Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2022-2023

Conference #17 **25 January 2023**

CASE I:

Signalment:
1 day old, female, commercial lineage, *Sus scrofa domesticus*, swine.

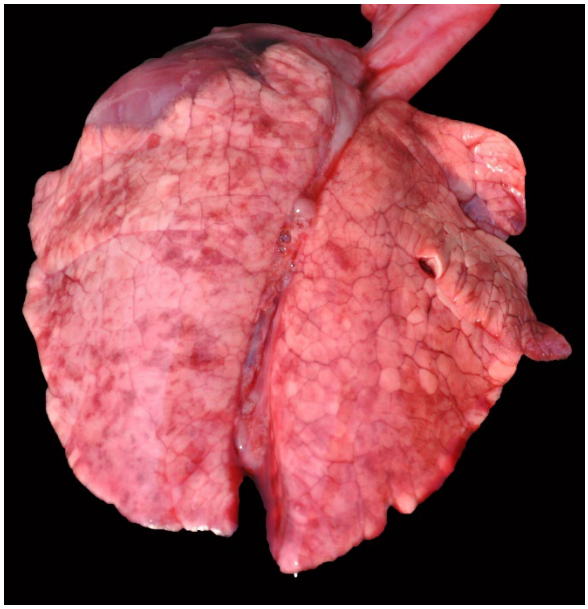
History:
Our research group was contacted to investigate a disease that have been affecting neonates piglets in a sow farm located in West of Santa Catarina, Brazil. The farm has 330 sows, in an intensive system. In the period between May 2020 and January 2021, the farm manager have reported the birth of piglets with large and caudally rotated ears ("Dumbo-like piglets"), with weakness, apathy and in most cases, dyspnea. The morbidity rate was 3,4%, the mortality was 3% and the lethality was 86%. Most of the affected piglets was born from gilts (77%), and with a bith average of 4.8 "Dumbo-like piglets" per litter. The piglets died 1 to 5 days after birth. We received 14 piglets for routine diagnosis investigation.

Gross Pathology:
Grossly, the lungs of all piglets did not collapse upon the opening of the thoracic cavity, and also had marked interlobular edema. Also, all piglets had large and caudally rotated ears. No other significant abnormalities were visible in the remaining organs.

Laboratory Results:
PCR:
The samples (pool of CNS, lung, heart, liver, and spleen) tested positive for PCV3 and negative for PCV1, PCV2, PPV 1, 2, 5, and 6, APPV, PRRSV, and OvHV-2.
ISH findings:
Replication of PCV3 was most commonly observed in lymphocytes and plasma cells in perivascular areas and in the smooth muscle of arteries (fig. 3). We also observed PCV3 replication in lungs (fig. 4), heart (fig. 5), and in neurons of the brain.

Microscopic Description:
The slides submitted for this conference contained tissues from only one affected piglet.
Vascular plexus and mesenteric lymph node: surrounding and disrupting the wall of vessels (mainly in arteries and arterioles), there is a severe and multifocal inflammatory infiltrate composed of lymphocytes, histiocytes and rare plasma cells. The

Presentation, piglets. Affected piglets had large, caudally rotated ears ("Dumbo piglets"). (*Photo courtesy of:* (*Photo courtesy of:* Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Setor de Patologia Veterinária, http://www.ufrgs.br/patologia)



Lung, piglet. The lungs of one affected piglet fail to collapse, and have marked interlobular edema.j.. (*Photo courtesy of:* (*Photo courtesy of:* Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Setor de Patologia Veterinária, http://www.ufrgs.br/patologia)

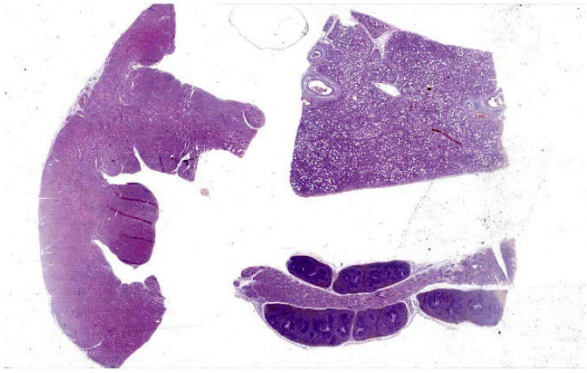
infiltrate is associated with mild fibrinoid degeneration. In the mesenteric lymph node, mild necrosis of the germinal centers is observed, in addition to lymphoid rarefaction and mild infiltrate of macrophages containing brown pigment in their cytoplasm (hemosiderin).

Heart: in the pericardium and myocardium there is a moderate and multifocal inflammatory infiltrate surrounding and disrupting the vessel walls (mainly in arteries and arterioles), composed of lymphocytes, histiocytes, and rare plasma cells. There is also mild fibrinoid degeneration. Some cardiomyocytes present mild necrosis and are surrounded by lymphocytes, macrophages, and plasma cells.
Lung: surrounding and disrupting the wall of vessels (mainly in arteries and arterioles), there is a severe and multifocal inflammatory infiltrate composed of lymphocytes, histiocytes, and rare plasma cells. A similar infiltrate is observed severely expanding the alveolar septa. There is also moderate and diffuse interlobular edema.

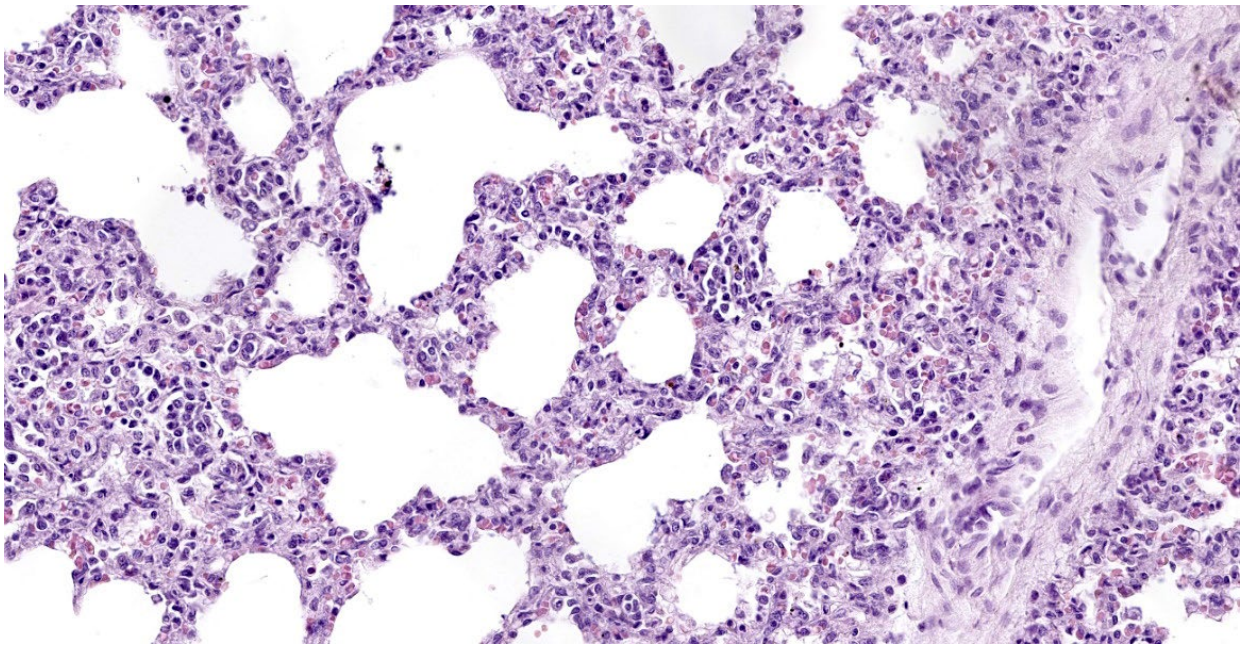
The other tissues presented a variable amount of lymphocytes, histiocytes, and plasma cells infiltrating and disrupting the wall of vessels, occasionally with fibrinoid degeneration, mainly in arteries and arterioles. Also, in the brain, there were multifocal areas of gliosis.

Contributor's Morphologic Diagnoses:
Mesenteric plexus: lymphohistiocytic periarteritis and arteritis, multifocal, marked and mild fibrinoid degeneration;
Lymph node: lymphoid rarefaction and necrosis, multifocal, mild, and hemosiderosis, multifocal, mild.
Heart: lymphohistiocytic periarteritis and arteritis multifocal, marked and lymphohistiocytic myocarditis, multifocal, mild.
Lung: lymphohistiocytic interstitial pneumonia, diffuse, marked, with mild lymphohistiocytic periarteritis and arteritis.

