CASE I: 10N0217 (JPC 3166543).

Signalment: 1-year-old male Thoroughbred equine, Equus ferus caballus.

History: Submitted was a 299 kg yearling Thoroughbred colt that lived on pasture with about ten other yearlings. He presented on emergency to the Equine Surgery Service at the Veterinary Medical Teaching Hospital (VMTH) at UC Davis on 28 Jan 2010 for colic of approximately 3 hours duration. The colt was initially found down and rolling in his pasture. His pasture mates were also yearlings. One...
pasture mate had presented with colic on the previous day and the others were clinically normal. The colt and pasture mates were fed a sweet feed and alfalfa twice daily. He was dewormed on 1 Dec 09 with ivermectin, and then on 1 Jan 10 with fenbendazole.

On presentation at the teaching hospital, the colt was depressed to obtunded. He continually attempted to lie down and exhibited severe discomfort regardless of analgesia administration. Mucous membranes were hyperemic and tacky, and the horse was tachypnic. Reduced borborygmi were ausculted in dorsal left quadrant, with excessive borborygmi in all other quadrants. Dried feces were pasted to the perianal area. Serum chemistry abnormalities included albumin 1.7 g/dL (reference: 2.7-4.2 g/dL), total protein 4.8 g/dL (reference: 5.8-8.7 g/dL), neutrophils 17,313 /uL (reference: 2600-6800 /uL), fibrinogen 700 mg/dL (reference: 100-400 mg/dL).

Nasogastric intubation resulted in no net reflux. Cloudy fluid was obtained via abdominocentesis and characterized by 19,880 total nucleated cells (89% neutrophils) and a lactate of 5.5 mg/dL. Due to unrelenting pain, immediate surgery was recommended and preparations were begun, but euthanasia was ultimately selected due to financial concerns.

Gross Pathologic Findings: The colt was in good post mortem and body condition. The oral mucosa was generally dull red, with a distinctive darker red-purple line along the gingiva of the maxillary incisors. The sclera of both eyes were injected. The haircoat was dirty and patches of hair were matted with mud. Approximately 5 L of yellow, cloudy fluid filled the...
peritoneal cavity. The serosal surface of the large colon was gray-purple and the colic arteries and veins were prominent and distended. The mesenteric lymph nodes associated with the large colon, most notably along the intercolonic mesothelium, were turgid, dark red, and embedded in edematous connective tissue. The large colon and cecum contained abundant green, liquid fluid ingesta with fewer than 100 free floating tapeworms. The mucosal surface of the large colon (ventral>dorsal) and cecum was thickened (up to 1.5 cm in width), pale pink to tan, and finely corrugated. Innumerable, pinpoint to 0.1 cm diameter, dark flecks cover the mucosal surface ("salt and pepper colon") and visualized by the dissecting scope, correspond to encysted larval worms. The apex of the cecum was inverted, extending proximally from the apical tip, with approximately 14 cm present beyond the ceco-colic junction and within the lumen of the right ventral colon. The base of the cecum was gas distended. The serosal surface of the intussusception was deep dark red, dull, and friable, and the associated mucosal surface was diffusely dark red, thickened to 1 cm, and oozed serosanguinous fluid on cut section. The lumen of the cecum near this intussusception within the base contained thick, mucoid, yellow material. The stomach contained abundant semi-dry, packed feed.

**Laboratory Results:** Cecal contents submitted for parasitology analysis revealed two endoparasites via flotation: filariform nematode larvae, consistent with the 3rd/4th stage of cyathostome larvae and adult *Anoplocephala* sp. tapeworms. No ova were found on flotation.

**Histopathologic Description:** Multiple sections of large intestinal mucosa were examined in which abundant cyathostome larvae were present. The majority of the cysts were embedded within the tubular glands of the mucosa, where curled single larvae resided in dilated glandular crypts either freely or surrounded by a thin fibrous capsule. Occasionally, the cysts penetrated beneath the basement membrane and submucosa where the larvae were surrounded with a more prominent fibrous capsule, and a variably dense circumferential band of inflammation. The lamina propria was expanded by edema, congested submucosal blood vessels, and an inflammatory infiltrate comprised of neutrophils, plasma cells, lymphocytes, and histiocytes. The number of eosinophils varied among sections of the colon. Multifocally, the superficial mucosal epithelium was eroded, and luminal contents included acellular eosinophilic material. Submucosal arteries had moderate medial hyperplasia and scattered foci of intimal mineralization ("intimal bodies").

**Contributor’s Morphologic Diagnosis:**
2. Large colon and cecum: Mucosal larval cyathostomiasis.
3. Peritoneum: Moderate peritoneal effusion.

