Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2022-2023

Conference #22 **14 April 2023**

CASE I:

Signalment:
Adult, male, Belgian blue, Ox (*Bos taurus)*

History:
An adult Belgian blue bull was severely depressed, with severe bilateral keratoconjunctivitis, mucopurulent oculo-nasal discharge, and multifocal to coalescing erosive lesions on the muzzle. As the animal's condition started to deteriorate rapidly, the decision to euthanize was taken by the owner.

Gross Pathology:
The animal was in poor body condition. There was bilateral conjunctivitis and multifocal, scattered, round, alopecic cutaneous lesions on the head. The oral mucosa exhibited multifocal, irregular to linear erosions, which were most pronounced on the tongue and the hard palate. The esophageal mucosa exhibited severe multifocal longitudinally oriented linear erosion and ulceration, with scattered multifocal hemorrhage. Generalized lymphadenomegaly and hyperemia of the vertebral rete mirabilis were also observed.

Laboratory Results:
PCR targeting the polymerase gene of AlHV-2 and OvHV-2 was carried out on samples from the tongue and lymph node. Both samples yielded a positive result for OvHV-2 DNA.

Microscopic Description:
Oral mucosa. Diffusely, affecting both small and larger vessels (both arteries and veins), there is mild to moderate perivascular inflammation, composed of lymphocytes, plasma cells and fewer macrophages. The endothelial cells are often hypertrophic with plump nuclei which protrude in the vascular lumen. The tunica media of medium and large-sized arteries is expanded by moderate to large numbers of inflammatory cells and, in some arteries, there is multifocal accumulation of bright eosinophilic material (fibrinoid necrosis). The overlying oral mucosa shows focally extensive moderate intracellular and intercellular edema and necrosis, with erosion

Figure 1-1. Presentation, ox. A Belgian blue bull was severely depressed, with severe bilateral keratoconjunctivitis, mucopurulent oculo-nasal discharge, and multifocal to coalescing erosive lesions on the muzzle. (Photo courtesy of: Department of Veterinary Pathology and Public Health, Institute of Veterinary Science, University of Liverpool, Leahurst campus, CH64 7TE, UK. https://www.liverpool.ac.uk/vetpathology/)

of the epithelial layer and sloughing of the epithelial cells and eosinophilic necrotic cellular debris. There is also mild to moderate multifocal lymphoplasmacytic infiltration of the epithelium. Inflammation and severe hyperemia are present in the superficial lamina propria, particularly extending between rete pegs.

Contributor's Morphologic Diagnosis:
Oral mucosa: Moderate to severe diffuse subacute lymphoplasmacytic vasculitis with erosive stomatitis.

Contributor's Comment:
Malignant catarrhal fever (MCF) is a viral disease which affects many different species of the order *Artiodactyla*, predominantly ruminants. Currently, several different viruses are considered causative agents of MCF; those which have been classified all belong to the genus *Macavirus* (malignant catarrhal virus), subfamily *Gammaherpesvirinae* and include *Alcelaphine gammaherpesvirus 1* (AlHV-1), *Alcelaphine gammaherpesvirus 2* (AlHV-2), *Ovine gammaherpesvirus 2* (OvHV-2) and *Caprine gammaherpesvirus 2* (CpHV-2) (https://talk.ictvonline.org/taxonomy/). These viruses, and others, including Ibex-MCF virus and another uncharacterized

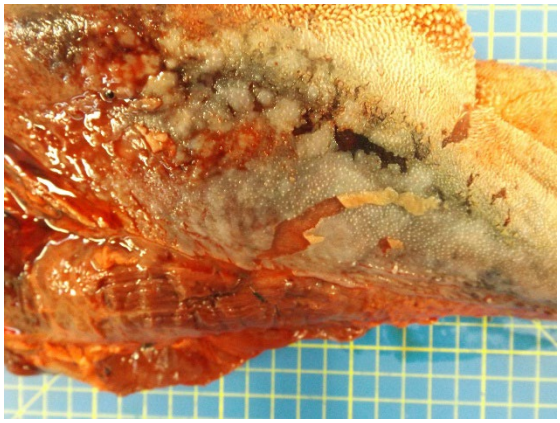

Figure 1-2. Tongue, ox. There are extensive ulcers on the tongue. (Photo courtesy of: Department of Veterinary Pathology and Public Health, Institute of Veterinary Science, University of Liverpool, Leahurst campus, CH64 7TE, UK. https://www.liverpool.ac.uk/vetpathology/)

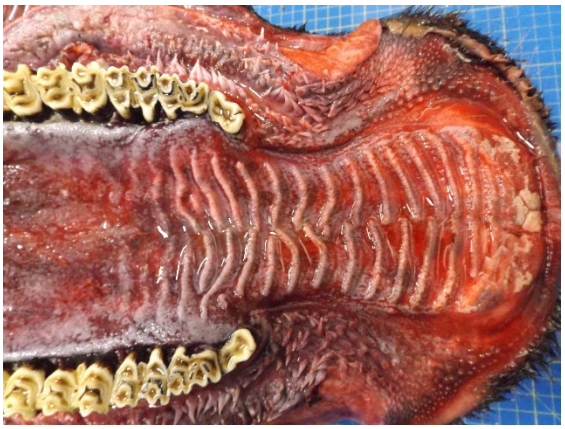

Figure 1-3. Palate, ox. There are extensive ulcers on the palate. (Photo courtesy of: Department of Veterinary Pathology and Public Health, Institute of Veterinary Science, University of Liverpool, Leahurst campus, CH64 7TE, UK. https://www.liverpool.ac.uk/vetpathology/)

virus isolated from clinically affected white-tailed deer (WTD-MCFV) are responsible for natural outbreaks of MCF.[4,8,13] Under experimental conditions, *Hippotragine gammaherpesvirus 1* (HipHV-1) induced MCF in rabbits, although naturally occurring infections are not reported in this species.[17] Gammaherpesviruses of ruminants are highly cell-associated lymphotropic herpesviruses. The key features shared among all the macaviruses, is the presence of the 15-A antigen epitope and high degree of similarity of the polymerase gene.[13]

The MCF-associated viruses have a recognized reservoir host, for example, sheep and wildebeest for OvHV-2 and AlHV-1, respectively. In the reservoir host, these viruses are usually clinically silent in contrast to susceptible species, in which infection causes clinical disease. The severity of the clinical signs varies greatly according to the species affected; some animals, for example bison (*Bison* spp.) or Pere's David deer (*Elaphurus davidianus*), show a high susceptibility to disease, while others such as the white-tailed deer (*Odocoileus virginianus*), appear to be more resistant.[11] The main source of infection

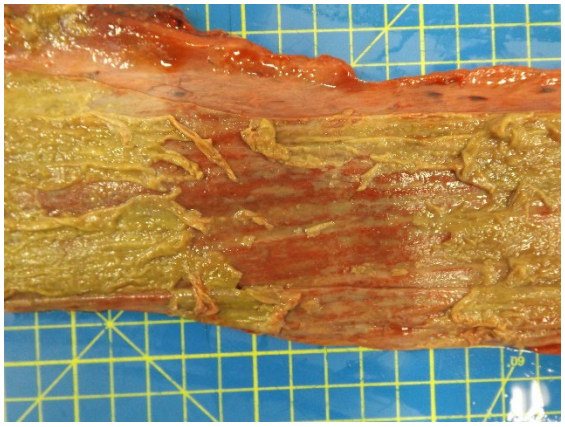

Figure 1-4. Tongue, ox. There are large areas mucosal ulceration in the esophagus. (Photo courtesy of: Department of Veterinary Pathology and Public Health, Institute of Veterinary Science, University of Liverpool, Leahurst campus, CH64 7TE, UK. https://www.liverpool.ac.uk/vetpathology/)

is the reservoir host, in which infection is transmitted from adults to young animals at 2-3 months of age, while clinically susceptible species usually act as dead-end hosts; in the latter, however, vertical transmission and abortion has sporadically reported.[5] Similar to other herpesvirus infections, the virus is shed through ocular and nasal discharges, which can then be inhaled or ingested via contaminated water or food. Even though the virus remains viable for only relatively brief periods outside the host animal, long distance transmission of OvHV-2 has been reported, causing MCF in bison.[9]

