Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #8 — 11 October 2023

CASE I:

Signalment:
22-year-old, gelding Appaloosa horse (*Equus caballus*)

History:
This horse had a history of chronic left front foot problems that started after a foot abscess caused by a penetrating foreign body. It had chronic recurrent abscesses with 3 hoof wall resection attempts. A CT scan showed evidence of osteomyelitis and radiographs showed laminitis and a distal displacement abscess in the left front foot. The patient also had a history of recurrent squamous cell carcinoma in the penile sheath and a new lesion in the left third eyelid. The patient also had unregulated pituitary pars intermedia dysfunction. The patient started dribbling urine and the clinician was concerned about renal, lower urinary tract, or neurologic disease. Humane euthanasia was elected.

Gross Pathology:
At necropsy, mucosal membranes were congested. The cranial margin of the left third eyelid contained a 3 mm focal raised, pink nodule. The skin at the base of the penis had multifocal to coalescing 4 to 13 cm raised plaques covered by yellow, friable, crusty material. Urine was oozing freely from the urethra. Multiple 1 to 4 cm diameter black

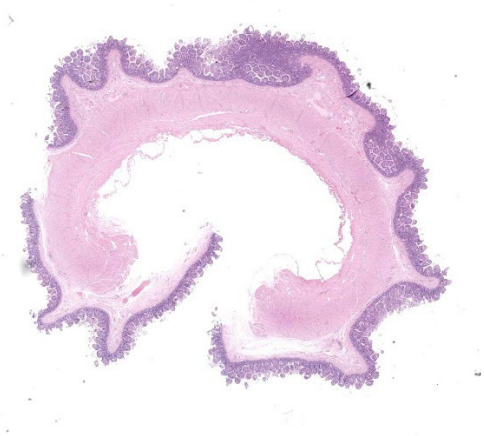

Figure 1-1. Small intestine, horse. One section of small intestine is submitted for examination. There is diffuse villar blunting. (HE, 5X)

nodules were disseminated within the subcutis of the neck and adjacent to the esophagus. One 1 cm diameter round ulcer was present at the base of the tongue. Petechiae and ecchymoses were observed on the pericardium. The spleen was enlarged, meaty, and oozed blood. There was a round 3 cm white, soft mass attached to the mesentery, interpreted as lipoma. The liver and kidneys were diffusely congested. The urinary bladder was filled with moderate amounts of opaque yellow urine and the urinary bladder wall was moderately thick and diffusely red. The rest of the urinary tract was unremarkable with no obstructions or uroliths present. Each hoof had variable degrees of fragmentation of the external corneal layer with cracks, with the left

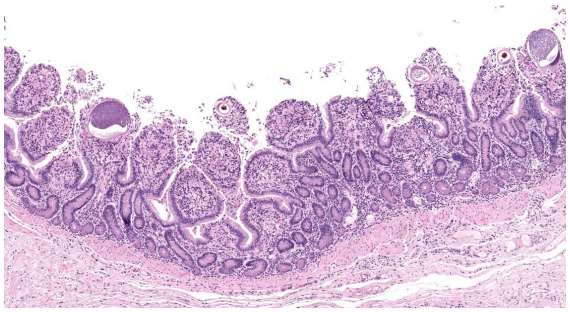

Figure 1-2. Small intestine, horse. There is diffuse villar blunting and scattered intraepithelial macro- and microgametes within the villi. (HE, 66X)

front hoof being the most severely affected. On section, the left frontal third phalange had severe deviation of the cranial margin with signs of laminitis and the hoof wall was markedly thickened and contained an air pocket. The pituitary gland was slightly bulged. Representative sections of observed lesions were collected for histopathology.

Microscopic Description:
Small intestine: Most villi are atrophic and blunted. The lamina propria is mildly to moderately expanded by lymphocytes, plasma cells, and eosinophils. A few eosinophils are observed within the submucosa. Within the lamina propria multiple host cells are markedly hypertrophied up to 30-250 µm in diameter with fibrillar cytoplasm and an enlarged peripheral nucleus that forms a crescent along one side of a thick eosinophilic parasitophorous vacuole. The parasitophorous vacuole contains various stages of development of *Eimeria leuckarti*, including microgamonts, macrogamonts, and occasional developing oocysts. Mature microgamonts contain myriad 1-2 µm basophilic microgametes. Intimal bodies are observed in small arteries and arterioles of the submucosa.

Other additional microscopy findings in this horse included pars intermedia pituitary adenoma, squamous cell carcinoma (prepuce and third eyelid), melanomas (neck), osteomyelitis (left front foot), chronic lymphoplasmacytic cystitis, multifocal mineralization of the brain (incidental finding), and eosinophilic preputial dermatitis of unknown etiology (habronemiasis suspected).

Contributor's Morphologic Diagnosis:
Small intestine: Enteritis, eosinophilic and lymphoplasmacytic, mild, subacute with intralesional coccidia (consistent with *Eimeria leuckarti*).

Contributor's Comment:
This horse was in declining health with multiple clinical problems that culminated in humane euthanasia. The intestinal coccidiosis was considered an incidental finding in this horse.

More than a thousand species of *Eimeria* are known to infect domestic and wild animals and birds.[5] *Eimeria leuckarti* is the only species of *Eimeria* consistently reported in horses and it has been consistently found worldwide.[3,10] Infection with *E. leuckarti* is more common in foals but it also affects adult horses.[3,10] Most infections are considered to be of no clinical relevance.[3,10] Clinical enteritis has been reported in very few cases.[3]

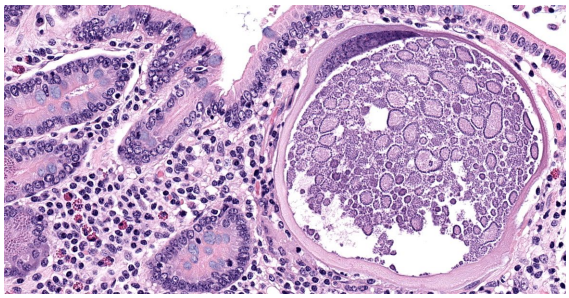

Figure 1-3. Small intestine, horse. Macrogamonts are contained within a hypertrophied enterocyte with a thick hyaline wall and a peripheralized, hypertrophied nucleus. (HE, 350X)

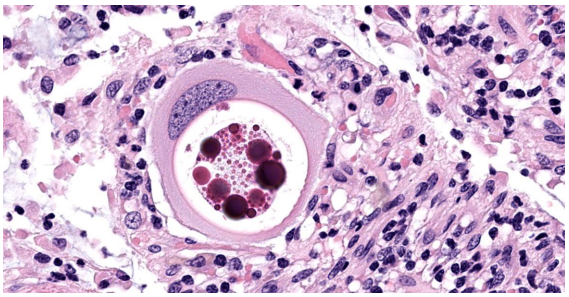

Figure 1-4. Small intestine, horse. A mature macrogamont resides within a hypertrophied epithelial cell. The eosinophilic globules are characteristic and will eventually contribute to the shell of the oocyst. (HE, 667X)

