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Department of Veterinary Pathology
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Conference Moderator:
Edward Stevens, DVM, Diplomate ACVP

CASE I: 2/09 (AFIP 3134519).

Signalment: 12-year-old female, mixed breed dog (*Canis familiaris*).

History: The dog was kept in a kennel and died spontaneously overnight.

Gross Pathology: Serous fluid was present around the nares and mouth. There was moderate pleural serous hemorrhagic effusion and severe consolidation of the lung lobes. Two adult filariae were noted in the right cardiac chambers. No other lesions were found in the abdominal cavity or trachea.

Laboratory Results: Adult nematodes were 18.5 and 23 cm in length and were brought to the parasitology laboratory where they were identified as *Dirofilaria immitis* adults.

Histopathologic Description: The pulmonary interstitium is expanded and partially effaced by multifocal to coalescing granulomatous foci associated with multifocal hemorrhages. The inflammatory foci are composed of macrophages, multinucleated giant cells (foreign body type), fibroblasts, lymphocytes and plasma cells, which are arranged concentrically around myriad parasitic larvae and eggs (consistent with those of *Angiostrongylus vasorum*). Larvae are elongated with a thin eosinophilic cuticle and a primitive intestinal tract. T he thin-walled eggs are ovoid, 50-60 µm in diameter, and contain either a morula or larva. Numerous *A. vasorum* larvae are present in the bronchial lumina, intermixed with few histiocytes and multinucleated giant cells, sloughed epithelial cells and abundant mucus.

Focally, a pulmonary artery is severely dilated with the tunica intima thickened by moderate amount of fibrous connective tissue and few lymphocytes and plasma cells. Within the lumen there is a cross section of an adult, 0.5-1 mm in largest diameter (varies among sections and not present in all the sections), degenerate nematode with a thin eosinophilic cuticle, lateral cords, tall coelomyarian/polymyarian musculature, and a small intestine (consistent with a *Dirofilaria immitis* adult). Additionally in some sections, within this vessel and in an adjacent small artery, there are one to three tranverse sections of a smaller degenerate nematode (0.1-0.25 mm in largest diameter) characterized by a very thin coelomyarian musculature and a variably evident large strongyloid intestine; one nematode has a uterus. Intravascular nematodes are surrounded by thrombi, which focally adhere to the vessel walls. The thrombus is composed of fibrillar eosinophilic material (fibrin) that is partially organized in fibrous connective tissue containing multiple small blood-filled channels (organization and recanalization). Within the thrombi and in some vessel lumina there are occasional nematode larvae, consistent with *Dirofilaria immitis* microfilariae. The tunica media of numerous pulmonary arteries is markedly thickened (smooth muscle hypertrophy).

Contributor's Morphologic Diagnosis: 1. Lung: severe, multifocal to coalescing, chronic, interstitial granulomatous pneumonia with intralesional nematode eggs and larvae.
2. Lung, pulmonary arteries: severe, focal, chronic, proliferative endarteritis with thrombus formation and an intralesional nematode adult and larvae.

Etiologies: 1. *Angiostrongylus vasorum*
2. *Dirofilaria immitis*

Contributor's Comment: Lesions were consistent with a double parasitic infection. Adult *D. immitis* and *A. vasorum* were present in the pulmonary vasculature (not in all sections) and myocardium. Most lesions of the pulmonary interstitium were attributed to *A. vasorum* laying morulated eggs in the alveoli in this case. Adults of *A. vasorum* are smaller than those of *D. immitis*. *D. immitis* adults have evenly-spaced lateral internal cuticular ridges, thicker, very well-developed coelomyarian musculature, and a smaller intestine than that of *A. vasorum*. These are the main useful morphologic features for differentiating the two parasites in dogs.(4,7) Adults of both *D. immitis* and *A. vasorum* live in the pulmonary arteries and right heart; however, *D. immitis* adult female worms are larger, are viviparous, and release microfilariae into the bloodstream.(7)

The first, second, and early third larval stages of *D. immitis* are obligate parasites of mosquitoes of the genera *Aedes*, *Culex*, and *Anopheles*. After maturation, larvae migrate into the cephalic spaces of the mosquito head and enter soft tissues of the new host when the mosquito feeds. The larvae reach the right ventricle 3-4 months after entering definitive host. Adults may live for years and microfilariae as long as 2.5 years. Clinical signs of canine dirofilariasis, such as cough and exercise intolerance, are related to cardiovascular dysfunction that may progress to congestive heart failure. The disease is seen more frequently in dogs older than 5 years, and clinical signs roughly correlate with the severity of infection. However, clinically normal dogs may harbor up to 30 worms. Adult heartworms are generally found in the pulmonary arteries and right ventricle; however, they may locate in vena cava or left ventricle in heavy infestations (i.e. 50 or more worms). The caudal lobar pulmonary arteries are generally most severely affected. Right heart failure may be caused by pulmonary hypertension due to vascular lesions.(7)

Immature and mature adult worms induce endarteritis with infiltration of eosinophils and neutrophils. Vascular lesions seem more severe in the presence of *Wolbachia*, a bacterial organism harbored by *D. immitis*.(5) The initial inflammation is followed by myointimal proliferation at sites of direct contact with worms; this reaction to chronic irritation is likely mediated by platelet-derived growth factor (PDGF). The lesions are followed by fibromuscular vascular wall hyperplasia. Pulmonary thromboembolism of viable or dead filarids (e.g. after therapy) may worsen hypertension, due to granulomatous interstitial inflammation elicited, and by occlusion of pulmonary vessels. However, pulmonary infarction seems an uncommon event.