**Contributor’s Comment:** The *Cyathostominae*, or small strongyles, are a subfamily of the class *Nematoda* that include four main genera. It is the larvae, typically, that are clinically significant, mainly in equid hosts. Larval stages (L3) migrate into the deep mucosa or submucosa of the large bowel (mainly cecum and ventral colon) from the gut lumen and enter the glands to molt and develop, before emerging into the lumen to molt again and mature. If larvae undergo a period of arrested development, most anthelmentics are ineffective. If arrested larvae synchronously emerge from these cysts (hypobiosis), edema, rupture of the muscularis mucosa and ulceration of the overlying mucosa may occur. Alternatively, larvae can complete development in the cecum and colon lumen, and shed eggs into the feces.
It is postulated that cyathostomes have become resistant to certain anthelmintic drugs. A combination of lack of penetration of anthelmintics during the encysted stage along with current inconsistent deworming practices practiced by horse owners and managers has likely led to these new resistant populations, which in turn has led to an increase in incidence of infection over the last 10 years. As a result, cyathostomes are considered the primary parasitic pathogen of horses.6

Cyathostomiasis has been associated with non-specific clinical signs of colic and chronic diarrhea, and more specifically with cecocoeal intussusceptions. Disrupted intestinal motility, which has been experimentally induced by infection with cyathostomes, could contribute to both diarrhea and/or cecocoeal intussusception. Given the large number of potential motility disorders in the horse, it is puzzling that cecocolic intussusception is commonly associated with only a limited number of etiologies. Some blood chemistry and hematology aberrations that have been associated with cyathostomiasis include neutrophilia and hypoalbuminemia. Histopathological changes associated with cyathostome larval infection of the colonic mucosa include a fibroblastic reaction to penetrating larvae, which results in distension and distortion of the glands as the larvae grow. Goblet cell hyperplasia and hypertrophy and a modest, predominantly lymphocytic, inflammatory infiltrate are associated with the encysted larvae.6

This colt had numerous clinical signs, hematologic changes, and post mortem findings characteristic of cyathostomiasis. The striking cecocolic intussusception was the probable source of colic and unrelenting pain that ultimately led to euthanasia. An additional sign supportive of intestinal dysmotility was pasting of feces in the perianal area, suggestive of diarrhea. Changes suggestive of cyathostomiasis in blood work parameters were found, including hypoalbuminemia and neutrophilia. Post mortem exam revealed abundant larvae encysted in the mucosa, with mucosal changes described above that are characteristic of cyathostomiasis.

**JPC Diagnosis:** Colon: Colitis, histiocytic and lymphoplasmacytic, diffuse, moderate, with numerous mucosal small strongyle larvae.

**Conference Comment:** This is an excellent case illustrating the severe reaction and associated clinical gastrointestinal problems which may ensue following a mass emergence from hypobiosis of the most pathogenically significant nematode of horses. The contributor highlights the important aspects of the Cyathostome spp. lifecycle and how it relates to anthelmintic resistance, an increasing problem among many domestic animals and their parasitic inhabitants. Conference participants agreed that the histologic lesions in this case are more acute than chronic, with little evidence of fibrosis and only 3rd stage and 4th stage larvae identified. This is consistent with a simultaneous, massive eruption of larvae in spite of recent anthelmintic treatment just over two weeks prior to presentation.

Adult small strongyles are essentially nonpathogenic; rather, it is the mass emergence of previously arrested larvae in a short period of time which causes clinical disease. The development of arrested larvae more often occurs from late winter to early summer, and the host and/or environmental factors which influence it are poorly understood. Some evidence suggests the presence of luminal worms provide negative feedback to mucosal larvae, which may correlate with their emergence after anthelmintic treatment and elimination of adults, as likely occurred in this case. Interestingly, arrested development of larvae has been documented for periods extending over two years.

Histologic classification of nematodes is often possible when organisms are well preserved like in the current case. The characteristic large intestine with few multinucleated cells is readily identifiable and is indicative of one of three strongyle subgroups. Cyathostomes are part of the subgroup Trichostrongylus, and all are found with platymyarian musculature and longitudinal ridges along their external cuticle. The ridges are faintly visible on some cross sections in this case as small and evenly-spaced. True strongyles also have platymyarian musculature, thick smooth cuticle and often vacuolated lateral chords. The third group, Metastrongylus, are the only strongyles with coelomyarian musculature. Also important to speciation in this example is the presence of numerous organisms, as small strongyles often occur in large numbers.
Conference participants discussed the absence of fibrin thrombi within vessels in the affected area and how that relates to intestinal diseases of displacement such as an intussusception, which often do not cause endothelial damage. This is in contrast to infectious diseases such as *Clostridium* spp. or *Salmonella* spp., where fibrin thrombi are commonly observed.

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**References:**
**CASE II: 070181-41/41A (JPC 4032316).**

**Signalment:**  Adult male cynomolgus macaque, *Macaca fascicularis*.

**History:**  This macaque was part of an experimental study to investigate the efficacy of a vaccine against *Yersinia pestis* (*Y. pestis*). This animal was administered the vaccine and was later challenged with aerosolized *Y. pestis*; the challenge dose of bacteria that was used has been previously shown to cause 100% mortality in unvaccinated control animals.