Contributor's Comment:
The diagnosis of PCV3-associated clinical disease in neonatal piglets was based on the molecular findings in association with the detection of PCV3 mRNA in microscopic lesions. PCV3 was first detected in 2015 in the United States, related to abortion cases, high mortality in sows, and clinical signs compatible with swine dermatitis and



Multiple organs, piglet: Sections of the heart (left) lung (top right), and mesenteric vascular plexus (bottom right) are submitted for examination. (HE, 381X)

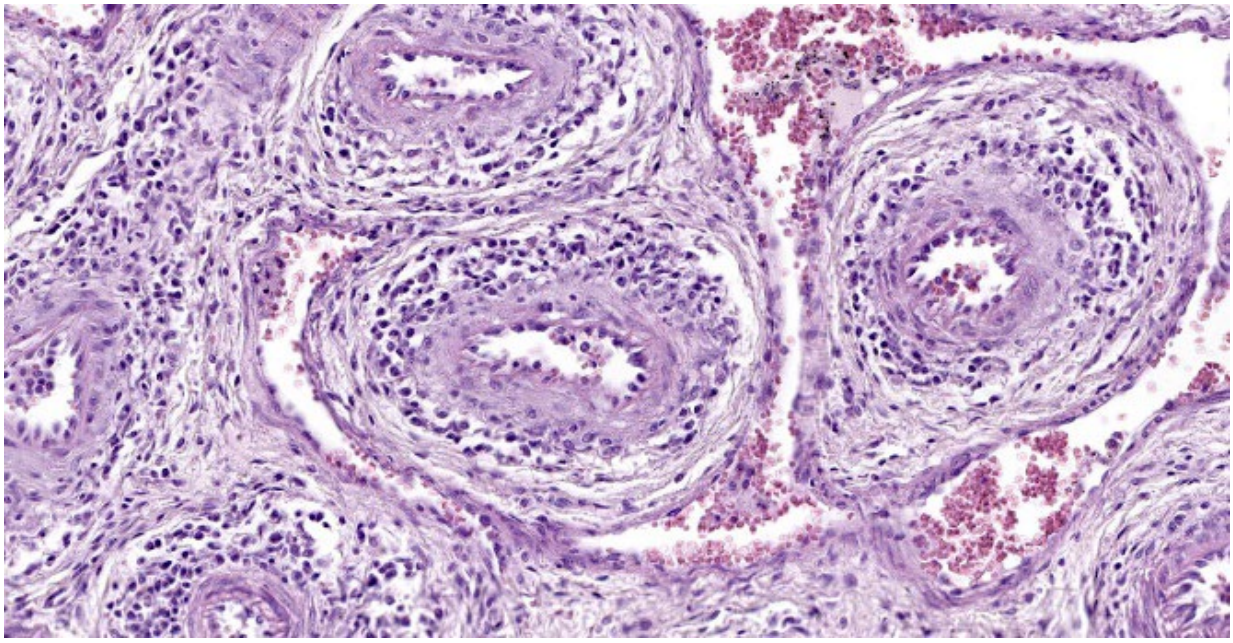


Lung, piglet. Alveolar septa are markedly thickened by macrophages, edema, congestion, and patchy Type II pneumocyte hyperplasia. Interlobular septa are expanded by edema. (HE, 282X)

nephropathy syndrome.[8] Also, PCV3 was described in three pigs from 3 to 9 weeks old, with multisystemic inflammation and myocarditis.[9] Other clinical/pathological presentations were also associated with PCV3 infection, such as gastrointestinal, respiratory, and neurological disturbances.[10] In most studies, the viral DNA has been detected in different tissues of pigs, however, without the demonstration of the agent in the lesions. PCV3 genetic material was detected through PCR in healthy piglet tissues,[5, 8] which makes it difficult to conclude the real pathogenic potential of the virus and its economic impact on swine production. Therefore, the diagnosis of PCV3 must be confirmed through detecting its DNA in affected swine lesions, using techniques such as ISH, or at least associating the histopathological lesions with qPCR.

The ear malformation was a consistent feature observed in piglets of this farm. This finding was described in PCV3 positive pigs, previously,[1, 6] however, it is necessary to perform more studies to characterize the pathogenesis of the large and caudally rotated ears and their relation to the PCV3 infection.
Multisystem vasculitis, myocarditis, and encephalitis were already PCV3-associated with the death of neonates,[2, 6] and post-weaning piglets.[9] Replication of PCV3 through ISH, was detected in the smooth muscle of arteries and arterioles.[2]

Lymphoplasmahistiocytic interstitial pneumonia was a constant pathological finding in the evaluated piglets, and through ISH it was possible to show the PCV3 viral replication within the lesions. This finding was described in a 19 day old piglet.[9] Interstitial pneumonia is a common finding in viral infections of the inferior respiratory tract. In the maternity phase (usually until 21-25 days old), the main causes of viral pneumonia include the Aujeszky virus and PRRSV. There are no reports of clinical disease related to PRRSV presence in Brazil, and this virus was excluded through a PCR test. Pneumonia related to the Aujeszky virus can occur in piglets up until 5 days old; however, in this



Mesenteric vascular plexus, piglet. The adventitia of arterioles and intervening fibrous connective tissue are infiltrated by moderate numbers of lymphocytes, macrophages, and histiocytes. (HE, 400X).

phase, neurologic signs are prominent, while respiratory signs are usually common in growing and finishing pigs.[3] PCV2 is an important cause of interstitial pneumonia, described mainly in nursery and finishing piglets,[4] and the lymphocytic and plasmacytic periarteritis is a common finding between PCV3 and PCV2 infection.[2, 7] All samples tested negative for PCV1, PCV2, PRRSV, APPV, PPV, and OvHV-2, which are other potential causes of myocarditis, vasculitis, and encephalitis in neonatal piglets.

Contributing Institution:
Faculdade de Veterinária
Universidade Federal do Rio Grande do Sul
Setor de Patologia Veterinária
http://www.ufrgs.br/patologia

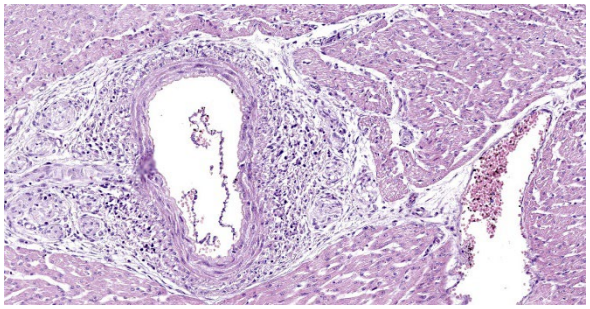
JPC Diagnosis: 1. Lung: Pneumonia, interstitial, histiocytic, diffuse, severe, with intralobular edema and histiocytic arteritis.

2. Mesenteric vascular plexus, heart: Arteritis, histiocytic, diffuse, moderate.

3. Lymph node, subcapsular and medullary sinuses: Hemosiderosis, moderate.

JPC Comment: As the contributor states, porcine circovirus 3 was first diagnosed in 2015; however, retrospective studies have identified PCV-3 in samples from as early as the 1960s.[7] And even though there is still much to be elucidated regarding this relatively young virus, PCV-3 is no longer the newest circovirus on the block: PCV-4 was discovered in 2019 in 7-week old pigs with respiratory disease, porcine dermatitis and nephropathy syndrome, and diarrhea.[7]

PCV-3 has been detected in a wide range of species and produces viremia in cattle, dogs, and laboratory mice.[7] Wild boar are thought to be a reservoir, with prevalence of up to 61.54%.[7] Mosquitos can also be infected, but studies have failed to support vector-borne transmission.[7] In fact, the precise mode of transmission has not been worked out, and virus has been identified in oral fluids, the salivary glands, colostrum, semen, and testicles.[7] The virus can infect a wide variety of cells and has been found



Coronary vessel, piglet: The intima of the coronary vessels is expanded by edema and moderate numbers of lymphocytes, plasma cells and macrophages. (HE, 279X)

in myocardiocytes, vascular smooth muscle, trophoblasts, adipocytes, ependymal cells. This suggests that the virus ligand binds to a widely expressed host receptor, which has yet to be identified.[7] Affected pigs may have a persistent infection with prolonged viremia.[7] Determining the effects of PCV-3 infection is challenging for multiple reasons: as the contributor states, there are a wide variety of syndromes associated with infection, but the virus has also been isolated in subclinical animals. Additionally, PCV-3 infection regularly occurs alongside other infectious agents, including PCV-2, porcine parvovirus, porcine reproductive and respiratory syndrome virus, making attribution of clinical signs with a specific agent difficult.[7]

