



WEDNESDAY SLIDE CONFERENCE 2015-2016

Conference 25

18 May 2016

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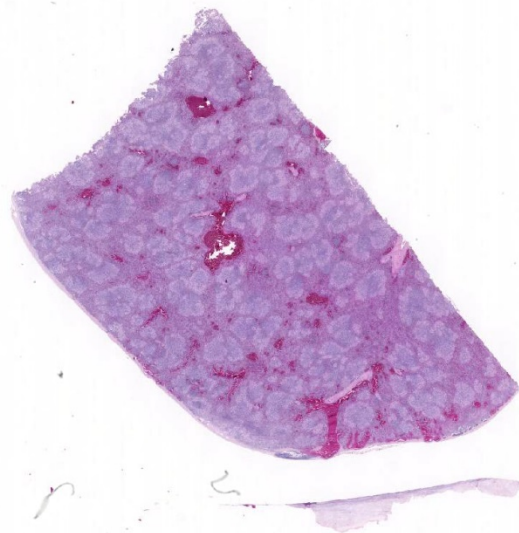
CASE I: H11-003673 (JPC 4021637).

Signalment: 10-week old, male, turkey,
(*Meleagris gallopavo*).

History: One of three turkeys found dead without premonitory signs on one day from a large flock of commercial fattening birds. Further individual birds also died over the following two weeks.

Gross Pathology: In good body condition, severe diffuse hemorrhagic enteritis and splenomegaly. Pale mottling of spleen by multifocal 0.5 to 3mm diameter, white, round to irregularly-shaped lesions.

Laboratory Results: No significant bacteria isolated from spleen or small intestine following aerobic and anaerobic culture on blood agar. PCR on splenic samples detected turkey hemorrhagic enteritis virus (THEV).⁷

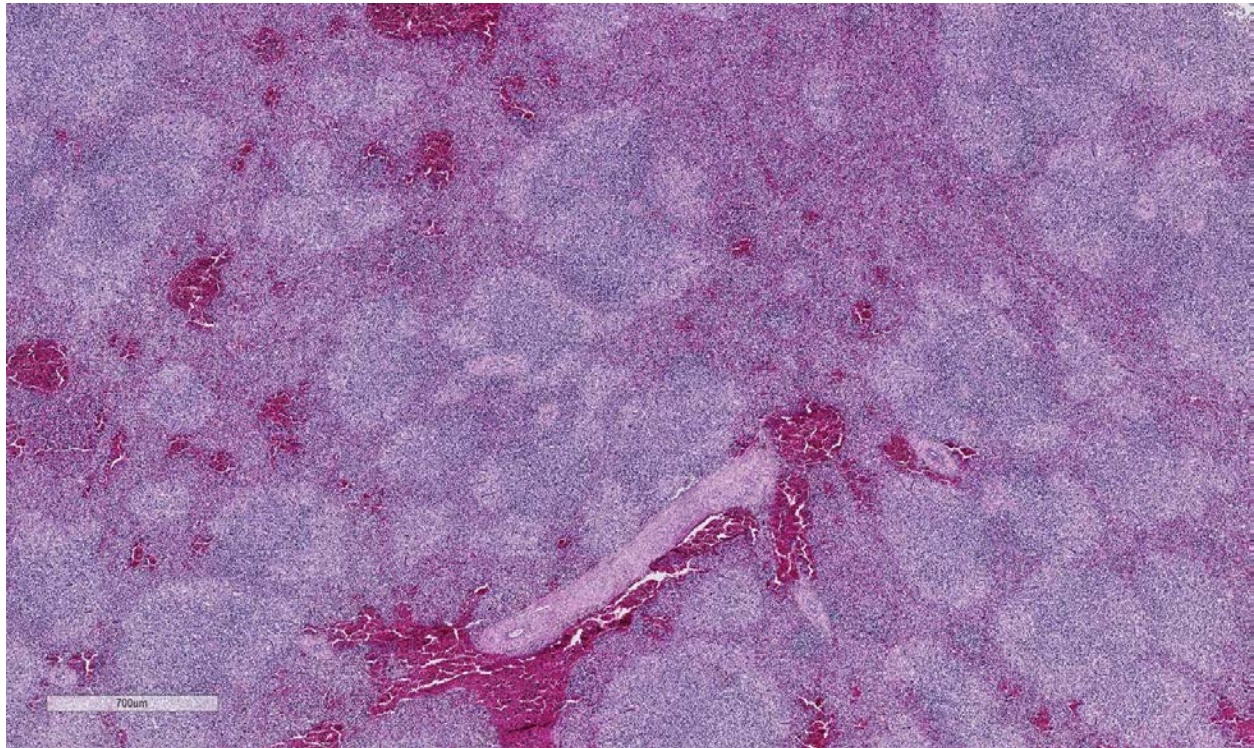


Spleen, turkey. At subgross, this section of enlarged spleen contains numerous coalescing areas of white pulp. (HE, 5X)

Histopathologic Description: Spleen: Multifocal to coalescing areas of necrosis in white pulp area containing eosinophilic amorphous material together with moderate

numbers of admixed heterophils, macrophages, lymphocytes and plasma cells. There is accompanying diffuse severe vascular congestion. Numerous large cells (approximately 15 μm in diameter) contain large nuclei (approximately 12 μm in diameter) which in turn exhibit large basophilic intranuclear inclusions with associated chromatin margination.

resulting in depression, bloody diarrhea, splenomegaly, immunosuppression and death.⁷ The most consistent postmortem finding is splenomegaly with a multifocal to coalescing splenitis with individual cell necrosis and large intranuclear inclusions affecting lymphocytes.^{1, 11} Intestinal lesions and the scale of the mortality depends at least in part on the virulence of the THEV strain.^{9,10} THEV is classified as a type II



Spleen, turkey. White pulp is expanded but hypocellular due to a marked loss of lymphocytes and an infiltrate of large histiocytes. (HE, 18X)

Contributor’s Morphologic Diagnosis:

Necrotizing splenitis: acute; multifocal to coalescing; severe, with basophilic intranuclear inclusions consistent with a diagnosis of turkey hemorrhagic enteritis.

Contributor’s Comment: The gross and histopathological findings are consistent with a diagnosis of turkey hemorrhagic enteritis (THE) disease of turkeys caused by infection with THEV.¹¹ This was confirmed by PCR. HE affects growing turkeys

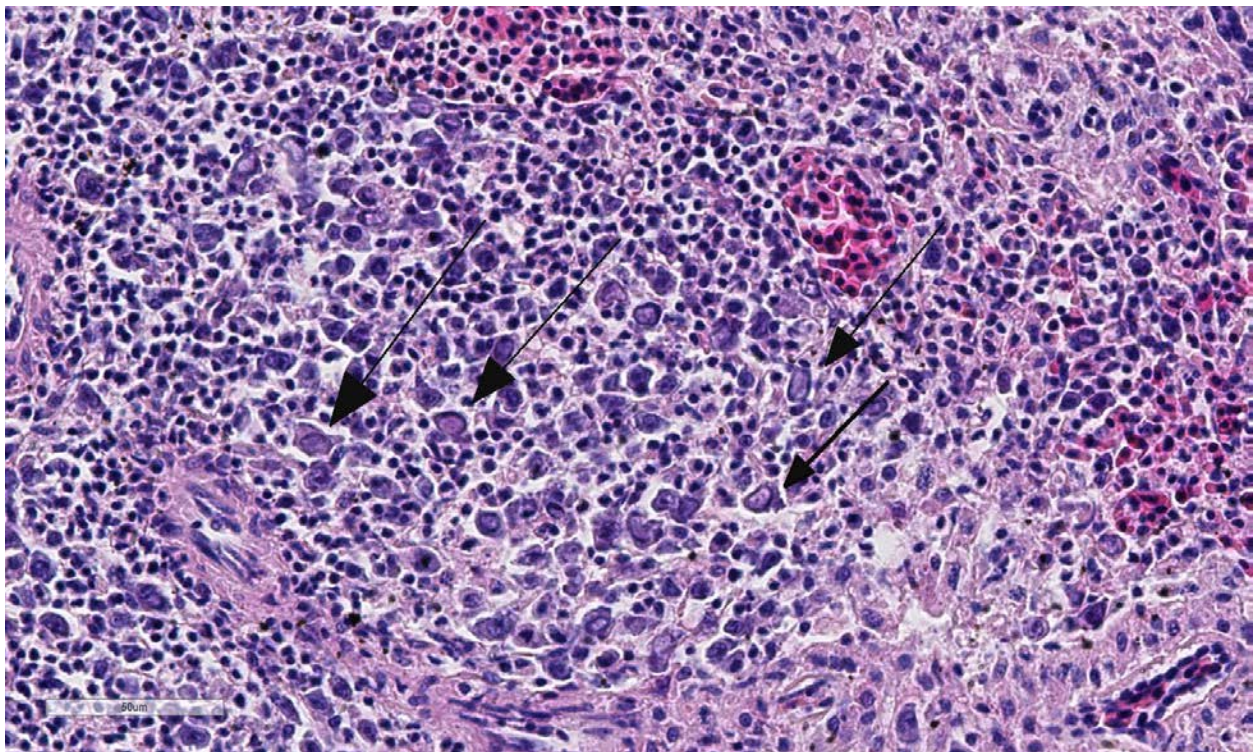
adenovirus, of the family Adenoviridae, and has been further classified as a member of the more recently formed genus *Siadenovirus*.^{1,4,11} It was originally identified in the USA, where it has been widely reported.¹¹ The virus spreads via horizontal transmission including the oral route and rapidly replicates in the spleen of poults.¹² A necrotizing splenitis is frequently described in the splenic white pulp¹³ with targeting of macrophages and particularly IgM-bearing B-lymphocytes.^{12,13} Infected B-lymphocytes and macrophages undergo both necrosis and

apoptosis.⁹ Although the pathogenesis is incompletely understood infection of turkeys with virulent strains of THEV results in hemorrhage into the lumen of the duodenum and jejunum by erythrocytes diapedesis without obvious attendant vascular injury.⁹