E. leuckarti was named after the German scientist Rudolf Leukart and was first recognized as a large-sized protozoan in sections of small intestine of a horse used for teaching anatomy in Switzerland. *E. Leuckarti* has been found in several species of equids including horse, donkey, mule, Asian wild ass, Mountain zebra, and Grant's zebra.[3]

In a study of parasites of horses in farms in Kentucky, *E. leuckarti* infection was common in foals as young as 28 days of age.[7] Prevalence of *E. leuckarti* oocysts in feces of foals in Kentucky from studies in 1986 and 2003 were similar at 41% and 41.6%, respectively, with *E. leuckarti* found in 100% of the farms in the 2003 study and 86% of farms in the 1986 study.[7,8] Foals can acquire the infection on the day of birth, most likely from the contaminated environment rather than from oocysts excreted by their mares.[3] In another study, the prevalence of foals affected by *E. leuckarti* was 59%.[9]

The life cycle for each species of *Eimeria* is host specific and direct.[5] Sexual stages identified as macrogametes (female) are uninucleate and contain peripheral PAS-positive granules. The immature male stage (microgamont, microgametocyte) is multinucleated. When each nucleus becomes incorporated into a sperm-like biflagellate structure (microgamete), the microgamont is considered mature.[5] So far, only gamonts and oocysts of *E. leuckarti* have been found in histological sections of small intestine; asexual stages of *E. leuckarti* have not yet been confirmed.[3]

E. leuckarti develop in the cytoplasm of hypertrophied host cells in the lamina propria of the small intestine.[3,5,6] An immunohistochemical study identified these cells as epithelial cells.[6] In this study, the cytoplasm of the host cells was immunopositive for cytokeratin AE1/AE3 and cytokeratin 13. Host cells did not react to vimentin, chromogranin A, neuron-specific enolase, desmin, alpha smooth muscle actin, or factor VIII. It was hypothesized that laminin may regulate the displacement of epithelial host cells parasitized by *E. leuckarti* into the lamina propria.[6] In addition, the expression of CK13 by the host cells implied that the lifespan of the host cells was possibly extended. How the host cells were dislocated to the lamina propria and achieved the extended life span was unclear.[6]

Usually there is no host inflammatory response to *E. leuckarti* and only a mild reaction to degenerate life stages can be seen.[3,10] Parasites are found in the lamina propria towards the luminal part of the villus but some occur throughout villi.[3] In most reports, infections with *E. leuckarti* in equids were considered incidental; however, occasionally it has been considered a cause or contributing factor to enteritis in foals.[3] In many cases, its pathogenicity is attributed to the distinctive large gamonts observed in the lamina propria of horses that died of enteric disease of undetermined cause, however the

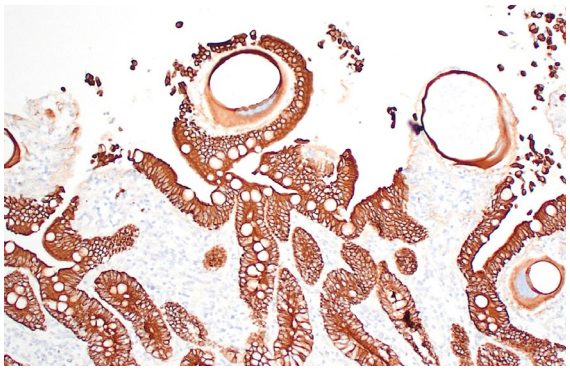

Figure 1-5. Small intestine, horse. A cytokeratin immunohistochemical stain demonstrates that, although the gamonts appear to be in the lamina propria, due to their size, they are actually contained within epithelial cells like other members of the genus *Eimeria*. (anti-AE1/AE3, 200X)

evidence for *E. leuckarti* causing enteric disease is rarely conclusive.[10]

Detection of *E. leuckarti* oocysts in feces is confirmatory for diagnosis; however, oocysts may be overlooked during routine fecal analysis that uses standard, low specific gravity floatation technique due to the large size and the heavy weight of oocysts.[3,9] The use of a sedimentation technique or floatation method using solution with a specific gravity of 1.3 or higher is recommended because *E. leuckarti* oocysts are large and heavy.[3,9] Saturated sodium chloride solution is not recommended.[3] Adding to the difficulty in diagnosing *E. leuckarti* is the short duration of patency and the relatively low oocyst output.[9]

In histological sections, the presence of gamonts and oocysts in the lamina propria of the jejunum and ileum is diagnostic.[3,10] There is a need for case-controlled studies to better understand the pathogenicity of *E. leuckarti* in equids.[3]

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JPC Diagnosis:
Small intestine: Enteritis, lymphoplasmacytic and eosinophilic, diffuse, mild, with marked villar atrophy and numerous intraepithelial coccidia consistent with *Eimeria leuckarti*.

JPC Comment:
As the contributor notes, *Eimeria leukarti* is a common parasite of the equine gastrointestinal tract; however, despite its ubiquity, it rarely causes significant disease and is most often encountered, as in this case, as an incidental finding at necropsy.

Eimeria spp. are not, however, universally benign. In sheep, *Eimeria gilruthi* forms megaloschizonts in the abomasal mucosa that are visible to the naked eye as multiple white, raised foci, and the clinical disease may include diarrhea, dehydration, anorexia, and weight loss.[1,4] In rabbits, *Eimeria stiedae* develops in the biliary epithelium of bile ducts and can cause significant hepatic changes, visible as large white foci on the surface of the liver, that often culminate in death.[4]

Eimeria spp. are coccidians, which are single-celled obligate intracellular parasites belonging to the phylum Apicomplexa. They keep phylogenetic company with other coccidian genera of veterinary importance, including *Klossiella, Cystoisospora, Hammondia, Besnoitia, Sarcocystis, Neospora,* and *Toxoplasma*, all of which cause disease by destroying their host cells.[2,4]

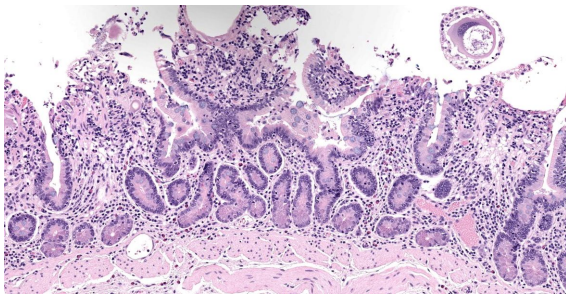

Figure 1-6. Small intestine, horse. There is mild expansion of the lamina propria with mild edema and slightly increased numbers of lymphocytes, plasma cells, and eosinophils. (HE, 144X)

Eimeria spp. exhibit the simplest form of the coccidian life cycle, which includes both asexual and sexual stages in the gastrointestinal tract of many vertebrate hosts.[2] Sexual reproduction produces oocysts which are released into the environment by rupture of host gastrointestinal epithelial cells and subsequent passage in the feces. Once in the environment, *Eimeria* oocysts develop eight infective sporozoites through the process of sporulation.[2]