This monkey survived the bacterial challenge and was euthanized at the end of the experiment. The carcass was then submitted for a complete necropsy.

This experiment and the necropsy were performed under biosafety level 3 (BSL-3) biocontainment conditions. This research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.

This research was sponsored by the Joint Science and Technology Office (JSTO), [Project No. R.R.0001_07_RD_B].

**Gross Pathology:**  There were no gross lesions attributable to *Y. pestis* infection. Within the omentum, there were three C-shaped pseudosegmented vermiform parasites, each measuring approximately 1 cm in length and 3 mm in diameter.

**Laboratory Results:**  Samples of lung, liver and spleen were collected at necropsy for bacterial culture; these were negative for *Y. pestis*.

**Histopathologic Description:**  Adipose tissue (omentum): At the peripheral margin of the tissue there is a coiled, encysted metazoan parasite measuring approximately 9 mm in length and 2 mm in widest diameter. The cyst wall consists of an eosinophilic to basophilic, 5-10 µm thick, hyaline membranous layer with multifocal undulations; this layer also contains multiple approximately 10 µm wide pore-like openings lined by refractile eosinophilic material. Adjacent to the cyst wall there is a 25-50 µm thick layer of well-vascularized fibrous connective tissue. Occasional infiltrates of low numbers of lymphocytes and plasma cells are located in the adjacent adipose tissue. Features of the metazoan parasite include: 1) a 3-5 µm thick cuticle containing multiple 8-10 µm wide pore-like openings lined by eosinophilic refractive material;
2) multiple annular bands of cuticle and underlying body wall (Figure 5); 3) skeletal muscle; 4) a multicellular digestive tract containing gray-brown granular material within the lumen; and 5) aggregates of large (up to 150 µm diameter) round to oval uninucleate cells containing eosinophilic finely granular cytoplasm that are located adjacent to the digestive tract. In some sections, one end of the parasite (i.e. the head) has 1 to 3 yellow refractile U-shaped to sickle-shaped structures, consistent with cephalic hooks.

**Contributor’s Morphologic Diagnosis:** Omentum; encysted pentastome nymph, with fibrosis and minimal multifocal lymphoplasmacytic inflammation.

**Contributor’s Comment:** The parasites encysted in the omentum of this monkey are incidental findings. Based on the gross morphology and microscopic features of these parasites, they are pentastome nymphs. Characteristic gross morphology includes the “C-shape” and the cuticular annulations which give it
Microscopic features of pentastomes include: a cuticle with sclerotized openings; cephalic hooks; striated muscle; and a digestive tract bordered by deeply eosinophilic “acidophilic glands”. Pentastomes are a unique group of animals, all of which are parasites. The adult parasites inhabit the respiratory tract of vertebrates. More than 100 species have been described and for approximately 90% of these, the definitive host is a carnivorous reptile. However, there is one species, Linguatula serrata, where the adult parasites live in the nasal passages of dogs and other carnivores. Adults in the genus Linguatula have a shape that resembles a mammalian tongue; this is the basis for the genus name and for the common term of “tongue worms” for pentastomes in general.

Each pentastome has two pairs of cephalic hooks adjacent to its mouth. These hooks are used to assist in attachment and burrowing. Early researchers mistook these hooks to be four a pseudosegmented appearance. Microscopic features of pentastomes include: a cuticle with sclerotized openings; cephalic hooks; striated muscle; and a digestive tract bordered by deeply eosinophilic “acidophilic glands”.

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additional mouths; this error is the basis for the name of this group of parasites (i.e., pentastoma).³

Although a few pentastome species have a direct life cycle, the great majority of species utilize intermediate hosts.⁴ Adult females lay eggs containing legged larvae that resemble mites.¹ The larvated eggs are passed into the environment either directly in respiratory secretions or are swallowed and passed in feces. Intermediate hosts become infected by ingestion of food or water contaminated with the eggs. Eggs hatch in the intestinal tract of the intermediate host and the larvae burrow through the intestinal wall. The larvae then develop into nymphs and encyst in the visceras and/or serous membranes of the intermediate host. After the intermediate host is consumed by the definitive host, the nymphs migrate to the respiratory tract of the final host and develop into adults.

A wide variety of animals (including humans) can serve as intermediate hosts for pentastomes. It is very difficult to accurately identify a pentastome species based solely on the morphology of nymphs.⁵ In a report about the massive pentastome nymph infection of a stray dog from the southern United States, the species of pentastome was identified as Porocephalus crotali using a combination of gross parasite morphology and DNA analysis.²

The species of pentastome present in this monkey was not identified. However, the definitive host is most likely a snake or crocodilian that inhabited the native environment of this macaque. In a recent report about a massive pentastome nymph infection of a cynomolgus macaque imported from China, the pentastome nymphs were identified as Armillifer agkistrodontis by DNA analysis.³ Armillifer agkistrodontis adults occur in the lungs of pit vipers in SE Asia and the usual intermediate hosts are small rodents that serve as the prey base for the snakes; the monkey described in the case report was thought to be an accidental, dead-end host.⁵

The parasites present in this WSC case submission elicited mild fibrosis and very little inflammation by its primate host; this is typical, even in cases of massive pentastome infections.²,⁵ However, fatal peritonitis has occasionally been associated with nymph penetration of the intestinal wall.⁶ Dead nymphs cause intense inflammation and may eventually become mineralized.