In the pulmonary parenchyma, the most common microscopic findings associated with *D. immitis* infection are arterial thrombosis and periarterial granulomatous inflammation.(7) Additional complications of heartworm disease include progressive chronic right heart failure with chronic passive congestion of the liver and occasional ascites. The vena caval syndrome is usually seen in young dogs with large numbers of adult worms that fill the right atrium and the vena cava, most likely as a result of retrograde migration from pulmonary arteries. In these instances, dogs develop sudden weakness, anorexia, bilirubinuria, hemoglobinuria and anemia. Shock derives from venous obstruction and decreased venous return. A common renal lesion associated with *D. immitis* infection is a membranoproliferative glomerulonephritis derived from the deposition of immune complexes from the circulation or formed *in situ*. The immune response leading to immune complex formation may be elicited by adults, immature adults, and microfilariae.(10) The most frequent ultrastructural lesions observed in kidneys of affected dogs are thickening of the glomerular basement membrane zone, presence of dense deposits in the glomerular basement membrane, and effacement of foot processes. In some dogs, electron dense deposits may be seen in the mesangium with expansion of mesangial matrix.(10)

Cats may become infected with *D. immitis* and are typically microfilaremic or afilaremic due to the low number of adults (often one) and high frequency of filarial male-only infection. Cats may develop cough and dyspnea, vomiting, and neurological signs, or sudden death may be the only event. A significant decrease in pulmonary intravascular macrophage activity in cats with *D. immitis* infection has been identified.(3)

Dirofilaria sp. may occasionally infect man.(9,11,13) The infection in America is more commonly attributed to *D. immitis*, while in Europe, pulmonary dirofilariasis is most commonly attributed to aberrant migration of *D. repens*.(11) There is no clear explanation for this geographical selective distribution. The distribution of reported human cases seems to reflect the prevalence of the disease in the canine population in different areas of the United States (13). These filarids induce a focal to multifocal pulmonary granulomatous reaction termed "coin lesions."(9,13) Coin lesions are the end-stage tissue result of the parasite's death in the vascular bed and the stimulation of a

granulomatous reaction. Although the disease still seems rare in humans, serology should be performed in regions where *D. immitis* is highly enzootic. *D. immitis* coin lesions present significant differential diagnostic problems since they may resemble the lesions of tuberculosis, fungal infections, or neoplasia.(13)

Angiostrongylus vasorum can be differentiated from *D. immitis* by histopathology, as described above, or by examination of intact adults. Intact adult *A. vasorum* females have a "barber-pole" appearance due to the helically-arranged red digestive tract and white ovaries. *A. vasorum* has a thin coelomyarian musculature, a large strongyloid intestine composed of few multinucleated cells, and a uterus with eggs.(2,4)

A. vasorum, termed "French heartworm" because it was reported first in France in the 1800s, is a metastrongylid nematode considered to be the most pathogenic lungworm of dogs.(1,12) Adults reside in the pulmonary artery and right heart ventricle in canids. Red foxes are the natural definitive hosts and are important reservoirs of infection for domestic dogs. Clinical signs may vary from mild coughing and exercise intolerance to fatal cardiopulmonary disease. *A. vasorum* has a worldwide distribution and the infection seems to be increasing in recent decades.(12) *A. vasorum* has an indirect life cycle in which aquatic and terrestrial snails serve as intermediate hosts.(1) Adult female nematodes shed eggs that are transported to the pulmonary parenchyma. Eggs develop and hatch, releasing first stage larvae that penetrate the alveoli. Larvae are coughed, swallowed, and excreted in the feces. Gastropods become infected with L1 larvae by eating contaminated plant material. In gastropods, L1 mature into L3 larvae. Final hosts become infected by ingestion of snails. Third stage larvae penetrate the gastrointestinal tract, migrate through visceral lymph nodes, and develop into immature adults. Juvenile worms migrate into the caudal vena cava trough the portal circulation and reach maturity in the pulmonary arteries.

A. vasorum may cause right heart failure and extensive pulmonary lesions as a consequence of egg embolization. Gross lesions consist of small, 1-2 mm diameter, red, firm, multinodular to confluent areas of hemorrhage and edema at the lung periphery. A variably severe, multifocal to coalescing, granulomatous to pyogranulomatous interstitial pneumonia with presence of eggs and larvae has been reported, often associated with prominent pulmonary arterial thrombosis. Vascular lesions are characterized by proliferative endarteritis, including thrombosis, thickening of tunica intima by fibromuscular tissue, and medial hypertrophy with infiltration of eosinophils, lymphocytes and plasma cells. Fibrosis and vascular recanalization of arterial thrombi may be seen in chronic cases. Granulomas have also been reported to occur in lymph nodes, brain, kidneys, and adrenal glands.(1,2)

The genus *Angiostrongylus* includes other important parasites, such as *A. cantonensis*, the rat lungworm. This parasite has an obligate neural migration cycle that may cause ascending paralysis and lumbar hyperalgesia in accidental hosts, such as dogs.

The differential diagnosis for pulmonary parasitic infections in dogs includes *Crenosoma vulpis, Eucoleus aerophilus* (formerly *Capillaria aerophila*), *Oslerus osleri* (formerly *Filaroides osleri*)*, Filaroides hirti and Andersonstrongylus milksi* (formerly *Filaroides milksi*).(2) *C. vulpis* infects bronchioli, bronchi and the trachea of wild canids and occasionally domestic canids, leading to chronic coughing.(2,12)

Eucoleus aerophilus is a trichurid nematode that parasitizes wild carnivores, domestic cats, and rarely dogs. The adult lungworms live embedded in the respiratory lining epithelium. Most infections are inapparent; more severe infection may result in catharral inflammation, coughing, secondary bacterial infection, and/or airway obstruction. The adult nematodes are slender, 2-3 cm long, and characterized by presence of bacillary bands (segmental thickenings of the hypodermis), a stichosome (basophilic esophageal gland), and embryonated eggs in the uterus. Eggs are oval with bipolar plugs; their morphology allows differentiation from other nematodes. Females lay eggs where larvae undergo initial development. Eggs are swallowed and are released via the feces. Animals become infected by ingesting embryonated eggs or earthworms bearing embryonated eggs. Hatched larvae migrate to the lungs, where they become adults in 3 to 6 weeks.(2,12)