A recent retrospective study reviewed formalin-fixed paraffin-embedded tissues from 587 pigs free of porcine circovirus 2 in Spain.[4] PCV-3 ISH and qPCR were conducted on a subset of cases which contained lesions reported to be associated with PCV-3, including reproductive disease, congenital tremors, porcine dermatitis and nephropathy syndrome, arteritis or periarteritis, myocarditis, encephalitis, and peri-weaning failure-to-thrive syndrome. [4] All evaluated cases of periarteritis were qPCR positive for PCV3, and most were positive via IHC. [4] The most consistently affected sites for periarteritis were the mesenteric arteries and the kidney, indicating they are likely suitable choices for diagnostic testing. [4] Additionally, PCV-3 was not detected in cases of myocarditis and encephalitis where periarteritis was lacking. [4]

The moderators and conference participants discussed a unique anatomic feature of pigs evident in this slide: the mesenteric arterial plexus. While the exact function is unknown, it may serve as a countercurrent mechanism to help regulate blood pressure. Conference participants also discussed the few foci of histiocytic infiltrates in the myocardium; the significance of these infiltrates is unknown.

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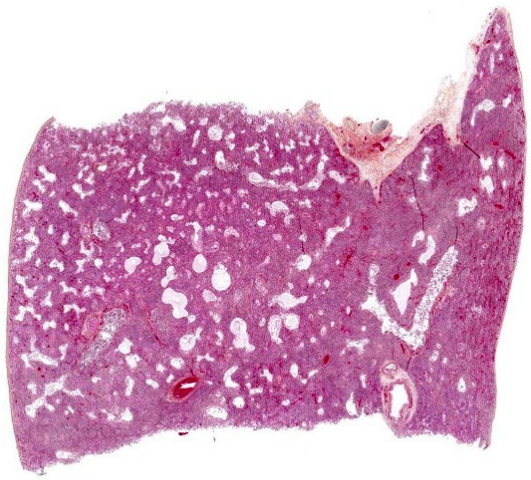
<u>CASE II</u>:

Signalment:

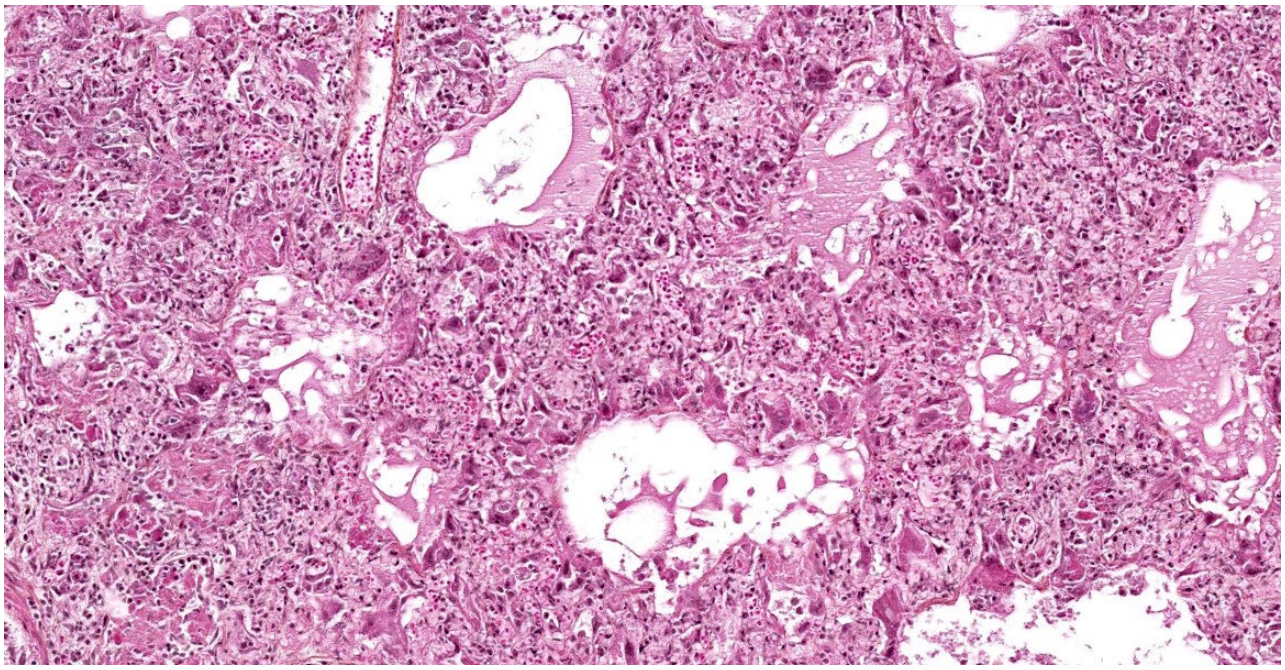
3½ month-old, intact female, Siberian Husky, Canis lupus familiaris, dog.

History:

This puppy was initially presented to our institution's veterinary teaching hospital with a history of anorexia, diarrhea and mild intermittent fever of 10 days duration. It had been vaccinated against CDV, CPV-2, CAV-1, *Leptospira spp.* and *Bordetella bronchiseptica* at 2 and 3 months of age, and administered anthelminthics (fenbendazole). At presentation, moderate dyspnea was observed and treated with antibiotics, anti-inflammatory drugs, IV fluids and oxygen. Hematology revealed moderate lymphopenia. The respiratory signs gradually worsened over the next 5 days, with seizures developing within 3 days after presentation; the seizures were well controlled with diazepam. Considering the progressive deterioration of the animal's condition, the owners elected for euthanasia. Littermates were unaffected.



Lung, dog. On subgross magnification, there is diffuse consolidation of the lung. (HE, 6X)



Lung, dog. Normal pulmonary architecture is lost. There is marked expansion of alveolar septa, and alveoli contain brightly eosinophilic hypertrophic Type II pneumoncytes and viral syncytia. Airways are often devoid of epithelium and filled with edema fluid. (HE, 148X)

Gross Pathology:

Body condition was assessed as 3/9. The lungs failed to collapse when the thorax was opened, and they were diffusely pale red to purplish with a rubbery to firm texture (interstitial pneumonia); there was no exudate on cut sections. The only other significant gross lesion was marked thymic atrophy.

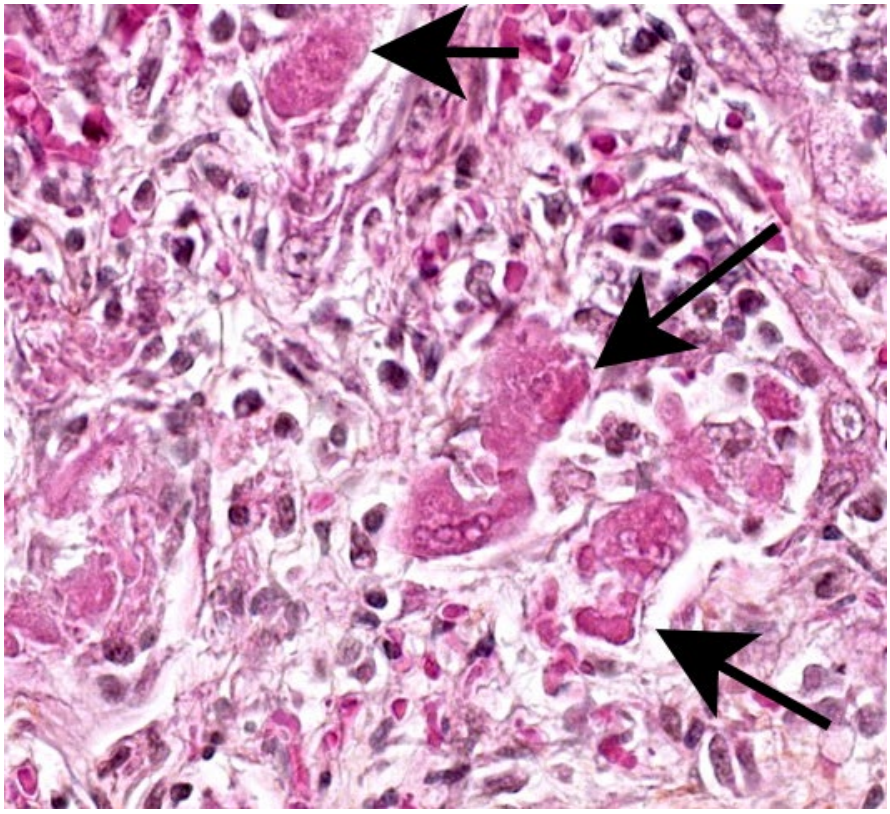
Laboratory Results:

PCR for canine distemper virus (CDV) on lung samples was positive (Ct: 17.00)

Microscopic Description:

The lesions are similar in all pulmonary lobes (only one section submitted). Diffusely, the pulmonary alveoli are lined by pleomorphic squamous to polygonal cells (type II pneumocytes), many of them multinucleated (syncytia). Multifocally and to variable degrees within the alveolar lumina, there are macrophages and desquamated pneumocytes with protein-rich edema and, to a lesser degree, fibrin and/or necrotic debris; small numbers of neutrophils are occasionally present. Minimal, mainly lymphoplasmacytic interstitial inflammation is present in the alveolar septa. Although there is significant post-mortem desquamation, some terminal bronchioles and alveolar ducts can be seen to be lined by squamous to cubic epithelial cells often with amphophilic cytoplasm (regeneration). Numerous variably sized, brightly eosinophilic, intracytoplasmic inclusion bodies are present in type II pneumocytes, alveolar macrophages and, to a lesser degree, bronchiolar epithelial cells.

Other relevant lesions observed in this pup were: 1) severe lymphoid depletion in the thymus, spleen and Peyer's patches, 2) inclusion bodies in the renal pelvic and vesical urothelium, 3) mild multifocal, mainly histiocytic cerebral leptomeningitis with intrahistiocytic inclusion bodies, 4) a few syncytial cells (uncertain cell type) with inclusion



Lung, dog. Viral syncytia contain irregular, brightly eosinophilic intracytoplasmic viral inclusions. (HE, 440X)

bodies in the ocular trabecular meshwork, and 5) a few small foci of myocardial necrosis associated with vascular fibrinoid necrosis and minimal lymphoplasmacytic inflammation. Inclusion bodies were not found in other organs/tissues (e.g. stomach, conjunctiva…), and there were no lesions in the upper respiratory tract and conjunctiva.

Contributor's Morphologic Diagnoses:
Marked, diffuse, subacute, proliferative and bronchointerstitial pneumonia with numerous epithelial syncytia (type II pneumocytes) and intracytoplasmic eosinophilic inclusion bodies, consistent with canine distemper virus (CDV) pneumonia.

Contributor's Comment:
Histopathological observations and PCR results were consistent with systemic canine distemper (CD), characterized by marked pneumonia and lymphoid depletion and minimal CNS and ocular lesions; the myocardial necrosis was also attributed to CDV.[2,5] Testing for other pulmonary pathogens (e.g. CPIV-5 or CAV-1 PCR; bacteriology) was not done as the lesions were typical for canine distemper virus (CDV) and there was no evidence of secondary bacterial infection. This case was submitted as an example of a CDV-induced proliferative pneumonia with numerous type II pneumocytes syncytia and inclusion bodies which, when present, are characteristic of the disease.[2] Canine distemper is a systemic disease caused by a single-stranded RNA virus in the family *Paramyxoviridae*, genus *Morbillivirus*. This genus presently includes, in addition to CDV, the *Measles virus* (MeV), the *Phocine distemper virus* (PDV), the *Cetacean morbillivirus* (CeMV), the *Peste des petits ruminants virus* (PPRV), the *Rinderpest virus* (RPV) and the more recently discovered *Feline morbillivirus* (FeMV); the RPV was eradicated in 2011.[3] All morbilliviruses share many virological, clinicopathological and epidemiological characteristics, with the exception of FeMV which is associated with renal tubulointerstitial disease.[3,10] Typical morbillivirus-induced disease is characterized mainly by profound and protracted immunosuppression, with respiratory, neurological and/or gastrointestinal signs, depending on virus/host.[3] As the range of hosts susceptible to MeV infection is very limited (primates),

CDV represents an interesting alternative model for the study of morbillivirus-induced disease such as measles.[3,4] The spectrum of hosts susceptible to CDV-induced disease includes all families of the order Carnivora, several seal species, javelinas and several macaque species;[1,4] CDV has also been isolated from many other terrestrial mammals and CDV is readily transmitted from one species to another.[7] In countries where vaccination is routinely done, CD incidence in dogs is low even though some vaccinated animals can develop the disease (possibly due to different strains).[1] However, CD is still very much a threat to domestic dogs and ferrets from countries where vaccination is not widespread and to wildlife in general. In the last decades, the known host range has expanded and several large outbreaks in different wildlife species have been documented; for instance, the much-publicized 1994 outbreak in large felids in Tanzania's Serengeti National Park killed about a third of its lion's population.[7]

Canine distemper is a highly contagious disease. Transmission of CDV is most frequently by inhalation of aerosols from the respiratory tract or direct contact with oronasal secretions, but all secretions are potentially infective.[1,2,7,9] The virus reaches the upper respiratory tract or pulmonary mucosa where it infects macrophages and monocytes and then reaches the tonsils and regional lymph nodes; it is highly lymphotropic. There is a subsequent first viremia and the virus is disseminated to all lymphoid organs/tissues (~2-6 days P.I.), either free or within leukocytes. The virus induces apoptosis in lymphocytes, mainly CD4+ T cells, resulting in lymphoid depletion, lymphopenia and immunosuppression. There is a second viremia in which the virus spreads to epithelial cells in many organs, mainly in the respiratory, gastrointestinal and urinary tracts and to the CNS (~10 days P.I).[1,2,7,9] The CDV H-protein (hemagglutinin) is considered the key protein in viral attachment to host cells.[3,7,11] It is also essential to cell fusion, responsible for the formation of syncytia which are characteristic of systemic morbilliviral diseases.[11] There are two major receptors, both with an immunoglobulin-like variable domain, for CDV on host cells: 1) CD150 or SLAM (signaling lymphocyte activation molecule) on B and T lymphocytes, dendritic cells and macrophages, and 2) nectin-4 (poliovirus-receptor-like-4) on epithelial cells.[4,7] The pathogenesis, pathology and clinical signs of the neurological form of CD are variable; it will not be discussed further (see reference 1). The severity of CDV-induced disease depends on virus strain (there is only one serotype), host immunity, age and species; in domestic animals, ferrets are considered the most susceptible species.[3] Animals with adequate humoral and cellular immunity will generally clear the infection after the first viremia, within 14 days of infection.[1]

In susceptible dogs, CD is generally a disease of dogs 3-6 months of age, when passive immunity declines.[2,9] The disease can be transmitted *in utero* (transplacental) in CDV-naïve animals, causing abortion, weak puppies and fatal neurological disease in newborn puppies; an unusual case with respiratory, but not neurological disease has been reported in 5-12 days puppies.[9] The pathology of CD in dogs is variable, but lesions are mostly found in the CNS (most frequently demyelinating leukoencephalomyelitis), the lower respiratory tract and lymphoid organs (lymphocytolysis and lymphoid depletion);[1,2] ocular and nasal discharge is relatively frequent and helpful in the clinical diagnosis of respiratory and/or neurological disease (e.g CD vs rabies). In the lungs, CD may cause minimal to no lesions, typical viral bronchitis/bronchiolitis or bronchointerstitial pneumonia with or without type II pneumocyte proliferation. Syncytial formation is variable and observed

in the lungs and occasionally in the CNS;[2] it has been reported that the more a CDV strain is attenuated, the more it will induce syncytial formation.[11] Inclusion bodies can be found in nearly all organs (epithelial cells, histiocytic cells, astrocytes, neurons…), with or without lesions, and are helpful for diagnosis. They are eosinophilic, mostly intracytoplasmic in epithelial and histiocytic cells, and mostly intranuclear in the CNS (astrocytes and neurons); a study found the tonsils to be the most consistently involved cells, which could be of interest for the ante-mortem diagnosis of CD (biopsy).[6] Some dogs have minimal CD-associated lesions, but because of immunosuppression develop opportunistic infections (e.g. bacterial and/or CAV-1 pneumonia, toxoplasmosis, cryptosporidiosis …). Other reported lesions include:

1. Pustular dermatitis and nasodigital hyperkeratosis/parakeratosis ("hardpad disease")[1,2]
2. Enamel hypoplasia/dysplasia[1,2]
3. Conjunctivitis ± keratitis, retinitis, optic neuritis[2]
4. Polioencephalomyelitis (can be concomitant with leukoencephalomyelitis) and "old dog encephalitis"[1,2]
5. Myocardial necrosis[2,5]
6. Growth retardation lattice (metaphyseal osteosclerosis)[1,2]

Contributing Institution:
Faculty of veterinary medicine, Université de Montréal. http://fmv.umontreal.ca/faculte/departements/pathologie-et-microbiologie

JPC Diagnosis: Lung: Pneumonia, bronchointerstitial, proliferative and histiocytic, diffuse, severe, with marked type II pneumocyte hyperplasia and hypertrophy, viral syncytia, and intracytoplasmic and intranuclear inclusions.

