photo courtesy of: Disease Investigations, Institute for Conservation Research, San Diego Zoo Global, PO Box 120551, San Diego, CA 92112, http://institute.sandiegozoo.org/disease-investigations)

ova that are swallowed and expelled from the definitive host in feces.[5] Intermediate hosts of various pentastomids are mostly fish and mammals, which can include humans, with additional animals able to serve as paratenic hosts.[7] Some definitive hosts can harbor nymph and adult stages.[7,9] The intermediate host ingests ova from contaminated water, vegetation, or fecal matter. Once ingested, the first stage nymph emerges from the egg, penetrates the gastrointestinal tract, and migrates through viscera. Here the nymph encysts and molts multiple times to become an infective nymph. Once the intermediate host is ingested by the definitive host, nymphs excyst and migrate to the lungs where they mature, completing the lifecycle.[5,7]

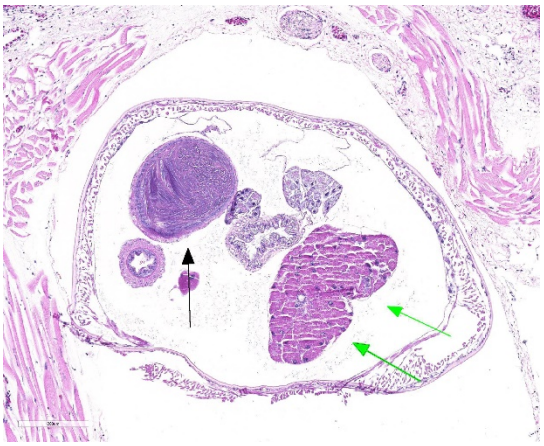
Infections in reptile hosts vary in severity from incidental to fatal. Adult pentastomes in their natural host often incite only mild inflammation; however, clinical signs in captive reptiles can include lethargy, anorexia, dyspnea, pneumonia, and sudden death.[1,4,5] The eggs can be detected in feces or lung wash samples.[2,4] Clinical signs are due to tissue destruction or inflammation associated with parasite migration, from parasite molting which can elicit a hypersensitivity reaction, from respiratory tract obstruction, or from secondary bacterial infections.[4,7,9] The turtle in this report had significant inflammation throughout the lungs, including a few heterophilic granulomas that appeared to be associated with molted cuticle, as well as obstruction of a primary bronchus by an adult worm.

The most defining characteristic of pentastomids in histologic tissue sections is the presence of sclerotic pores that open into the cuticle within the body wall. These pores produce cuticle during molts.[2] Better visualization of the pores can be achieved by using a Movat pentachrome stain that stains the typically eosinophilic sclerotic pore black. Additional identifying features include two paired hooks surrounding the mouth, striated musculature, acidophilic glands encircling the intestinal tract, and pseudosegmentation.[2]



Lung, turtle. Cross-section of a female pentastome demonstrates a uterus with developing ova and multiple cross-sections of a uterus with fully developed eggs (HE, 100X)

Malayan snail-eating turtles are carnivorous turtles with diverse diets including items such as fish, earthworms, aquatic insects, and, of course, snails. Its diet in captivity consisted of earthworms, processed turtle brittle and gel, and occasional snails. Fish were not reported to be component of its diet, although mosquito fish were sometimes present in the water system in which it was housed. The pentastomid species recovered from this turtle was *Sebekia mississippiensis*, for which fish (including mosquito fish) are intermediate hosts.[1,6]



Lung, turtle. Cross-section of a male pentastome demonstrates sperm- filled testis (black arrow) and eosinophilic glandular cells (green arrows). (HE, 115X)

Sebekia sp. are pentastomids of crocodilians, and *Sebekia mississippiensis* is specifically of the American alligator.[1, 6] High mortality rates among hatchling crocodilians infected with *Sebekia* sp. has been previously reported, along with a single report of a dermal infection in a woman.[1,6,7] Pentastomes are less frequently reported in turtles than other reptiles, but infections with *Diesingia* (family Sebekidae) have been seen.[4,7,9] Both adults and nymphs were present in this turtle, which could indicate autoinfection or a continued source of reinfection. It could also act as both a definitive and intermediate host. A second snail-eating turtle in the collection was subsequently screened for pentastome infection and was negative. Screening of fish and proper freezing protocols prior to feeding can help reduce incidence of this parasite in susceptible captive animals.[1,7]

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JPC Diagnosis: Lung: Pneumonia, bronchointerstitial, granulocytic, mild to moderate, chronic, with intra- and extrapulmonary pentastomes.
2. Lung: Granuloma, heterophilic, focal.