Oslerus osleri is commonly reported in wild canids.(2) The infection is generally asymptomatic. The parasite induces the formation of single to multiple, 1-10 mm diameter, firm, submucosal nodules in the trachea and bronchi that are most prominent at the tracheal bifurcation. Coiled worms may be grossly visible trough the overlying mucosa. Dead worms may cause a granulomatous to pyogranulomatous reaction. Nodules are circumscribed by fibrous tissue and contain adult parasites or fifth stage larvae. Diagnosis is based on adult worm identification by histology or from crush preparations of tracheal nodules. The adults have coelomyariam musculature, an intestine composed of few multinucleated cells with indistinct microvilli, and embryonated eggs or larvae in the uterus.

Filaroides hirti has been reported in colonies of laboratory beagles with cases described in pet dogs.(2) *F. hirti* has a direct life cycle wherein infective first stage larvae are passed in the feces. Most infections are apparently acquired from the dam. Adults live in the alveoli and respiratory bronchioles. Mostly incidental findings at necropsy, lesions are 1-5 mm diameter gray-tan to black-green nodules in subpleural regions of lungs. Nodules may be white or have cystic centers. Rarely, fatal cases occur in immunosuppressed dogs; in these cases, a severe granulomatous pneumonia has been reported. Histopathology consists of minimal inflammation in response to live adult worms; however, dead parasites induce severe interstitial granulomatous and eosinophilic inflammation.

Andersonstrongylus milksi is a metastrongylid nematode. The literature regarding this parasite is confusing (2) and identification of the adult is necessary to distinguish *A. milksi* from *F. hirti*. Adults inhabit bronchioles and alveoli and the gross and histologic lesions are similar to those caused by *F. hirti*. Larvae have been reported in the brain and internal abdominal organs.

AFIP Diagnosis: 1. Lung, arteries: Endarteritis, proliferative, chronic, multifocal, marked, with organizing thrombi and few intravascular adult metastrongylid and filarid nematodes, etiology consistent with *Angiostrongylus vasorum* and *Dirofilaria immitis*.
2. Lung: Pneumonia, granulomatous, multifocal coalescing, marked, with hemorrhage, fibrosis, and many nematode larvae and eggs.

Conference Comment: This case was reviewed in consultation with Dr. Christopher Gardiner, Consulting Parasitologist for the AFIP's Department of Veterinary Pathology. Much of the discussion during the conference was devoted to the morphological features that distinguish *A. vasorum* from *D. immitis*, as described in the contributor's exceptionally complete review of the entities above. Unlike true strongyles and trichostrongyles, which have platymyarian musculature, metastrongyles, such as *A. vasorum*, have coelomyarian musculature; however, it is greatly attenuated in comparison to the robust coelomyarian musculature that typifies *D. immitis*. As compared to *A. vasorum*, *D. immitis* possesses a much thicker cuticle with distinct lateral internal cuticular ridges, and a much smaller intestine. Finally, the developing and mature larvae in the lungs are readily distinguished from microfilariae by having more developed internal structure; microfilariae, which were not noted in any conference participants' sections, lack internal structure and are therefore often described as a cuticle-bound bag of nuclei.(4)

Conference participants reviewed the classic immune response to invasive nematodes, in which a T_H2 response generally predominates. Briefly, cytokines produced by cells of the innate immune system in response to microbes activate and direct the differentiation of helper T cells in the adaptive immune system. In response to invasive nematodes, interleukin (IL)-4 initiates the differentiation of naïve T cells into T_H2 cells. This differentiation requires the lineage-specific transcription factors GATA3 and c-Maf; differentiated T_H2 cells characteristically produce IL-4, IL-5, and IL-13. Interleukin-4 acts on B cells to stimulate class-switching to IgE, which mediates the cross linking of Fc receptors on mast cells and their activation. Additionally, IL-4 further amplifies the differentiation of T_H2 cells in an autocrine loop, and inhibits the differentiation of T_H17 cells, potent recruiters of neutrophils and monocytes involved in the host defense against extracellular bacteria and fungi. Interleukin-5 activates eosinophils, and IL-13 enhances IgE production, stimulates mucus production by epithelial cells, and stimulates the synthesis of proline, an important constituent of collagen, thereby stimulating fibrosis.(6,8) Conference participants noted the paucity of eosinophils present in this case and speculated on possible explanations, such as glucocorticoid administration or immunosuppression.

Contributor: Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Sezione di Anatomia Patologica e Patologia Aviare, Facolta' di Medicina Veterinaria, Via Celoria 10, 20133 Milano, Italy
http://www.anapatvet.unimi.it/

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CASE II: 09-11870-9 (AFIP 3135619).

Signalment: 5-year-old male, castrated domestic shorthair cat (*Felis catus*).

History: This cat had a history of fever and clinical signs consistent with acute liver failure.

Gross Pathology: Not provided.

Histopathologic Description: Duodenum with pancreatic duct: Diffusely, the villi are short, clubbed, regionally fused, and lined by non-ciliated attenuated epithelium. There is a segmental loss of crypts and the lamina propria within these attenuated areas is collapsed and contains karyolytic and karyorrhectic nuclear debris admixed with small numbers of lymphocytes, plasma cells, and rare macrophages and neutrophils. Crypts are occasionally collapsed and their lumina contain sloughed epithelium admixed with necrotic cellular debris (crypt abscess). Occasionally the crypts are dilated and are lined by attenuated to disorganized hyperplastic epithelium characterized by 2-3 layers of nuclear piling and increased numbers of mitotic figures. Often macrophages contain multiple, intracellular 2-4 um, round basophilic, intracytoplasmic protozoal zoites. Similar individualized tachyzoites are present free within the lamina propria. The underlying submucosa is similarly infiltrated by moderate numbers of lymphocytes, plasma cells, and macrophages.