Clinical signs of MCF are similar, regardless of the causative gamma herpesvirus, but can be highly variable between individual animals. The most common and well-known expression of clinical disease is the "head and eye" form, in which the main clinical signs comprise depression, fever, keratoconjunctivitis, oral ulceration, lymphadenopathy and rhinitis with nasal discharge. However, gastro-intestinal, urinary, and neurological signs can be observed.[18]

Postmortem examination can reveal a wide range of lesions; bilateral keratoconjunctivitis, erosion of the nares with adhered crusts, and bilateral oculo-nasal discharge are typical gross findings. Cutaneous lesions consisting of exanthemata with overlying crusting are described on the thorax, abdomen, inguinal regions, perineum, and, less often, on the head.[13] In the gastrointestinal system the most common lesions are observed in the oral cavity. Erosive lesions develop first on the lips, especially close to labial commissure, then develop on the tongue before extending over the oral mucosa, from the gingiva to the palate.[13] Similar lesions can be observed also in the esophagus, the forestomachs and abomasum. The liver may show mild diffuse enlargement with multifocal pinpoint foci of white discoloration. Petechiae and erosions have also been described on the gall bladder mucosa. Respiratory lesions may be present, especially in the upper airway, ranging from mild congestion to fibrinous tracheobronchitis. Lesions in the urinary system are very characteristic and specific but are not always present; these consist of multifocal interstitial nephritis with infarcts giving a mottled appearance to the kidneys. In the lower urinary tract, erosion and hemorrhage are reported on the mucosa of the renal pelvis and the urinary bladder. Lymphadenomegaly is very common in most hosts and may be generalized or grossly affecting only a proportion of lymph nodes. Likewise, hemolymph nodes are affected and appear swollen. Edema of the meningeal vessels is also reported.[18]

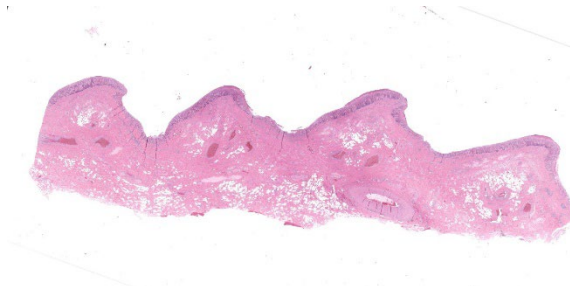

Figure 1-5. Oral mucosa, ox. A section of multifocally ulcerated oral mucosa is submitted. (HE, 6X)

The gross findings may be variable in distribution and severity, depending on the progression of the disease: acute MCF with rapid mortality is more likely to be associated with fewer grossly-evident lesions. In these cases, histopathology is an invaluable key diagnostic tool. Histologically, diffuse accumulation of mononuclear inflammatory cells around arteries and veins with fibrinonecrotizing vasculitis constitutes the hallmark of the disease; the distribution of these changes can be variable, affecting some tissues more severely than others, but are always present.[13,18] The infiltrate is mainly composed of lymphoid cells with large, open nuclei and prominent nucleoli, with occasional plasma cells and small lymphocytes.[18] Alongside these lesions, lymph nodes exhibit diffuse hyperplasia of the paracortical areas and necrosis and/or apoptosis can be observed in the skin, urinary and gastrointestinal tracts. Autolysis, accelerated by hyperthermia which occurs before death, can hamper diagnosis, especially in those cases where the lesions are mild.[13]

The gross and histopathological features are well recognized, however, the pathogenesis of MCF is not fully understood.[7] Viral infection of susceptible hosts induces a lymphocytic proliferation, mainly targeting vessels, with the development of severe arteritis/phlebitis and consequent necrosis affecting several organs. The majority of these lymphocytes show a CD8+ immunophenotype, but only a small fraction show evidence of viral infection.[13] Furthermore, large granular lymphocyte-like morphology and non-MHC restricted killing activity is observed in infected lymphocytes;[1] *in vitro* experiments indicated that the cells are resistant to concanavalin A while responsive to cyclosporin A.[7] Taken together, these findings seem to indicate that viral infection causes an autoimmune disease provoked by lymphocytic dysregulation, rather than a direct effect of the viral infection of lymphocytic cells. However, in experimental infection of bison an unexpectedly large number of virus-infected T cells was detected, suggesting that both direct viral effects and indirect immune responses may play a role in MCF pathogenesis.[2,7,12] Further studies are needed in order to solve this discrepancy. Recently, the role of AlHV-1 sema, a member of the semaphorin protein family, has been studied. An immune-escaping activity has been shown *in vitro*; however, absence of viral AlHV-1 sema had did not impair MCF induction and associated lymphoproliferative lesions in experimentally infected rabbits.[11]

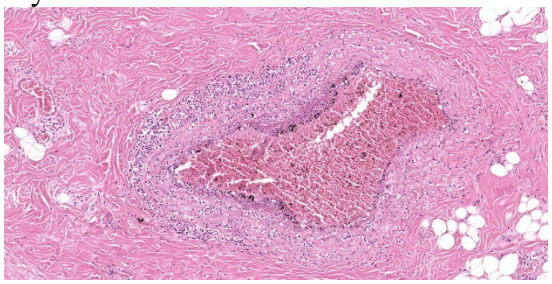

Figure 1-6. Oral mucosa, ox. There is a moderately dense cellular infiltrate within the adventitia and to a lesser extent, the media of affected vessels. (HE, 114X)

Contributing Institution:
Department of Veterinary Pathology and Public Health, Institute of Veterinary Science, University of Liverpool, Leahurst campus, CH64 7TE, UK
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JPC Diagnosis:
Hard palate mucosa: Arteritis, lymphocytic and necrotizing, multifocal to coalescing, moderate, with ulceration.

JPC Comment:
This contributor provides a great description of this classic case of OvHV-2 malignant catarrhal fever in an ox. This week's contributor, Dr. Patricia Pesavento of the University of California at Davis, discussed that when there is mucosal or epithelial ulceration with-

out an evident cause on the surface, it is important to examine the arteries subjacent the ulcer in a search for vascular lesions. In this case, there is subtle vacuolation to the endothelium of the large artery within the section, and, more superficially, the medium caliber arteries are more severely affected with transmural, predominantly T-cells disrupting the arterial wall and lifting the intima, presumably arising from the capillary bed of the adventitia. The moderator also stressed the importance of being specific in vascular lesions: in this case, the lesion specifically affects medium caliber arteries, thus arteritis was included in the morphologic diagnosis as opposed to vasculitis. This localization is important in sorting other potential causes.

The moderator and conference participants discussed bovine viral diarrhea virus as a differential which would cause vascular lesions, and for that the gross images of the palate and esophagus, these would be reasonable differentials. The moderator also described how the herpesviruses which contribute to MCF are characteristically lymphoproliferative, so a pleomorphic population of lymphocytes may weigh more in favor of MCF. Aside from PCR for OvHV2 (which was conducted in this case and is the most common cause of MCF in the United States), IHC for BVDV or ISH for the MCF agent would allow for differentiation of these two entities.

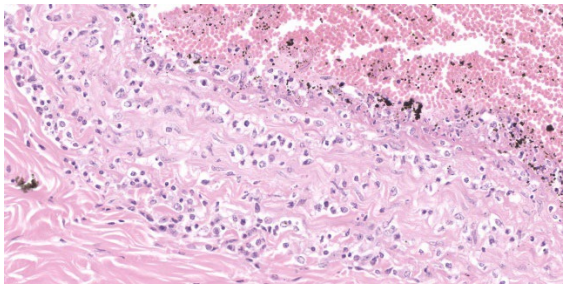

Figure 1-7. Oral mucosa, ox. The arteriolar mural infiltrate is a mixed cellular population of large and small lymphocytes and macrophages. (HE, 374X)

There are some histologic features which point to a location more specific than oral mucosa: the distribution of submucosal adipose, the presence of an elastic artery close to the mucosal surface, and especially the undulating surface point to the hard palate.