JPC Comment: The contributor has presented an excellent review of pentastome infections in reptiles. To expand on their histology, pentastome have two pairs of hooks surrounding the mouth which led early researchers to believe that they had five heads (leading to the name "pentastomes").[2] The presence and staining of sclerotized openings (unique to pentastomes) with Movat's pentachrome is important as these structures are present in all life stages, and is maintained even in degenerate or calcified specimens.[2]

Pentastomes utilize a wide range of intermediate hosts, and occasionally end up in human intermediate hosts. Most human infections involve the species *Armillifer armillatus* (whose definitive host is the python) or *A. grandis.* Intermediate hosts are infected by eating water and vegetation contaminated with eggs passed in the feces of respiratory secretions of snakes. Human infections are most common in regions of African where snake meat is eaten, although some infections are likely acquired by ingesting water or vegetation contaminated with the feces or respiratory

secretions of snakes. Autopsy studies have demonstrated a 22% infection rate in certain African countries, and a 45% incidence in autopsy is some parts of Malaysia.[8]

Poorly cooked snake meat may be purchased at markets and eaten by inhabitants of certain parts of Africa, and adult pentastomes (referred to locally as "snake springs") are spit out.[10] Chewing the pentastomes may result in liberation of eggs. After ingestion, 4-legged primary larvae migrate through the viscera, and following several molts, transformed in the legless nymphs which are characteristically found on serosal membranes and rarely within viscera.[10] While most infections in these regions are the result of *A. armillifer*, a significant number of cases of pentastomiasis may be seen with *A. grandis*.[8] While cases of *A. armillifer* result in more traditional peritoneal encysted nymphs, *A. grandis* has a predilection for ocular infections, with blindness as a common result.

References:

1. Adams L, Isaza R, Greiner E. Fatal Pentasomiasis in Captive African Dwarf crocodile Hatchlings (Osteolaemus tetraspis). Journal of Zoo and Wildlife Medicine. 2001;32(4): 500-502.
2. Gardiner, CH, Poynton, SL. *An Atlas of Metazoan Parasites in Animal Tissues*. Washington DC: Armed Forces Institute of Pathology; 1999 59-60.
3. Ioannou P, Vamvoukaki R. Armillifer infections in humans: a systematic review. *Trop Me and Inf Disease* 2019; 16;4(2). pii: E80. doi: 10.3390/tropicalmed4020080.
4. Jacobson ER. *Infectious Disease and Pathology of Reptiles.* Boca Raton, FL: CRC Press, Taylor & Francis Group; 2007 590-592.
5. Meyers WM, Neafie RC. Pentastomiasis. In Meyers, WM, ed: Topics on the Pathology of Protozoan and Invasive Arthropod Disease. Ebook; 2011: 1-10.
6. Moreland, AF, Forrester, DJ,Delany MF. Sebekia mississippiensis (Pentastomida) from Juvenile American Alligators in North Central Florida. The Helminthological Society of Washington. 1989;56(1): 42-43.
7. Paré JA. An Overview of Pantastomiasis in Reptiles and Other Vertebrates. Journal of Exotic Pet Medicine. 2008;17(4): 285-294.
8. Potters I, Desaive C, Van Den Broucke S, Esbroeck MJ, Lynen L. Unexpected infection with *Armillifer* parasites. *Emerg Inf Dis* 2017; 23(12): 2116-2118.
9. Riley J. The Biology of Pentastomids. Advances in Parasitology. 1986;25: 45-128.
10. Tappe D, Sulyok M, Riu T, Rozsa L, Bodo I, Schoen C, Muntau B, Babocsay G, Hardi R. Co-infections in visceral pentastomiasis, Democratic Republic of the Congo. *Emerg Inf Dis* 2016; 22(8):1333-1339.