Pancreas: Rarely the endocrine islets are effaced and the cells are individualized by a homogenous, pale, eosinophilic material (amyloid).

Immunohistochemistry for parvovirus was positive in the duodenum. Immunohistochemistry for *Toxoplasma gondii* was positive in the duodenum, lung, liver, spleen, and mesenteric lymph nodes. The liver, spleen, and mesenteric lymph nodes are not submitted.

Contributor's Morphologic Diagnosis: 1. Duodenum: duodenitis, lymphohistiocytic, diffuse, with crypt necrosis and crypt abscesses, epithelial loss and regeneration, collapse of lamina propria, and protozoal tachyzoites.
2. Pancreas: amyloidosis, islet, mild.

Etiologies: Feline panleukopenia virus and *Toxoplasma gondii*

Contributor's Comment: The histologic findings and positive IHC within the duodenum are consistent with feline panleukopenia virus (FPV) and concurrent infection with *Toxoplasma gondii*. Parvoviruses are single stranded DNA viruses. Unlike papillomaviruses, which are double stranded DNA viruses and possess DNA polymerase and genes for inducing mitosis, parvoviruses do not possess DNA polymerase and other genes for inducing mitosis;

therefore, parvovirus infects only actively dividing cells, particularly in the S (synthetic) phase of cell division. The enteric, lymphoid, and hematopoietic systems are predominantly affected. Large intranuclear inclusions can sometimes be seen within infected cells.

The mode of FPV exposure is typically oronasal, allowing the virus to infect the tonsillar epithelium. Free virus released into the lymph as well as dissemination of infected lymphocytes results in infection of other lymphoid organs, such as the thymus, spleen, Peyer's patches, and bone marrow. Lymphocytolysis results in lymphopenia as well as viremia. The enteric system and bone marrow are infected following viremia and by circulating infected lymphocytes. Destruction of crypt cells results in villus atrophy and mucosal erosion or ulceration. Infection of the bone marrow causes depletion of myeloid and erythroid precursors. The lymphopenia and secondary bone marrow destruction can result in significant immunosuppression, allowing the animal to become susceptible to normally harmless infectious agents.

Although infection with *Toxoplasma gondii* typically results in seroconversion without clinical disease, immunosuppression due to concurrent FPV infection can allow the protozoa to disseminate systemically, resulting in necrotizing lesions in a multitude of organs, such as the liver, heart, spleen, eye, or brain. Cats are easily infected by ingestion of tissues containing the latent asexual stage (bradyzoite) in latently infected prey animals. Another possible but more difficult route of infection of cats is by direct transmission from ingestion of oocysts passed in the feces of another cat (after exposure to oxygen and time for sporulation).

Once *Toxoplasma* organisms penetrate the intestinal mucosa, the protozoa can spread via lymphocytes to regional lymph nodes, lymph, and the bloodstream, or from the intestine they may pass directly into the portal circulation, allowing dissemination to a variety of organs. Tachyzoites invade (or are phagocytosed by) host cells, proliferate and spread to neighboring cells, causing lysis of host cells upon egress.

AFIP Diagnosis: 1. Small intestine, duodenum: Enteritis, necrotizing, diffuse, moderate, with crypt regeneration, few crypt abscesses, and many intracellular and extracellular protozoal tachyzoites.
2. Pancreas, endocrine: Islet amyloidosis, focal, mild.

Conference Comment: There is substantial slide variation with respect to the severity of islet amyloidosis in this case. As noted by the contributor, the presence of disseminated toxoplasmosis in an adult cat should trigger an investigation for a cause of immunosuppression. In this case, most conference participants appropriately suspected underlying immunosuppression, and considered feline panleukopenia virus (FPV) and feline leukemia virus (FeLV) as the most likely etiologies. During the conference, attendees discussed the histomorphologic features used to distinguish these entities.

This case is unique in that FPV classically affects younger animals after maternal antibodies have waned. Gross lesions of FPV are most consistently discovered in the thymus (e.g. marked involution and reduced mass in young kittens) and intestines (e.g. dry, nonreflective serosa; mucosal, submucosal, and/or muscular petechiae; and segemental dilation and/or increased turgidity). Because the gross intestinal lesions may be subtle, histopathology is indispensable to achieve a definitive diagnosis of FPV enteritis. Microscopic lesions are most consistently found in the intestines, bone marrow, and lymphoid organs. As noted by the contributor, the rapidly dividing crypt epithelial cells are chiefly affected, and early in the course of infection may contain intranuclear inclusion bodies, particularly within cells sloughed into crypt lumina. The likelihood of visualizing inclusions is increased if tissues are fixed in Bouin's solution rather than formalin.(1) Villar enterocytes, which are not actively dividing, are not affected; in the present case, necrosis of villar epithelium is attributed to toxoplasmosis, and crypt regeneration is attributed to FPV.