While OvHV-2 typically does not cause clinical signs in the host-adapted species, in a 2018 *Vet Pathol* report, Dr. Pesavento et. al showed that OvHV-2 is associated with systemic necrotizing vasculitis and can sporadically cause arteritis in sheep similar to MCF in cattle.[14] This study included a collection of cases previously diagnosed as idiopathic polyarteritis ("polyarteritis nodosa") and using the in-situ hybridization probe specific for OvHv-2, demonstrated viral nucleic acid in lymphocytes associated with vasculitis in small to medium caliber arteries in all cases.[14] This work showed that OvHv-2 driven MCF may occur in the adapted host.[14]

OvHV-2 associated MCF in cattle is generally fatal, and chronic infection and recovery are both considered rare.[10,16] A histologic hallmark of chronic MCF in cattle is neointimal hyperplasia characterized by proliferation of spindle (myofibroblast) cells between the endothelium and internal elastic lamina; this may be accompanied by obliterative arteriopathy due to luminal narrowing.[10,16] Other arterial lesions described in chronic MCF include disruption of the internal elastic lamina and attenuation or loss of the tunica media. A 2022 report in the *Journal of Veterinary Diagnostic Investigation* also reported lymphocytic hypophysitis in a chronic case of OvHV-2 associated MCF, and the lymphocytes positively labeled for OvHV-2 ISH.[10]

T-cells are described as the predominant inflammatory cell in the vascular lesions of

MCF; however, a recent study evaluating rete mirabile lesions of 34 cases of OvHV-2 MCF in cattle, water buffalo, and bison found that macrophages were present in equal or greater numbers to T cells in all cases.[16] This raises the possibility that the pathogenesis of vascular damage and necrosis may be secondary to macrophage activity.[16] This study also evaluated the expression of viral protein oLANA and ov-IL10 mRNA, products of latency-associated genes, and demonstrated that the virus infects many more cells than just T-cells: endothelial cells, smooth muscle cells, and macrophages all demonstrated immunohistiochemical staining for oLANA and in-situ hybridization for ov-IL10 mRNA.[16]

As the contributor describes, there are a group of MCF viruses from the genus Macavirus, sub-family Gammaherpesvirinae, family Herpesviridae, that cause MCF disease in susceptible hosts, and a recent report described an Ibex-MCF virus outbreak in captive duikers (small antelope).[2] The virus was presumed to be spread indirectly from captive ibex in an enclosure 35 meters away, and cases in duikers were associated with times of parturition in the ibex.[2] The disease was fatal, and clinical signs included anorexia, ataxia, and diarrhea or sudden death.[2] On necropsy, mucosal ulceration was lacking, however, most of the animals had pulmonary ecchymoses, lymphadenomegaly, renomegaly, mucosal hemorrhage in the urinary bladder, and ascites.[2] Half of the cases also had hydrothorax and hepatomegaly.[2] Histologically, there were dense and wide perivascular lymphocytes and scattered (rare) fibrinoid vascular necrosis in multiple organs, demonstrating remarkable and chronic lymphoproliferation, with ibex-MCF identified in all cases using PCR and demonstrated in lymphocytes using ISH.[2] Most of the animals were deficient in copper, and the authors raised the possibility of copper deficiency

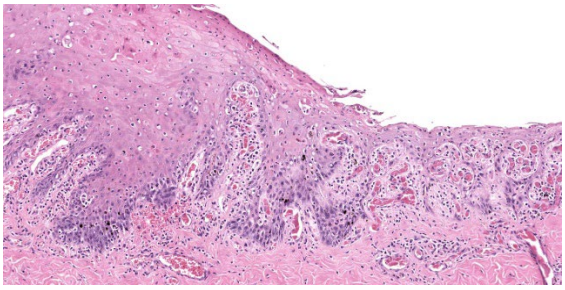

Figure 1-8. Oral mucosa, ox. There is ulceration of the mucosa overlying affected vessels. (HE, 340X)

compromising the duikers' immune responses, predisposing the animals to overwhelming viral infection.[2] Clinically normal but infected ibex were culled and lacked any gross or histologic lesions associated with the virus.[2]

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CASE II:

Signalment:
1-year-old, male, Domestic Shorthair Cat (*Felis catus*)

History:
A 1-year-old indoor male Domestic Shorthair cat was found deceased with no premonitory signs. The history indicated a prolapsed rectum had been surgically repaired in the past.

Gross Pathology:
Grossly, the lungs were diffusely edematous, mildly consolidated, and mottled dark red to red with petechial hemorrhages. The distal trachea and bronchi contained moderate amounts of yellow mucus and foamy fluid. There were two 4-5 cm long white worms in the right cardiac ventricle, located at the tricuspid valve. An oval 1 cm area of intimal hemorrhage was present in the base of the pulmonary artery. The heart was within normal limits for size and shape.

Laboratory Results:
No laboratory findings reported.

Microscopic Description:
Multifocally, the tunica intima of the small and medium-sized pulmonary arteries are markedly thickened and have villous-like proliferations that protrude into the lumens, partially or completely occluding the arteries. The intimal proliferations are often lined by hypertrophied endothelial cells and consist of fibrous connective tissue with hyperplastic fibroblasts and smooth muscle cells. Moderate to marked infiltration by eosinophils, macrophages, fewer lymphocytes, and plasma cells are present in the affected tunica intima, occasionally associated with minimal hemorrhage. In some arteries, the lumen is completely obliterated by endothelial proliferation, with a few small blood vessels lined by hypertrophied endothelial cells. Often, the internal elastic lamina is effaced and obscured by the intimal proliferation and inflammation. Most of the pulmonary arteries and arterioles, with or without intimal changes, are narrowed and have moderate hypertrophy of the tunica media. The alveolar septa adjacent to the affected arteries are moderately thickened by infiltration by eosinophils, macrophages, and fewer lymphocytes. Occasionally, the alveoli contained increased numbers of foamy macrophages, fewer eosinophils, hemorrhage, rare edema, and minimal amounts of fibrin. There are multiple granular, linear, or round concentric basophilic mineralized deposits in the pulmonary capillaries, bronchiolar smooth muscles, and arterial media. Round concentric deposits, some resembling corpora amylacea, are 15-20 microns in diameter and often located in the connective tissue around arteries and bronchi. The pulmonary capillaries are diffusely congested.

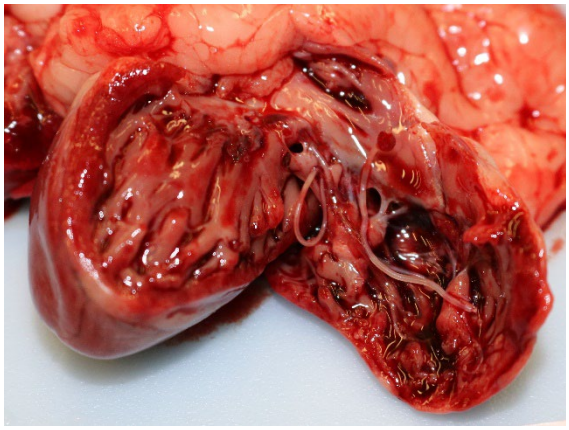

Figure 2-1. Heart, cat. Two heartworms were in the right ventricle. (Photo courtesy of: Connecticut Veterinary Medical Diagnostic Laboratory, http://cvmdl.uconn.edu/)

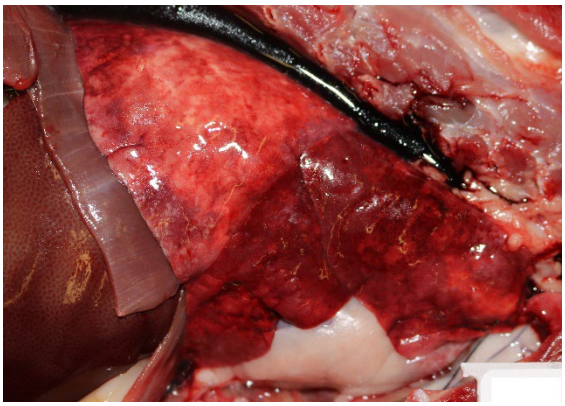

Figure 2-2. Lung, cat. Grossly, the lungs were diffusely edematous, mildly consolidated, and mottled dark red to red with petechial hemorrhages. (Photo courtesy of: Connecticut Veterinary Medical Diagnostic Laboratory, http://cvmdl.uconn.edu/)

Contributor's Morphologic Diagnoses:
Lung, arteries: (1) Endarteritis, proliferative and eosinophilic, chronic, multifocal, moderate to marked, with occasional luminal occlusions and re-canalizations; (2) Medial hypertrophy, diffuse, mild to moderate.