CASE IV: S1601842 (JPC 4101199).

Signalment: Two week old, male Arabian foal (*Equus caballus*)

History: The foal was found in right lateral recumbency. It had appeared in good health

the day before, although it was observed to fall once while nursing when the mare moved; however it immediately stood back up and resumed nursing. On physical examination the foal was afebrile, and had a heart rate of 100, and respiratory rate of 28. The foal was unable to rise, remain standing when assisted to rise, or maintain sternal recumbency. Front limb movement appeared hypermetric, the foal intermittently flailed with a rigid head and neck, and exhibited intermittent horizontal nystagmus. No external evidence of trauma was detected. The umbilical stump and limb joints appeared within normal limits, the abdomen appeared taut, and the sclerae were slightly yellow-tinged. Abdominal ultrasound revealed an intact urinary bladder, and possible thickening of the small intestinal wall.

The foal was euthanized the same day due to a poor prognosis.

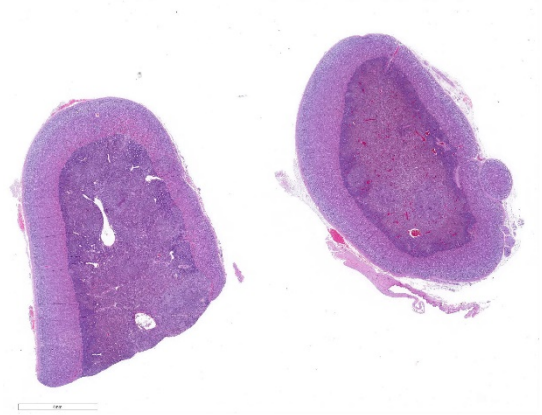
Gross Pathology: The carcass was in good postmortem condition and well-fleshed. Conjunctival mucosa, sclera and gingival mucosa were slightly yellow tinged, and there was multifocal, moderate subcutaneous hemorrhage of the subcutis of right upper eyelid, and caudodorsal cranium (overlying interparietal and occipital bones). Cranial meninges were congested with two small, focal hemorrhages on the dorsolateral aspect of the right cerebral hemisphere.

The trachea contained scant stable foam, and the lungs were spongy and pink with mild, multifocal red mottling. There was a small dark red nodule of approximately 0.3cm in diameter on the cardiac right atrioventricular valve. The stomach contained a small amount of fragmented roughage (hay) and no milk curds. The cecum and large colon contained plentiful sand and the large intestinal mucosa was diffusely red. The small colon contained loose, malodorous feces.