Because FeLV can cause crypt necrosis in some cats, distinguishing it from FPV depends on examination of the lymphoid and hematopoietic tissues. In early FPV, lymphocytolysis is characteristic in lymphoid organs such as the thymus, lymph nodes, and Peyer's patches; by 7-8 days post-infection, regenerative lymphoid hyperplasia may instead be found. Similarly, early bone marrow lesions include marked depletion of all cell line precursors; this results first in neutropenia, because of the relatively short circulating half-life of neutrophils, followed next by lymphopenia, then finally thrombocytopenia. Bone marrow changes later progress to marked stem cell hyperplasia. In FeLV, by contrast, lymphoid hyperplasia, rather than lymphocytolysis, is typical of early disease. Additionally, enteric FeLV is characterized by associated mononuclear cell inflammation in the mucosa, whereas the enteric lesions of FPV infection contain a paucity of inflammatory cells. Antemortem hematology may also be helpful in distinguishing FPV from FeLV; the former elicits panleukopenia, as suggested by its name and as described above,

while the latter results in a nonregenerative anemia. Finally, the detection of viral antigen by immunohistochemistry, as employed in this case, is also useful for distinguishing enteric FeLV from FPV.(1)

Conference participants briefly reviewed several other parvoviruses of importance in veterinary medicine, including canine parvovirus (CPV)-2; canine minute virus (CPV-1); mink enteritis virus; Aleutian mink disease virus; bovine parvovirus; porcine parvovirus; and the parvoviruses of rats, mice, and Syrian hamsters. Receptor binding to the transferrin receptor TfR determines the host ranges of FPV and CPV infection.(1)

In most domestic animal species, with the possible exception of placentitis and abortion in sheep and goats, infection with *Toxoplasma gondii* only rarely causes overt disease. In immunocompetent hosts, cell-mediated immunity, dominated by a T_H1 response, is sufficient to maintain the organism as quiescent tissue cysts, which are non-pathogenic. Systemic toxoplasmosis is generally only seen in neonates or immunocompromised hosts, such as the cat in this case. Specifically, decreased levels of interferon-γ, and resultant impaired ability to activate macrophages, are associated with increased susceptibility to systemic disease.(1) During the conference, the moderator drew parallels between this case and canine toxoplasmosis secondary to canine distemper virus infection. For another example of toxoplasmosis and more detailed review of the entity, readers are referred to WSC 2008-2009, Conference 1, case I.

Contributor: University of Illinois College of Veterinary Medicine, Pathobiology Department, 2522 VMBSB, 2001 South Lincoln Avenue, Urbana, IL 61801
http://vetmed.illinois.edu/path/

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CASE III: 08 B 14569 10 (AFIP 3138188).

Signalment: 10-month-old, Angus-cross steer (*Bos taurus*).

History: These calves were purchased as stocker calves between 6 and 9 months of age. The calves were being fed a ration composed of millet hay, wet beet pulp, and whole corn, with access to protein blocks containing monensin. At 10 months of age, seven calves demonstrated abrupt onset of neurological signs, including behavioral changes (apprehension and aggression), staggering, muscle twitching/fasciculation, recumbency, and opisthotonos, followed shortly by death. Several calves were treated with broad-spectrum antibiotics, thiamine, calcium gluconate, and a variety of corticosteroids with no response. Three calves were examined postmortem by the referring veterinarian and samples were collected on two calves for histopathology and associated testing.

Gross Pathology: Per the submitting veterinarian, all three calves demonstrated some combination of the following: subcutaneous edema, mild subcutaneous hemorrhages, visceral congestion, ascites, pulmonary edema and emphysema, and hepatomegaly, with enlarged congested livers having a prominent "mottled" or lobular pattern on surface and section.

Laboratory Results: Fresh tissues were received from two calves. Bacterial culture attempts did not yield growth of any significant aerobic or anaerobic pathogens from the lung, liver, or small intestine. Fluorescent antibody tests for a variety of viral pathogens (i.e. infectious bovine rhinotracheitis, parainfluenza-3, and bovine respiratory syncytial virus) were negative and virus isolation attempts on pooled tissue homogenates were negative (bovine embryonic testis cells). A serum chemistry panel and complete blood count performed on one calf revealed the following abnormalities: elevated ALP, AST, CK, GGT and LDH; evidence of mild dehydration (elevated PCV and hemoglobin); and a mild leukocytosis with neutrophilia. Tissue (liver) levels of lead, arsenic, mercury, copper, selenium, and several other metals were within normal limits. A developmental (not commercially available) thin-layer chromatography assay detected a "significant" (not quantified) level of carboxyatractyloside in rumen contents from both calves tested.

Histopathologic Description: Striking lesions are confined to the liver for both calves, and include diffuse acute hepatocellular necrosis most severe in centrilobular, and to a lesser extent, midzonal areas (zones 3 and 2), generally

sparing periportal (zone 1) hepatocytes. In some sections there also is patchy sinusoidal congestion and pooling of blood, mild vacuolar change of remaining hepatocytes in zone 1, and variable, but light infiltrates of inflammatory cells (predominantly neutrophils) in sinusoids and attending necrotic hepatocytes. Other changes observed microscopically include diffuse pulmonary congestion and edema with multifocal emphysematous bullae and subtle changes suggestive of cerebral edema.

Contributor's Morphologic Diagnosis: Hepatocellular necrosis, marked, acute, diffuse, centrilobular and midzonal (zones 3 and 2), with multifocal congestion/hemorrhage and multifocal mild hepatocellular vacuolar change.

Contributor's Comment: Zone 3 (periacinar or centrilobular) necrosis is the most common form of zonal hepatocellular necrosis observed in domestic animals, including cattle, and is a relatively stereotyped response/ lesion that may be caused by a variety of infectious, inflammatory, metabolic, and toxic insults.(4) Given the clinical history and microscopic findings in this case, a toxic etiology was suspected, particularly a toxic plant incorporated into the millet hay. Other etiologies, such as cyanobacteria (blue-green algae) and molybdenum toxicosis, were considered less likely given the controlled diet, season (winter), and location (western Nebraska). Examination of rumen contents from two affected calves and of the millet hay incorporated into the feed ration revealed abundant mature burs or fruits of the common cocklebur plant (*Xanthium strumarium*), and thin-layer chromatography analysis of rumen contents from both calves demonstrated a "significant" amount of the cocklebur toxic principle, the diterpenoid glycoside carboxyatractyloside.(4,5)