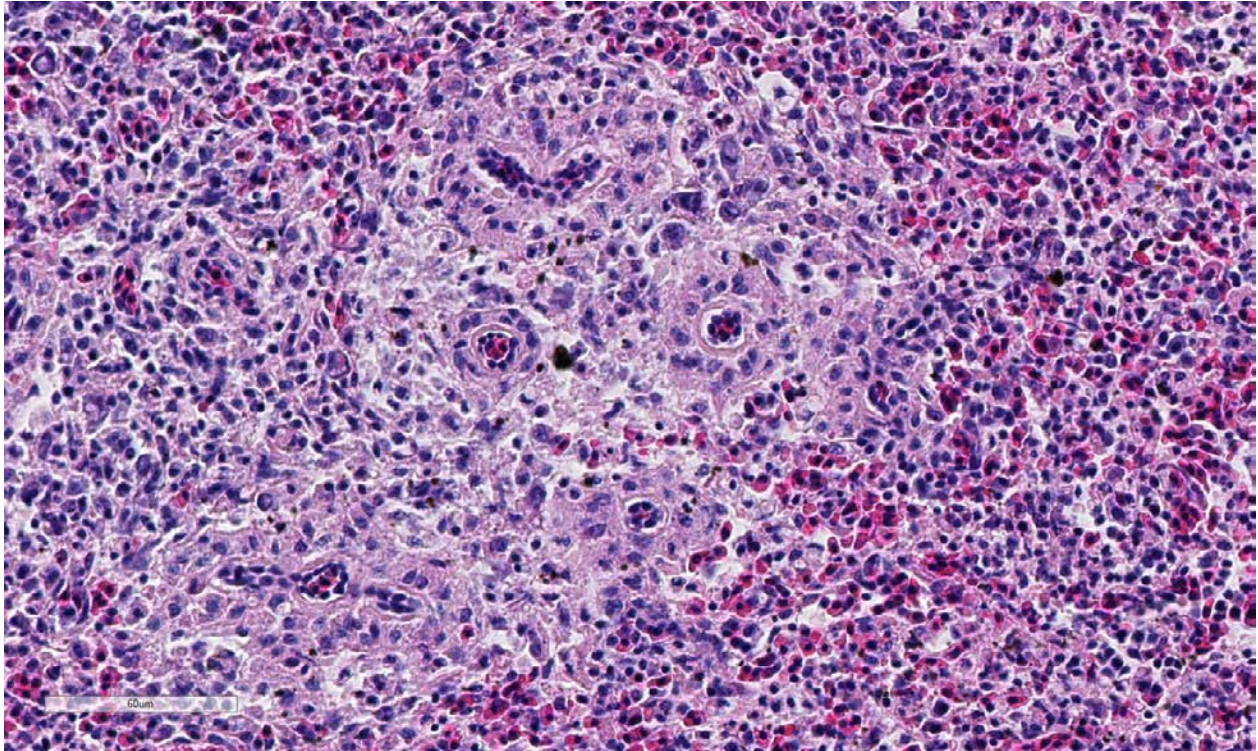
JPC Diagnosis: Spleen: Splenitis, histiocytic, diffuse, severe with lymphoid depletion and intrahistiocytic, intranuclear viral inclusion bodies.

Conference Comment: Adenoviruses in avian or mammalian species may occur as asymptomatic infections or as primary pathogens as seen in this case of THEV. Previously two genera of adenovirus existed, *Mastadenovirus* and *Aviadenovirus*, but the adenoviridae family was revised in 2005 and now includes two additional genera *Siadenovirus* and *Atadenovirus*, the former of which includes THEV.⁶ The *Aviadenovirus* genera, which was previously designated as

group I avian adenovirus, includes many of the pathogenic avian adenovirus diseases including inclusion body hepatitis, quail bronchitis virus and hydropericardium syndrome. *Siadenovirus*, which was previously designated as group II avian adenovirus, in addition to THEV includes MSD in pheasants and “splenomegaly virus” of chickens, another disease very similar to THEV and MSD. This genera is distinguished genetically by the presence of a gene that encodes for sialidase (for which it is named), and also includes an adenovirus of frogs.⁵ *Atadenovirus*, which was previously designated as group III avian adenovirus, includes egg drop syndrome, which is characterized by decreased egg production and thin-shelled eggs; ducks and geese are the natural hosts. *Atadenovirus* also includes viruses which affect mammals and reptiles.^{5,6} Adenoviruses of the *Mastadenovirus* genera include the majority of common mammalian adenoviruses and do not cause disease in avian species.



Spleen, turkey. Histiocytes within splenic white pulp contain large oblong amphophilic to basophilic adenoviral inclusions which peripheralize the chromatin (arrows) (HE, 40X).



Spleen, turkey. Penicilliary vessels within the red pulp are cuffed by several layers of infiltrating histiocytes. (HE, 400X)

The age of affected turkeys is approximately 4 weeks and older, with most infections occurring between 6 – 11 weeks; the course of clinical disease is approximately 7-10 days. Mortality rates may exceed 60%. Due to immunosuppression and secondary bacterial infections or co-infections, such as with *E. coli*, the course of disease and severity of losses can be exacerbated. Infection and recovery, as well as vaccination, provides protection against subsequent challenge.⁸ It is postulated that persistent infection or latency may occur within macrophages or B-lymphocytes in birds infected with avirulent strains of THEV, which are used in live virus vaccines.² The differential diagnosis list for the splenic lesions includes lymphoid neoplasia and bacteremia associated with infections such as *E. coli*, *P. multocida* and *E. rhusiopathiae*.⁸ A similar condition occurs in confinement-raised pheasants of approximately 3-8 months of age due to

infection with an indistinguishable virus, and the condition is known as marbled spleen disease (MSD). These two viruses are nearly genetically identical, although the presentation of the disease in these respective species has some distinctions. In pheasants, MSD is characterized by diffuse necrotizing splenitis with hemorrhage and loss of architecture, but generally presents as a respiratory condition in naturally infected birds. Gross lesions may include congested lungs in addition to the splenic changes.⁸ THEV and MSD affect a slightly different age range of fowl but retain many similar features in regards to their effect on the spleen. Macrophages and B lymphocytes are the main target of both THEV and MSD as discussed above and although viral replication chiefly takes place within the spleen, virus may also be found in the intestine, bursa of Fabricius, thymus, liver, kidney and lung.

Conference participants described multifocal areas of pallor characterized by necrosis and loss of B lymphocytes within follicles / white pulp. There is extensive histiocytic infiltration of the spleen, primarily surrounding the vascular tree within the white pulp, and histiocytes frequently contain pale basophilic intranuclear viral inclusion bodies. Inclusion bodies are identified also within morphologically recognizable lymphocytes, although no IHC was performed to definitively establish the range of cell targets. The moderator commented about the histiocytic infiltration around splenic arteries, which is suggestive of the pathogenesis of THEV. The virus has a cytopathic effect on B lymphocytes and also replicates within macrophages, and transient immunosuppression occurs in infection by both virulent and avirulent strains.^{2,8} In response to infection, there is a large influx of macrophages, as well as T-lymphocytes in an attempt to clear the virus.⁸ Conference participants discussed the diagnosis of inflammatory splenitis vs. infiltrative and or necrotizing disease of the spleen. The moderator led a discussion around other features of THEV infection including the rapid clinical course and characteristic presence of hemorrhagic feces just prior to death, for which it is appropriately named. The hemorrhage is thought to be secondary to endothelial disruption and not due to viral destruction.² Grossly, the spleen appears enlarged, friable and mottled.

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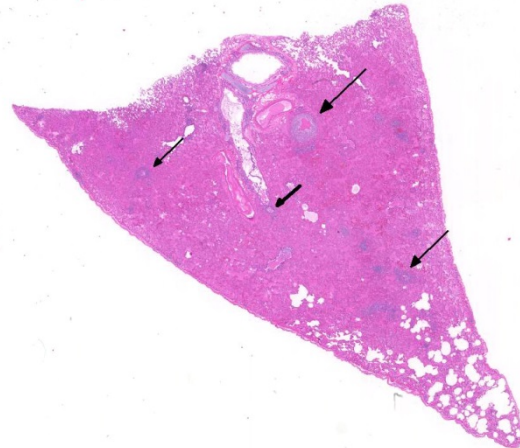
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CASE II: 10-01139 (JPC 3167330).

Signalment: 7-month-old spayed female domestic shorthair cat (*felis catus*).

History: Chronic lethargy, anemia, icterus; presented with low body temperature.

Gross Pathology: Lungs were diffusely reddened, firm and did not collapse. Throughout all liver lobes are 1mm to 3mm pale tan nodules. Similar pale tan nodules from 1mm to 7mm diameter were scattered



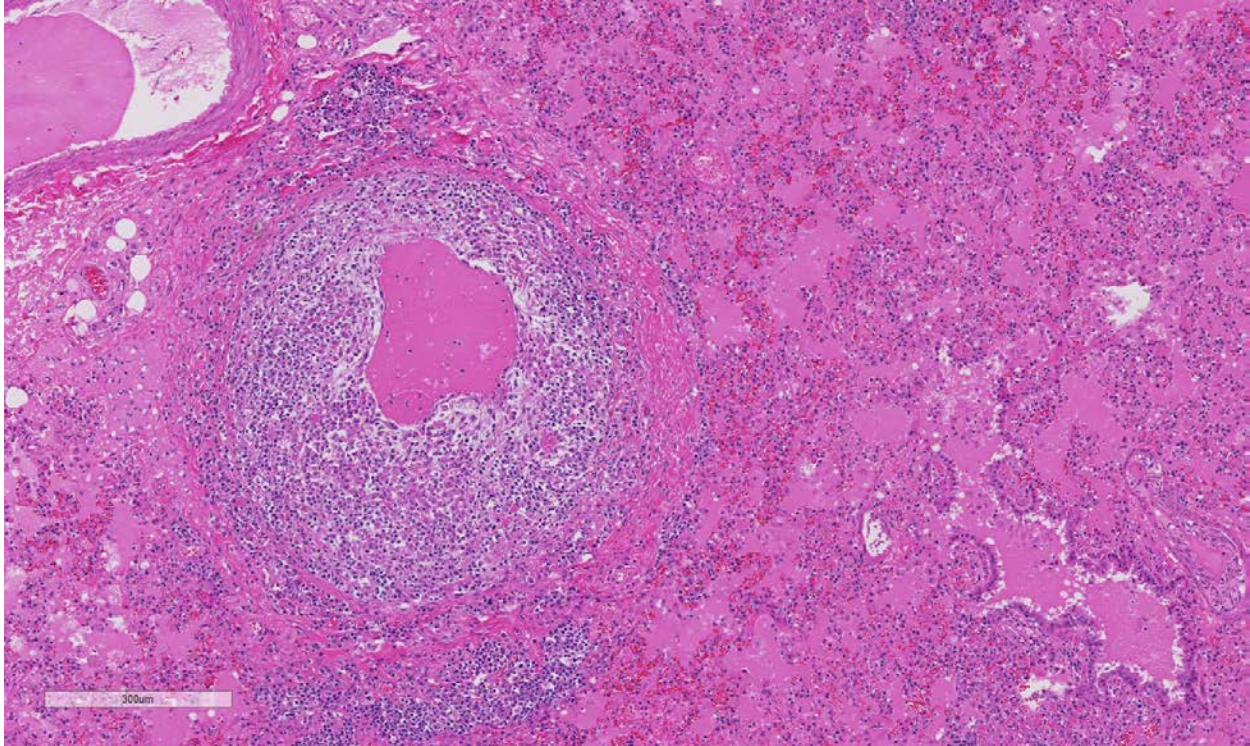
Lung, cat. Subgross examination of the submitted section of lung reveals marked diffuse intra-alveolar edema, and the walls of vessels are prominently expanded by a cellular infiltrate (arrows). (HE, 5X).

throughout the cortex of both kidneys. Mesenteric lymph nodes were enlarged.