Once the infective sporulated oocyst is ingested by a suitable host, the sporozoites emerge and enter epithelial cells or cells of the lamina propria, where they round up and become trophozoites in a membrane-bound parasitophorous vacuole formed by the host cell membrane.[2] The trophozoites grow larger and multiply asexually within host cells via schizogony (also known as merogony), in which the apical complex is replicated, the nucleus lobulates with portions associated with each apical complex, and the cell membrane contracts and divides to form many individual merozoites encased in a schizont.[4] Depending on the *Eimeria* spp., there may be multiple generations of schizont and merozoite formation; however, the key outcome of schizogony is an exponential increase in the number of zoites and the destruction of host cells in proportion to the degree of infection.[2]

Merozoites produced by the final round of schizogony enter host cells and develop into either microgamonts (male) or macrogamonts (female) through the process of gametogony.[2] Microgamonts undergo repeated nuclear divisions with each nucleus finally incorporated into a flagellated microgamete, which fertilizes the macrogamete to form a zygote.[4] Eosinophilic globular wall-forming bodies present in the macrogamete then coalesce to form a wall around the zygote, forming an oocyst.[2,4] This oocyst is released by rupture of the host cell and the cycle begins anew. This basic life cycle pertains, albeit with increasing complexity and nuance, to the other coccidian genera of veterinary importance.

Our moderator for parasite week was Dr. Chris Gardiner, PhD, self-proclaimed "parasitologist to the stars". Discussion of the parasite in this case focused on identifying the various life stages in tissue section. Several conference participants identified organisms in section as oocysts, though Dr. Gardiner thought they represented trophozoites, the gamont precursor. We noted that the contributor also described oocysts, but none were present in the examined section.

The discussion of pathologic changes centered on the villi, which are substantially blunted, and the degree of inflammation present in the lamina propria. Dr. Bruce Williams noted that intestinal crypts should be closely apposed and should rest on the muscularis mucosae; however, in this case, crypts were widely and irregularly separated and were multifocally elevated off the underlying muscularis mucosae. Though the lamina propria normally hosts a substantial population of inflammatory cells, these crypt changes

are important clues that the inflammation (and edema) is more florid than normal, even when, as in this case, the enteritis is considered mild.

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CASE II:

Signalment:
6-month-old, female Yorkshire pig (*Sus domesticus*)

History:
Seven 6-month-old pigs were shipped to a research facility and all animals developed a high-grade fever and high respiratory rate one week after arrival. The animals were treated with antibiotics and nonsteroidal anti-inflammatories to reduce the fever. One died overnight and was submitted for necropsy.

Gross Pathology:
The lungs were diffusely mottled red to dark red, with patchy areas of consolidation on the right side.

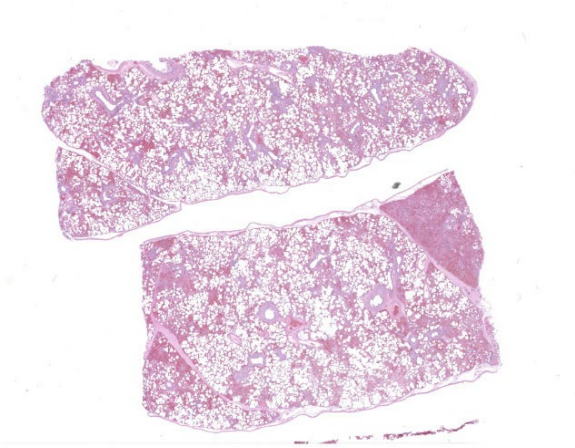

Figure 2-1. Lung, pig. Two sections of lung are submitted for examination. There is patchy to lobular inflammation scattered throughout the sections. (HE, 5X)

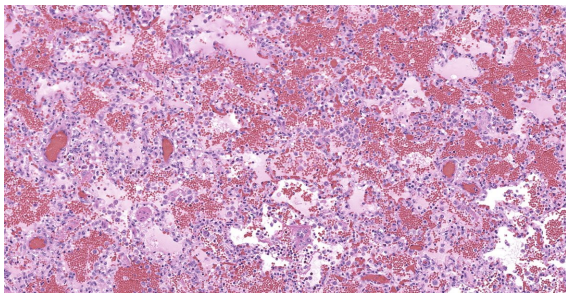

Figure 2-2. Lung, pig. In inflamed areas, septa are markedly expanded and alveoli are hypercellular with abundant hemorrhage, edema, and occasionally, aggregates of polymerized fibrin. (HE, 152X)

Laboratory Results:
PCR positive for Porcine Reproductive and Respiratory Syndrome Virus.

PCR negative for Porcine circovirus-2, PCV-3, and Influenza A.

Lung culture resulted in moderate amounts of mixed bacterial growth.

Microscopic Description:
In multifocal to coalescent foci affecting approximately 30% of the examined area of lung, alveolar spaces are filled with erythrocytes, fibrin, large numbers of macrophages, fewer multinucleated giant cells, and scattered eosinophils and neutrophils. Adjacent alveolar septa are moderately to markedly widened by fibrin and similar inflammatory infiltrates, and occasionally form confluent ruptured spaces (emphysema). Multifocally, alveolar macrophages are hypereosinophilic and contain pyknotic nuclei (necrosis). The inflammatory infiltrate often extends into and fills terminal bronchioles, with expansion and disruption of the epithelium by inflammation. Affected bronchioles are lined incompletely by attenuated epithelium. Multifocally throughout affected and unaffected regions of lung are numerous nematode larvae within both alveoli and bronchioles. Nematode larvae are 40-60 µm in diameter, with a 3 µm thick smooth cuticle, prominent lateral alae, large lateral chords, and coelomyarian-polymyarian musculature. The pseudocoelom contains a polycytous intestine lined by uninucleate epithelial cells. Some bronchioles that contain nematodes are lined by variably hyperplastic to eroded epithelium infiltrated by increased numbers of eosinophils. Interlobular septa are expanded by edema.

Contributor's Morphologic Diagnosis:
Lung: Interstitial pneumonia, histiocytic and eosinophilic, acute, multifocal to coalescing, moderate, with intra-alveolar macrophage necrosis, and intra-alveolar and intra-bronchiolar ascarid larvae consistent with *Ascaris suum*.

Contributor's Comment:
While the patchy inflammatory infiltrate is occasionally centered on nematode larva in this case, interstitial pneumonia in pigs has several viral and bacterial differential diagnoses. The presence of necrotic alveolar macrophages increased the suspicion for Porcine Reproductive and Respiratory Syndrome (PRRS) virus, and the virus was detected in the lung by PCR.

PRRS virus is a porcine arterivirus with a wide variety of clinical manifestations in pig herds depending on the endemic nature of the virus in the herd, age, immune status, and presence of coinfecting pathogens.[2] Most notably, the virus causes reproductive and respiratory disease. The virus infects macrophages at the point of entry and disseminates to local lymphoid tissue, resulting in viremia and systemic infection.[7] Pulmonary lesions are characterized by interstitial pneumonia with expansion of the alveolar septa by increased numbers of mononuclear leukocytes.