In humans, three types of histologic lesions caused by pentastomid nymphs have been described.⁷ The first lesion consists of an encysted and viable nymph surrounded by little to no inflammation (as in this monkey). In the second type of lesion, a dead nymph is surrounded by granulomatous and usually eosinophilic inflammation, encapsulated by concentric rings of fibrosis. In the third type of lesion, known as the granulomatous scar or cuticle granuloma, fibrous tissue surrounds a central mass of amorphous and/or mineralized material consisting of remnants of the long-dead parasite.

The cuticle of a pentastome often remains long after the rest of the dead parasite has degenerated. Finding parts of a cuticle with sclerotized openings within a focus of inflammation proves that the lesion was caused by a pentastome because no other parasites have sclerotized openings.³ Visualization of the sclerotized openings can be greatly enhanced with a Movat pentachrome or Masson’s trichrome stain.³,⁷ In this case, the cyst wall also has sclerotized openings. This means that the wall is composed of a molted cuticle from the parasite.

The taxonomic classification of pentastomes was a subject of debate for many years. It has been long thought that they should be classified as arthropods or arthropod-like animals. Based on more recent DNA analyses, it is now generally accepted that pentastomes constitute a unique subclass (i.e., Pentastomida) of crustacean arthropods and are most closely related to the crustacean subclass Branchiura -- species of which are ectoparasites of fish and are commonly known as “fish lice”.⁴

Acknowledgement: The author wishes to thank Dr. Kathleen Cashman for providing photography assistance and the gross photographs.

Note: Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

JPC Diagnosis: Omentum: Encapsulated pentastome nymph.
Conference Comment: The contributor eloquently discusses the history, life cycle and clinical implications of pentastome infections. Routine sectioning and submission for histopathology of an encysted pentastome is likely a rare event due to its gross diagnosis, making this case a rare opportunity to see the organism in such exquisite detail. As mentioned, the observation of sclerotized openings are specific for pentastomes and of diagnostic importance in cases which lack intact specimens and appear as otherwise nonspecific inflammation. These become distinctly dark and readily identifiable with a Movat pentachrome stain, and portions of cuticle can remain in tissue for long after the rest of the parasite has died and been reabsorbed.³

Conference participants discussed the peculiarity of consistently observing the shed cuticle, or exuvia, lining the cystic cavity which encloses the pentastome. The exuvia is never observed floating freely, but rather always tightly adhered to tissue forming the cyst wall. This exuvia is from a previous nymph, and as nymphal stages undergo multiple molts before becoming a mature adult, it is possible the exuvia becomes a protective barrier from the immune defenses of the host. This is supported by the finding that intact cysts with viable nymphs are usually found with little or no adjacent cellular infiltration of the host tissue as antigenic compounds are largely sequestered.⁷

Contributing Institution: US Army Medical Research Institute of Infectious Diseases, Pathology Division, Fort Detrick, MD http://www.usamriid.army.mil/

References:
**CASE III: NCI/MPU 2012-2 (JPC 4019853).**

**Signalment:** Adult woodchuck, *Marmota monax.*

**History:** The tissue derives from a woodchuck killed and field dressed by a hunter in early summer in the state of Maryland. An approximately 4 cm diameter mass was identified in the muscles of the shoulder. The hunter saw similar masses in multiple woodchucks throughout the spring.

**Gross Pathology:** The excised nodule is approximately 4 cm in diameter and encased in thick, fibrous connective tissue. On cut section, numerous fluid-filled cysts ranging from 5 to 20 mm in diameter are separated by a variably thick fibrous stroma. Cysts contain multiple, 0.5-1 mm diameter, white cysticerci that are variably attached to the inner cyst wall or are floating within the cyst fluid.