Laboratory Results: Total protein - 10.0, albumin 2.1, globulins 7.5, phosphorous 6.8, potassium 3.4, elevated ALP, CK and total bilirubin (1.4).

Histopathologic Description: Blood vessels in the lung are accentuated by inflammatory infiltrates that greatly expand the vessel wall. These infiltrates are composed of macrophages, plasma cells, neutrophils, lymphocytes and occasional Mott cells. Mild fibroplasia is present in the infiltrate also. Vessel lumens are narrowed by the infiltrates. Diffusely, alveoli contain edema fluid and low numbers of macrophages and occasional neutrophils.

Contributor's Morphologic Diagnosis: Lung: Vasculitis/perivasculitis, multifocal, pyogranulomatous with alveolar edema.



Lung, cat. The walls of vessels are markedly expanded up to 10 times by an infiltrate of histiocytes and fewer lymphocytes, neutrophils and neutrophils which effaced the tunica intima and media and extend into the surrounding adventitia and pulmonary parenchyma. Alveoli are filled with edema fluid which is refluxed into adjacent airways. (HE, 71X)

Contributor's Comment: Gross findings in this cat were suggestive of the dry form of feline infectious peritonitis (FIP), and the vascular lesions in the lung were suggestive of those described in cases of FIP. Coronaviral antigen was identified in the vessels by immunohistochemistry.

FIP is one of the leading infectious causes of death in cats from shelters and catteries.⁷ FIP is caused by infection with feline coronavirus (FeCoV) that has mutated to become a pathogenic virus (FIPV). FCoV infections are very common, with up to 90% seropositivity in cats, while FIP morbidity is low, rarely above 5% of infected cats. Most infections with feline coronavirus are sub-clinical, although mild diarrhea and vomiting can occur. The FCoV mutants that cause FIP are either generated within the individual cat, or possibly are acquired externally.

Persistently infected, healthy carriers are believed to be most important in the epidemiology of FIP.⁶ Mutation of the FCoV in the carrier animal generates the FIP virus that is capable of replicating in monocyte/macrophages, resulting in transportation to organs outside of the GI tract, and development of FIP disease. The FCoV mutants also apparently lose their ability to replicate in intestinal epithelium, as they are not recovered from intestinal tissues.² For this reason, it is believed that cat to cat transmission of FIP virus is infrequent; the virus does not readily infect cats under natural conditions because it does not replicate in enterocytes, even though the virus readily infects cats when they are experimentally inoculated by routes other than oral.

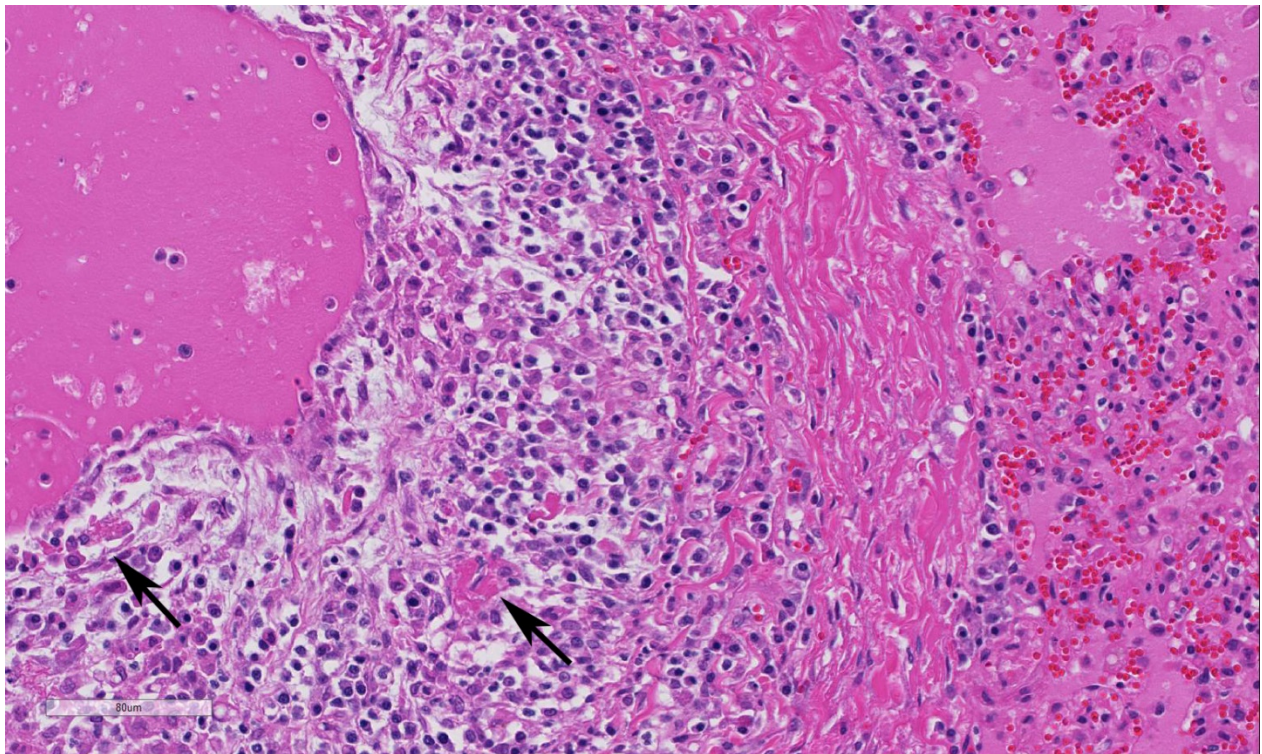
The host immune response largely dictates the lesions of FIP. The immune response to FIP virus is presently understood as follows:

humoral immunity is not important for protection while protective immunity is largely cell mediated.⁷ The type and strength of immunity determines the form that FIP virus infection will take. Cats that develop FIP will have either the wet or dry form depending on whether ineffective cell-mediated or humoral immunity dominates the clinical disease. Strong humoral immunity with very weak or non-existent cellular immunity leads to effusive (wet) FIP. With the effusive form, cats will have up to 1 L of viscous abdominal fluid while pleural effusion is present in about 25% of cases. Humoral immunity with intermediate cellular immunity will manifest as the non-effusive FIP (dry form). With the dry form, the kidneys may be enlarged and nodular with white, firm nodules protruding from the cortex. Foci of inflammation may also be seen in other organs, including the liver and pancreas. The gross lesions of wet versus dry forms are often not distinctly separate,

and much overlap occurs.

Vasculitis and perivasculitis characterize the microscopic lesions of FIP. FIP-induced granulomatous vasculitis occurs in small to medium-sized veins predominantly in the leptomeninges, renal cortex, and eye, but also frequently in the lung and liver.⁴ Vasculitis is characterized as venous and perivenous, macrophage-dominated, circular infiltrates in small veins, and focal infiltrates in larger veins. Neutrophils and T cells represented minorities among inflammatory cells, and B cells mainly occur as peripheral rims around circular granulomatous infiltrates. FIP virus-infected monocytes that become activated and emigrate from vessel lumens into perivenous locations are reported to be unique to the development of the periphlebitis that occurs.

JPC Diagnosis: Lung: Vasculitis,



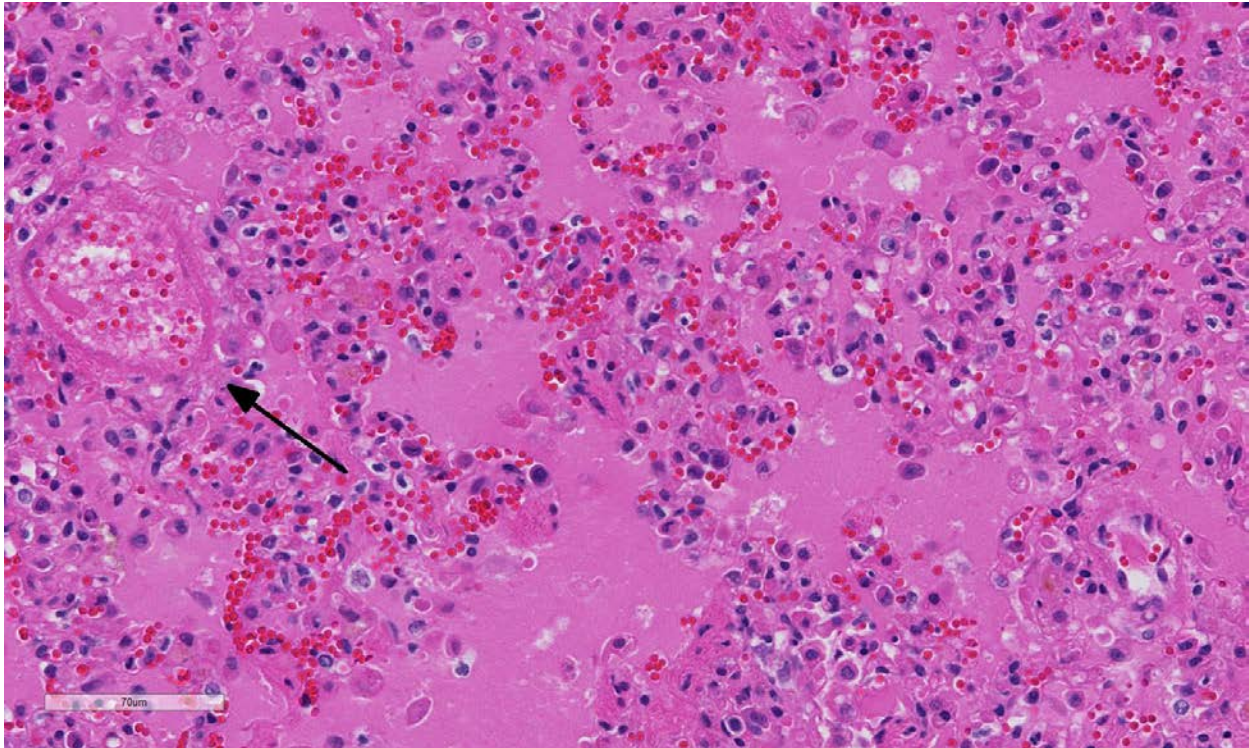
Lung, cat. Higher magnification of the vessel showing the number of infiltrating neutrophils, histiocytes, and lymphocytes as well as the proliferation of fibroblasts and small amounts of brightly eosinophilic extruded protein within the wall (arrows). (HE, 240X)