Carboxyatractyloside and other atractylosides are inhibitors of cellular oxidative phosphorylation, and act specifically by binding to and inhibiting ADP/ATP carriers, leading to ATP depletion and subsequent mitochondrial dysfunction, ion pump failure, lipid peroxidation, and glutathione depletion resulting in cellular apoptosis and/or necrosis.(4,5) All members of the *Xanthium* genus seem to produce carboxyatractyloside, and other plants in the families Asteraceae and Compositae may produce the same or similar glycosides causing similar clinical syndromes in susceptible species, including cattle, sheep, swine, and humans.(2,3,5-8)

The common cocklebur, a coarse herbaceous annual, is common throughout much of the United States; it grows to a mature height of 2-5 feet, with an erect, often angled stem, and alternate, triangular or heart-shaped rough leaves. The plant produces hard, prickly, oval fruits or burs approximately 0.75 inches long containing two seeds; these can be found entangled in the coats of livestock and long-haired dogs. The plants are invasive and are often found growing in pastures and meadows (especially those with a history of previous or seasonal flooding), along fencerows, in roadside ditches, along stream and pond banks, in dried out ponds or stock reservoirs, and occasionally in disturbed areas in feedlots.(7)

Cocklebur poisoning is most common in the spring or early summer, associated with the ingestion of germinated seeds and palatable young dicotyledon seedling plants that are high in the toxic principle, carboxyatractyloside; adult plants contain relatively little toxin, other than in seeds or burs. Most poisonings seem to occur in pigs foraging naturally, but the plant is toxic to a wide range of animals, including ruminants, horses, dogs, rats, and humans; most cases of toxicity in these species are caused by incorporation of seedlings or mature plants with seeds into feed rations (hay, haylage, silage, or grain rations).(2,3,6,8) As with the submitted case, there are several reports of poisoning in cattle associated with the presence of mature cocklebur plants and seeds (burs) in hay.(8)

Common clinical signs observed with cocklebur poisoning include anorexia, depression or other behavioral changes including apprehension or excitability, blindness, ataxia, twitching progressing to spasmodic muscle contractions or convulsions, recumbency, opisthotonos, and rapid progression to death. Clinical signs may follow ingestion of the plant by as short a period as several hours in monogastric animals and may be delayed for a day or so in ruminants. Characteristic gross lesions of cocklebur poisoning are not specific, but can include ascites and various effusions, hemorrhages (associated with consumption of clotting factors), hepatic swelling, congestion and mottling, fibrin tags on serosal surfaces of viscera, renal congestion, and gastrointestinal congestion.(2,4,8) Microscopic lesions generally are confined to the liver, with characteristic centrilobular/periacinar (zone 3) to midzonal (zone 2) or rarely panzonal hepatocellular degeneration, necrosis, and apoptosis with congestion and/or hemorrhage; however, lesions also may be observed in the kidney (e.g. tubular epithelial degeneration and necrosis) and brain (e.g. neuronal degeneration/necrosis and cerebral edema) on occasion.(2,4,5,8) Diagnosis of cocklebur poisoning generally requires some combination of the following: 1) evidence of ingestion of cotyledonary seedlings or seeds/burs, 2) appropriate history and clinical signs, 3) characteristic clinical pathology findings, and 4) consistent gross and

microscopic lesions. Diagnostic assays that detect the toxic principle in tissues or other biological samples have been or are being developed, but none are routinely or widely available to veterinary diagnosticians.

Treatment of affected animals is generally unrewarding once clinical signs have progressed to the neurological stage, and there is no antidote for the toxic principle; supportive care and therapy aimed at increasing gastrointestinal clearance of ingested plants, decreasing gastrointestinal absorption of the toxin, and treating metabolic and neuromuscular complications all have been shown to be effective on occasion. Prevention of poisoning is more effective than treatment of clinical cases, and can be achieved by elimination of plant populations (e.g. mowing before seed production begins, use of herbicides, limiting access to contaminated pastures and meadows, manual removal of plants from hay fields, etc.).

AFIP Diagnosis: Liver: Hepatocellular necrosis, coagulative, centrilobular and midzonal (submassive), acute, diffuse.

Conference Comment: In WSC 2009-2010, Conference 12, case IV, we discussed the reasons why centrilobular hepatocytes are particularly susceptible to hypoxic injury and to indirect-acting toxins that undergo biotransformation through cytochromes P450. This superb example of centrilobular to midzonal hepatocellular necrosis due to cocklebur ingestion provides a timely reminder of the importance of pattern recognition in the evaluation of hepatic lesions. We thank the contributor for a concise overview of the entity.

The bulk of the discussion during the conference was focused on toxic plants that cause centrilobular to midzonal hepatocellular necrosis. Several of these are summarized in the table below:(1)

Hepatotoxic Plants Causing Centrilobular Necrosis			
Plant Family	**Plants**	**Species Affected**	**Toxic Principle**
Compositae	*Xanthium* spp.	Pigs, cattle	Carboxyatractyloside
Myoporaceae	*Myoporum* spp.	Pigs, cattle, sheep, horses	Furanosesquiterpenoid oils (ngaione)
Leguminosae	*Cassia* spp.	Cattle	Unknown
Ulmaceae	*Trema aspera*	Cattle, sheep, goats	Trematoxin
Solanaceae	*Cestrum parqui*	Cattle, sheep	Saponins
Zamiaceae Cycadaceae Strangeriaceae		Cattle, sheep, goats, dogs	Methoxymethanol
Fabaceae	*Indigofera linnaei*	Cattle, dogs	Indospicine
Cyanophyceae	*Microcystis* spp., *Aphanizomenon* spp.	Cattle, sheep, goats, horses, dogs	Microcystins, others

As mentioned by the contributor and well-illustrated by this case, the investigation of a suspected plant intoxication often involves linking a number of pieces of evidence, including clinical signs, pathological and clinical pathological findings, examination of the feed and/or environment for the offending plant(s), and ancillary diagnostics, when available. In this case, the use of an investigational assay to detect carboxyatractyloside in rumen contents proved helpful in substantiating the diagnosis.