JPC Comment:
The contributor provides a thorough review of this classic case of canine distemper virus infection. As previously stated, CDV infects a wide range of host species, and a 2020 *Vet Pathol* report detailed the first documented cases of CDV infections in sloths.[8] The outbreak occurred in Tennessee, and five of eight sloths within the quarantine enclosure died after a short history of lethargy and hyporexia.[8] Crusting and ulceration over the tongue, oropharyngeal mucosa were present in three animals.[8] All cases had lingual and oropharyngeal mucosal erosions and ulcerations, necrotizing bronchointerstitial pneumonia, multifocal splenic necrosis, random hepatic necrosis.[8] Intranuclear and intracytoplasmic inclusions were observed in numerous tissues, including oropharyngeal mucosa, trachea, alveolar macrophages, type II pneumocytes, esophagus, urinary bladder, and hepatocytes.[8] IHC confirmed CDV presence in the epithelial cells of the tongue, hepatocytes, and less frequently in the meningeal vessel walls, ependymal cells, and choroid plexus.[8] The infection also spread to three kinkajous in the same enclosure who died after developing the same clinical signs. [8]

The moderators, Dr. Eric Lee and Dr. Juliana Lee, and conference participants discussed the amount of autolysis in this specimen, as evidenced by sloughing of individualized epithelial cells in the bronchioles, which obscured evidence of epithelial necrosis. Ultimately, the group decided there was enough convincing evidence of bronchiolar involvement to diagnose bronchointerstitial pneumonia.

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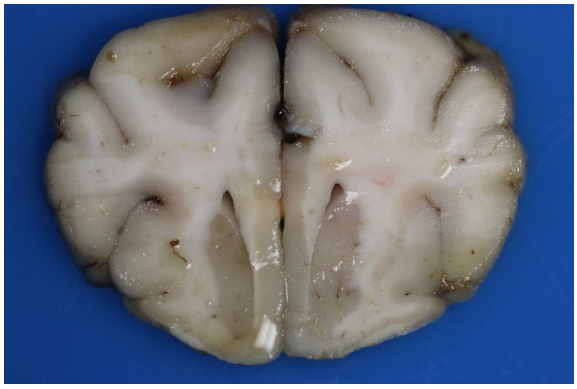
CASE III:

Signalment:
Estimated 3-year-old, spayed-female, Beagle dog (*Canis lupus familiaris*)

History:
The patient was originally adopted from Puerto Rico and was estimated to be 3-years-old. The patient was reportedly up-to-date on routine veterinary care, including vaccination (last vaccination was given Sept 2018 - about 1 year prior to presentation).

Patient initially presented to the Neurology Service for a one-month history of progressive ataxia and lethargy. At that time, neurolocalization included the thalamic cortex and cerebellar vestibular system. MRI did not reveal any abnormalities and CSF analysis showed normal protein levels with a slightly elevated mononuclear cell count. Tick-borne

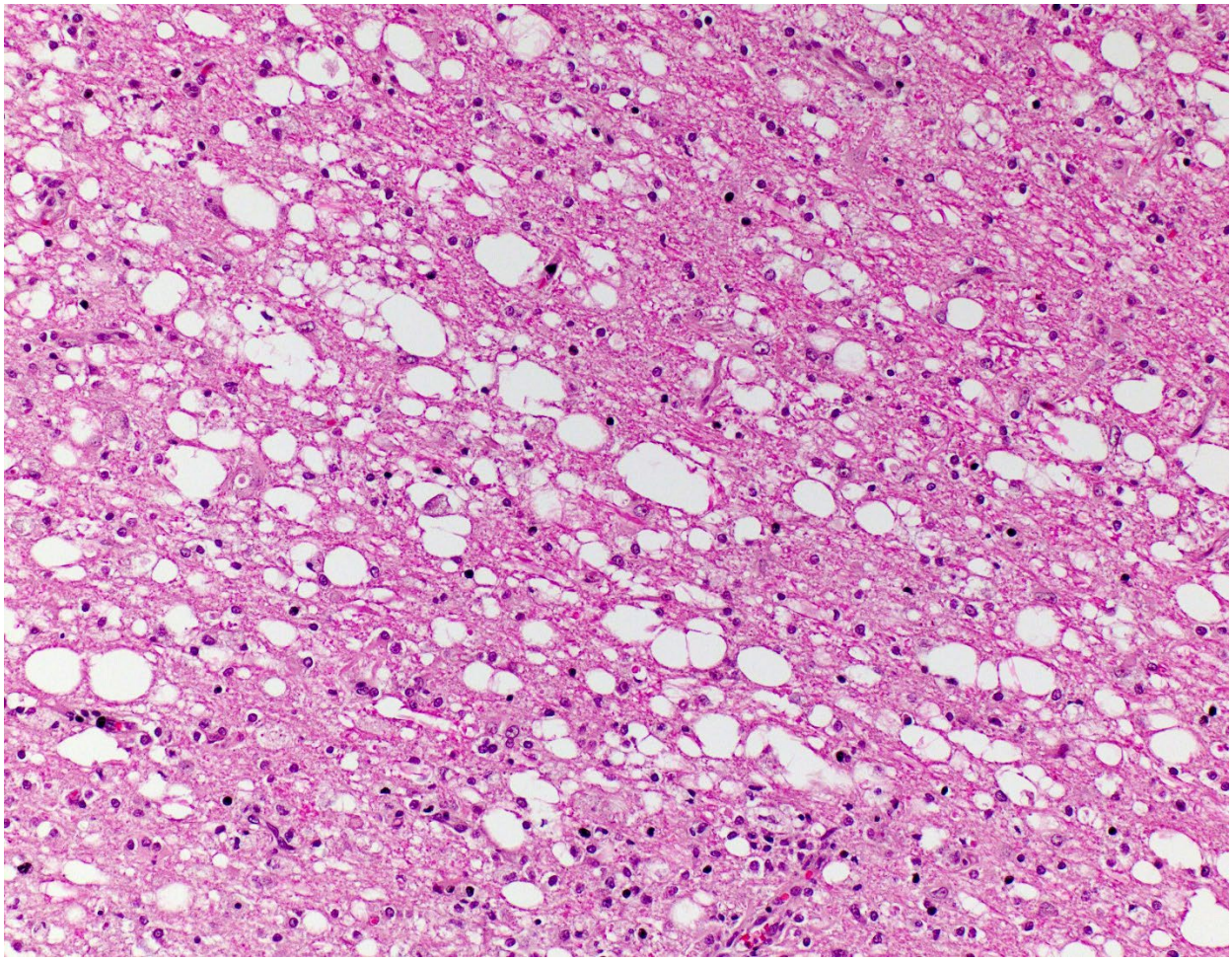


Cerebrum, dog: There is multifocal loss of the sharp linear distinction between grey and white matter (Photo courtesy of: Animal Medical Center, 510 E. 62nd Street, New York, NY 10065.)

disease panel was negative. The patient was managed over one month with doxycycline and corticosteroids. Over the following month, the patient continued to decline with progressive ataxia and mentation changes, resulting in euthanasia.