necrotizing, lymphocytic and histiocytic, diffuse, severe with fibrinoid necrosis and alveolar and interstitial edema.

Conference Comment: Feline coronavirus (FCoV) belongs to the genus *Alphacoronavirus* and species *Alphacoronavirus-1*, along with canine coronavirus and transmissible gastroenteritis virus of pigs. There are two serotypes of FCoV (based on antigenicity) types I and II, and while both may cause feline infectious peritonitis (FIP), type I is more common in the cat population. The serotypes differ primarily in growth characteristics in cell

between type I FCoV and CCoV. Along with homologous recombination, the propensity for frequent variation and mutation of coronaviruses is also based on a high mutation rate (2.0×10^{-6} mutations per site per round of replication) and the sheer size of the genome (26–32 kb).⁹ As is true with many viruses, even a single amino acid mutation and/or recombination events can change viral properties, host range and pathogenicity.

Both feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV) can infect monocytes, but FIPV's are able to sustainably replicate in much higher

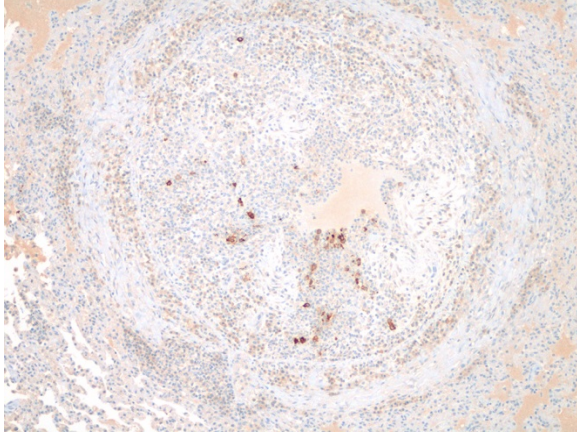


Lung, cat. The walls of thrombosed smaller vessels are diffusely necrotic (arrow), with necrosis within adjacent alveolar walls and flooding of alveoli with edema fluid. (HE, 400X)

culture and in receptor usage, and it is notable that most, if not all, of the experimental work so far has been done on the Type II strains because they grow well in cell culture. Type II FCoVs arose as a consequence of a double recombination

numbers.⁵ However, not all monocytes are permissive to replication of FIPV⁸ and there is variation in individual cats regarding the susceptibility of monocytes to infection and replication, which influences disease susceptibility. It has also been suggested that

monocytes may potentially be the cells where mutation from FECV to FIPV occurs.^{5,8} The precise genetic and mechanistic differences that define changes in viral replication and virulence have not yet been clearly elucidated, but various proteins such as 3c, S, S1 fusion peptide and 7a/b,



Histiocytes within the walls of affected vessels stain positively for feline coronavirus antigen. (anti-FECV, 100X)

among others, appear to play a role.^{5,8} In many cases, these various proteins appear to influence virus infection of, and replication in, monocytes. At least three key events are known in the development of FIP including systemic infection with FIPV, effective FIPV replication in monocytes and activation of those monocytes,⁵ highlighting the critical role of the monocyte response in development of FIP.

There was conference discussion around the use of the terms “wet” and “dry” forms of FIP. Some researchers believe these are a temporal continuum, with the latter being a chronic manifestation, or a post-manifestation of the former. The terms are useful clinically, but we know little about what contributions of the virus and/or host are the bases of the two types of presentations. Lesion distribution in cases of FIP is rather consistent although some degree of individual variation may be observed.

Peritoneal involvement was seen in 75% of cases, often associated with abdominal effusion, and the kidneys, followed by eyes and brain, were most often affected according to one study, and ocular involvement was frequently bilateral.⁵

Antemortem diagnosis of FIP can be particularly challenging. Cytology of abdominal effusion suggestive of FIP contains nondegenerate neutrophils, macrophages, lymphocytes and few plasma cells on a proteinaceous background. Using immunofluorescence or immunohistochemistry to visualize the virus within monocytes of the effusion is considered diagnostic, excepting cases of sequestered granulomas, such as this case. A positive reaction in the Rivalta’s test, used to differentiate transudates from exudates, may increase diagnostic sensitivity when accompanied by cytology of abdominal effusion.³ As discussed above, vascular lesions are generally limited to the small and medium sized veins in affected tissues due to interaction between activated macrophages and endothelium.⁵ In some cases, vascular lesions, which are generally dominated by macrophages, may be replaced by B cells and plasma cells.⁵ This is commonly observed in ocular disease where plasma cells predominate. The clinical course of disease in the wet form is generally much faster than for the dry form and subclinical as well as a protracted or multiphasic course of disease may also be seen. The conference attendees discussed a recent publication that demonstrates theoretical reversal of viremia using pro-tease inhibitors, and clinical trials that are underway to establish whether a rigorous anti-viral therapy could be helpful.¹⁰

Conference participants described the inflammatory infiltrate as being vascular and perivascular in distribution, effecting small

and medium sized vessels, and remarkably circumferential in most affected vessels. The loss of vascular architecture included the entirety of the vessel wall which was markedly thickened by fibrin, both hyalinized and fibrillar, edema, and a mixed population of inflammatory cells, predominantly macrophages. The endothelium is hypertrophic and vascular lumina are narrowed. The moderator stressed the importance of describing vascular changes in detail, specifically with reference to vasculitis; the description should characterize changes within each layer of the effected vessel. Edema fluid fills alveoli in this case, and has refluxed into small and terminal bronchioles, a change which needs to be distinguished from an airway-centric disease (i.e., bronchopneumonia). The alveolar septa and interstitium are mildly expanded by fibrin and edema. There are multifocal areas of alveolar emphysema and few areas of mild fibrinous pleuritis.

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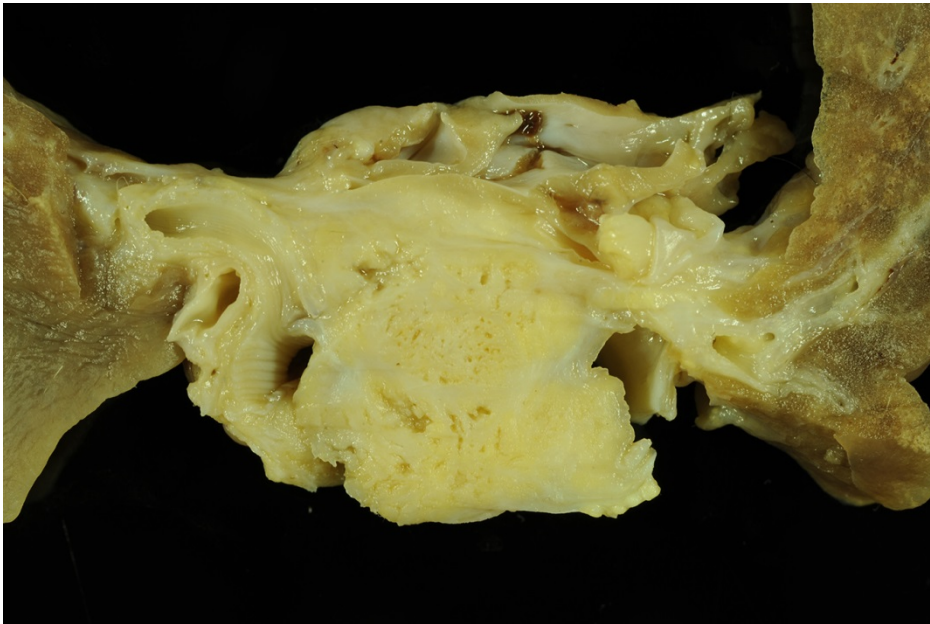
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CASE III: S 307/14 (JPC 4048846).

Signalment: Few week old, female mongrel dog, canine (*Canis familiaris*)

History: The dog was originally born in Romania. The bitch and her litter were brought to Germany by an animal rights group. The pup showed a 4-week-history of diarrhea and apathy. After 3 weeks the dog developed additionally central nervous signs



Lung, dog: A 3cm yellow-gray mass was present within the pulmonary parenchyma at the level of the tracheal bifurcation. (Photo courtesy of: Setor de Patologia Veterinária, UFRGS, <http://www.ufrgs.br/patologia/>)

including torticollis and ataxia. Finally, it died spontaneously. The mother showed no clinical signs. One other puppy of the same litter developed also diarrhea and apathy and died one week prior to the pup described here. Both puppies received a single vaccination against canine distemper five days before occurrence of the first clinical signs.

Gross Pathology: The dog was in a moderate to poor nutritional condition. In the lung, there was a single white to yellowish-grey mass of 3 cm in diameter at the level of the bifurcation. It was firm with

a granulated cut surface. Focally, there was a yellow greenish suppurative focus. The kidneys showed multiple white foci on the cortical surface. Furthermore, the skin of the abdomen caudally to the umbilicus had multifocal red spots of 0.3 cm diameter and few pustules of approximately 0.2 cm diameter. Brain, spinal cord and intestine were grossly unremarkable.