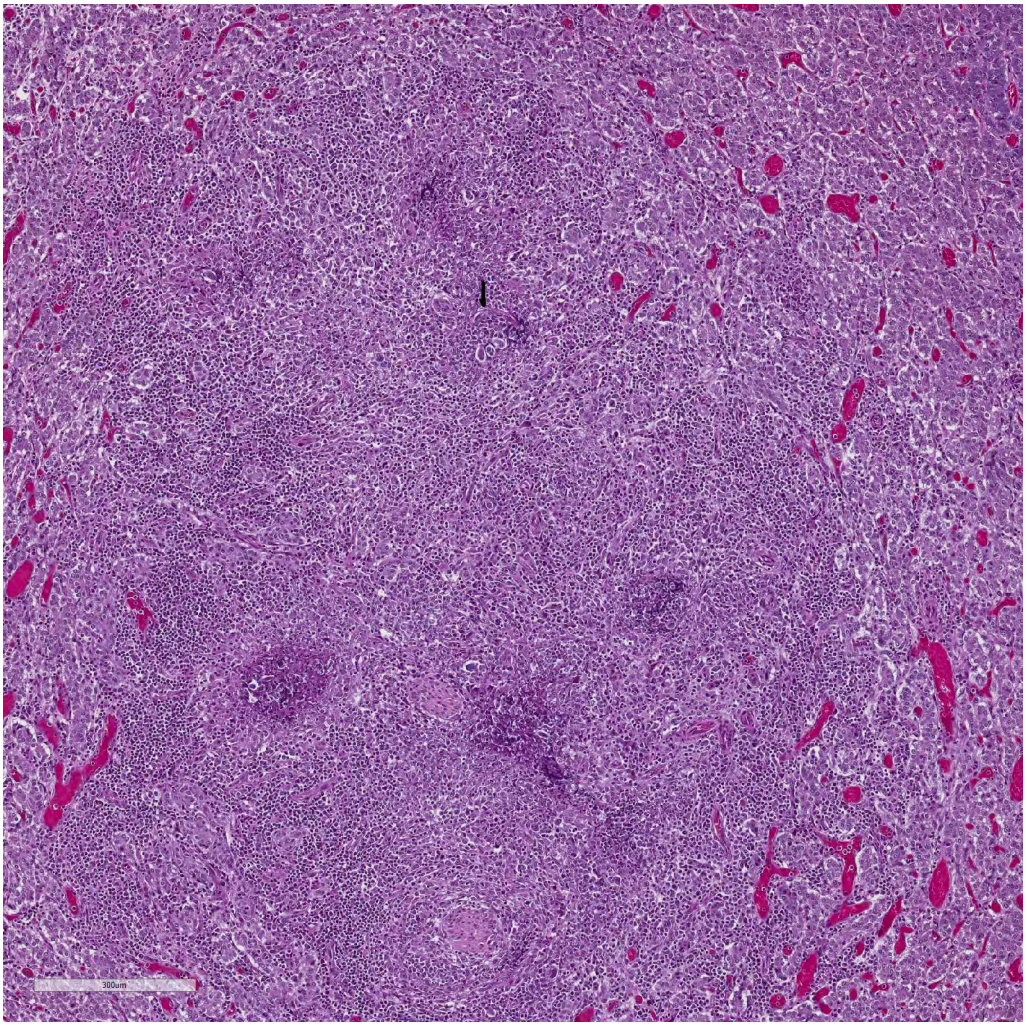
Laboratory results: Rare mixed bacterial flora was isolated from liver on aerobic culture, and large numbers of *Escherichia coli* and mixed bacterial flora were isolated from small intestine and large colon. Salmonella PCR was negative on liver, small intestine and colon, and ELISA testing for both *Clostridium perfringens* toxins and *Clostridium difficile* toxins, was negative on small intestinal and cecal contents.

No parasite eggs were detected in feces. Heavy metal analysis of the liver detected a marginally deficient selenium concentration, while other tested minerals were within acceptable ranges for the species.

No aerobic bacterial pathogens were isolated from a submitted sample of dam's milk, and



Adrenal gland, foal. Approximately 50% of the adrenal medulla is replaced by coalescing areas of necrosis and inflammation which extend into the adrenal cortex. (HE, 7X)



Adrenal gland, foal. Approximately 50% of the adrenal medulla is replaced by coalescing areas of necrosis and inflammation which extend into the adrenal cortex. (HE, 7X)