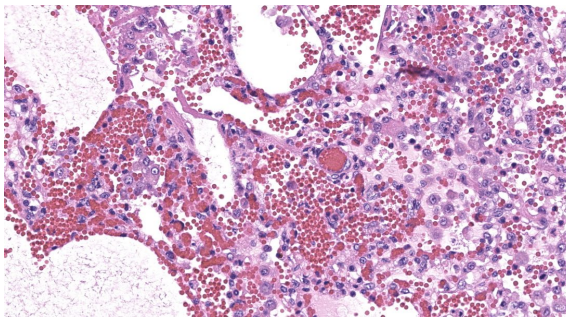

Figure 2-3. Lung, pig. Two sections of lung are submitted for examination. In inflamed areas, septa are expanded by macrophages, neutrophils, fibrin, and type II pneumocytes, and similar inflammatory cells are present within alveoli where they are admixed with abundant hemorrhage, fibrin, and edema. (HE, 381X)

Necrotic macrophages and smudged chromatin within alveoli are consistent findings in PRRS infection.[2]

PRRS virus infection has been reported to have immunosuppressive effects that enable secondary viral and bacterial infections.[7] Multiple mechanisms of immunosuppression have been described, including reduction in NK cell cytotoxic activity, promotion of immunosuppressive cytokines IL-10 and TGF-B, and impairment of pulmonary macrophage functional activity.[2,5,7] *Streptococcus suis, Glaesserella parasuis,* and *Salmonella* spp. are common co-infecting pathogens, but an association with pulmonary parasitism or parasite migration has not been described.[2]

The intrapulmonary helminths in this case are morphologically consistent with ascarid nematodes. In combination with the signalment, the histologic features of the ascarid nematode (diameter, presence of prominent lateral alae, and appearance of the hypodermis, musculature, and intestinal tract) are compatible with larval *Ascaris suum*.[4]

Ascaris suum is ubiquitous in swine populations and infection results in production losses and potentially severe pathologic consequences.[1] The life cycle, as with most ascarids, involves ingestion of the ascarid egg which hatches in the gastrointestinal tract. The larvae gain access to the portal vasculature, leading to larval migration through the hepatic parenchyma. From there, larvae access the pulmonary vasculature and enter alveoli. Eventually larvae ascend the bronchiolar tree and are coughed up and swallowed back into the gastrointestinal tract where they develop into sexually mature adults. Mechanical damage by the migrating larvae in the lung results in hemorrhage, edema, and eosinophil infiltration. As the larvae mature, an eosinophilic bronchiolitis ensues, with reactive hyperplasia or denuding of bronchiolar epithelium and invasion of eosinophils into bronchiolar walls.[1,2]

While an immunologic association between the PRRS infection and the ascarid infection could not be determined, this case highlights the importance of ruling out primary viral causes of pneumonia in pigs with pulmonary ascariasis.

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https://vmp.vetmed.wsu.edu/

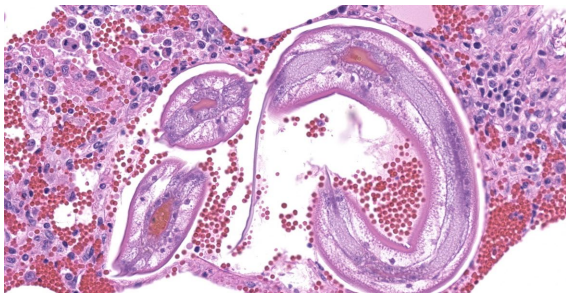

Figure 2-4. Lung, pig. Cross and tangential sections of ascarid larvae are present within the parenchyma. The larvae (which at this stage possess neither gonads nor eggs) have lateral alae, polymyarian-coelomyarian musculature, prominent lateral chords, and a uninucleate intestine. (HE, 450X)

JPC Diagnoses:

1. Lung: Pneumonia, interstitial, lymphohistiocytic, multifocal, marked, with type II pneumocyte hyperplasia and peribronchiolar and perivascular lymphoid hyperplasia.

2. Lung: Pneumonia, interstitial, histiocytic and eosinophilic, multifocal, mild, with eosinophilic bronchiolitis and ascarid larvae.

JPC Comment:

Ascarids are nematodes of extremes. They are among the largest of the worms found in domestic animals, with members of the order Ascaridida ranging from several inches up to 2 feet in length.[1] Ascarid eggs are also remarkably resistant to chemical and physical insults in the environment and can remain infective in soil for years.[1]

The life cycle of the terrestrial ascarids tend to be direct, and larvae undergo two molts within their hardy eggshell, emerging within the host as infective L3 larvae.[1] Ascarids also tend to be relatively host specific, and most domestic species have at least one ascarid to call their very own; *Parascaris equorum* infects horses, *Toxocara vitulorum* infects cattle, *Taxocara canis* infects dogs, *Toxocara cati* infects cats, and, of course, *Ascaris suum* infects swine.[1]

There are exceptions to this "one ascarid, one host" rule. Historically, *Ascaris suum* was thought to be identical to *Ascaris lumbricoides* in humans, though these two organisms are now considered distinct species. Nevertheless, *A. lumbricoides* can mature in swine and *A. suum* can mature in humans, as evidenced by one recent study where 50 *A. suum* eggs were fed to healthy, human research subjects.[1,3] Once they emerged from the required regulatory paperwork, researchers found that experimental infection produced clinical symptoms identical to *A. lumbricoides* infection, including respiratory discomfort and radiographic evidence of pulmonary larval migration, and *A. suum* eggs were recovered from the feces of the intrepid volunteers.[3] *A. suum* may also infect sheep, causing mild respiratory disturbances in young lambs. Calves exposed to yards contaminated with infective pig feces may develop severe acute interstitial pneumonia with millions of *A. suum* larvae present in the lungs.[6]

The contributor provides an excellent summary of the winding walkabout taken by the typical *A. suum* larva after emerging from its egg in the small intestine of its porcine host. In young pigs, extensive pulmonary damage can lead to severe respiratory disease, with rapid, shallow breaths and audible expiratory efforts ("thumps") that may lead to death.[1] As larvae wander through the liver, the mechanical damage and ensuing inflammation heal by fibrosis, producing "milk spots" that cause condemnation of the liver at slaughter.[1] While less pathogenic, infection by adult worms may cause diarrhea, impaired nutritional uptake and growth, and rare disorders such as perforated bowels or bile duct occlusion.[1] Taken together, these effects make

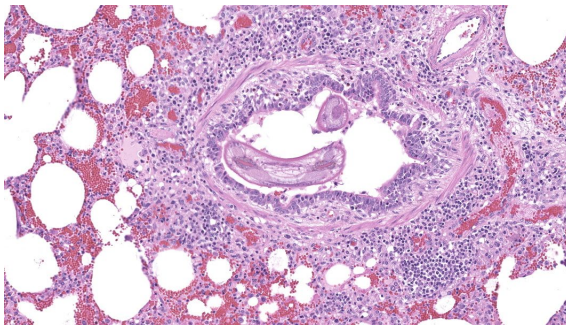

Figure 2-5. Lung, pig. Ascarid larvae are also present in airways. (HE, 175X)

Ascaris suum the most economically important nematode of swine.

Dr. Gardiner reminded conference participants that nematode larvae are typically coiled; thus, when assessing parasite load on histology, it is helpful to remember that a close grouping of cross sections typically represents only one larva, not multiple. Dr. Gardiner also noted that conference participants failed to describe the nematode excretory cells, visible as eosinophilic material within the larval lateral cords.