Lung: (1) Interstitial pneumonia, eosinophilic and histiocytic, multifocal, mild; (2) Mineralization, multifocal, moderate

Contributor's Comment:
Dirofilaria immitis commonly causes clinical diseases in dogs, but can infect other mammals including cats, wild felids, wild canids, and rarely humans.[15] Typical histological lesions in lungs in both dogs and cats caused by

D. immitis are villous proliferation of the arterial intima with medial hypertrophy and eosinophilic infiltrates.[12,15]

Mosquitoes play the main role in the heartworm lifecycle. The first, second, and early third stage larvae of *D. immitis* are obligate parasites of mosquitoes of the genera *Aedes, Culex* or *Anopheles*. In mosquitoes, larvae develop to the infective stage in the malpighian tubules and then migrate to the proboscus. When mosquitoes feed on hosts, the infective third stage larvae deposit on the skin and enter through the bite wound. The third stage larvae molt to the fourth stage and become immature adults in the connective tissue. Finally, immature adults enter the veins and migrate to the pulmonary arteries, 70-90 days postinfection. The immature adults mature in 3 months to adults, after which time female worms produce microfilariae. The average pre-patent period is 6-8 months for *D. immitis*.[10,11,15]

Dogs are the natural definitive host of *D. immitis*, and canine dirofilariasis occurs when heartworms become adults and produce microfilariae. In dogs, the most common clinical presentation is a chronic congestive right cardiac failure with more than 30 adult worms, and it is usually seen in dogs older than 5 years with continuous or multiple infections. Mechanical irritation by adult worms and microfilariae can cause pulmonary arterial sclerosis and hypertension, leading to chronic heart failure. Also, large numbers of adult *D. immitis* (usually more than 100) infecting the right atrium and vena cava can result in acute venous obstruction and shock, known as vena cava syndrome.[15]

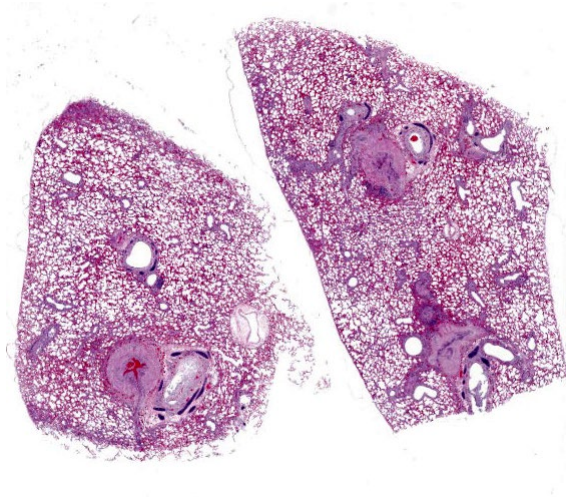

Figure 2-3. Lung, cat. Two sections of lung are submitted for examination. Multifocally, pulmonary arteries are markedly thickened and tortuous. (HE, 6X)

The prevalence and pathogenesis of feline dirofilariasis is somewhat different from dogs.[10,11] The distribution of feline dirofilariasis parallels that of dogs, but the overall prevalence is between 5-10 % of that in dogs in any given area.[16] Mosquitos feeding preferences and the poor suitability of cats as definitive hosts may be reasons for this low prevalence.[10] Since cats are atypical hosts for *D. immitis*, the majority of worms are cleared in the immature adult stage, and there are typically only 2-4 adult worms infect the heart. Due to low numbers of adult worms and poor suitability, cats tend to be amicrofilaremic or have only a short (2-3 months) microfilaremic period.[6,10,11] Vena cava syndrome caused by a large burden of *D. immitis* infection has been described rarely in cats.[12] Similar to dogs, chronic heartworm disease with adult worms occurs in cats, but congestive right cardiac failure or pulmonary hypertension are rare.[17]

Chronic feline dirofilariasis caused by adult worm infection can cause death,[10,11] as seen in this case. Cats may be asymptomatic or show chronic respiratory signs such as coughing or intermittent dyspnea during the infection.[1,6] Vomiting is also a relatively common finding, reported in about half of the cases with unknown pathogenesis [1]. Unlike most cases of canine dirofilariasis, acute respiratory distress and sudden death with adult

heartworms in cats are attributed to embolization of dead worms causing pulmonary arterial infarction and circulatory collapse.[6,10,11] Adult heartworms are believed to secrete a product that suppresses the activity of the pulmonary intravascular macrophage (PIM), the main component of the reticuloendothelial system in cats; PIM are not present in normal dogs.[8] Decreased PIM activity results in an anti-inflammatory reaction and minimizes the clinical signs of cats having adult worms, but the death of adult worms triggers an intense inflammatory reaction.[8,10]

Additionally, there is a condition known as heartworm-associated respiratory disease (HARD) in cats, that is caused by the death of larvae in caudal pulmonary arteries before they migrate into the heart.[7,10,15,] Clinical respiratory signs associated with HARD are similar to chronic feline dirofilariasis, but occur around 3-6 months post-infection without any adult worms.[7,10] The strong inflammatory response induced by larval death is also hypothesized to be due to the activity of PIM only seen in cats.[8,10]

Histological presentation of chronic feline dirofilariasis and HARD is similar, but HARD has less severe lesions.[3,17] The fibromuscular intimal proliferation has been considered a reaction to heartworms, but a response to intracellular bacteria *Wolbachia* harbored by *D. immitis* may be involved.[10,12,14] Arterial medial hypertrophy is another common finding in both cats and dogs infected with *D. immitis*.[12,15] In cats, this change can be spontaneous, and also can be caused by other parasites such as *Aelurostrongylus abstrusus* and *Toxocara cati*.[12,14] However, a recent experimental study revealed a strong association of smooth muscle hypertrophy of arteries, bronchi, and bronchioles in cats infected with *D. immitis*.[7] Without any adult heartworms, pulmonary proliferative and/or eosinophilic endarteritis is suggestive for larval *D. immitis* infection. As with hypertrophy of the arterial tunica media, migration of other larval worms (including *Toxocara* spp), is known to cause eosinophilic endarteritis in feline lungs.[14]

Aberrant migration of *D. immitis* occurs with greater frequency in cats than in dogs.[9,14] Aberrant migration of larvae to body cavities, eyes, and central nervous system are more common in cats, inducing an inflammatory response at the site of migration.[9,10] Recently, aberrant migration of heartworms to the femoral artery causing hind limb paresis was described in a cat.[13] Migrating *D. immitis* should be considered for any nematodes found anywhere in cats.

There was conversation about concentric mineral deposits in the pulmonary connective tissues in this cat as to whether they were dead larvae or spontaneous mineralization. Some of deposits had fragmented or concentric structures but were not associated with any inflammation, and there were no histologically identifiable larvae. It is known that dead *D. immitis* tend to induce strong inflammation.[9] No other cause for arterial mineralization was identified on anatomic examination, and antemortem bloodwork was not available. The cause of this widespread mineralization was not identified.

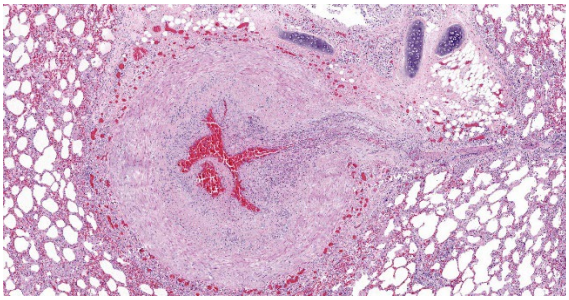

Figure 2-4. Lung, cat. A cross section of a pulmonary artery shows a recanalized lumen with marked intimal hyperplasia, mural thickening, and marked inflammation of the intima and adventitia. (HE, 42X)

Contributing Institution:
Connecticut Veterinary Medical Diagnostic Laboratory
http://cvmdl.uconn.edu/

JPC Diagnosis:
Lung, arteries, and arterioles: Endarteritis, proliferative and eosinophilic, diffuse, severe, with marked smooth muscle hypertrophy.