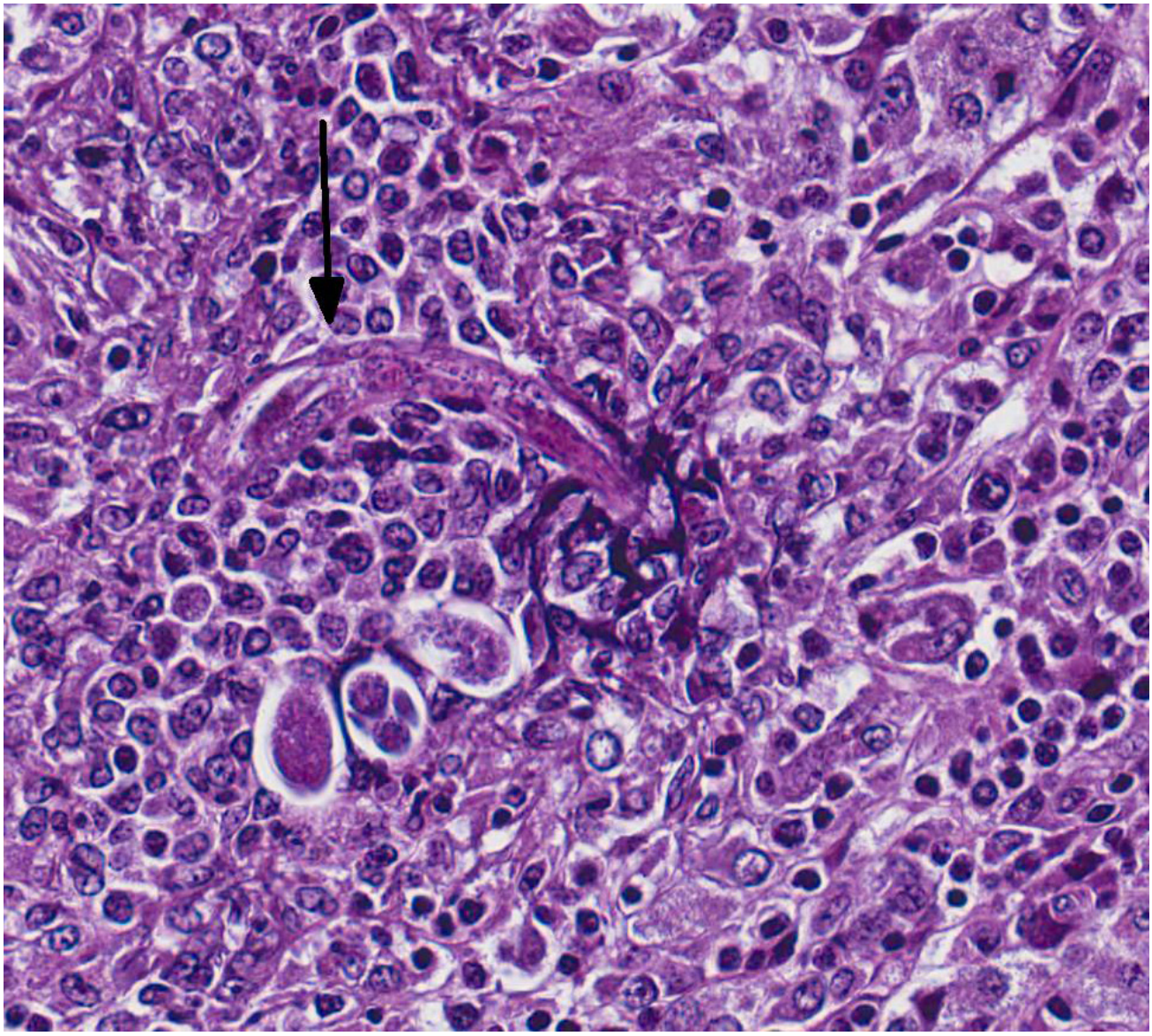
Clostridium botulinum toxin testing was negative on a submitted feed sample (mare and foal pellet).

Microscopic Description: Adrenal gland: The medulla is extensively disrupted by multifocal to coalescent, variably sized foci of granulomatous inflammation characterized by densely cellular aggregates of Langhans-type multinucleated giant cells and epithelioid cells, and large numbers of admixed and/or peripheral lymphocytes and plasma cells. Some of the granulomas are centrally necrotic and there are scattered tangentially and cross-sectioned larval and adult rhabditid nematodes with a diameter of 10-25um, and fewer thin-walled, multicellular to larvated, ovoid eggs. In a small number of tangentially sectioned nematodes it is possible to identify anatomical features such as a rhabditiform esophagus with isthmus and terminal bulb, dark basophilic granular structures of 2-3 um within the pseudocoelom, and a smooth cuticle.