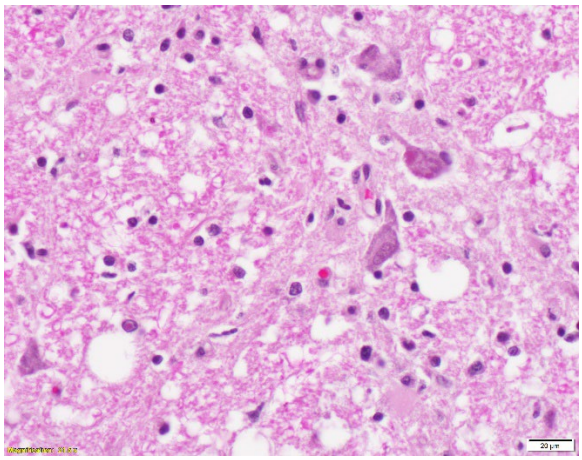
Gross Pathology:
There is multifocal loss of grey-to-white matter distinction. Randomly the white matter contains poorly demarcated, soft, tan regions/streaks (tinctorial change; decreased parenchymal whiteness), which is more evident in the rostral cerebrum. Throughout the cerebral cortex, the grey and white matter there are dozens of poorly demarcated, soft, light brown-yellow foci. The meninges over the spinal cord are mildly to markedly thickened, mottled tan, brown, and dark red (hemorrhages).

Laboratory Results:
Touch imprints of the meningeal surface are of good cellular and stain quality. Sample is comprised of rafts of cohesive mildly pleomorphic epithelial cells mixed with small numbers of macrophages, lymphocytes, and plasma cells on a hemodiluted background.



Within the subcortical white matter tracts, white matter tracts of the pons, and interspersed white matter within metencephalon are foci of spongiosis with myelin sheath loss, severely dilated myelin sheaths that infrequently contain macrophages or fragmented myelin (digestion chambers), and multifocal swollen axons (spheroids). (Photo courtesy of: Animal Medical Center, 510 E. 62nd Street, New York, NY 10065.)

Microscopic Description:
Brain, cerebral cortex -or- metencephalon at the level of the cerebellar peduncles: Subcortical white matter tracts, white matter tracts of the pons, and interspersed white matter within metencephalon are multifocally pale and vacuolated (spongiosis). Within these regions, there is myelin sheath loss, severely dilated myelin sheaths that infrequently contain macrophages or fragmented myelin (diges tion chambers), and multifocal swollen axons (spheroids). Affected white matter tracts contain moderately increased numbers of reactive astrocytes. Multiple, inconsistently coalescing, poorly demarcated regions of grey matter display mild neuropil rarefaction and contain moderately to severely increased numbers of activated, reactive glial cells and gemistocytic astrocytes and few infiltrating lymphocytes and plasma cells. Astrocytes are commonly hypertrophied. Regional neurons are degenerate, multifocally display central chromatolysis, and are less frequently are shrunken, angulated, and hypereosinophilic (necrotic neurons). Neurons and astrocytes often contain intranuclear and/or -cytoplasmic 3-7µm, round to oval, hyaline, pink or magenta inclusion bodies that in nuclei are associated with chromatin margination and clearing. There are rare syncytial cells. Regional cerebral blood vessels are occasionally tortuous and are often lined by swollen, hypertrophied endothelial cells. Multifocally, the meninges are mildly infiltrated by lymphocytes, plasma cells, and macrophages. Following along Virchow-Robbin spaces and surrounding multiple cerebral blood vessels are moderate cuffs of lymphocytes, plasma cells, and lesser macrophages.



Cerebrum, dog: Affected white matter tracts contain moderately increased numbers of reactive astrocytes. (Photo courtesy of: Animal Medical Center, 510 E. 62nd Street, New York, NY 10065.)

Immunohistochemical stain for canine distemper virus (Animal Health Diagnostic Center/Cornell University) was placed on sections of the brain and revealed strong cytoplasmic immunoreactivity within neurons and astrocytes and highlights axonal projections.

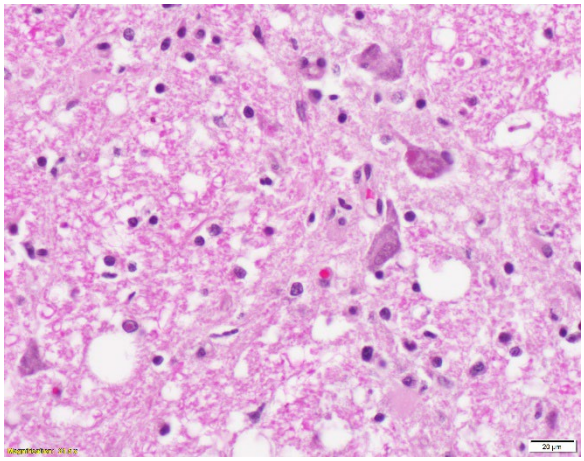
Contributor's Morphologic Diagnoses:
1) Demyelination and axonal degeneration, multifocal to locally extensive, moderate to marked with spongiosis, swollen myelin sheaths with fragmented myelin and macrophages (digestion chambers), and multifocal distended axons (spheroids)
2) Leukoencephalitis, lymphoplasmacytic, histiocytic, chronic, multifocal, moderate with regional moderate to marked gliosis, neuronal degeneration and necrosis with mild neuronophagia, numerous intranuclear and -cytoplasmic inclusions, rare syncytial cells, and moderate lymphoplasmacytic perivascular cuffs

Contributor's Comment:
Histologic features in this case were consistent with a demyelinating leukoencephalitis. Other findings in this dog were a single *Dirofilaria immitis* adult in the pulmonary artery with expected reactive changes in the blood vessel and a single metazoan parasite larva in one kidney. In dogs, differentials for leukoencephalopathy include viral (rabies, CDV, canine herpes virus) and parasitic (*Neospora caninum*) infections, as well as non-

infectious etiologies (e.g., polyneuritis, allergic encephalitis, necrotizing encephalitis).[16] Profound demyelination and the presence of both intracytoplasmic and -nuclear inclusion bodies was suggestive of paramyxovirus infection. Lesions were most prevalent and severe in the cerebral cortex and the caudalmost focus was in the metencephalon at the level cerebellar peduncles. The cerebellum, caudal brainstem, and spinal cord were spared. Immunohistochemistry for CDV confirmed intense immunoreactivity in neurons and astrocytes. Based on this patient's signalment and history, progressive neurologic disease over two months, and histologic lesion distribution, the current case was most consistent with "old dog encephalitis" (ODE). Like other reported cases of ODE, this patient was up-to date on vaccinations.

Canine distemper virus is a highly contagious disease of carnivores with a worldwide distribution.[1-21] Infections and outbreaks have been reported in numerous families within the order Carnivora**,** including Canidae (fox, wolves), Mustelidae (ferret, mink), Hyaenidae (hyena), Procyonidae (raccoon), Ailuridae (red panda), Ursidae (bear), Viverridae (civet, mongoose), Mephitidae (skunks), Odobenidae (walrus), Otariidae, and Felidae.[3, 5, 9-11, 13, 19, 21] Most infections involve carnivores, but infections have been reported in non-carnivore species in the orders Rodentia, Primates, Artiodactyla (pigs, cervids), Pilosa (tamandua and sloth), and Proboscidea (elephants).[5, 9, 10, 11, 17] Outbreaks have been described in multiple wildlife species and hence CDV is a threat to conservation efforts worldwide.[5, 10, 11] CDV can circulate in wildlife species even when infection rates are low in domestic dogs, suggesting that the virus persists in a complex reservoir system[10] and thus both asymptomatic domestic and wildlife reservoirs play an important role in driving epidemics.[5] In one review, the majority of cases outside of domestic dogs were reported in captive animals, suggesting that conditions of captivity (e.g., housing multiple mammalian species in close proximity) is a risk factor for infection.[10] CDV should be a considered a differential diagnosis of disease and extinction events, even in species outside of the order Carnivora.[10]