Dr. Williams noted that you rarely find only one process in pig lungs; they tend to reward careful evaluation with additional revelations. In this case, while the nematode larvae may initially hog the spotlight, careful examination reveals multiple areas within the lungs that are devoid of nematodes but rich in macrophages, fibrin, hemorrhage, edema, and multinucleated cells. These lesions are not entirely attributable to nematode migration, and a young pig with many macrophages and multinucleated cells in the lungs should raise suspicion for PRRSV and PCV-2. Differentiating the two can be difficult, though large numbers of necrotic macrophages, while not apparent in the section examined at conference, are characteristic of PRRSV infection.

There was spirited discussion about whether to combine the nematode and PRRSV lesions into one morphologic diagnosis, particularly since assigning histologic effects to one etiology or another is somewhat artificial in this case. The separatists carried the day, however, as the majority of participants felt the etiologic agents were unrelated, each notable, and each deserving of their due.

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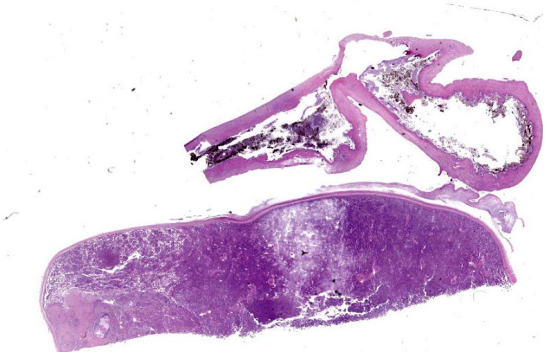

Figure 3-1. Esophagus and digestive gland, octopus. One section of the esophagus (above) and digestive gland (below) are submitted for examination. (HE, 5X)

CASE III:

Signalment:
Age unknown, adult male octopus (*Octopus vulgaris*)

History:
An experimentally naïve octopus was found dead in the morning. The animal was caught from the shores of the Florida Keys and had been doing well for up to a month when it started displaying periods of lethargy and decreased appetite. One night, the animal went into one of its tunnels and never re-emerged. Water quality parameters were within normal limits during this period.

Gross Pathology:
A dead, frozen male octopus with mottled pale brown to gray skin was received for examination in severely autolyzed post-mortem condition. The skin had numerous foci of subtle white discoloration.

Microscopic Description:
Digestive gland: Approximately 20% of the digestive gland mucosa is effaced by multifocal to coalescing nodules of fibrosis, necrosis, and inflammation centered around cestode larvae of up to 650 µm in diameter, with a 4-6 µm thick, homogeneous, eosinophilic tegument, spongy parenchyma, numerous peripheral calcareous corpuscles, suckers, and bilaterally symmetrical bothria with multiple tentacles that were anchored by prominent bulbs encircled by striated retractor muscles that attached to everted or invaginated hooks. Confident assessment of mucosal and cellular details is hampered by a high degree of postmortem autolysis and freeze-thaw artifacts.

Esophagus: Occasionally within the lumen or embedded in the esophageal wall are cestode larvae with similar features as previously described.

Contributor's Morphologic Diagnosis:
Digestive gland and esophagus: Moderate, multifocal to coalescing, chronic cestodiasis, with hemocytic infiltrates, necrosis, and fibrosis.

Contributor's Comment:
Despite the severe degree of postmortem autolytic and freeze-thaw artifacts, this octopus had unequivocal cestodiasis in the digestive tract, widespread coccidiosis in all examined skin tissues (including arms, funnel, and dorsal mantle), eyes, and gills, consistent with *Aggregata* spp. infection, and varying degrees of systemic hemocyte infiltration throughout the body.

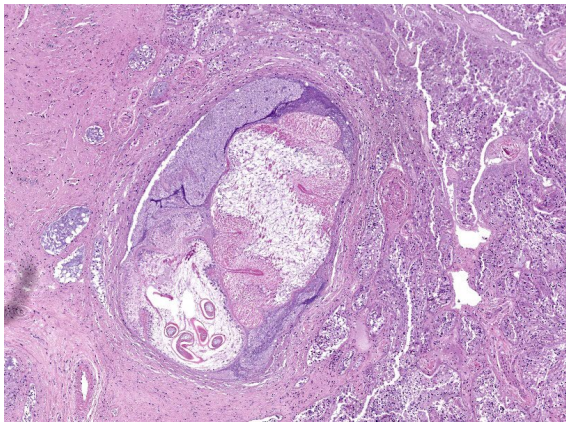

Figure 3-2. Digestive gland, octopus. A larval cestode (left) is embedded within an area of fibrosis within the digestive gland. (HE, 52X)

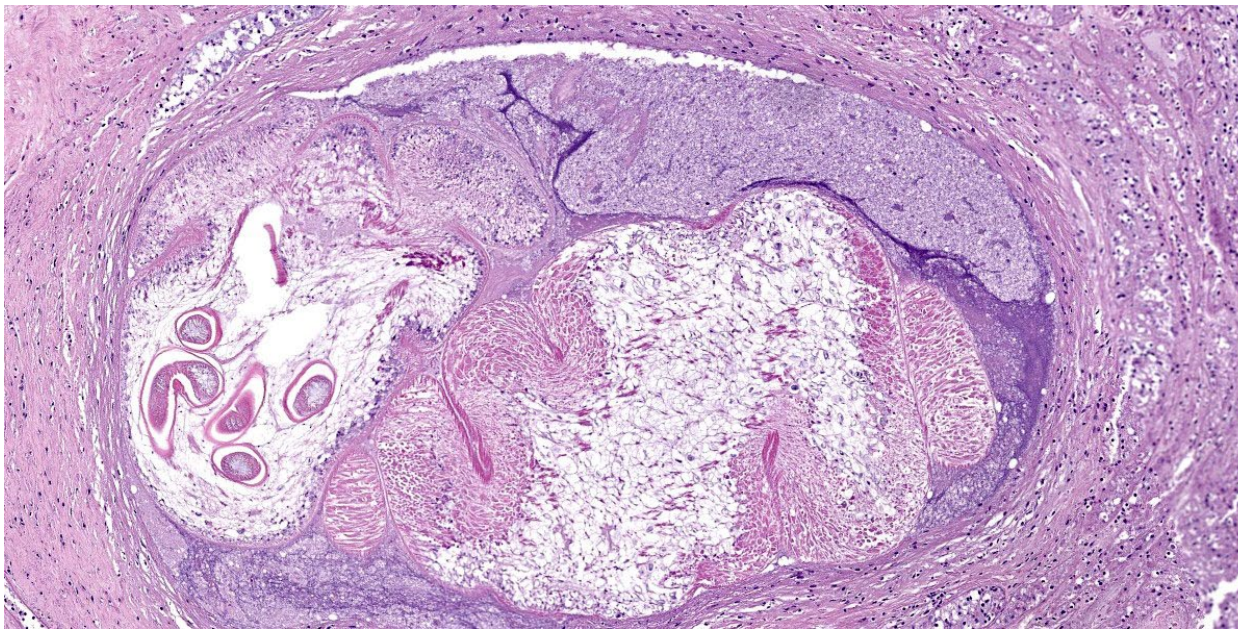

Figure 3-3. Digestive gland, octopus. The cestode has an armed rostellum (suckers with birefingent hooklets), a spongy parenchyma, and numerous calcareous corpuscles. (HE, 106X)