Contributor's Morphologic Diagnosis:

Adrenalitis, granulomatous, lymphoplasmacytic, multifocal to coalescent, moderate to severe, with multifocal necrosis and rhabditiod nematodes, etiology presumptive *Halicephalobus gingivalis*.

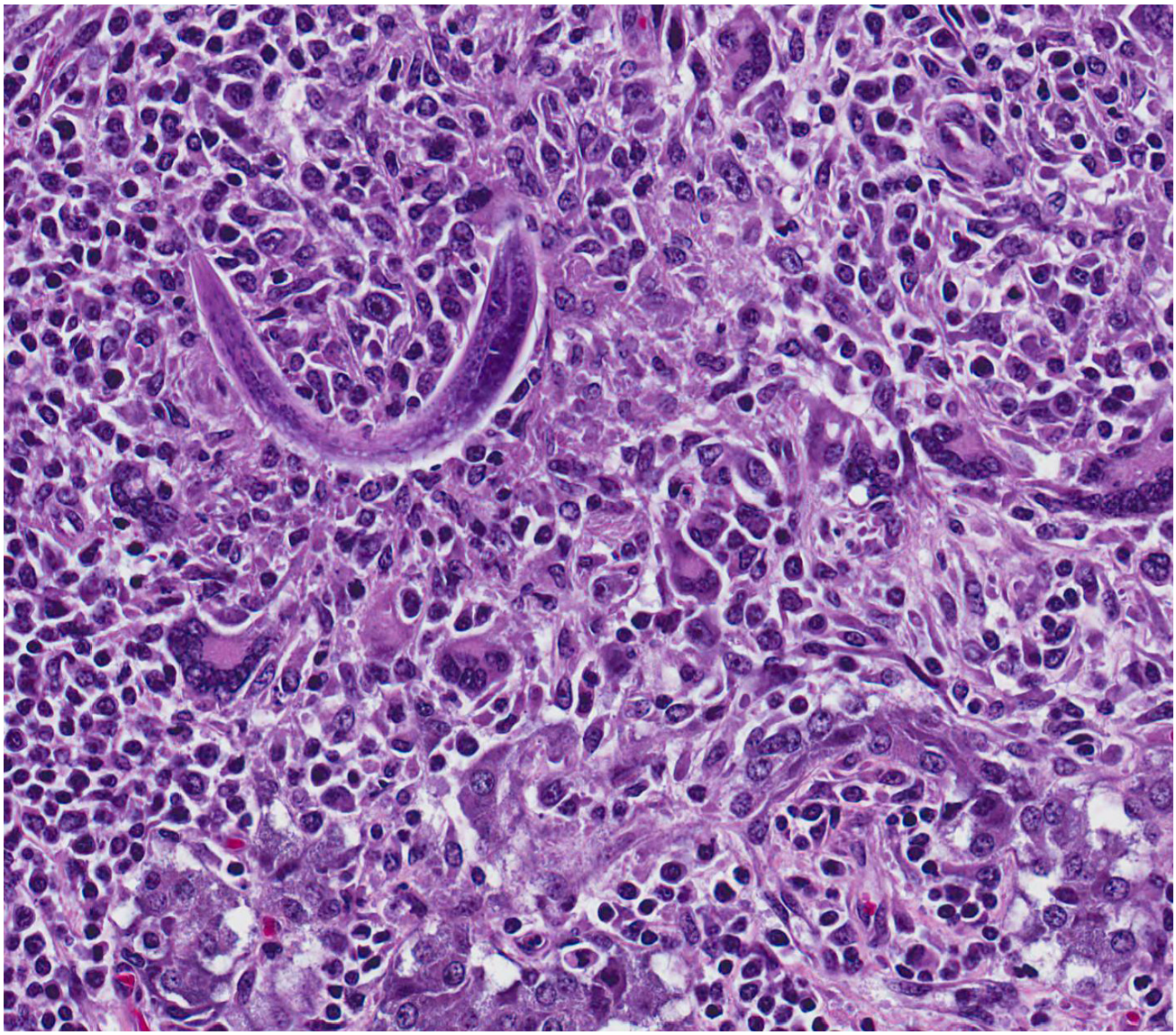
Contributor's Comment: In addition to the adrenalitis, histologic examination of the brain sections from this foal identified the presence of granulomatous to