Laboratory Results: Microbiological culture of lung tissue revealed a moderate content of *Escherichia coli* and mild contents of *Nocardia*-like bacteria and coagulase-negative staphylococci. Identification of the *Nocardia*-like bacteria by sequencing of the 16S rRNA showed 100% similarity to the sequence of *Nocardia veterana*.

Histopathologic Description:

The histologic preparation shows a cross section of the cerebellum and brain stem at the level of the corpus trapezoideum. Multifocally, there are randomly distributed pyogranulomatous foci with centrally destroyed neuroparenchyma. The lesions are composed of moderate amounts of centrally-located degenerated and viable neutrophilic granulocytes surrounded by macrophages with a moderate amount of pale eosinophilic and mildly granulated to foamy cytoplasm with indistinct cell borders. These cells contain one prominent, paracentral, oval to kidney-shaped, basophilic nucleus and are interpreted as epithelioid macrophages. Adjacent to the granulomas moderate pe-

rivasascular infiltration (up to 3 layers) composed of lymphocytes and plasma cells are noted (perivascular cuffing). Vascular endothelial cells display hypertrophy. The meninges also show multifocal, mild lymphocytic infiltration. Mild to moderate microgliosis is characterized by rod-shaped microglial cells. Furthermore, single periventricularly located swollen astrocytes contain intranuclear and/or cytoplasmic, brightly eosinophilic, round inclusion bodies with a diameter of up to 4 μm and occasionally surrounded by a clear halo.

Contributor's Morphologic Diagnosis:

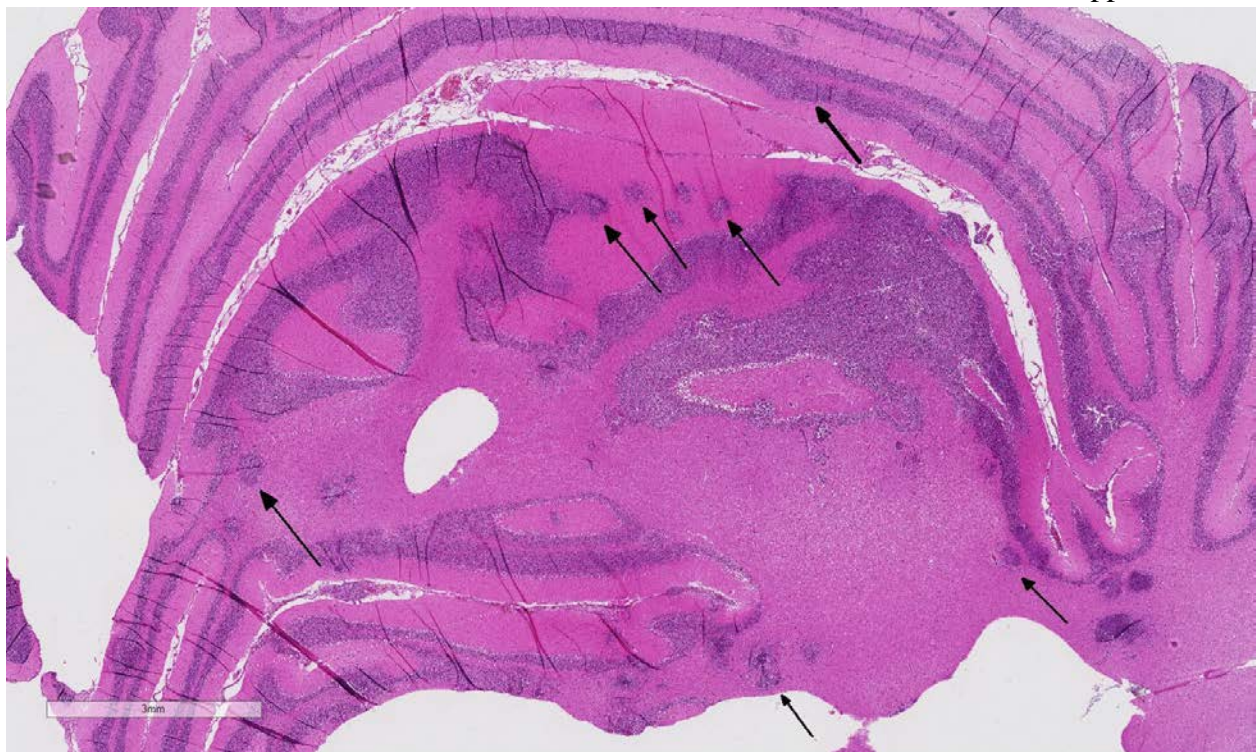
Cerebellum and brain stem:

1. Encephalitis, pyogranulomatous to necrotizing, severe, multifocal, chronic with microgliosis;
2. Periventricular astrocytosis with intranuclear and/or cytoplasmic, viral inclusion bodies;
3. Meningitis, lymphocytic, mild, multifocal, chronic.

Contributor's Comment: The morphologic

findings are consistent with a concurrent infection of canine distemper virus (CDV) and a mixed bacterial infection with the involvement of *Nocardia veterana*.

In addition to the alterations of the brain, the lung revealed a focally severe, chronic pyogranulomatous pneumonia and a non-suppurative interstitial pneumonia with single intranuclear and cytoplasmic, eosinophilic viral inclusions in epithelial cells of bronchioles. The kidneys exhibited a severe, multifocal, chronic, granulomatous to pyogranulomatous nephritis with multifocal arteritis and extensive necrosis. Moderate, multifocal, chronic, pyogranulomatous inflammation was also present in the adrenal gland and the myocardium. However, Ziehl-Neelsen's stain did not reveal convincing evidence of intralésional acid-fast organisms consistent with *Nocardia* sp. However, Grocott's methenamine silver impregnation displayed filamentous bacteria. The skin of the abdomen caudally to the umbilicus revealed a moderate to severe, multifocal, subacute, suppurative to



Cerebellum, dog. There are numerous cellular foci distributed randomly throughout all levels of the cerebellum. (HE, 12X).

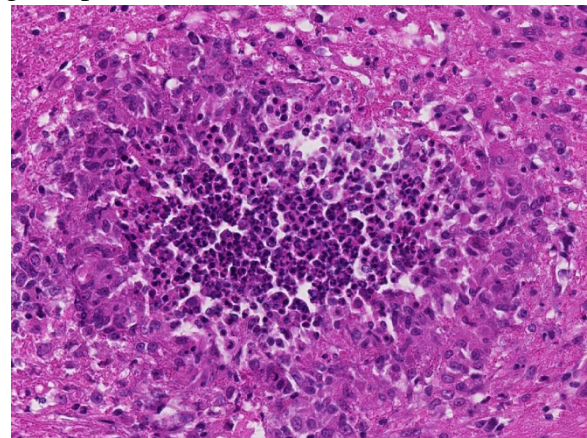
necrotizing dermatitis and inflammation of sweat glands with multifocal formation of pustules and single cytoplasmic, eosinophilic viral inclusions in epidermal cells. Mesenteric lymph nodes displayed severe depletion of lymphocytes and suppurative lymphadenitis. At the time of necropsy, the intestine was unremarkable.

Immunohistochemistry for CDV antigen was positive in epithelial cells of the lung, affected abdominal skin, and in periventricular astrocytes of the brain stem.

Canine distemper virus is a morbilliviral disease of the family *Paramyxoviridae*, including measles virus and rinderpest virus. The host spectrum comprises various species of *Canidae*, *Mustelidae*, *Procyonidae*, *Phocidae*, *Felidae* and other. The virus represents an important infectious disease in many parts of the world. CDV is usually transmitted through inhaled aerosols or close contact. First steps of the pathogenesis include infection of macrophages of the upper respiratory tract or the lung, which migrate to local lymph nodes and tonsils. Afterwards the virus replicates in local lymphoid tissue and spreads throughout the body within 2-5 days after exposure. Manifestations include bronchointerstitial pneumonia, demyelinating disease of the central nervous system, thymus atrophy, ocular disease including conjunctivitis, keratitis, retinitis and optic neuritis, pustular and/or hyperkeratotic cutaneous lesions, dental defects, bone lesions, and abortions.^{4,18} Furthermore, CDV infection leads to profound inhibition of cellular and humoral immune functions resulting in immunosuppression, lymphocyte loss and leukopenia, what boosts susceptibility for opportunistic infections.⁶ Common secondary infections following CDV infection include *Bordetella* sp., adenovirus and *Pneumocystis* sp. infections of the lung as well as toxoplasmosis, Tyzzer's disease, sarcocystosis and

encephalitozoonosis. Secondary enteric infections with *Cryptosporidium* or attaching-and-effacing *Escherichia coli* are also well known.¹⁸ In this case, a secondary mixed bacterial infection with involvement of *Nocardia veterana* led to the pyogranulomatous and necrotizing meningoencephalomyelitis. However, a primary bacterial infection followed by canine distemper cannot be excluded, but is even more unlikely because cases of *Nocardia veterana* described so far in man almost all occurred in severely immunocompromised patients.² The detection of coagulase-negative staphylococci and *Escherichia coli* from lung tissue results most likely from secondary infection with ubiquitously existing bacteria.