Contributor: Wyoming State Veterinary Laboratory, University of Wyoming, 1174 Snowy Range Road, Laramie, WY 82070
http://uwadmnweb.uwyo.edu/vetsci/
http://wyovet.uwyo.edu/

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CASE IV: V08-28214 (AFIP 3141624).

Signalment: 1-year-old, female, spayed, Labrador retriever dog (*Canis familiaris*).

History: The dog, which lived in Kansas for a period, presented to the referring veterinarian in respiratory distress and was sent to the emergency clinic. The dog deteriorated significantly and was supplemented with nasal oxygen. The dog died later and was submitted for necropsy examination.

Gross Pathology: The dog was in normal body condition with mild postmortem autolysis. There was a linear midline incision on the ventral abdomen. The lungs were dark red and firm with a granular texture, and did not collapse when the thoracic cavity was opened. The hilar lymph nodes were moderately enlarged, congested and "wet" on cut surface.

Laboratory Results: Bacterial culture from the lung, liver and blood was negative. *Blastomyces dermatitidis* and *Penicillium* sp. were isolated by fungal culture.

Histopathologic Description: Lung: In multifocal to coalescing areas, the alveoli contain moderate to large numbers of intact and degenerate neutrophils and macrophages, fewer foreign body type multinucleated giant cells, lymphocytes, plasma cells, fibrin, edema fluid and eosinophilic cellular and karyorrhectic debris (necrosis). The inflammatory infiltrate surrounds large numbers of round to oval, 8-25 µm yeasts with a thick, double-contoured wall, basophilic central zone and broad-based budding. A few macrophages and/or multinucleated giant cells contain organisms within their cytoplasm. Multifocally alveolar septa are thickened and infiltrated by inflammatory infiltrate similar to that described previously. The pulmonary capillaries and larger blood vessels are congested and there are multiple hemorrhages scattered throughout some sections. Multifocally, perivascular and peribronchiolar spaces are distended by edema. Although the organisms are readily visible in H&E staining, PAS and GMS stains more selectively demonstrate the outer wall.

Contributor's Morphologic Diagnosis: Lung: Pneumonia, pyogranulomatous and necrotizing, multifocal to coalescing, severe with intra- and extracellular budding yeasts, etiology consistent with *Blastomyces dermatitidis*, Labrador retriever, canine.

Contributor's Comment: North American blastomycosis is a systemic disease caused by *Blastomyces dermatitidis*, a dimorphic fungus that mainly affects dogs and humans. The disease in humans was first described in 1894; since then it has been documented in many species of vertebrate animals such as cats and horses, as well as captive non-domestic animals such as sea lions, wolves, ferrets, polar bears, tigers, cheetahs and snow leopards.(6) More recently blastomycosis was identified in a rhesus monkey.(9)

Geographically, the disease mainly occurs in North America, with occasional infections in Africa, Europe, Asia and Central America. In North America, the Mississippi, Ohio and St. Lawrence river basins; northern Ontario; the Mid-Atlantic states; and the Canadian provinces of Quebec and Manitoba have had endemic infections.(1) Sporadic cases have been described in New York. The prevalence of blastomycosis is 10 times higher in dogs than in humans, with occasional simultaneous infections; therefore, dogs are considered sentinels for human disease.(2)

Blastomyces dermatitidis is thought to originate from the soil; however, the organism is not commonly recovered at the suspected exposure sites. In the environment it grows as mycelia, requiring sandy, acidic soil and proximity to water; it causes disease to animals by inhalation of the spores.(1) The mycelial form converts to the yeast form in the terminal bronchioles of the lung, then disseminates to other preferred sites in the body via the blood and lymphatic vessels. In dogs these include the skin, eyes, bones, lymph nodes, subcutaneous tissues, external nares, brain and testes. Less commonly the mouth, nasal passages, prostate, liver, mammary gland, vulva and heart also might be affected. Skin lesions may be caused by local inoculation. Intestinal lesions are uncommon in dogs with systemic disease. Subclinically infected dogs are very rare. Experimentally infected dogs develop mild lesions, but with much higher frequency than for other systemic mycoses. However, the disease in experimentally infected dogs is mostly mild and the animals recover without treatment.(4) Lung lesions may resolve by the time other organs present with signs of infection. The lesions normally present as either granulomas with many epithelioid and giant cells, or pyogranulomatous foci that consist of necrotic neutrophils and macrophages.(6)

The virulence factors of *B. dermatitidis* remain mostly unknown and naturally infected animals may have a suppressed immune response, the mechanism of which is not clear. Moreover, it has not yet been determined whether dissemination of the disease depends upon a compromised immune system.(1) An effective immune response requires T lymphocytes targeting a surface adhesion virulence factor *Blastomyces* adhesion 1 (BAD-1) immunodominant antigen (formerly called Wisconsin 1 (WI-1)), a molecule that mediates attachment to the host cells and blocks tumor necrosis factor (TNF)-α production. Antibody responses to BAD-1, although associated with reduced disease severity, were not able to entirely protect against the infection. Moreover, the cell wall polysaccharide α-glucan was also shown to be associated with virulence and to inhibit elimination by macrophages. (6)

Cytologic or histologic evaluation can be used to identify the organism. Usually, the diagnosis is made by demonstrating the yeast bodies in tissue sections or cytologic preparations. Safety measures must be taken because laboratory personnel can become infected by the cultured mycelia.(1,6) Serologic tests are also available but commonly produce false negative results. Measurement of *Blastomyces* antigen in urine or serum by antigen enzyme immunoassay (EIA) is more sensitive than measurement of anti-*Blastomyces* antibodies for the diagnosis of blastomycosis in dogs.(8)