**Histopathologic Description:** A large, multilocular mass effaces and distorts skeletal muscle. The mass is composed of cystic cavities that contain multiple cysticerci and are surrounded by thick bands of fibrous connective tissue and inflammatory infiltrates. In some areas, there is complete loss of the cyst wall (cyst rupture) with neutrophils, eosinophils, macrophages, multinucleated giant cells, and lymphocytes infiltrating degenerate cysticerci with loss of distinct architecture and variable parenchymal mineralization. Cysticerci measure up to 1 mm in diameter and have a thick tegument that surrounds a large bladder and an inverted neck. They lack a pseudocoleom, a digestive tract, and reproductive organs. The parenchyma of the neck contains numerous calcareous corpuscles, excretory ducts, and an inverted scolex covered by a thick tegument and associated with large muscular suckers. Rostellar hooks are present in few cross-sections. The surrounding mature fibrous connective tissue is infiltrated by many neutrophils, eosinophils, macrophages, and fewer lymphocytes and plasma cells admixed with necrotic cell debris. Surrounding skeletal muscle fibers are in varying stages of degeneration, ranging from swollen, hypereosinophilic myocytes to myocytes with fragmented, flocculent sarcoplasm that lack cross-striations. Few myocytes in the surrounding skeletal muscle are distended by intracytoplasmic apicomplexan cysts containing myriad bradyzoites that measure approximately 5 µm in diameter (*Sarcocyst spp.*, presumptive). Postmortem bacterial overgrowth is prominent.

**Contributor’s Morphologic Diagnosis:**

1. Skeletal muscle: Cysticercosis with eosinophilic, granulomatous myositis, fibrosis, and myodegeneration.

2. Skeletal muscle: Sarcocytosis.

**Contributor’s Comment:** Cysticercosis has been reported several times in woodchucks, most

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3-1. Skeletal muscle, woodchuck: Cut section of the fixed mass taken with a stereomicroscope showing multiple cystic cavities containing numerous small, white cysticerci. (Photo courtesy of: Comparative Molecular Pathology Unit, Laboratory of Cancer Biology and Genetics, Center for Cancer Research, National Cancer Institute; http://ccr.cancer.gov/staff/staff.asp?profileid=8472)

3-2. Skeletal muscle, woodchuck: This section of skeletal muscle contains an unusual concentration of cysticerci. A small amount of atrophic skeletal muscle is present at the top and bottom of the section (arrows). (HE 6.3X)
commonly due to infection with *Taenia crassiceps*. The axillary subcutis is most commonly affected, though other subcutaneous regions may also be affected as well as the peritoneal cavity, thoracic cavity, nasal sinuses, liver, lung, and brain.\(^1\,3\,5\)

Feral woodchucks may bear heavy cysticerci burdens that may be particularly prominent after emergence from hibernation.\(^2\) Laboratory woodchucks may also develop cysticercosis, especially if the animals are of wild-caught origin.\(^1\,3\,5\)

The species of tapeworm affecting this woodchuck is not definitively known. Previously reported cases of woodchuck cysticercosis were consistent with *T. crassiceps* larvae; the fox was considered the likely definitive host in these cases.\(^1\,3\,5\) Morphologic features of this parasite are consistent with *T. crassiceps* and include an anterior scolex with four suckers, an apical rostellum that contains large and small rostellar hooks, and a posterior bladder.\(^1\,4\,5\)

The inflammatory response in this case is relatively mild and composed of a mixed population of inflammatory cells. Inflammation is more intense in areas of cyst rupture. Previous reports of the inflammatory reaction woodchuck cysticercosis describe a similarly mild response with more prominent lymphocytic infiltrates and a similar variability in the degree of reactive fibrosis.\(^1\)

**JPC Diagnosis:** Skeletal muscle: Multiple cysticerci with fibrosis and moderate histiocytic and atrophic rhabdomyositis.

**Conference Comment:** The interesting aspect of this case is its unique presentation. As is consistent with previously reported cases highlighted by the contributor, the cysticerci were found in a focal aggregate in the skeletal muscle of the shoulder.

There are two orders in the phylum *Platyhelminthes* which comprise tapeworms.\(^4\) The order of pseudophyllideans grows into much larger adults, such as *Diphyllobothrium* spp., and never shed their proglottids. This is contrast to the order of cyclophyllideans, as observed in this case, which shed gravid proglottids each containing thousands of infectious eggs. The cyclophyllideans are more readily transmissible and as a result, are the most significant cause of
CNS disease in people in South America. When the eggs are ingested, the larvae migrate into various tissues resulting in significant pathology. Conference participants speculated on what drove these larvae to all migrate to the same location in this woodchuck.

We agree with the contributor that, although *T. craciseps* is the most often reported and most likely species in this case, the presence of an anterior scolex with four suckers and a posterior bladder is only indicative of the type of larval cestodes known as a cysticercus and cannot be differentiated further. However, some references indicate the possibility of species identification based on the length of small and large hooks in the rostellum. Other groups of larval cestodes include the cysticercoids which have a tiny bladder and a scolex surrounded by parenchymous arms, coenurus which has more than one scolex, and hydatid cysts with a bladder and numerous small protoscolices each with a scolex and suckers. Solid-bodied cestodes are plerocercoids (lack suckers) or tetrathyridium (has suckers).

**Contributing Institution:** Comparative Molecular Pathology Unit, Laboratory of Cancer Biology and Genetics, Center for Cancer Research, National Cancer Institute; http://ccr.cancer.gov/staff/staff.asp?profileid=8472

**References:**

CASE IV: 2 (JPC 4048512).