Adrenal gland, foal. Approximately 50% of the adrenal medulla is replaced by coalescing areas of necrosis and inflammation which extend into the adrenal cortex. (HE, 7X)

lymphohistiocytic meningoencephalitis, with multifocal malacia and rhaditid nematodes that were morphologically consistent with *Halicephalobus gingivalis.*

Halicephalobus gingivalis is a free-living nematode of soil, decaying organic matter and horse manure, and a facultative parasite primarily of horses and rarely of humans.[5,7,9] although there is one published report of an outbreak of halicephalobiasis producing meningoencephalitis in a group of dairy calves in Denmark.[3] Little is known about the life cycle and method of transmission; however it has been speculated that the portal of entry is mostly penetration of mucous membranes (particularly oropharyngeal following ingestion) and skin wounds, with subsequent, predominantly hematogenous spread to other organs.[3,5,6,7,9]



Adrenal gland, foal. Some areas of inflammation surrounding nematodes contain numerous foreign body type giant cells. The posterior end of the female rhabditoid nematode contains a deeply basophilic genital tract. (HE, 400X)

In one documented case of a fatal *H.gingivalis* infection in a 5 year old boy, he developed meningoencephalitis 8 days after falling into a horse manure spreader, acquiring manure-contaminated facial lacerations.[7,9] Only female nematodes, larvae and eggs have been detected in tissues, and as such nematode reproduction in the host is thought to be asexual with parthenogenetic females. [3,5,6,7,9] Sexual reproduction is believed to occur during the free-living part of the life cycle, and both males and females have been found in the environment.[3]

In horses, the organs/tissues most commonly affected are the meninges/brain, with or without involvement of the spinal cord and central/spinal nerves, kidney, mandibular bone and maxillary bone/sinuses, gingiva, eye, and prepuce, with less frequent reports

of involvement of adrenal gland, lung, mammary gland and testicle.[5,7,9]

Halicephalobus gingivalis infection of young foals is very rare.[3,9] Strong evidence of a transmammary route of infection was documented in one case[8] in which a 3-week-old foal presented with neurologic signs and was diagnosed postmortem with leptomeningoencephalitis caused by rhabditiod nematodes morphologically compatible with *H.gingivalis. A* biopsy of the dam's mammary gland taken the previous year was diagnosed at the time as a nematode infection, and retrospectively as mastitis caused by infection with *H.gingivalis.*

Diagnosis of halicephalobiasis is mostly performed postmortem by histologic identification of consistent lesions and morphologically compatible rhabditoid nematodes; however the nematodes can also be extracted from macerated and sieved fresh tissue, washed, centrifuged and examined by light microscopy.[3] If there is renal involvement, the nematode may also be shed in the urine, and can be collected by centrifugation of a urine sample. These methods enhance the ability to identify the characteristic features of adult female *H.gingivalis* including the rhabditiform esophagus with a corpus, isthmus and 2 esophageal bulbs, and the didelphic reproductive tract with reflexed ovary at the posterior end.[7] Identification of the nematode can be confirmed by sequencing of extracted ribosomal DNA.[3,6,7]

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JPC Diagnosis: Adrenal gland: Adrenalitis, granulomatous, multifocal to coalescing, severe, with adult and larval rhabditoid nematodes.

JPC Comment: The contributor has provided an excellent and comprehensive review of disease associated with *Halicephalobus* gingivalis. The genus *Halicephalobu*s comprises eight species of small nematodes belonging to the Family Rhabditoida, which also includes *Rhabdias, Strongyloides,* and *Pelodera*.