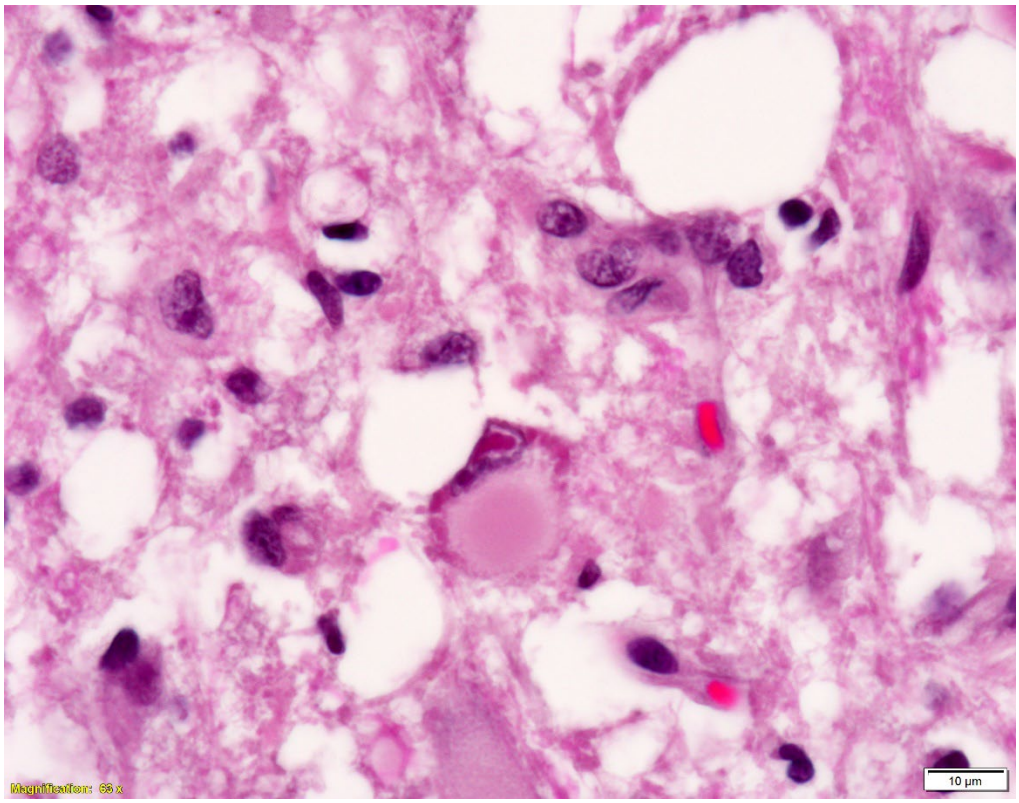
Canine distemper virus (CDV) belongs to the family Paramyxoviridae within the morbillivirus genus.[1-3, 5-11, 13, 14, 16, 17, 20, 21] Other important morbilliviruses in veterinary medicine include Rinderpest Virus, Peste des Petits Ruminants Virus, Phocine Distemper Virus, Cetacean Morbillivirus, and Equine Morbilivirus.[8-11, 16, 19, 21] Important veterinary pathogens within the larger Paramyxoviridae family include those of the genera henipavirus (Hendra and Nipah Viruses), avulavirus (Newcastle Disease Virus and other avian paramyxoviruses), rubulavirus (Porcine Rublavirus, mumps), respirovirus (Sendai virus, Bovine Parainfluenza Virus 3), Metapneumovirus (Avian Rhinotracheitis Virus), ferlavirus (Fer-de-lance), and aquaparamyxovirus (Atlantic Salmon Paramyxovirus), as well as several unclassified paramyxoviruses (Bottle-nosed Dolphin paramyxovirus, Salem Virus). [8, 9, 11, 12]



Cerebrum, dog: Affected white matter tracts contain moderately increased numbers of reactive astrocytes. (Photo courtesy of: Animal Medical Center, 510 E. 62nd Street, New York, NY 10065.)

Paramyxoviruses are large (150-300 nm), pleomorphic, enveloped viruses with a negative-sense, single-stranded RNA genome.[3, 5, 6, 8, 9, 11, 14, 17, 20, 21] Viral proteins include two viral envelope glycoproteins (fusion (F) and hemagglutinin (H)), a nucleocapsid (N) protein, two transcription-associated proteins (P protein and viral polymerase (L)), and a membrane (M) protein. [3, 5, 9-12, 20] Infection is initiated through binding of the viral hemagglutinin (H) protein to SLAM (signaling lymphocyte activation molecule) and nectin-4 receptors on host immune and epithelial cells, respectively.[5, 9, 10, 20] A potential 3rd host receptor, GliaR, is suggested on glial cells.[5] Thus, the highly variable H glycoprotein is the main determinant of cellular and host tropism. [5, 9, 10, 12, 20] Virulence is modulated via the fusion (F) glycoprotein, which mediates union between the virus and host plasma membranes allowing for viral penetration.[5, 20] Characteristic syncytial cells due to host cell fusion is likewise facilitated by the F glycoprotein.[9, 20] Replication occurs in the host cell cytoplasm and virions are released by budding from the plasma membrane through interactions with the membrane (M) protein.[5, 11, 12] Infection is cytolytic with the virus killing host cells during replication and release.[11, 20] Virus is shed primarily in respiratory secretions, but other body secretions and fluids are infectious to a lesser extent.[3, 5, 7] The primary modes of transmission include close contact and aerosol.[3, 5-7, 9-11, 20, 21] As this virus is relatively labile in the environment, [9, 11] fomite transmission is less frequent. [3, 5-7, 9-11, 20, 21]

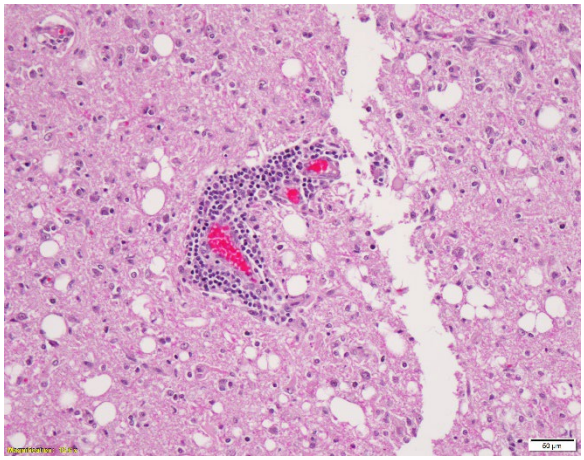
After inoculation, CDV first colonizes the upper respiratory tract mucosa, where it infects local lymphocytes and is phagocytized by local macrophages and dendritic cells.[6, 9, 11, 13, 19-21] Infected immune cells then travel to the regional lymph nodes and tonsils, where viral replication occurs resulting in lymphoid necrosis and depletion.[3, 6, 9, 11, 13, 19-21] Primary viremia results allowing for dissemination through the hemolymphatic system.[3, 6, 9, 11, 13, 19-21] The resulting immunosuppression facilitates the development of opportunistic infections, [3, 6, 9, 11, 13, 19 21,



Neurons and astrocytes often contain intranuclear and/or -cytoplasmic 3-7µm, round to oval, hyaline, pink or magenta inclusion bodies. (Photo courtesy of: Animal Medical Center, 510 E. 62nd Street, New York, NY 10065.)

and CDV-associated thymic atrophy further drives immunosuppression.[3, 16] Secondary viremia develops through leukocyte and platelet trafficking and cell free viremia.[5, 6, 9, 11, 13, 19-21] The extent of viral dissemination and thus clinical disease depends on the rapidity and effectiveness of the individual's immune response against the specific viral strain.[3, 9, 11-13, 21] Patients with an adequate immune response will go on to clear infection, while those that mount a poor to absent response display widespread systemic disease with high mortality.[3, 9, 13, 21] With an intermediate immune response, minor to absent mucosal disease and variable neurologic disease may be seen.[3, 9, 13, 21]

Canine distemper virus is pantropic with a high affinity for lymphoid, epithelial, nervous, and ocular tissues.[13, 20, 21] Severity of disease depends on the viral strain, age and immune status of the host, and rapidity of the immune response. [3, 6, 9, 11-13] Clinical disease in dogs is most common in puppies of 12-16 weeks of age when maternally derived, passive immunity wanes. [3, 6, 9, 11-13, 20] In these young naive dogs, clinical disease typically manifests as a combination of CNS, respiratory, and/or gastrointestinal signs. [9, 13, 20] Infection typically starts with fever and conjunctivitis and rapidly progresses to respiratory and gastrointestinal signs.[3, 9] Extra-nervous signs typically develop about a week post inoculation and include pyrexia, oculonasal discharge, pharyngitis, tonsilitis, otitis interna/labyrinthitis, cough, vomit, diarrhea, skin rash and pustules, hyperkeratosis of the nose and foot pads, enamel hypoplasia (due to ameloblast degeneration and necrosis), lower urinary tract signs, ocular disease (due to viral retinitis, non-suppurative optic neuritis, and optic nerve demyelination), and abortion.[3, 5, 6, 9, 11-17, 19-21] Histologic changes in the lungs include necrotizing bronchointerstitial