**Signalment:** Juvenile female Springer Spaniel, *Canis familiaris.*

**History:** This was a stray dog found dead on February 2014 and sent for necropsy to evaluate the circumstances of death.

**Gross Pathology:** Mucosae were icteric and severely anemic. In the abdominal cavity, liver was enlarged and fibrotic with severe passive congestion. Abdominal aorta was severely focally dilated (aneurysm) and yellow (jaundice). Elevated numbers of trichurids (whipworms) were observed in the rectum and cecum. In the thoracic cavity, severe pneumomediastinum and pneumothorax was observed. Moderate hemothorax was also present.

Severe, diffuse pleural thickening and fibrinous pleuritis affected all lung lobes. Severe diffuse pulmonary edema and fibrino-necrotizing pneumonia were present. Multifocal areas of necrotizing and gangrenous pneumonia with colliquative necrosis were evidenced on cut section of lung lobes.

Mild pericardial effusion and severe right ventricular and atrial dilation were observed.

**Laboratory Results:** Parasitologic isolation of adult nematodes from lung specimens identified the parasites as *Angiostrongylus vasorum.*

Parasitologic examination of intestinal nematodes identified the species as *Trichuris vulpis.*

**Histopathologic Description:** Lung: Approximately 80-90% of the pulmonary parenchyma (severity of extension varies among tissue sections) is affected by severe degenerative and inflammatory changes involving pulmonary arteries, pulmonary interstitium and to a lesser extent alveolar spaces.

Occasionally, in arterial lumens or embedded in the endoluminal fibrin thrombi there are variable numbers of transverse and longitudinal sections of both viable and degenerated/necrotic adult nematodes associated in some instances with larvae, occasionally with deep basophilic granular material (dystrophic mineralization). Intravascular adult nematodes are approximately 450-550 µm in length and 200-250 µm in diameter, with a smooth eosinophilic 4-5 µm thick cuticle, coelomyarian musculature and a pseudocoelom, in which are detectable tracts of the gastrointestinal tract with an intestine lined by multinucleated cells and of the reproductive male and female tracts, the latter with intrauterine embryonated eggs.

Pulmonary arteries multifocally are occluded by laminated meshwork of pale eosinophilic finely beaded to fibrillar material adherent to the endothelium (fibrin thrombi), occasionally characterized by ingrowth of endothelial cells, smooth muscle cells, fibroblasts and slit-like

![Image 4-1: Lung, dog: Cut sections of lung contained multifocal areas of necrotizing and gangrenous pneumonia with colliquative necrosis. (Photo courtesy of: C DIVET- School of Veterinary Medicine, Milano – Italy, http://www.anapatvet.unimi.it/)](image1)

![Image 4-2: Lung, dog: Subgross of submitted slides. The one at top has a large area of suppuration and necrosis, likely second to an infarct. The larger section at the bottom has a large thrombosed artery and extensive hemorrhage. (HE 6.3X)](image2)
clefts (capillary channels; thrombus organization and recanalization) and extravasated erythrocytes (hemorrhages). Some arterial lumens contain necrotic debris and moderate numbers of both viable and degenerated (karyorrhectic) neutrophils (thrombus dissolution). Multifocally, arteries have medial hypertrophy/hyperplasia with intima bulging within the vascular lumen (luminal narrowing) and endothelium lined by plump reactive endothelial cells (proliferative endarteritis). The walls of pulmonary arteries are multifocally expanded by extravasated erythrocytes (intramural hemorrhages) or by bright eosinophilic amorphous material (fibrinoid necrosis), in which are embedded degenerated neutrophils (leukocytoclastic vasculitis) with disruption of the wall.

Multifocally expanding alveolar septa, peribronchial interstitium and filling alveolar lumens there are nematode eggs and larvae, or more rarely on adult nematodes. Eggs are round to oval, 40-50 µm in diameter, filled with eosinophilic granular material and containing a single basophilic often eccentric, 10 µm in diameter nucleus (nonembryonated eggs) or are oval, 150x50 µm and are multinucleated (embryonated eggs). Larvae are 150x50 µm and are composed of numerous, round, 4-6 µm in diameter, basophilic nuclei with scant eosinophilic cytoplasm and a smooth 1 µm wide amphophilic cuticle.

Larvae and eggs are occasionally free in the alveoli but more frequently are surrounded by inflammatory cells composed by a prevalence of epithelioid macrophages, foamy reactive macrophages, occasionally containing golden granular material (hemosiderin), and by lesser numbers of multinucleated giant cells with up to 7 haphazardly arranged nuclei (foreign body-type) and rare eosinophils and granulocytes. Granulomas are multifocally surrounded by dense fibrous tissue.

Multifocally the lung is characterized by locally extensive areas of coagulative necrosis (pulmonary infarcts) associated often with hemorrhages and/or hematoidin (chronic hemorrhages). Alveoli not affected by the inflammatory process are characterized by edema, rupture of alveolar septa and blunted clubbed ends (alveolar compensatory emphysema) or atelectasis. Diffusely, alveolar capillaries are engorged by erythrocytes (alveolar septa hyperemia).