Nocardia veterana has been described in 2001 for the first time when it was isolated from bronchial lavage of a human patient with a history of tuberculosis in a veteran's hospital in Australia.¹⁵ *Nocardia* spp. are gram-positive, nonmotile aerobic



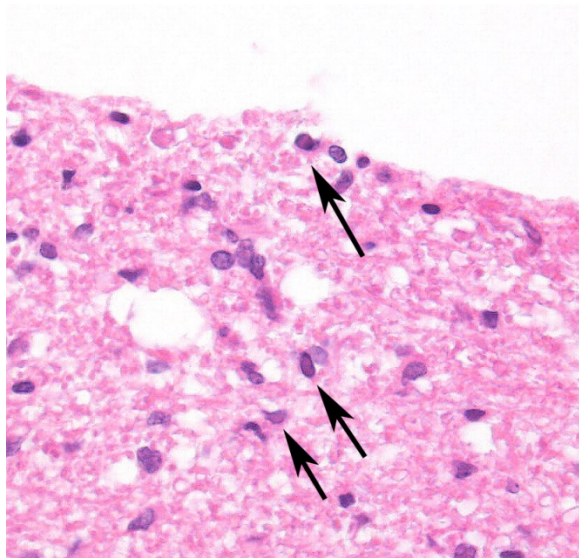
Cerebellum, dog. Hypercellular foci are pyogranulomas, composed of a central core of degenerate neutrophils surrounded by a thick rim of epithelioid macrophages. (HE, 252X)

actinomycetes, which are ubiquitous in the environment and cause a variety of suppurative and granulomatous infections, ranging from cutaneous mycetomas to disseminated systemic diseases.⁵ The

majority of infections are caused by members of the *Nocardia asteroides* complex, which includes *Nocardia asteroides sensu strictu*, *Nocardia abscessus*, *Nocardia cyriacigeorgica* and two clusters closely related to *Nocardia carneae* and *Nocardia flavorosea*.²⁰

Organisms of the *Nocardia asteroides* complex are capable to cause pulmonary, systemic, central nervous and localized cutaneous nocardiosis in man and animals. Infections of bone, eyes, heart, joints and kidneys have also been reported in man as well as mammary gland infections of cows.⁵

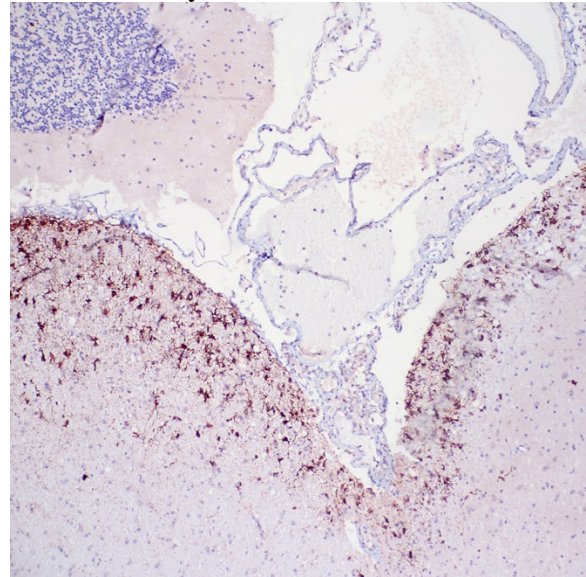
In contrast to *Nocardia asteroides*, infections caused by *Nocardia veterana* are rare and have previously only been reported in man and cows with mastitis in Brazil.¹⁰ In man, *Nocardia veterana* infection is a rare event that is mostly promoted by



Cerebellum, dog. Periventricular astrocytes contain a glassy eosinophilic intranuclear viral inclusion which peripheralizes the chromatin. (HE, 400X)

preliminary immunosuppression.¹⁴ Reported cases include pneumonia in a HIV-infected patient, nodular lymphangitis in a man who was in remission from non-Hodgkin's lymphoma, and a brain abscess in a patient with type 2 diabetes.^{3, 13, 17} Furthermore,

Nocardia veterana infection had caused cutaneous mycetoma in a woman with



Cerebellum, dog. Periventricular astrocytes are strongly cytoplasmically immunopositive for canine morbillivirus antigen. (anti-CDV, 100X).

systemic lupus erythematosus.¹⁶ Single cases of ascitic fluid infection and bloodstream infection in immunocompromised man have also been described in the literature.^{2,10} In cows, *Nocardia* infection of the mammary gland induces severe suppurative pyogranulomatous mastitis.¹⁰ *Nocardia veterana* shows a high rate of multi-resistance to commonly used antibiotic drugs resulting in a failure of conventional antimicrobial agents.^{2,10} It has been shown that high rates of resistance for commonly used drugs in many other *Nocardia* spp. isolated from man exist.²¹

To our knowledge, this is the first report of a systemic bacterial infection associated with *Nocardia veterana* in a dog. These findings emphasize the risk of nocardiosis caused by *Nocardia veterana* in immunocompromised companion animals and a possible transmission from companion animals to immunocompromised man must be considered. Furthermore, sequencing of the 16S rRNA is a suitable tool for defining different *Nocardia* species.^{9,20}

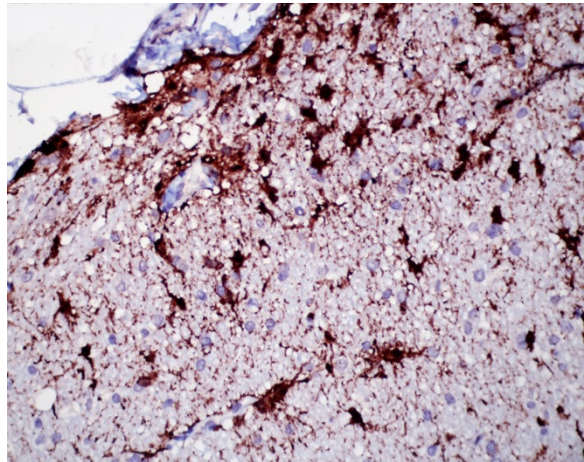
JPC Diagnosis:

1. Cerebellum: Meningoencephalitis, pyogranulomatous, multifocal, moderate with fibrinoid vasculitis.
2. Cerebellum, periventricular astrocytes: Intranuclear viral inclusion bodies, numerous.

Conference Comment: This case serves to highlight the markedly immunosuppressive nature of CDV and the susceptibility to secondary or opportunistic infections, which are quite common as discussed above. CDV infection in dogs has many similarities with measles virus infection in humans. Both viruses enter through alveolar macrophages and dendritic cells, as well as lymphocytes, via the CD150/SLAM molecule and both may result in severe neurologic disease, although neurologic disease is more commonly seen in canine distemper than measles.¹ The demyelinating CNS disease that occurs with CDV infection is said to resemble multiple sclerosis in humans. Additionally, epithelial entry is mediated by the nectin-4 receptor in both viruses.¹ Research has shown that for measles virus infection the period of immunosuppression is generally thought to be weeks to months following infection. The precise mechanisms responsible for this immunosuppression include processes involved in both functional impairment and depletion of immune cells.^{11,12} Interestingly, a recent study using population level data demonstrated that the period of immunosuppression following measles infection may actually be much longer than previously thought, for a period of up to three years where recovered individuals are more susceptible to infectious disease.¹⁹ The cause of this phenomenon is postulated as due to loss of immune memory

lymphocytes due to measles virus infection, which erases previously acquired immunity and has been referred to as “immune amnesia.”^{11, 12,19} This idea is supported by the finding that measles infection resulted in a 2-3 year increase in mortality from other infectious diseases in a population, post measles virus infection.¹⁹ Given the similarities between CDV and measles virus, one wonders if a similar long-term immunosuppressive phenomenon may be observed in CDV infected dogs.

Various neurologic conditions, that are distinctive based on lesion localization and age of onset, may be associated with canine distemper virus (CDV). When the virus infects older dogs, ranging in age from 4-8 years, it can result in a chronic progressive disease referred to as multifocal distemper encephalomyelitis. Histologic lesions



Cerebellum, dog. Higher magnification of CDV-antigen-laden astrocytes. (anti-CDV, 400X).

include a demyelinating leukoencephalomyelitis in the cerebellum and spinal cord.⁷ Old dog encephalitis is separate and rare condition that can slowly progress over a period of 3-4 months and is thought to be due to chronic, subclinical, non-replicating (persistent) infection with CDV. In this condition lesions are localized to the

cerebral cortex, thalamus and midbrain and consist of a necrotizing, non-suppurative encephalitis with prominent perivascular cuffing by lymphocytes in both the grey and white matter. There is demyelination and atrophy of the cerebral white matter, and in some cases intranuclear and intracytoplasmic inclusion bodies may be found in astrocytes. Neuronal changes such as chromatolysis and swelling may be seen in some locations.⁷ Typical lesions of the CNS in classic CDV infection include white matter demyelination which is prominent in the cerebellum and surrounding the fourth ventricle. Lesions are multifocal and there is vacuolation of the neuropil along with myelin loss, and inflammatory infiltrates are rare. Astrocytes often contain intranuclear inclusion bodies, which may be present prior to encephalomyelitis and often persist in the CNS longer than other tissues. Grey matter lesions occur less commonly and may include neuronal necrosis and mononuclear cell infiltration/nonsuppurative encephalitis; inclusion bodies may be identified within neurons of the grey matter when affected.⁸

The conference histologic description was similar to the contributor's description above. The salient features of the section include multifocal pyogranulomas, fibrinoid vasculitis and the presence of intranuclear viral inclusion bodies in periventricular astrocytes. Additionally, the meninges are multifocally expanded by lymphocytes, plasma cells and macrophages and there is mild multifocal expansion of Virchow-Robin space by mononuclear inflammatory cells. There is slide variation with not all slides containing choroid as well as variability in the presence and severity of fibrinoid vasculitis. Not all conference participants identified viral inclusion bodies in periventricular astrocytes, and in general, the distemper lesions in these sections were

extremely subtle. Inclusions were most predictable in the periventricular cells, and were demonstrated in low numbers during the conference. Various differential diagnoses for pyogranulomatous meningoencephalitis were discussed including systemic mycotic infections such as blastomycosis and cryptococcosis. Other considerations include protothecosis and mycobacteriosis.⁷ Silver and acid-fast stains repeated at JPC on the section failed to identify microorganisms. Regardless of the cause for the granulomas in this particular case, pathologists should be concerned about immunosuppression as a predisposing factor in the dissemination of potentially any "secondary" pathogen. The true nature of dual infections (opportunistic vs. polymicrobial) has not been elucidated in most cases.

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CASE IV: 11N2549 (JPC 4032260).