JPC Comment:
The fundamental histologic finding in this case is neointimal proliferation of pulmonary arteries. During the conference, the moderator and conference participants discussed presence of visible small caliber blood vessels in the expanded region of the tunica inwhich represents either neovascularization or hypertrophy of existing, penetrating vasa vasorum. Indeed, immunohistochemical and special stains were helpful to evaluate the character of the intimal proliferation: negative reactivity to smooth muscle actin and abundant factor 8 immunoreactivity confirmed that the majority of the proliferation is from small caliber vessels. Conference participants also discussed the presence of interstitial pneumonia, but felt the changes were mild and did not warrant morphologic diagnosis. The fatal outcome of such a low parasite load in cats is enigmatic, in this case there were no filaria, nor evidence of emboli (in sections examined)

The contributor provides a good summary of the histologic lesions of the pulmonary vasculature in cats, as well as the parasite life cycle, clinical signs, and pathogenesis of this disease. Heartworm infection may also lead to membranoproliferative glomerulonephritis due to deposition of antigen-antibody complexes, or to glomerular damage fraying and thickening glomerular basement membrane, both of which lead to proteinuria.[5,15]

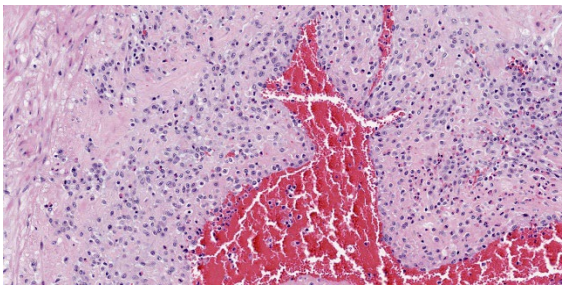

Figure 2-5. Lung, cat. Higher magnification of the pulmonary artery show marked intimal hyperplasia with segmental loss of the endothelium, and infiltration of the markedly thickened intima by moderate numbers of macrophages and eosinophils. (HE, 215X)

Dirofilaria can cause similar lesions in dogs. Dogs also can harbor *Angiostrongylus vasorum*, which can cause proliferative endarteritis, intimal proliferation, and medial hypertrophy in the lung.[4] This metastrongyle differs from *D. immitis* in both life cycle and pathogenesis. The intermediate host of *A. vasorum* is a mollusk (snail or slug), which is ingested by a dog. Larvae migrate through the lymph nodes, where they molt, and adults reside in the pulmonary arteries and right ventricle.[2,4] Eggs pass into the pulmonary vessels, hatch, and penetrate into alveolar lumen, eventually to be coughed up, swallowed, and passed into the feces. These eggs and larvae can cause granulomatous pneumonia, occasionally forming larger granulomas containing mixed inflammatory cells, while the adult worms cause characteristic proliferative endoarteritis.[4]Histologically, the presence of eggs in the granulomatous inflammation and within adult female nematodes is a helpful differentiating feature between *A. vasorum* and *D. immitis*; other differences include a thin coelomyarian musculature and a large intestine in *A. vasorum* compared to tall coelomyarian musculature and a small intestine in *D. immitis*.[4]

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CASE III:

Signalment:
8-week-old, male intact, Australian Heeler dog (*Canis familiaris*)

History:
This puppy presented with seizures and was euthanized on the same day. The puppy was unvaccinated, and the vaccination status of the dam was unknown.

Gross Pathology:
Unremarkable

Laboratory Results:

Sample	Test	Results
Brain (unfixed)	Direct fluorescent antibody testing for rabies virus	Negative
Lung (unfixed)	Aerobic culture	No growth

Microscopic Description:
Brain (cerebrum, hippocampus, and thalamus): Widespread throughout the white and gray matter of the cerebrum, hippocampus, and thalamus, there is mild gliosis. Many distinct glial nodules composed of aggregates of mononuclear cells including oligodendroglial cells and astrocytes are present. Many glial cells within these nodules have karyorrhectic or pyknotic nuclei. Multifocally, there are occasional neurons that are surrounded by small numbers of glial cells (satellitosis). Rare neurons are hypereosinophilic and shrunken with pyknotic nuclei (neuronal necrosis) and surrounded by small numbers of glial cells (neuronophagia). Occasional neuroparenchymal and meningeal blood vessels are surrounded and partially obscured by a variably thick layer of lymphocytes, plasma cells, fewer macrophages, and rare neutrophils (perivascular cuffing). Affected blood vessels are lined by hypertrophied endothelial cells. Throughout the section, there are rare, small, randomly distributed foci of hemorrhage. Occasional endothelial cells, rare glial cells, and rare intravascular mononuclear cells have chromatin margination and a solitary, large, approximately 4-6µm in diameter, intranuclear, basophilic inclusion body that often fills the entire nucleus. The leptomeninges, typically adjacent to blood vessels, are multifocally infiltrated by small to moderate numbers of plasma cells and lymphocytes.

Brain (cerebellum and brainstem) [not provided]: The histological changes are similar to those described in the cerebrum, hippocampus, and thalamus.

Liver [not provided]: There are many widely disseminated hepatocytes and rare, scattered Kupffer cells with chromatin margination and intranuclear inclusion bodies similar to those described in the brain. There are scattered small aggregates of lymphocytes with rare plasma cells and neutrophils, some of which are concentrated around blood vessels. There are rare hepatocytes with individual cell necrosis and/or apoptosis characterized by hypereosinophilia and karyorrhectic nuclei.

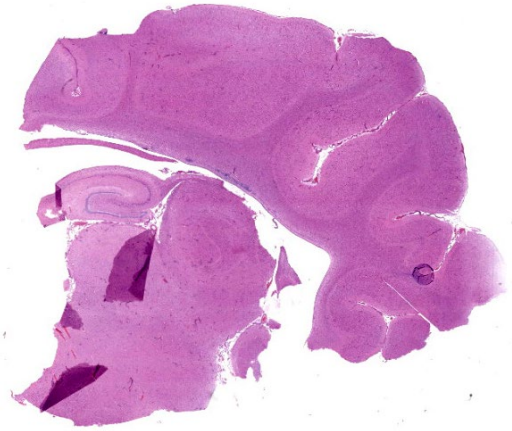

Figure 3-1. Diencephalon, dog. A single section of thalamus, hippocampus, and overlying cerebral cortex is submitted for examination. There are no apparent lesions at subgross magnification. (HE, 6X)

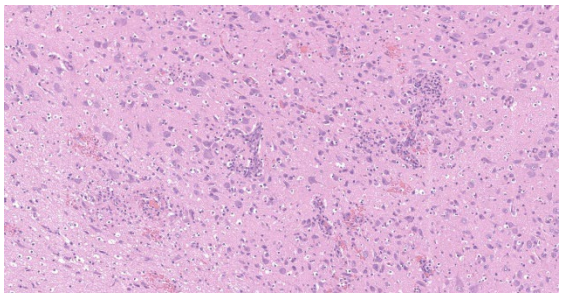

Figure 3-2. Diencephalon, dog. Predominantly in the thalamus, there is perivascular hypercellularity and hemorrhage, and diffuse gliosis of the intervening neuroparenchyma. (HE, 140X)

Immunohistochemistry:

1. Brain:
    a. Canine adenovirus-type 1 (CAV-1): There are scattered rare endothelial cells that have strong intracytoplasmic and occasional intranuclear immunoreactivity.
    b. Adenovirus: There are scattered rare endothelial cells that have strong intracytoplasmic and occasional intranuclear immunoreactivity.
    c. Canine distemper virus: There is no immunoreactivity.
2. Liver:
    a. CAV-1: There are occasional Kupffer cells, hepatocytes, and rare endothelial cells, with strong intracytoplasmic and occasional intranuclear immunoreactivity.
    b. Canine herpesvirus: There is no immunoreactivity.