Cestodiasis in cephalopods is common, and many cephalopod species serve as intermediate or paratenic hosts and act as vectors for other intermediate or definitive hosts. Adult cestodes are not frequently reported, but the diversity of larval and post-larval stages found in cephalopods suggests that they are important intermediate hosts for the development of adult stages that parasitize cartilaginous and bony fish. In cephalopods, larval cestodes most often infect the digestive tract, but may be found free in the mantle cavity or encysted within the mantle musculature. The most commonly reported cestode to infect cephalopods is *Phyllobothrium* spp, but cestodes from other genera have also been identified in the common octopus (*Octopus vulgaris*) and include the onchoproteocephalidean *Acanthobothrium* spp, the tetraphyllidean *Anthobothrium* spp, and the trypanorhynch *Nybelinia* spp. Adult stages of Tetraphyllidea and Trypanorhynchea are found within the gastrointestinal tract of sharks, skates, and rays, and their larval forms are some of the most commonly identified cestodes in cephalopods.[3]

Coccidiosis is a common, chronic disease in cephalopods, caused by an obligate, intracellular protozoa in the phylum Apicomplexa, family Aggregatidae. To date, 10 species have been described worldwide in octopus, squid, and cuttlefish. All 10 species are considered pathogenic. This disease primarily affects the digestive tract of cephalopods, but extraintestinal coccidiosis, as seen in this case, have been reported when it harbors an intense infection. Damage to the host includes mechanical, biochemical, and molecular effects, and infection severely weakens the host's innate immunity making it vulnerable to secondary infections. Notable signs of disease include malabsorption syndrome, decrease in the number of hemocytes, plasmatic protein, and iron in hemolymph, as well as up- regulation of immune genes.[1]

Senescence can also cause immunosuppression and make the octopus more susceptible to secondary diseases. This octopus also had evidence of cataract in one eye, similar to what has been described in the literature, in

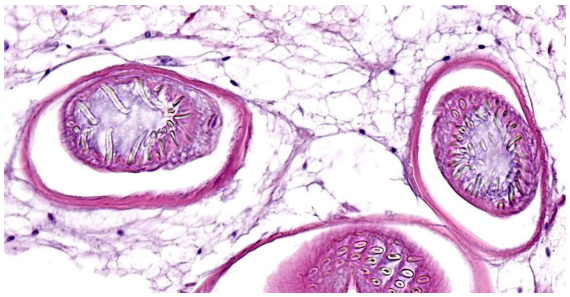

Figure 3-4. Digestive gland, octopus. High magnification of the hooked tentacles of the cestode. (HE, 660X)

which possible underlying causes for the intraocular lesions included water quality, ocular manifestation of a systemic disease, intraocular infection, or natural senescence. Senescence is a natural pre-death process in octopuses and other species. While senescence is not a disease or a result of illness, diseases can be a symptom of senescence. Clinical signs of senescence in octopuses are observed over a couple of months and are reported to include loss of appetite, weight loss, retraction of skin around the eyes, uncoordinated movements, and increased undirected activity level. In octopuses, senescence-like symptoms can also be triggered by collection stress, systemic diseases, and improper water temperature or water quality.

Octopuses lack a humoral immune system, and their innate immune system with cellular factors is their primary mechanism of defense against disease. The octopus' hemocytes respond to infection with phagocyntesis, encapsulation, infiltration or cytotoxicity, aiming to destroy or isolate pathogens.[2] In summary, senescence and/or stress may have facilitated parasitic burden, inflammation, other infectious processes, and potential sepsis as contributory factors to the demise of this octopus.

Contributing Institution:
Laboratory of Comparative Pathology
(Memorial Sloan Kettering Cancer Center, The Rockefeller University, and Weill Cornell Medicine)
http://www.mskcc.org/research/comparative-medicine-pathology-0

JPC Diagnoses:
1. Digestive gland and esophagus: Larval cestodes, multiple, with fibrosis and hemocytic inflammation.
2. Digestive gland: Atrophy, diffuse, moderate.

JPC Comment:
This case provides an excellent example of cestodiasis in an unusual species, and the contributor provides a good summary of the histologic lesions that typify cestode infections in cephalopods.

An additional finding in this case was atrophy of the digestive gland, the histologic correlate for which is loss of eosinophilic cytoplasmic globules. Although this is a common lesion in senescence, it can be observed in any animal with negative energy balance.

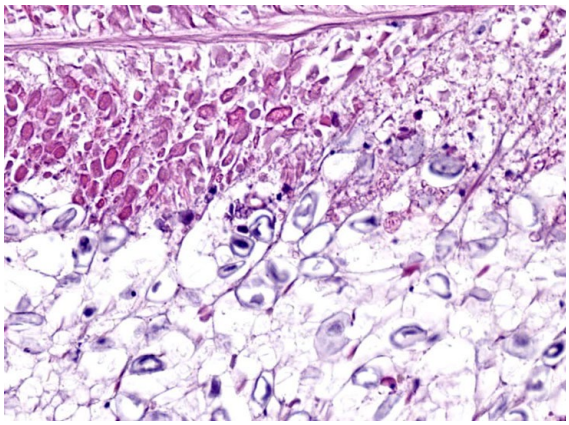

Figure 3-5. Digestive gland, octopus. There are oval amphophilic calcareous corpuscles within the spongy body parenchyma. (HE, 570X)

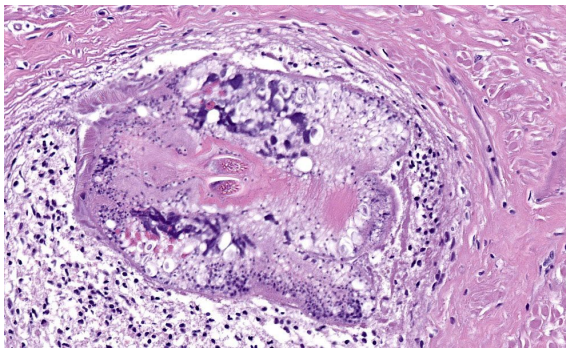

Figure 3-6. Esophagus, octopus. A similar larval cestode is present within the muscular wall. Nuclei of hemocytes are present adjacent to the cestode. (HE, 315X)

In this case, the reported severe coccidiosis and resultant malabsorption likely led to negative energy balance and digestive gland atrophy. While infections are reportedly more common and more abundant in senescent animals, the primary lesion of senescence is gonadal atrophy.[4]

Coccidia were not observed in the examined slide, which included esophagus and digestive gland. While the gills and the intestines are commonly infected, esophagus and digestive gland can be infected with coccidia in severe cases. Of all cephalopod species, common octopuses are one of the most commonly infected with *Aggregata* spp.