Signalment: 2-month-old intact female Dachshund-cross dog (*Canis familiaris*)

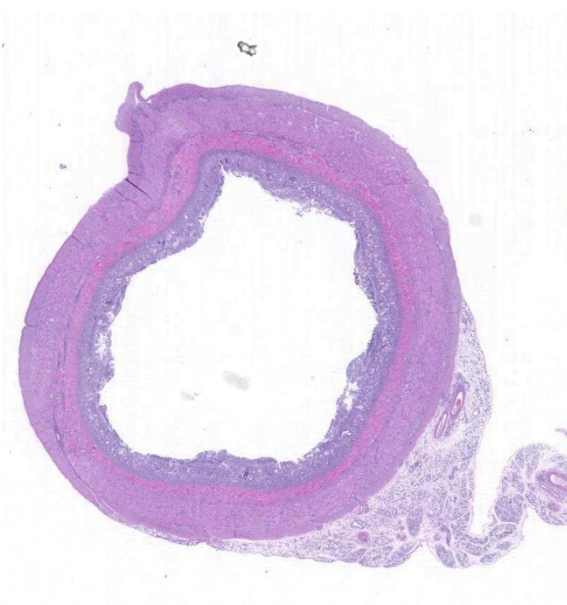
History: The patient was presented to the VMTH with three day history of vomiting, diarrhea, hematemesis and hematochezia. The puppy was adopted few days earlier from a rescue group and received one DHPP vaccine seven days prior to adoption. At admission the patient was severely dehydrated (8-10%) and had pink and tacky mucous membranes. The puppy was weak and ambulatory with diarrhea staining on the perineum. A small amount of bloody diarrhea was expressed during abdominal palpation. The dog was administered IV LRS fluids qs 20 mEqKCl/L and 2.5 % dextrose at 8mls/hr 0.07 mg Ondansetron IV q 12 hrs, 1.4 mg Ranitidine IV q 12hrs, 0.03 mg Buprenorphine IV q 8 hrs, and 70 mg Unasyn IV slow q 12 hrs. The patient did not respond to dextrose boluses and struggled to maintain adequate perfusion. Within 72 hours the dog still had no interest in food, and became stuporous with evidence of severe cranial abdominal pain. At this point, hypoglycemia, hypovolemic shock and possible DIC were suspected. The owners elected humane euthanasia.

Gross Pathology: On gross postmortem examination the dog had depleted fat stores and oral and ocular mucous membranes

were pale. The dorsal aspect of the tongue was multifocally eroded /ulcerated and areas between the erosions were covered by thin, friable, loosely adhered white tan (cotton like) plaque. The abdominal cavity contained approximately 10ml of clear blood tinged watery fluid. The intestinal serosal surface was slightly granular and opaque. The small and large intestine contained yellow-tan mucus.

Laboratory Results: Blood smear demonstrated severe leukopenia with neutropenic left shift. The PCV was 34% and total protein of 5.8. A SNAP test for canine parvovirus was positive on the day of admission.

Histopathologic Description: In a full-thickness transverse section of small intestine, the mucosal architecture was diffusely and circumferentially collapsed with attenuation, blunting and fusion of villi. The superficial mucosa was replaced by a band of eosinophilic cellular and karyorrhectic debris (necrosis). A nearly continuous thick mat of mixed bacteria and yeast covered the



Intestine, dog. At subgross examination, the mucosa is thin and villar architecture is lost. (HE, 5X)

necrotic mucosa. Yeast pseudohyphae had parallel walls and were 2.5 μm in diameter with budding oval to round yeast cells ($\approx 7.5 \times 5.0 \mu\text{m}$ in diameter). The pseudohyphae invaded into various depths of the lamina propria. Crypts were often absent and where present were ectatic and variably lined by attenuated enterocytes and packed with sloughed epithelial cells (crypt necrosis) or lined by hypertrophic and hyperplastic epithelial cells, up to 4 cell layers deep, with increased amounts of cytoplasm, vesicular nuclei, and occasional mitotic figures (regeneration). The lamina propria was expanded by reactive fibroblasts, neutrophils, and rare lymphocytes and plasma cells. Multifocally, intestinal crypts herniated into the severely depleted lymphoid follicles (GALT). The serosal and mesenteric vessels were moderately

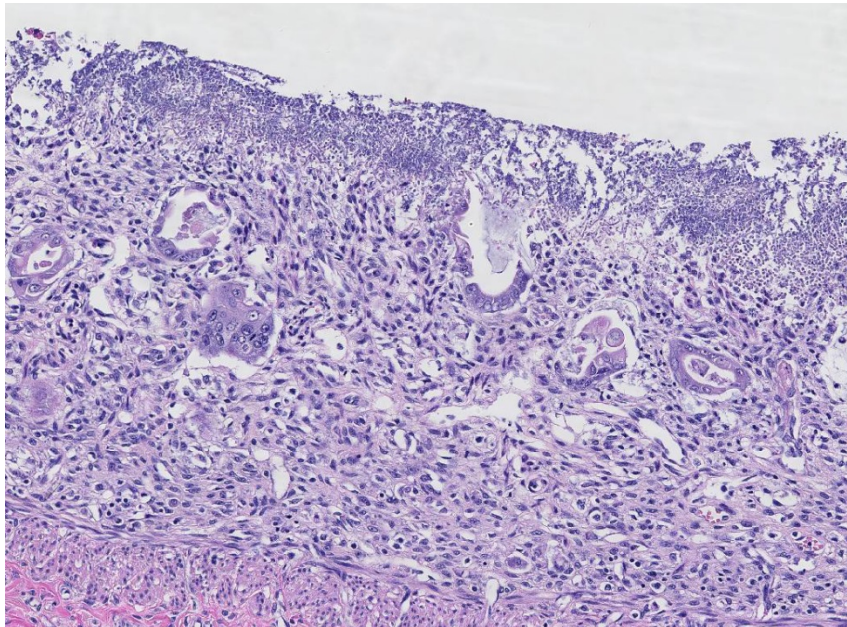
congested, lined by reactive endothelium and contained marginating neutrophils.

Contributor's Morphologic Diagnosis:

1. Small intestine (duodenum, jejunum, ileum): Severe, diffuse, subacute necrotizing enteritis, with colonization of yeast (*Candida* presumed) and mixed bacteria
2. Small intestine (Peyer's patches): Severe diffuse lymphoid depletion

Contributor's Comment: In addition to the necrotizing enteritis, significant findings in this patient also included severely depleted primary and secondary lymphoid organs and severe necroulcerative glossitis with *Candida* yeast and mixed bacterial colonization. The Brown-Brenn (modified Gram) stain highlighted gram-positive bacterial cocci and gram-negative bacterial

rods that colonize the affected areas. A Gomori metheniamine silver stain reveals abundant yeast pseudohyphae and spores. Immunohistochemistry for canine parvovirus (CPV2) was strongly positive in the sections of affected tongue and occasional immunoreactive cells were observed within the necrotic crypts and occasionally in the depleted lymphoid follicles.

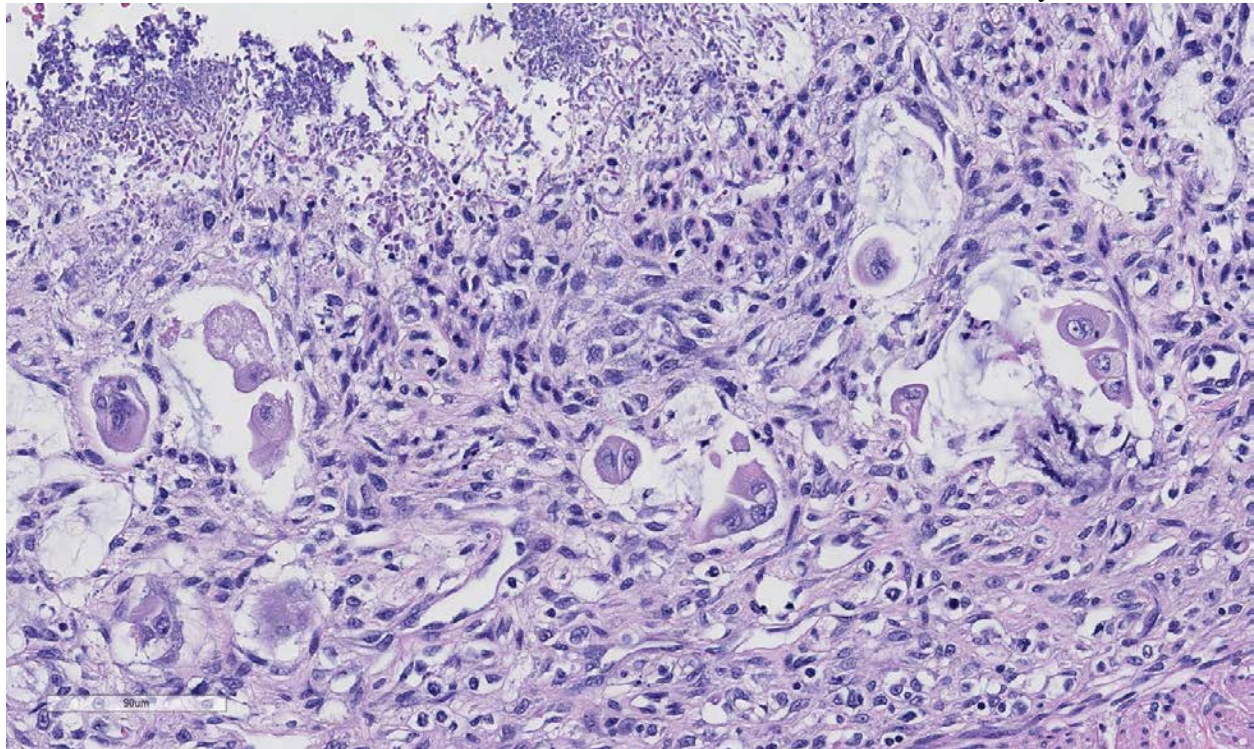


Intestine, dog. There is diffuse villar loss and marked crypt loss, with stromal collapse. There is marked regenerative change and atypia within remaining crypt epithelium. (HE, 88X)

Canine parvoviruses are small non-enveloped DNA viruses that require rapidly dividing cells for replication. They are divided into CPV-1 and CPV-2. CPV-2 is one of the most common causes of infectious enteritis in dogs.⁵ Acute CPV2-enteritis can be observed in dogs of any breed, age, or sex. Long list of predisposed breeds is provided in referenced text.⁷ Since it first appeared in dogs in the 1970s, CPV-2 has a progressive series of recognized antigenic variants that are based on single

host range compared to its predecessor, CPV2.