In some of the examined slides are also detectable:
intrabronchial and intrabronchiolar nematode larvae, admixed with sloughed epithelial cells and a moderate amount of eosinophilic cellular and karyorrhectic necrotic debris;
bronchioles lined by a multifocally ulcerated epithelium, with denuded lamina propria;
-thickening of the pleura, expanded up to 8-10 times normal by collagen bundles (fibrosis);
-alveolar septa lined by plump type II pneumocytes (type II pneumocytes hyperplasia).

Contributor’s Morphologic Diagnosis: 1. Pulmonary multifocal to locally extensive necrosis (pulmonary infarcts) and hemorrhage with multifocal occlusive arterial thrombosis with variable numbers of adult nematodes, larvae and eggs and multifocal moderate subacute proliferative endoarteritis. 2. Multifocal to coalescing severe chronic granulomatous and eosinophilic pneumonia, with intralesional nematode adult, larvae and eggs, with multifocal severe subacute pulmonary coagulative necrosis (infarcts), hemorrhages and hemosiderosis.

Contributor’s Comment: The cause of death in this case was ascribed to severe massive pulmonary thromboembolism (acute and chronic) with pulmonary infarction, locally extensive necrotizing and gangrenous pneumonia, all findings associated with vascular occlusion by *A. vasorum* adults.

In the first part of 2014 (February, March, April), an increased number of dogs (at least one every week) were referred for necropsy at our institution and were diagnosed with massive pulmonary nematodiasis, with parasitological identification of *A. vasorum*. Italy is considered one of the European countries where this nematode is spreading rapidly.7 Northern Italy this year has been, for the most part, a warm winter with mild temperatures that have caused an increased survival/vitality rate of intermediate hosts and larvae of *A. vasorum*.

An increased emergence of canine heartworms and lungworms has been reported in Europe. This increase may have many drives including global warming, changes in vector epidemiology and movement of animal populations.19 Survival rates of *A. vasorum* L1 larvae have been demonstrated to be influenced by temperatures and temperatures over 10° celsius influencing positively larval development into infective L3 stage larvae.8,9 Also, larval burdens within slugs are highly dependent on temperatures.9

Additional risk factors in dogs include age (higher risk in younger dogs), season (more cases earlier in the calendar year), and worming history (lower risk if given milbemycin oxime in the past 12 weeks).13 No history was available in this dog; however, young age and the time of year (February) were compatible with these observations.

*Angiostrongylus vasorum*, French heartworm, is a metastrongyloid parasite found in the pulmonary
arteries and right ventricle of wild and domestic canids and various other animal species. The natural definitive hosts are foxes. The geographic distribution of the parasite includes various countries of Europe, Africa, South America, and North America. In North America, autochthonous A. vasorum infection occurs only in the Canadian province of Newfoundland-Labrador. Angiostrongylosis is considered an emerging disease in dogs in Europe and North America. At present, angiostrongylosis is considered endemic in parts of France, southwestern England, Ireland, Denmark, Italy, Spain, Germany, Hungary, Finland, Switzerland, and Turkey, in South America in Brazil and Colombia, and in Uganda. However, in the last decade, a relatively new endemic focus of A. vasorum infection has emerged in eastern Canada, in the provinces of Newfoundland and Labrador. The reasons of increased emergence are little known but many drivers such as global warming, changes in vector epidemiology and movements in animal populations may be taken into account.

Clinical signs associated with angiostrongylosis may be diverse and a range of signs is described in the above reports. However, two prevalent clinical syndromes predominate; respiratory disease caused by the inflammatory response to eggs and migrating larvae, and hemorrhagic diatheses manifesting as local or diffuse haemorrhages. Clinical features include foraging on infected feces or on contaminated plant material. In the intermediate host, the larvae mature and develop into second-stage and third-stage larvae (L2 and L3). The final host, usually a fox or dog, becomes infected by eating an intermediate host containing L3. The slug or snail is digested within the stomach or intestine of the canid, releasing L3 that penetrate the gastrointestinal tract wall, migrate to visceral lymph nodes, and eventually develop into immature adults. The juvenile worms migrate via portal circulation to the liver, caudal vena cava, the right atrium, right ventricle, and pulmonary arteries where they reach maturity approximately 33 to 35 days post-infection. The prepatent period for A. vasorum is approximately 38 to 57 days.
Cardiorespiratory (63%), coagulopathic (71%) and other (63%) signs. Cough, dyspnea and tachypnea have been reported as the most common cardiorespiratory abnormalities. Of animals with evidence of coagulopathy, excessive hemorrhage from wounds, airway hemorrhages, epistaxis, hematomas, haemarthrosis, and hematuria have been reported. Vague signs of exercise intolerance, lethargy and weakness may be present.