Hypercalcemia has been associated with blastomycosis, and is likely caused by abnormal vitamin D metabolism. The kidneys provide the site of hydroxylation of 25-hydroxy-cholecalciferol to produce active 1,25- dehydroxy-cholecalciferol. Studies suggest, however, that granulomas composed primarily of monocyte derived phagocytes can metabolize 25-hydroxyl-cholecalciferol to calcitriol *in vitro*, providing the extrarenal source for hypercalcemia associated with blastomycosis. The production of calcitriol by the granulomas seems to be de-regulated or mediated differently than by the kidneys.(3)

Multinodular lesions in the lung and other organs must be differentiated from those of other systemic mycoses and metastatic neoplasia that can be distinguished by examination of impression smears or histologic sections. Neoplastic nodules tend to be larger and more variable in size. Other fungi are normally differentiated from *Blastomyces* based on morphologic features. *Blastomyces* cells are yeast-like, multinucleated, spherical, 8-20 µm in diameter, possess thick double walls, and produce single, broad-based buds. *Histoplasma capsulatum* is much smaller (2-4 µm) and found in the cytoplasm of macrophages. *Cryptococcus neoformans* has a thick capsule and cause a mild inflammatory response. Mutant forms of *Cyptococcus* lacking the typical thick capsule might resemble *Blastomyces* and induce a similar granulomatous response, but this fungus displays narrow-based budding. *Coccidioides immitis* is much larger (20-200 µm) and contains endospores. *Blastomyces*, *Cryptococcus* and *Histoplasma* can be differentiated in culture by the presence and morphology of mycelia, conidia, and yeast, respectively.(1) *Histoplasma capsulatum* var. *duboisii* (African histoplasmosis) has narrow-based buds and is not multinucleated. *Aspergillus* organisms are septate, thin, and parallel walled with acute-angle dichotomous branching. *Paracoccidioides braziliensis* causes South American blastomycosis and reproduces in tissues by multiple budding.(4)

AFIP Diagnosis: Lung: Pneumonia, pyogranulomatous, diffuse, severe, with edema, hemorrhage, many Langhans and foreign body type giant cells, and myriad intrahistiocytic and extracellular broad-based budding yeasts, etiology consistent with *Blastomyces dermatitidis*.

Conference Comment: The contributor provides an excellent overview of blastomycosis and most of the other etiologies that conference participants considered in the differential diagnosis. Other, less likely considerations

mentioned during the conference included protothecosis, chlorellosis, zygomycosis, pythiosis, sporothricosis, mycobacteriosis, and neoplasia (e.g. histiocytic origin or metastatic carcinoma).

Careful consideration of the molecular basis for histomorphologic changes is often useful in their proper interpretation. Both the T_H1 and T_H2 responses are important in cell-mediated immunity to invasive fungi; however, they result in dissimilar histomorphologic changes. The former is typified by the formation of discrete granulomas with the hallmark being epithelioid macrophages, while in the latter neutrophils and activated monocytes and macrophages predominate.(5,7) Conference participants reviewed the mechanisms responsible for T_H1 and T_H17 responses in detail.

In response to intracellular bacteria and fungi, dendritic cells present fungal antigen to naïve T cells and secrete interleukin (IL)-12 which, along with interferon (IFN)-γ, initiates the differentiation of naïve T cells into T_H1 cells. This differentiation requires the lineage-specific transcription factor T-bet; differentiated T_H1 cells characteristically produce IFN-γ and IL-12. Interferon-γ further amplifies the differentiation of T_H1 cells in an autocrine loop, and inhibits the differentiation of T_H17 cells, potent recruiters of neutrophils and monocytes involved in the host defense against extracellular bacteria and fungi. Interferon-γ induces isotype switching in B cells to favor IgG production, and causes a number of functional and morphological alterations in macrophages, resulting in their activation, e.g. enhanced phagocytosis and killing ability; increased class II major histocompatibility complex expression; and increased production of TNF-α, IL-12, and other proinflammatory cytokines. Interleukin-12, acting synergistically with IL-18, further amplifies the T_H1 response. Tumor necrosis factor-α is an important cytokine in phagocyte-mediated killing of yeast; thus, as mentioned by the contributor, its depressed production in the presence of the BAD-1 antigen impairs the cell-mediated immunity that is vital to the host defense against *Blastomyces dermatitidis*. (5,7)

In response to extracellular bacteria and fungi, naïve T cells under the control of transforming growth factor (TGF)-β plus IL-6 and IL-1, or TGF-β plus IL-21 followed by IL-23, undergo differentiation into T_H17 cells. Transforming growth factor-β, classically characterized as an immunosuppressive cytokine, induces the expression of forkhead box P3 (Foxp3) in naïve T cells, driving the induction of regulatory T cells that suppress inflammation. Interleukin-6 is a potent inhibitor of this pathway, and in combination with TGF-β and IL-1, drives the expression of IL-17 by naïve T cells, committing them to the T_H17 lineage. Activation of the transcription factor ROR-γt in cells committed to the T_H17 lineage causes expression of the receptor for IL-23. Interleukin-23 exposure to these developing TH17 cells enhances IL-17 expression, induces IL-22 expression, and suppresses the expression of IL-10 and IFN-γ, thus stabilizing the T_H17 phenotype. Unlike the signature cytokines produced by cells of the T_H1 and T_H2 pathways (i.e. IFN-γ and IL-4, respectively), IL-17 produced by T_H17 cells does not amplify T_H17 responses in an autocrine loop; rather, IL-21, produced by mature T_H17 cells, in combination with TGF-β, amplifies T_H17 differentiation. Notably, the differentiation of T_H17 cells is inhibited by IFN-γ (T_H1 pathway) and IL-4 (T_H2 pathway).(5,7)

Contributor: NMDA-Veterinary Diagnostic Services, 700 Camino de Salud NE, Albuquerque, NM 87106-4700
http://www.nmda.nmsu.edu/animal-and-plant-protection/veterinary-diagnostic-services

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