Conference discussion was facilitated by JPC's very own Dr. Elise LaDouceur, who discussed cephalopod anatomy generally, before diving into the histologic lesions. The digestive gland is analogous to the mammalian liver and the normally abundant eosinophilic globules within the digestive gland are typically packed with storage products such as glycogen and lipid. Dr. LaDouceur noted that the digestive gland was extremely autolyzed, but despite the autolysis, the lack of eosinophilic globules within the digestive gland was notable. This led to a discussion of senescence, a feature of many invertebrates' life history, where metabolism is shut down after release of gonads.

Conference participants discussed the large cestode larvae within the digestive gland and the accompanying multifocal areas of necrosis in the adjacent parenchyma, interpreted as migration tracts. Dr. Gardiner believed the organism to be an encysted cestode, which would typically grow in place without moving through the tissue, and questioned whether the histologic features represented true migration tracts. These histologic features do, however, align with recent published reports in cephalopods that feature cestodes invading tissues of organs with non-chitinized epithelium, leaving behind trailing necrotic foci.

Finally, Dr. LaDouceur pointed conference participants to an excellent, open-source resource by Gestal, *et al* (see reference below), which provides excellent gross and histologic images of common cephalopod pathogens, along with discussion of the unique biology of these interesting Molluscans.

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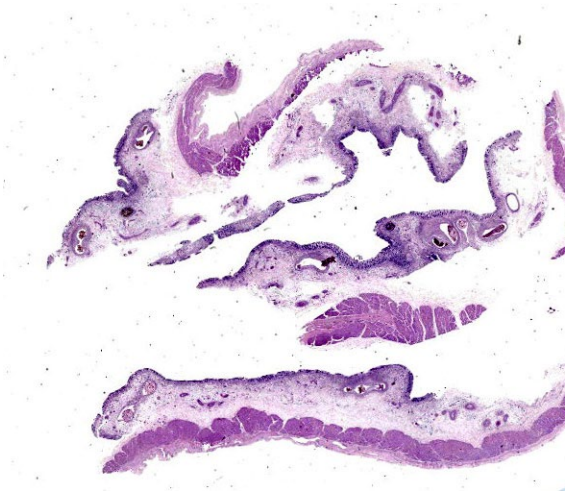

Figure 4-1. Colon, horse. Marked edema is evident in the submucosa and, to a lesser extent, the mucosa of the submitted sections. (HE, 5X)

CASE IV:

Signalment:
1-year-old, Gypsy Vanner colt (*Equus caballus*)

History:
The patient presented for acute recumbency and was treated in the field with antimicrobials, plasma, and IV fluids. He was unable to rise but would sit in sternal recumbency and would eat when offered hay. On presentation, he was in lateral recumbency on the trailer, but was able to stand when assisted with the sling. He was severely underweight with muscle wasting. His heart rate was 80 bpm with a systolic murmur, his respiratory rate was 40 bpm, and his rectal temperature was 98.5 ºF. Mucous membranes were pale with a capillary refill time of 2 seconds.

Thoracic ultrasound revealed mild pneumonia (comet tailing cranioventrally) with a small amount of pleural fluid. Abdominal ultrasound revealed edema in the wall of the colon, small intestine, and cecum along with a moderate amount of fluid within the cecum and colon. A small amount of peritoneal fluid was present.

Medical therapy consisted of IV fluids, ceftiofur, flunixin, vitamin E, and supportive care. Two days after presentation, the patient was found colicking and abdominal radiographs at that time revealed a large amount of intestinal sand. He began passing large amounts of diarrhea but continued to have a good appetite and remained standing (assisted with sling). A plasma transfusion was given, but the patient continued to have profuse watery diarrhea and began to slowly decline over the next 36 hours. Two days later he became increasingly dull and spontaneously died.

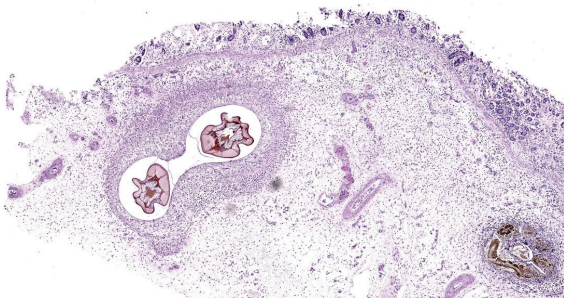

Figure 4-2. Colon, horse. Large larval nematodes are surrounded by poorly formed granulomas within the submucosa. (HE, 47X)

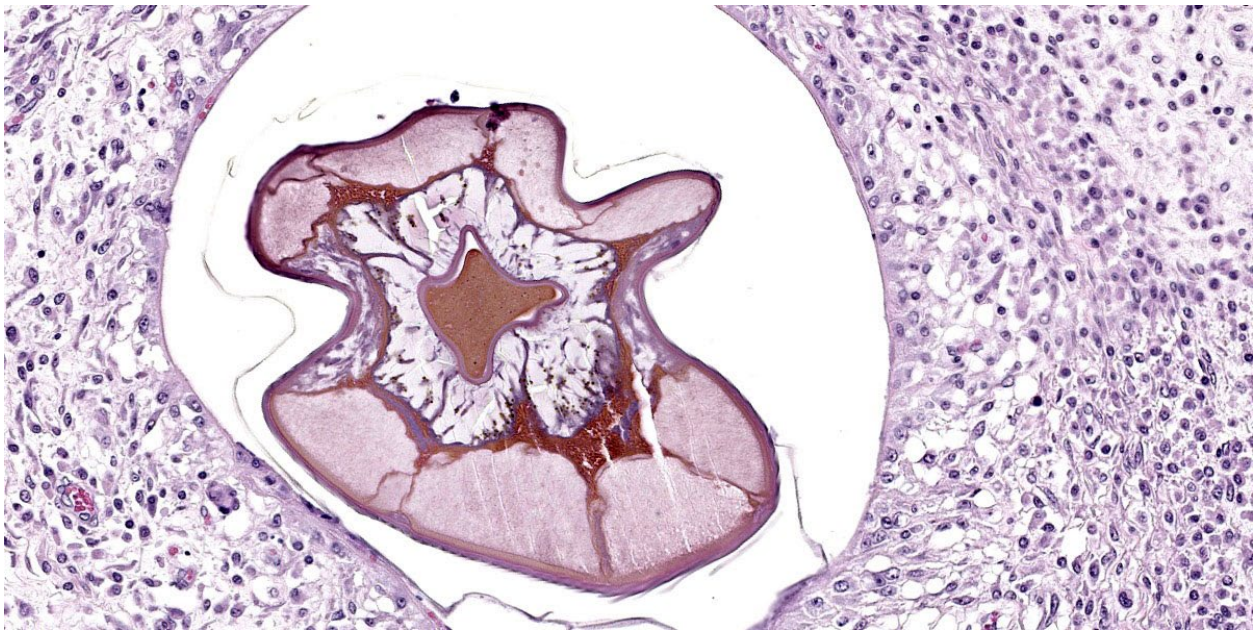

Figure 4-3. Colon, horse. Embedded nematodes have a thin cuticle, pseudocoelom, platymyarian-meromyarian musculature, large lateral chords, and a central large intestine with uninucleated, tall columnar cells which contains luminal blood pigment. (HE, 278X)

Gross Pathology:
The cecal and ventral colonic mucosa contains numerous widespread multifocal, brown to red, 1-2 mm diameter circular nematodes. In the region of the diaphragmatic flexure, the right dorsal colon contains a moderate amount of wet, packed, tan, granular material (sand) admixed with numerous 8-5 x 1 mm red round nematodes and a small amount of green fibrous ingesta. The wall of the cecum and ventral colon is mildly to moderately expanded by clear gelatinous material. Numerous pinpoint red foci are present within the mucosa of the dorsal colon.