The mechanism of injury in parvoviral infection is death of rapidly dividing cells such as crypt epithelial cells, lymphocytes and bone marrow cells. Specificity for these mitotically active cells occurs because parvoviruses require a host cell-derived duplex transcription template, which is only available when cells divide, during the S-phase of the cell cycle. Parvoviruses are unable to turn on DNA synthesis in host



Intestine, dog: Higher magnification of bizarre attempts at crypt epithelial regeneration. (HE, 284X)

amino acid substitutions in VP2 gene (most recently CPV2c have emerged and is now distributed worldwide). In dogs, etiologic diagnosis through fecal ELISA is relatively straightforward and effective in recognition of the more recent strains.⁸ The current vaccine series is generally effective regardless of the CPV strain type. It is controversial whether CPV2c is more virulent in dogs, but it does decidedly have an extended

cells, so they must wait for host cells to enter the S-phase of the cell cycle before infecting these cells.⁴

Virus probably infects macrophages or dendritic cells migrating in the mucus layer and on the surface of mucosa.⁴ Virus replicates in these cells and is then spreads via leukocyte trafficking to the lamina propria of the tonsils. Here, additional macrophages and lymphocytes are infected and spread the virus via leukocyte trafficking in

lymphatic and blood vascular systems to regional lymph nodes and systemically to the spleen, thymus, lymph nodes, bone marrow, and mucosa-associated lymphoid nodules such as Peyer's patches of the small intestine.⁴ Virus may also spread as cell-free viremia in lymph via lymphatic vessels to regional lymph nodes. Experimental data suggests that virus spreads to the intestine via the vascular system and not in ingesta via peristalsis.⁴

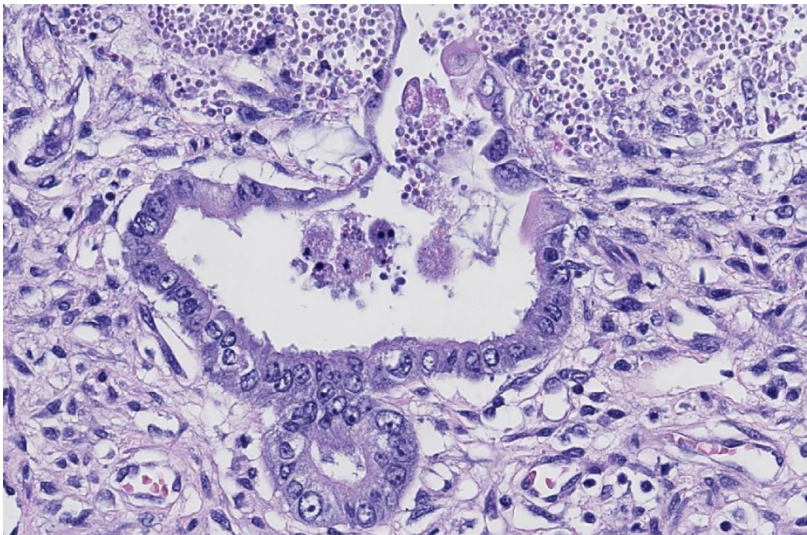
Clinically, CPV in dogs most commonly presents as hemorrhagic enteritis. However myocarditis, thrombosis, bacteremia, and neurological disease have also been reported.^{4,5,7} Two cases of cutaneous disease (erythema multiforme) induced by CPV-2 were recently published.^{3,10} Typically, parvovirus infection peaks after weaning at the age of 4 to 12 weeks, when maternally acquired antibody wanes.² Viral tropism to rapidly dividing cells leads to profound leukopenia and immunosuppression which predispose infected individuals to opportunistic bacterial or fungal infections.⁵ Specifically, in this case, candidiasis and surface bacterial colonization was present.

Candida spp. normally inhabit the alimentary, upper respiratory and genital

mucosae of mammals.⁵ *Candida albicans* and *Candida parapsilosis* are the most common. The mechanism of injury in candidiasis is disruption and death of cells in mucosae caused by inflammation and the concurrent proliferation and invasion of filamentous pseudohyphae and hyphae. *Candida albicans* persists in two forms: yeast (commensal) and filamentous pseudohyphae and hyphae (pathogenic).⁴ Yeast persists in the oropharyngeal cavity by adhering to and colonizing mucosae via ligand-receptor and/or hydrophobic interactions. Yeast ligands include cell wall components, such as mannose, C3d receptors, and mannoproteins, whereas mucosal receptors include fibrinogen, fibronectin, thrombin, collagen, laminin, and vitronectin-binding proteins. The balance between commensalism and disease is tenuous and perturbations of the mucosae and/or changes in the physiologic status of the animal may shift this balance in favor of disease (filamentous pseudohyphal and/or hyphal forms).⁴

Through a process called morphologic (phenotypic) switching, the yeast phase switches to the invasive filamentous phase. Switching appears to occur through inducible chromosomal rearrangements in the genome of the yeast in response to changes in the mucosal environment. Switching is reversible. Under normal conditions, the temperature of mucosae in the oral cavity is near room temperature (25° C). This temperature favors the growth of yeast, whereas growth of the filamentous phase prefers 37° C. Yeast is able to switch this temperature dependence for growth so that the filamentous phase can grow at 25° C.⁴

Pseudohyphae and hyphae of the filamentous phase express



Intestine, dog: Few crypts are widely dilated and contain sloughed necrotic enterocytes admixed with cellular debris (crypt abscesses). (HE, 280X).

new adhesin ligands, secrete hydrolytic aspartyl pro-teinas that injure the mucosa, and invade the mucosae and submucosa in which new groups of adherence receptors are encountered. It appears that a large group of virulence determinates are involved in the process of infection and invasion, but no single factor accounts for virulence and not all expressed virulence determinates may be necessary for a particular stage of infection.⁴

JPC Diagnosis:

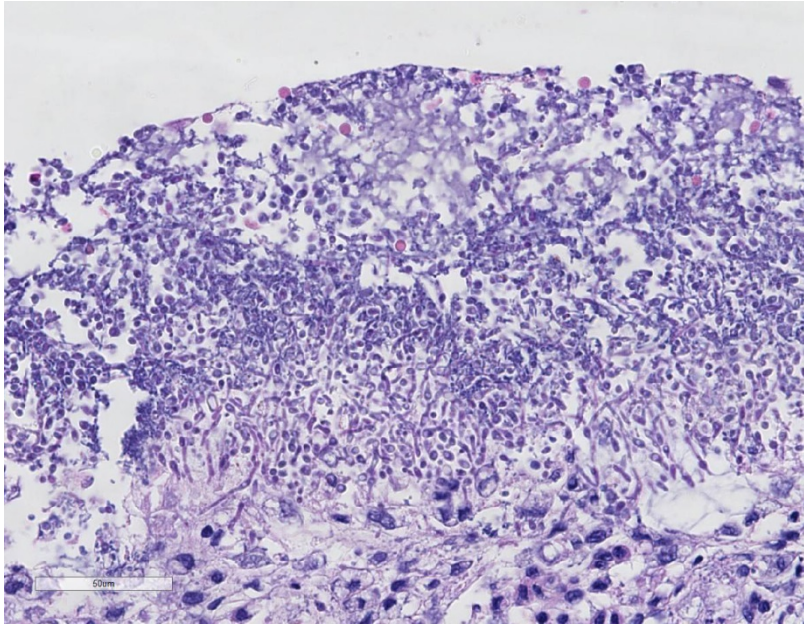
1. Small intestine: Enteritis, necrotizing, diffuse, severe with stromal collapse, crypt loss, abscessation and regeneration.
2. Small intestine, lumen: Yeasts, numerous.

Conference Comment: The contributor provides an excellent review of CPV above. Parvoviruses are common in many wild carnivore populations and result in similar symptomatic disease as seen in domestic animals. However, factors such as virus strain, host and species variations and differences in tissue tropism can result in alterations in disease manifestation and severity.⁶ In general, host ranges are determined by receptor binding, specifically to the transferrin receptor.⁹ Parvoviruses are associated with enteric disease in cats, dogs, mink and calves and are associated with reproductive losses in swine.⁹ In cases of neonatal or *in utero* infection, virus may reproduce in various tissues depending on the replicative status of the organ. This is demonstrated in the cerebellum of infected kittens where infection results in cerebellar hypoplasia and in the myocardium of neonatal puppies, which results in myocarditis. Infection of neonates typically does not result in gastrointestinal disease.⁶

CPV has also been isolated from cats as well as a few species of wild animal such as

raccoons, mountain lions, and cheetahs. In general the disease caused by CPV in cats is much less severe than in dogs and has even been isolated from clinically healthy cats.⁶ Additionally, results from at least one study have suggested that asymptomatic cats can shed CPV for extended periods and may serve as reservoirs for infection of dogs in some cases. In that study cats were documented to be shedding CPV-2a or 2b and not feline panleukopenia virus.¹ CPV-2c, a more recently emerging variant, is able to infect dogs, cats, skunks, foxes and raccoons.⁹ It has become widespread in some areas although there is disagreement regarding the relative virulence as compared to other variants.

Conference participants noted the conspicuous absence of inflammatory cells which is incongruent with the degree of tissue damage and necrosis, and characteristically results from parvo-viral lymphocytolysis and lymphoid depletion as well as lysis of myeloid precursors in the bone marrow. Leukopenia may be extensive in severe cases but a neutrophilia with left shift is often observed during recovery.⁹ Conference participants described mucosal stromal collapse with complete, cataclysmic loss of crypts. Canine parvovirus (CPV) is relatively unique among enteric viruses with its predilection for intestine crypts, and this tropism may be referred to as radiomimetic,⁴ referring to its imitation of the effects of radiation. Other causes of canine enteric disease such as coronavirus or rotavirus, both attack the villus tips and are generally associated with mild, non-fatal disease. Canine distemper virus can infect crypt epithelium but is not described as producing the degree of tissue destruction seen in CPV infection. Pathogenic *Clostridium* spp. generally produce a more hemorrhagic lesion with the presence of bacilli in the necrotic tissue, and the most severe lesions



Intestine, dog. The necrotic mucosal surface is covered by a layer of innumerable yeasts and pseudohyphae (most consistent with Candida sp.) admixed with bacteria. (HE, 368X)

will be in the large intestine.⁹ Conference participants discussed the presence of yeast in this case and most agreed they are an overgrowth or opportunistic infection, secondary to the immunosuppressive virus and change in bacterial flora, and play a minimal role in tissue damage. Classic gross lesions include segmental necrotizing enteritis producing a “red gut” with multifocal Peyer’s patch necrosis. The moderator commented that virus may be identified within germinal centers of Peyer’s patches or mesenteric lymph nodes, but in many severe cases of enteritis virus will be absent from necrotic intestinal epithelium at time of death due to sloughing and loss of enterocytes.

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