Most cases develop chronic heart failure, but acute interstitial pneumonia occurs in heavily infected dogs. Pulmonary arterial hypertension seems to develop rarely despite the severe lung lesions in chronic cases. Other uncommon manifestations include disseminated intravascular coagulation, uveitis in response to aberrant migration to the anterior chamber of the eye, hemotherax and hemoabdomen.

Acute neurological signs caused by hemorrhages in the central nervous system (brain and/or spinal cord) have been reported. In these cases, results of coagulation assays are inconsistent for certain authors while others find consistently coagulopathy and associated pulmonary hemorrhages.

Neurological signs reflect the site of lesions and may include seizures, various cranial nerve deficits, vestibular signs, proprioceptive deficits, ataxia and paraplegia. Thoracic radiographs (n = 19) identified abnormalities in 100% of cases. A variety of changes were observed, the most typical being a patchy alveolar-interstitial pattern affecting the dorso-caudal lung fields.

Dogs may exhibit increased total white blood cell counts with transient neutrophilia and eosinophilia. An increase in serum globulins and a decrease in serum fructosamine have also been found. Variably severe anemia (hematocrit ≤ 0.20 L/L) and thrombocytopenia characterize dogs with hemorrhagic diatheses. Prolonged prothrombin time and/or activated partial thromboplastin and BMB values have been observed.

The mechanism behind the coagulation defects is poorly understood but most authors have suggested that antigenic factors secreted by the parasites cause excessive intravascular coagulation and a consumptive coagulopathy. Dogs with neurological signs may demonstrate high concentrations of protein and evidence of erythrophagia in the cerebrospinal fluid.
Lesions during the prepatent period are mild. Adult, 14-21 mm long worms are present in the pulmonary arteries, and the lungs contain a few 1-2 mm red nodules, consisting of aggregates of eosinophils and mononuclear cells. More severe lesions develop at the time of patency, including proliferative endoarteritis in response to the adult worms in the pulmonary arteries, and granulomatous to pyogranulomatous pneumonia as a consequence of embolized eggs and larvae. Arterial lesions include thrombosis, thickening of the tunica intima by fibromuscular tissue, medial hypertrophy, and lymphoplasmacytic aggregates in the adventitia. Pulmonary lesions consist of red or golden-brown, nodular or confluent areas of hemorrhage, edema, and firmness at the periphery of the lung. Histologically, coalescing granulomas formed by macrophages and giant cells are centered on parasite eggs and larvae in the lung are present with variable association with necrosis, neutrophils and eosinophils. There is mild proliferation of type II pneumocytes, alveolar hemorrhage, hemosiderin-laden macrophages, and arteriolar thrombosis. Fibrosis and recanalization of arterial thrombi develop as the lesion ages. Similar granulomas developing around dead or degenerating worms have been reported in brain, kidney, and various tissues. Foci of granulomatous interstitial pneumonia can often be found when worm remnants may no longer be identified. Central nervous system hemorrhages have also been observed. Definitive ante-mortem diagnosis is by detection of L(1) in feces, sputum, or bronchoalveolar lavage samples. Baermann fecal examination is the most reliable method for fecal detection. However, a false negative result can occur due to the typical erratic/sporadic fecal larval shedding pattern of A. vasorum. Furthermore, this method has been reported to have low sensitivity. Only recently has an increased repertoire of techniques for A. vasorum infection diagnosis been reported, such as detection of L1 in broncho-alveolar lavage fluid and the development of a sandwich ELISA to detect circulating antibodies and worm excretory/secretory antigens.

Diagnosis has been achieved by testing fecal samples and blood of foxes and domestic dogs by PCR. Definitive diagnosis requires extraction of intact adults from the lungs, as the histologic appearance is similar to Andersonstrongylus milksi. Angiostrongylus can be differentiated from Dirofilaria by examination of intact adults, or by histologic examination. Angiostrongylus has thin coelomyarian musculature, a large, strongyloid intestine composed of few multinucleate cells, and eggs in the uterus. The females have a "barber-pole" appearance due to the helically arranged red gut and white ovaries. The larvae in the lungs are wider and more developed than the microfilariae of Dirofilaria. In contrast, Dirofilaria has well-developed coelomyarian musculature, a smaller intestine, and a uterus containing microfilariae.

Current treatment options include moxidectin, milbemycin oxime, and fenbendazole. However, killing the worms with an anthelmintic may incite severe response.

**JPC Diagnosis:** Lung: Pneumonia, interstitial, granulomatous, chronic, multifocal, marked, with severe arterial hypertrophy, villar endarteritis, thrombosis, and numerous nematode adults, larvae and eggs.

**Conference Comment:** With the multitude of lesions present, this case provides an exercise in delivering a thorough histopathologic description. In addition, the contributor’s exhaustive review of pathogenesis highlights the important elements of this emerging disease. The details of this case serve as a reminder of resilience of these parasites despite effective anthelmintics and adequate medical infrastructure.

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**References**