Laboratory Results:
Complete blood count:
WBC = 12,700/µL
Fibrinogen = 600 mg/dL

Serum chemistry:
Total protein = 5.6 g/dL (6.1-8.4)
Albumin = 2.2 g/dL (2.7-4.5)
Potassium = 5.6 mEq/L (2.2-5.3)
Sodium = 130 mEq/L (136-144)
Chloride = 94 mEq/L (96-105)

Fecal float: Negative.

Microscopic Description:
Randomly distributed throughout the large intestinal submucosa and rarely within the mucosa, there are encapsulated 20-400 µm diameter larval nematodes. The nematodes have a ridged eosinophilic cuticle, platymyarian-meromyarian musculature, vacuolated lateral cords, and an intestine that is lined by few multinucleated cells and contains red to brown granular material. Occasional nematodes are mineralized. The nematodes are often surrounded by a moderate number of macrophages and few fibroblasts. The submucosa is markedly edematous and contains a low number of lymphocytes and fewer plasma cells. The lamina propria contains a low to moderate number of plasma

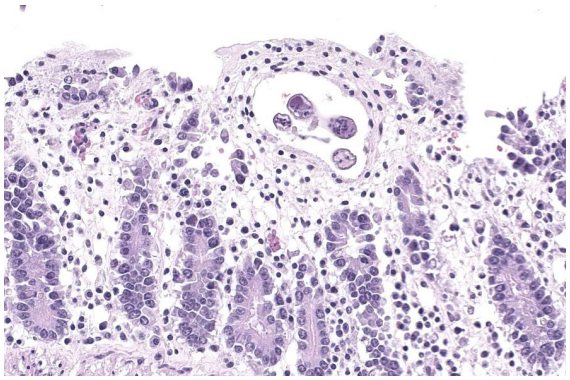

Figure 4-4. Colon, horse. The overlying edematous mucosa contains 3rd stage larvae. Colonic glands are separated by edema and moderate numbers of lymphocytes and plasma cells. (HE, 166X)

cells, including occasional Mott cells, fewer lymphocytes and occasional macrophages and neutrophils. There are rare small superficial mucosal erosions.

Contributor's Morphologic Diagnosis:
Colon: Colitis, granulomatous, chronic, with edema and intralesional encapsulated nematodes consistent with small strongyles.

Contributor's Comment:
Referred to as small strongyles or red worms, the more than 50 species of cyathostomins can infect horses of any age, but more severe clinical disease is typically noted in young horses.[2] Cyathostomins have a direct life cycle and horses become infected with cyathostomins by ingestion of the early L3 stage, which burrows into the mucosa of the hindgut and forms a fibrous capsule within

two weeks, cloaking it from the host's immune system.[2,4] Development can arrest for as long as 2 years in the early L3 stage.[2]

Late fourth stage larvae exit the mucosa to mature to the adult stage in the intestinal lumen.[4] The emergence of a large number of L4 larvae from the hindgut, in late winter and early spring in temperate regions and late summer and early fall in tropical regions, triggers the syndrome of larval cyathostomiasis.[3] Clinically, horses present with rapid weight loss, colic, leukocytosis, hyperglobulinemia, hypoproteinemia, rough hair coat, and severe diarrhea containing a large number of larval cyathostomins detectable by dilution of feces with water and viewing under a microscope.[3,4] Edema of the limbs and ventrum can also be seen.[3]

Contributing Institution:
University of Florida
College of Veterinary Medicine
Department of Infectious Diseases and Pathology
Gainesville, FL 32611-0880
http://idp.vetmed.ufl.edu/

JPC Diagnosis:
Colon: Colitis, granulomatous, multifocal, mild to moderate, with marked submucosal edema and small strongyle larvae.

JPC Comment:
As the contributor notes, there are approximately 40-50 species of cyathostomins that parasitize the cecum and colon of horses, and as many as 15 to 20 of these species commonly colonize the same host at the same time.[1] Luckily, cyathostomin larvae do not migrate far beyond the mucous membranes of the cecum and colon and feed mainly on intestinal contents, so their pathogenic effect can be minimal.[1,5] However, infection by large numbers of arrested cyathostomin larvae and their simultaneous emergence from the gut wall can cause the clinical disease of larval cyathostominosis described by the contributor.

Larval cyathostominosis is a significant cause of morbidity and mortality in horses and can affect horses of any age.[5] Grossly, the

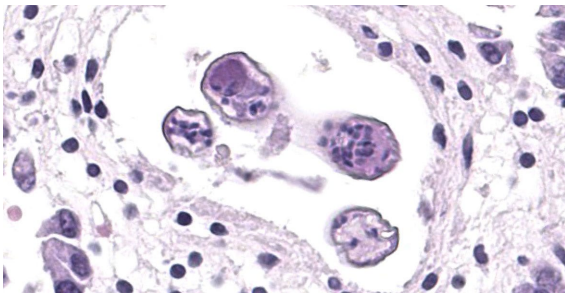

Figure 4-5. Colon, horse. 3rd stage larvae do not have numerous nuclei lining developing internal organs. (HE, 576X)

colonic mucosa is studded with 2-5 mm diameter nodules that are slightly raised, red or black, and contain encysted third or fourth stage hypobiotic (developmentally arrested) larval nematodes.[5] Histologically, the mucosa and submucosa are edematous, as in this case, and may contain a mixed inflammatory response either centered on encysted larvae in the submucosa or more diffusely throughout the lamina propria.[5]

Cyathostomins are widespread throughout the world, and even apparently healthy horses may be infected with tens to hundreds of thousands of their larvae. As anthelmintic resistance of cyathostomins is a large and growing problem, many current preventive measures are focused on pasture and herd management solutions.[1] It has been said (by a parasitologist, naturally) that "the king's horses probably had fewer worms" owing to the immediate removal of fecal material after deposition, and this concept has inspired the development of pasture vacuums and pasture sweepers.[1] Other suggested preventive measures include rotation of administered anthelmintics, orchestrating field plowing to reduce the spreading of infective fecal material, and the composting of horse manure.[1]

Conference discussion focused initially on tissue identification. The significant amount of autolysis in section complicated the issue, but the lack of identifiable villi placed this solidly in the colon. The examined section was notable for the lack of fibrosis around the larvae, leading conference participants to speculate that this tissue section was procured early in infection or that the horse's immune response to the larvae might have been impaired. Participants also discussed how simultaneous eruption of myriad larvae can cause loss of the enteric mucosal barrier, providing fertile soil for the development of a potentially profound enterotoxemia.

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