Contributor's Morphologic Diagnoses:

1. Brain (cerebrum, thalamus, hippocampus, brainstem, and cerebellum) - encephalitis, lymphohistiocytic, multifocal, moderate to marked, subacute, with gliosis, glial nodules, perivascular cuffing, lymphoplasmacytic vasculitis, endothelial and glial cell intranuclear basophilic inclusion bodies, and mild lymphoplasmacytic leptomeningitis
2. Liver [not provided] - hepatitis, lymphocytic, multifocal and random, mild, subacute, with individual cell necrosis/apoptosis and intranuclear basophilic inclusion bodies

Contributor's Comment:

Canine adenovirus type-1 (CAV-1) is a non-enveloped DNA virus, and is the causative agent of infectious canine hepatitis. The virus affects various canids, including domestic dogs, foxes, coyotes, and wolves, as well as skunks and bears.[1,3,4,9] Clinical signs are typically more severe in younger animals (<1-2 years of age), ranging from asymptomatic to severe, and may include pyrexia, inappetence, lethargy, vomiting, diarrhea, abdominal pain, corneal edema, or death often without premonitory clinical signs.[1,3,4] The virus has affinity for endothelial cells, hepatocytes, and mesothelium, resulting in edema, serosal hemorrhage, and hepatic necrosis.[1-3] The neurological manifestation of CAV-1 infection in domestic dogs has rarely

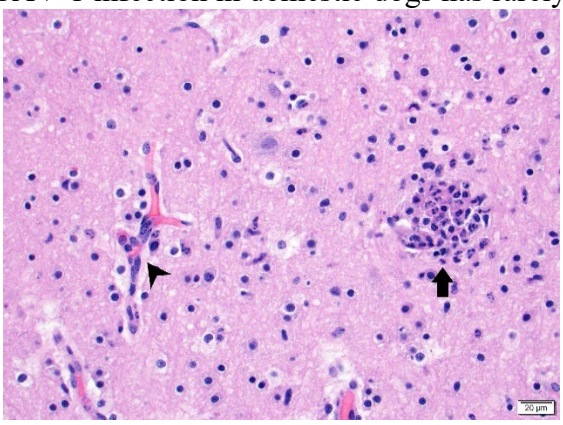

Figure 3-3. Diencephalon, dog. There is mild gliosis and ran-domly distributed glial nodules (arrow). Blood vessels are surrounded by perivascular cuffs of variable thickness, with as many as three layers of lymphocytes, fewer macrophages, and plasma cells. Affected blood vessels are often lined by re-active/ hypertrophic endothelial cells (arrow-head). (Image courtesy of: The University of Minnesota, College of Veterinary Medicine, Veterinary Diagnostic Laboratory. https://www.vdl.umn.edu) (HE, 400X)

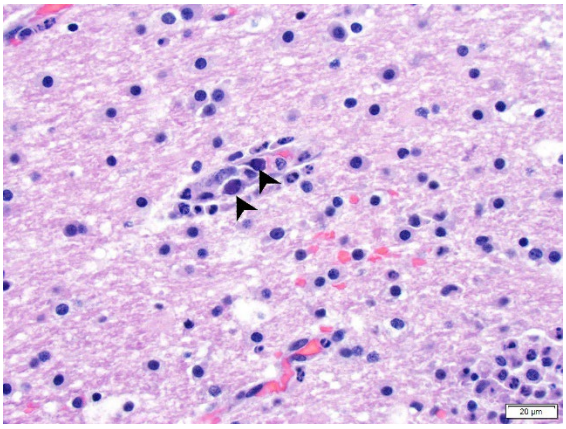

Figure 3-4. Diencephalon, dog. Affected endothelial cells (as well as rare intravascular leukocytes and mono-nuclear cells within glial nodules) have large, 4-6 µm in diameter, basophilic, intranuclear inclusion bodies that often fill the entire nucleus (arrow-heads). (Image courtesy of: The University of Min-nesota, College of Veterinary Medicine, Veteri-nary Diagnostic Laboratory. https://www.vdl.umn.edu) (HE, 400X)

been reported, and is most commonly seen in non-domestic animal species.[6,10]

In this case, a diagnosis of CAV-1 infection was made based on the characteristic histologic changes and immunohistochemical results involving the liver and brain. In previously reported CAV-1 cases in domestic dogs involving the brain, characteristic lesions included hemorrhage, vasculitis, and perivascular accumulation of mononuclear cells.[2,6,10] The lesions are typically seen in the brainstem, thalamus, and caudate nuclei, often sparing the cerebral and cerebellar cortices,[8] although in one report, the changes were most commonly observed in the corona radiata, caudate nucleus, thalamus, pons, and leptomeninges.[6]

CAV-1-associated encephalitis has been more commonly associated with foxes.[5,10] Under experimental conditions, the foxes developed hemorrhage predominantly affecting the brainstem and spinal cord, and variable mononuclear meningoencephalitis with perivascular cuffing.[5] In the present case, the lesions differed slightly from those previously reported in dogs and foxes, with evidence of encephalitis with gliosis and glial nodules, in addition to the previously reported vascular changes. In addition, the lesions were more widespread, involving the cerebral cortex, thalamus, hippocampus, brainstem, and cerebellum.

Neurological signs associated with CAV-1 have been attributed to vascular damage within the central nervous system,[3,4,10,11] although other possibilities include hepatic encephalopathy and/or concurrent infection with canine distemper virus (CDV).[11] It has been suggested that different CAV-1 strains may have tropism for the endothelium of the central nervous system.[2,10] However, to our knowledge, sequence analysis has not been performed to confirm this hypothesis. In this case, the cause of the reported seizures likely involved a combination of encephalitis and central nervous system vascular involvement. Although speculative, it is possible that the differences in the lesions and distribution of

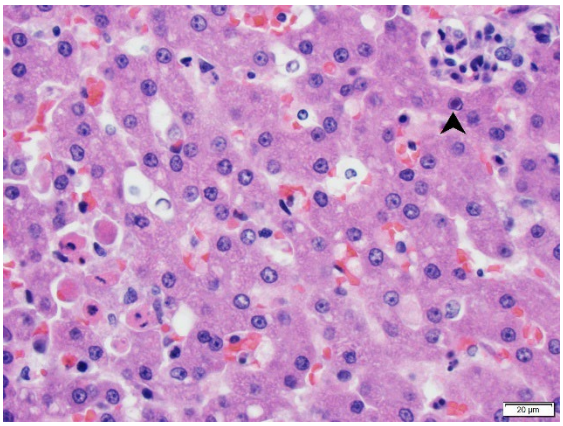

Figure 3-5. Liver, dog. There is individual hepatocyte necrosis, and scattered hepatocytes, Kupffer cells, and endothelial cells have intranuclear inclusion bodies similar to those observed in the brain (arrowhead). (Image courtesy of: The University of Minnesota, College of Veterinary Medicine, Veterinary Diagnostic Laboratory. https://www.vdl.umn.edu) (HE, 400X)

the lesions from the previously reported cases may have been due to the characteristics of the CAV-1 strain and/or the host immune response.

Susceptible dogs are exposed to CAV-1 via oronasal route through contact with infectious saliva, feces, urine, or respiratory secretions, or through direct animal-to-animal contact.[1,4,10,11] The virus initially replicates in the tonsillar crypts resulting in tonsillitis, followed by spread to the local lymph nodes and systemic circulation.[1,3,8] Viremia typically lasts 4-8 days and the virus spreads to the liver, endothelial cells, and mesothelial cells.[1,3,10] The virions exit the cells by cell lysis, resulting in cell death and tissue injury.[1,9] Characteristic histological lesions typically involve the liver, and range from randomly scattered foci of hepatocellular necrosis to widespread centrilobular necrosis.[1,3] Intranuclear inclusion bodies are often found in hepatocytes, vascular endothelium, and Kupffer cells.[1,3] Other lesions that may be seen include corneal edema/iridocyclitis (typically between 14 to 21 days post infection), gallbladder wall edema, interstitial nephritis, hemorrhagic enteritis, laryngitis, tracheitis, pneumonia, and ecchymotic and petechial hemorrhages.[1-4,6,11]