The morphology of *Halicephalobus* is unique and characteristic. Rhabditoids are one of three families which have platymyarian-coeloyarian musculature, with the others being the strongyles and oxyurids.[4] All stages of the parasite possess the characteristic "rhabditiform esophagus" – a unique structure with a corpus, isthmus and bulb. *Halicephalobus* adult females possess a single genital tract as opposed to the paired tracts of *Strongyloides.* For this reason, only one egg is seen per cross-section of an adult female.[4] A fortuitous section can also demonstrate the dorsoflexed ovary mentioned by the contributor, which is also unique to the genus.[4]

Only females of *Strongyloides* and *Halicephalobus* are parasitic. *Halicephalobus* females (which are the only gender recovered from lesions) are not hermaphroditic and likely not parthenogenetic,– males live in decaying plant material, soil, and fresh and salt water, as do both genders of the other seven species.[2,5] Vectors have not been identified for this particular parasite; infections are presumed to be the result of ingestion, inhalation, or direct contact with infected plant matter or water with wounds or mucous membranes. Parasitic females migrate along vessels to numerous organs – in domestic equids, the brain, lymph nodes and kidneys are commonly infected.[2]

Less than ten cases of human halicephalobiasis have been reported, making this a very uncommon disease in humans. Halicephalobiasis in humans manifests as encephalitis and myelitis, and cases have been invariably fatal, due to not only the non-specific signs of disease but also the unfamiliarity of human physicians with this parasite.[8]

In humans, neurological disease associated with helminth infection may occur with a number of nematodes, including *Toxocara canis*, *Angiostrongylus cantonensis, Baylisascaris procyonis, Strongyloides stercoralis,* and nematodes lesser known to veterinarians, such as *Gnathostoma spinigerum* and *Lagochilascaris minor.*[8] Humans are atypical hosts for *Gnathostoma* sp., which utilizes a copepod of the genus *Cyclops* as a primary intermediate hosts and a wide range of vertebrates as second intermediate hosts. Humans may contract gnathostomiasis from eating undercooked seafood containing the larval nematodes (Asian swamp eels are a common source in areas of SE Asia. .As humans are aberrant hosts for this parasite, larva will migrate through a number of tissues, never completing their life cycle. *Lagochiascaris minor* is an ascarids which uses the mouse as an intermediate host and a cat for a definitive host. Accidental ingestion of undercooked rodent meat may result in visceral larval migrans in the human.[1]

References:

1. Campos DMB, Barbosa AP, De Oliveira JA, Tavares GG, Cravo PVL, Ostermayer AL. Human lagochilascariasis – a rare helmintic diseas. *PLoS Neglect Trop Dis*; s11(6): e0005510.
2. Cantile C, Youssef S. Nervous system. *In:* Maxie MG, ed. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. Vol 1. 6th ed. Philadelphia, PA: Elsevier; 2016:390.
3. Enemark HL et al. An outbreak of bovine menigoencephalomyelitis with identification of *Halicephalobus gingivalis*. Vet Parasitol 2016; 218: 82-86.
4. Gardiner CH, Poynton SL. Morphologic characteristics of rhabditoids in tissue section. *In*: An Atlas of Metazoan Parasites in Animal Tissues. Washington DC, AFIP Press, pp. 14-16.
5. Henneke C et al. The distribution pattern of *Halicephalobus gingivalis* in a horse is suggestive of a

haematogenous spread of the nematode. *Acta Vet Scand* 2014; 56: 1-4.
6. Jung JY et al. Meningoencephalitis caused by *Halicephalobus gingivalis* in a Thoroughbred gelding. *J Vet Med Sci* 2014; 76(2): 281-284.
7. Lim CK et al. First human case of fatal *Halicephalobus gingivalis* meningoencephalitis in Australia. *J Clin Microb* 2015; 53(5): 1768-1774.
8. Papadi B, Boudreaux C. Tucker JA, Mathison B, Bishop H, Eberhard ME. Case report Halicepahlobus gingivalis: a rare cause of fata meningoencehalomyelitis in humans. *Am J Trop Med Hyg* 2013; 88(6):1062-1064.
9. Wilkins PA et al. Evidence of *Halicephalobus delatrix* (*H. gingivalis*) from dam to foal. *J Vet Intern Med* 2001; 15: 412-417.