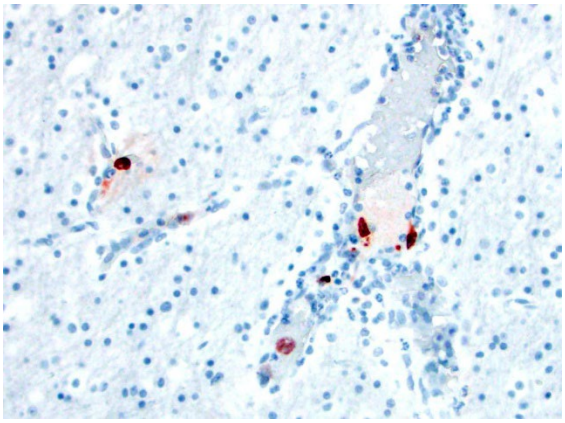

Figure 3-6. Brain (cerebral cortex), dog. Canine adenovirus type-1 immunohistochemistry. Scattered endothelial cells have strong intracytoplasmic and rare intranuclear immunoreactivity. (Image courtesy of: The University of Minnesota, College of Veterinary Medicine, Veterinary Diagnostic Laboratory. https://www.vdl.umn.edu) (anti-CAV-1, 400X)

Differential diagnoses for viral encephalitis in dogs include CDV, rabies virus, and canine herpesvirus. Coinfection is possible, with reports of CDV and CAV-1 resulting in an increased mortality rate.[6] CDV infection may be characterized by both intranuclear and intracytoplasmic inclusion bodies. CDV infection was excluded in this case based on the negative immunohistochemistry results. Rabies was considered unlikely based on negative direct fluorescent antibody testing result for rabies antigen. Canine herpesvirus (CHV)-associated meningoencephalitis has been reported in natural and experimental conditions.[7] In one case series involving natural canine herpesvirus encephalitis, lesions were characterized by randomly distributed glial nodules throughout the white and gray matter of the cerebrum, brainstem, and cerebellum, as well as lymphocytic meningitis and cerebellar cortical necrosis.[7] Although CHV immunohistochemistry was not performed on the brain in this case, the liver was immune-negative, and CHV infection was considered unlikely.

Contributing Institution:
The University of Minnesota, College of Veterinary Medicine, Veterinary Diagnostic Laboratory. https://www.vdl.umn.edu

JPC Diagnosis:
Thalamus and cerebrum: Vasculitis, lymphocytic, multifocal to coalescing, moderate, with glial nodules and intraendothelial intranuclear viral inclusions.

JPC Comment:
The moderator and conference participants discussed the presence of glial nodules, which are multifocal proliferation of microglial cells in reaction to injury in the CNS and neuronal necrosis. Canine adenovirus 1 is not

apparently associated with the glial nodule formation, and glial nodule formation is not an expected finding in canine adenovirus-1 infection. All agreed that the contributor did an excellent workup to rule out canine distempervirus and canine herpesvirus-1. Both viruses can cause neuronal necrosis with glial nodule formation and would be top differentials based on this H&E section.

Two helpful histologic features which indicate that this animal is juvenile are the diffuse high concentration of glial cells and the prominence of the undifferentiated glial and neuronal precursors ("rests") in the periventrular tissue. Both of these findings are normal in puppies.

Most vertebrate species can be infected by viruses of the Adenovirus family, but individual viruses have narrow host ranges, are persistent, and generally are subclinical or associated with mild respiratory infections.[8] There are notable exceptions such as CAV-1, deer adenovirus, and several avian adenoviruses, which cause important diseases in their host species.[8] There are five genera in the *Adenoviridae* family: *Mastadenovirus, Aviadenovirus, Atadenovirus, Siadenovirus,* and *Ichtadenovirus.* The name adenovirus is derived from how the virus was initially identified: as a cause of cellular degeneration in human adenoid cultures.[8]

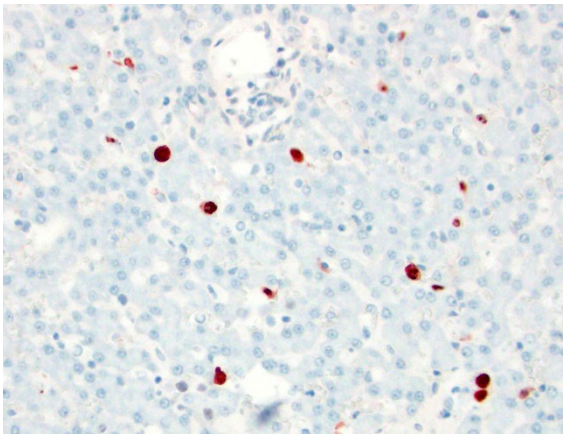

Figure 3-7. Brain (cerebral cortex), dog. Canine adenovirus type-1 immunohistochemistry. Occasional Kupffer cells, hepatocytes, and rare endothelial cells have strong intracytoplasmic and occasional intranuclear immunoreactivity. (Image courtesy of: The University of Minnesota, College of Veterinary Medicine, Veterinary Diagnostic Laboratory. https://www.vdl.umn.edu) (anti-CAV-1, 400X)

Canine adenovirus 1, which affects domestic and wild canids, skunks, coatis, and bears, was discovered in 1954 as the causative agent of fox encephalitis.[8,9] The contributor provides an excellent overview of CAV-1 infection in dogs and non-domestic animals. A recent report by Pereira et al described natural CAV-1 infection and death in captive maned wolf puppies.[9] On necropsy, findings mirrored those seen in dogs, with nonsuppurative meningoencephalitis, necrotizing hepatitis and splenitis, and intranuclear inclusion bodies in hepatocytes (1 of 2 pups) and kidneys (2 of 2 pups).[9]

Canine adenovirus 2, another virus in the *Mastadenovirus* family, is a member of the canine respiratory disease complex and infection is limited to the respiratory system.[9]

Significant adenoviruses in avian species fall within the *Aviadenovirus, Atadenovirus,* and *Siadenovirus* genera.[9] Fowl adenovirus 1, a member of *Aviadenovirus* genus, causes quail bronchitis and is characterized by necrotizing tracheitis, air sacculitis, conjunctivitis, and gastrointestinal disease, with a high mortality in young quail.[9] Another member of the *Aviadenovirus* genus is fowl adenovirus 4, which causes hydropericardium syndrome in chickens, with the most severe disease occurring around one month of age.[9] In addition to the pericardial effusion, pulmonary edema and enlargement of the kidneys and liver may be seen.[9] Within the *Siadenovirus* genus, turkey adenovirus 3 causes hemorrhagic enteritis in turkeys over a month of age, and two

closely related (serologically indistinguishable) viruses cause marble spleen disease in pheasants and avian adenovirus splenomegaly in chickens.[9]

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CASE IV:

Signalment:
8-month-old male American mink (*Neogale vison*) (non-Aleutian)

History:
This mink was a non-inoculated experimental control animal. All mink were acclimated to the facility after arrival for a period of 3 weeks prior to initiation of the experiment. No clinical signs were observed in this mink.

Gross Pathology:
Bright red lungs that did not collapse normally.

Laboratory Results:
Other mink from this cohort were PCR positive for Aleutian Mink Disease Virus (AMDV) in lung and spleen samples. Frozen samples from this case were not immediately available for molecular testing at the time of conference submission.

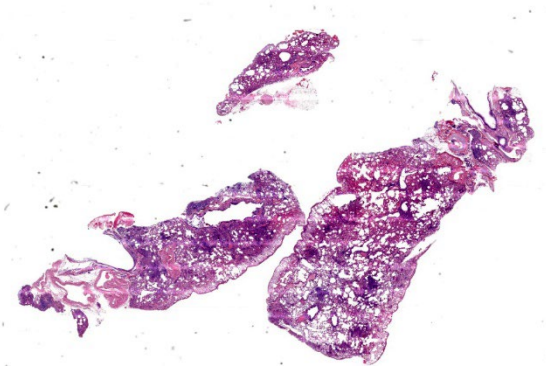

Figure 4-1. Lung, mink. Foci of hypercellularity can be seen even at subgross magnification. (HE, 6X)

Immunohistochemistry for B cell, T cell, and macrophage markers. Most perivascular mononuclear cells stained with CD3 antibody with immunohistochemistry.

Microscopic Description:
Lung: The alveolar septa are diffusely thickened due to increased numbers of lymphocytes and enlarged round to cuboidal epithelium (type II pneumocyte hyperplasia) lining the alveoli. There are many lymphocytes with fewer plasma cells around pulmonary vasculature. There are multifocal areas of low numbers of neutrophils within alveoli and alveolar septa along with numerous alveolar macrophages. Alveolar macrophages have two distinct morphologies. The first type is 20-35 microns in diameter with a round to reniform 10-20 microns in diameter; the cytoplasm is amphophilic with an eosinophilic perinuclear clear zone. The second population of macrophages have prominent 2–3-micron intracytoplasmic vacuoles. There are occasionally multinucleated macrophages. Alveolar lumens also contain sloughed epithelial cells multifocal areas of eosinophilic proteinaceous fluid. Alveolar septa are lined by a curved cup-shaped eosinophilic material. Capillaries are congested with red blood cells and occasionally filled with dense eosinophilic material that expands the capillary. Near the lung periphery, capillaries are largely devoid of red blood cells.

Pneumocytes occasionally have vacuolated cytoplasm. Rarely within intra-alveolar cells, there are oval to round magenta intranuclear inclusion bodies 3-4 µm in diameter with peripheralized chromatin. There are multifocal areas where alveolar septal walls are absent, and alveoli conjoin (microbullae).

Contributor's Morphologic Diagnoses:
Interstitial pneumonia, lymphohistiocytic, diffuse, moderate, chronic with perivascular lymphocytes.

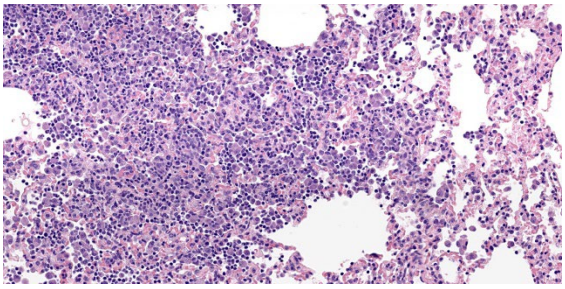

Figure 4-2. Lung, mink: Hypercellular foci consist of alveoli filled by numerous macrophages with basophilic cytoplasm, fewer neutrophils and lymphoplasmacytic infiltrate of peribronchial and perivascular connective tissue. There is also Type 2 pneumocyte hyperplasia contributing to the mix. (HE, 200X)

Contributor's Comment:
The microscopic findings are consistent with Aleutian Mink Disease Virus (AMDV). Previous mink from this shipment died acutely upon arrival and were positive for AMDV by PCR in the spleen and lungs and had concomitant severe hepatic lipidosis.

Aleutian mink disease virus (AMDV) is the archetype and prototype member of Amdoparvoviruses that infects domesticated and wild mink. Individual cases of infection with AMDV in other species is reported, but some historic reports did not include evaluation of sequence. The viral family within the carnivores has recently grown, with distinct, host-specific *amdoparvoviruses* (APVs) discovered in fishers, grey foxes, red foxes, raccoon dogs, red pandas, and striped skunks.[8] In AMDV infected mink, the host age and immune status affects the clinical outcome. In mink kits, the disease classically manifests with interstitial pneumonia and viral replication is predominantly within type II pneumocytes.[3,7] In adult mink, the virus is found in macrophages in lymphoid tissue. Plasma cells proliferate and infiltrate the liver, spleen, kidneys, and lymph nodes. The disease has also been called mink plasmacytosis because of this. Mink have a hypergammaglobulinemia and develop renal disease char-

acterized by glomerulonephritis and interstitial nephritis. Glomerulonephritis and vasculitis are due to viral-antibody complexes (type III hypersensitivity) in affected mink.[3,6]

Aleutian mink are named for a hair coat color dilution due to a genetic mutation. The mutation, termed Chédiak-Higashi syndrome, results in defective lysosomes, melanosomes, cytolytic granules, and platelet rich granules.[6] Aleutian mink are more susceptible to AMDV and appear to be affected by all isolates or strains; whereas, non-Aleutian mink, may be less susceptible to some virus strains resulting in non-progressive persistent infections.[3]

Contributing Institution:
National Animal Disease Center
https://www.ars.usda.gov/midwest-area/ames/nadc/

JPC Diagnosis:
Lung: Pneumonia, interstitial, lymphohistiocytic, diffuse, moderate, with type II pneumocyte hyperplasia, peribronchial and perivascular lymphoplasmacytic infiltrates, and intranuclear viral inclusion bodies.

JPC Comment:
As the contributor mentions, a critical component of the pathogenesis of Aleutian mink disease is the exuberant production of antibodies by plasma cells leading to a type III hypersensitivity reaction (immune complex hypersensitivity). These reactions occur when immune complexes (typically containing IgM and IgG) accumulate in tissues, activate complement, and recruit neutrophils, leading to local damage.[6] Type III hypersensitivity reactions generally occur when there is slightly more antigen than antibody; if antibody is present in large quantities, the insoluble complex is easily cleared by tissue macrophages and hypersensitivity does not develop.[5,6] Complexes may deposit in vessels, joints, glomeruli, or choroid plexus.[4,6] Type III hypersensitivity reaction typically occur in diseases that are the result of persistent infection (i.e. Aleutian mink disease), autoimmunity (i.e. systemic lupus erythematosus), or inhalation of environmental antigens (chronic obstructive pulmonary disease).[6] The Arthus reaction is a localized, cutaneous version of a type III hypersensitivity reaction that can be induced experimentally via intracutaneous injection of antigen into an animal with a preformed antibody; the resulting reaction produces fibrinoid necrosis in vessel walls and subsequent thrombosis.[4]

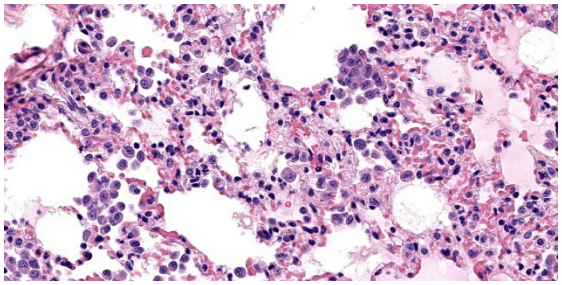

Figure 4-3. Lung, mink: There is diffuse expansion of alveolar septa by congestion, edema, macrophages, lymphocytes and scattered hyperplastic Type II pneumocytes. (HE, 580X)

Aleutian mink disease virus is single stranded, negative-sense DNA virus of the genus *Amdoparvovirus*.[2,5] Dr. Pesavento described that diseases associated with other carnivore APVs (Red Panda and Skunk) share many of the features of AMDV in mink, including tissue distribution, but the well described immune complex disease of the infected mink is not present with other APV infections so far.

The *amdoparvovirus* isolated from endangered red pandas (RPAV) is widespread in captive populations in the United States, with the virus isolated from at least 50% of 104 animals tested.[1] Infection appears to be persistent, as well: the virus was consistently isolated from 6 animals serially sampled for up to 6 years.[1,2] In the index case, the red

panda had virus associated by ISH with significant pyogranulomatous inflammation in multiple sites, including the peritoneum, pancreas, and heart.[1] It appears that a subset of pandas is susceptible to viral associated disease, with heavy loads of virus, and lesions consistently in kidneys, but also variably in other tissues (lung, heart, brain, eggs). In pandas positive for the virus, but dead from other causes, virus was identified in normal lymphoid tissues and within normal gastrointestinal mucosa. The intestinal tract, given the consistent shedding and the ISH results, is a likely site of persistence in Red Pandas.

Classically, parvoviruses are thought to infect cells only which are actively replicating (in S-phase, i.e. crypt enterocytes), since the virus requires cellular machinery to replicate, as the contributor mentions. Dr. Pesavento described, however, that human parvovirus B19, the causative agent of fifth disease in humans, induces a DNA damage response (DDR) that facilitates viral DNA replication.

In addition to AMDV, mink are susceptible to another parvovirus, mink enteritis virus, which is closely related to feline panleukopenia virus and an important disease of farmed mink.[5,8] Similar to feline panleukopenia, mink enteritis virus targets the enterocytes, causing crypt epithelial necrosis; lymphocytes, causing lymphoid necrosis; and bone marrow progenitor cells, causing leukopenia.[8] Curiously, mink enteritis virus is not associated with cerebellar hypoplasia.[5]

Conference participants discussed euthanasia artifact due to barbiturate salt precipitation as a possible cause for the thickening and eosinophilic debris in the pleura.

References:

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5. MacLachland NJ, Dubovi EJ, eds. Parvoviridae. In: *Fenner's Veterinary Virology.* San Diego, CA: Elsevier. 2017; 251-255.
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