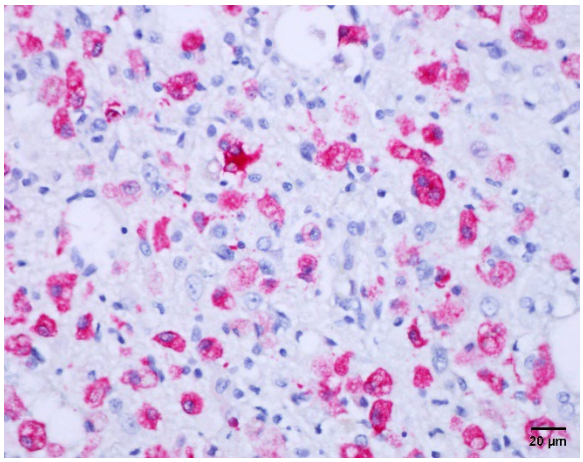


Cerebrum, dog: Regional cerebral blood vessels are occasionally tortuous and are often lined by swollen, hypertrophied endothelial cells and contain 2-3 layers of lymphocytes and plasma cells. (Photo courtesy of: Animal Medical Center, 510 E. 62nd Street, New York, NY 10065.)

pneumonia with type II pneumocyte hyperplasia and syncytial cell formation, pulmonary edema, and inclusion bodies in pneumocytes and alveolar macrophages.[3] Enteric lesions attributable to the gastrointestinal signs are not well described, but virus is thought to infect the intestinal crypt epithelium.[14] CDV less consistently causes an infectious myocarditis, which depending on chronicity can be accompanied by frank myocardial necrosis and/or fibrosis. [3, 19] In both experimental and natural infections growth retardation lattices can be seen in the bones, which in experimental models was related to transient impaired osteoclastic resorption of the primary spongiosis.[4] In an outbreak in Linnaeus's 2-toed sloth (*Choloepus didactylus*), marked hepatic tropism was an atypical striking feature.[17] Affected dogs may die at this stage, recover fully, or go on to develop neurologic signs 1-6 weeks later.[3, 6, 9, 11, 21]

CDV is associated with several distinct nervous system manifestations in dogs[1] that include: 1) canine distemper encephalomyelitis in immature dogs, 2) old dog encephalitis, 3) multifocal distemper encephalomyelitis in mature animals, and 3) post-vaccinal enceph-

alitis.[1, 7] The latter three are uncommon manifestations confined to the CNS only.[1, 2, 7] The syndrome that develops is directly dependent on the viral strain, host age and immune status, and neuroanatomic location of pathologic lesions.[7] The most common CNS manifestation is canine distemper encephalomyelitis in immature dogs that is part of the above-described pantropic infection. [1, 2, 7, 13] In these naive animals, neurologic disease can occur with or without preceding or concurrent gastrointestinal and/or respiratory signs.[9, 13, 16, 20, 21] Approximately 30% of dogs infected with systemic disease ultimately develop neurologic dysfunction observed 1-6 weeks after the onset of initial signs.[6, 9, 20, 21] Hematogenous spread to the CNS allows for initial infection of and viral replication in vascular pericytes and astrocyte foot processes, endothelial cells, microglia, and choroid plexus epithelium.[13, 20, 21] Involvement of the choroid plexus epithelium and ependyma allows for the virus to be shed into and disseminated by the CSF.[3, 20, 21] With dissemination, virtually all cells of the CNS are susceptible to infection.[3, 11, 13, 20, 21] Neurologic signs include cerebellar or vestibular ataxia, "gum chewing", paresis to paralysis, myoclonus, tremors, circling, hyperesthesia, and epileptic seizures. [3, 5, 6, 9, 11, 16, 19-21] In a recent single case report, CDV infection presented as focal lower motor neuron signs and hyperesthesia to one forelimb, which rapidly progressed to diffuse lower motor signs of all limbs and seizures.[6] This unique presentation was related to lesion distribution to the C5 and lumbar spinal cord segments.[6] The main histologic lesion in naïve patients is a demyelinating leukoencephalopathy related to viral-induced injury to the neurons and olidodendroglia.[9, 13, 16, 17, 20, 21] Acute lesions in the CNS are not overwhelmingly inflammatory and are likely related to metabolic dysfunction, decreased myelin synthesis by damaged oligodendrocytes, and microglia activation.[9] With lesion progression CDV-specific immune responses and viral persistence induce an inflammatory reaction.[9] Viral-induced damage to oligodendrocytes leads to highly characteristic, primary demyelination predominantly distributed to the cerebellum, periventricular white matter, spinal cord, and optic tracts.[3, 6, 9, 13, 16, 18, 20, 21] Additional white matter changes depend on chronicity and include intra-myelinic edema, vascular proliferation with hypertrophied endothelial cells, infiltrating macrophages associated with myelin debris, white matter necrosis, gliosis.[3, 9, 16, 18, 20, 21] Alterations to the grey matter target the cerebral cortex, cerebellum, brainstem, and spinal cord and include non-suppurative encephalitis with perivascular cuffs.[3, 16, 20, 21] Neuronal injury is demonstrated by central chromatolysis and degenerative neurons that progress to eventual necrosis with inconsistent neuronophagia.[3, 16, 20, 21] Intracytoplasmic and -nuclear inclusions are seen in neurons and astrocytes.[11, 16, 21] Sclerotic astrocyte foci and myelin loss may be seen in survivors.[3, 11, 20, 21]



Cerebrum, dog: Immunohistochemistry for CDV antigen reveals strong cytoplasmic immunoreactivity within neurons and astrocytes and highlights axonal projections. (Photo courtesy of: Animal Medical Center, 510 E. 62nd Street, New York, NY 10065.)

This patient's signalment and history (i.e., middle-age, progressive neurologic disease

over two months, and up-to-date vaccination status) and pathologic findings (i.e., lack of findings consistent with systemic CDV infection, histomorphologic features confined to the CNS, and lesion distribution) were most consistent with "old dog encephalitis". Old dog encephalitis (ODE) is a rare, unique, invariably fatal CNS-only presentation in older dogs.[1-3, 7, 9-12, 16, 21] A diagnosis of ODE necessitates a combination of clinical history and findings, typical histologic lesions and distribution, and absence of CDV-induced lesions in other organs.[7] Affected animals are typically completely vaccinated with no evidence of previous systemic CDV infection.[1, 7, 9, 12, 20] Clinical disease is insidious characterized by chronic, progressive cortical disease with motor and behavioral disturbances (i.e., circling, swaying, weaving, compulsive walking, postural reaction deficits, head pressing, and pushing on fixed objects) .[1-3, 7, 11, 21] In one experimentally-induced ODE case, the dog exhibited multiple episodes of relapsing cortical and subcortical signs and epileptic seizures were part of this dog's disease.[1] Gross lesions are non-specific with ODE and include dilation of the ventricles, loss of grey-white matter distinction, and grey-brown regions in the forebrain.[2,7] Histologic changes are confined to the cerebral cortex, thalamus, and mid-brain, with sparing of the caudal brainstem and spinal cord.[1, 2, 7, 16, 21] Histomorphologic features include demyelination; vacuolization of the subcortical white matter, non-suppurative encephalitis with profound perivascular cuffs; astrocytosis and astrogliosis; neuronal loss, degeneration, and necrosis; and neuropil rarefaction.[1, 2, 7, 16, 21] Inclusion bodies are numerous.[1, 2, 7, 16, 21] Ultrastructural changes include mononuclear cuffs, viral nucleocapsids and viral budding in the grey matter, electron-dense granular material in Virchow-Robbins spaces (unclear origin), and white matter changes suggestive of abortive and inadequate axonal remyelination (i.e., naked axons surrounded by astrocyte processes and remyelinated axons with thin myelin sheaths) and chronic demyelination (i.e., loosely arranged demyelinated axons separated by inflammatory cells).[1] The pathogenesis of old dog encephalitis is unclear and the host-to-virus relationship is not understood.[1-3, 7, 9-12, 16, 21] Previously proposed theories have included: 1) wild-type viral persistence in a rare replication-defective state, 2) terminal result of chronic subclinical CDV encephalitis, 3) re-infection, and 4) a viral strain predisposed to persistent infection.[1, 2, 9, 11, 12, 21] At this time, ODE is believed to be the result of virus persistence in the CNS in a replication-defective form.[1-3, 7, 9-12, 16, 21] Evidence to support this theory include: 1) ODE isolates are antigenically similar to the wild-type virus and don't display a mutation rate beyond what is expected for an RNA virus,[1, 7] 2) unique histomorphologic features of ODE that are less consistent with progression of previous infection (i.e., profound angiogenic distribution and atypical lesion localization),[1, 7] 3) difficult post-infection isolation of the inoculating virus (i.e., prolonged culture and coculture with susceptible Vero cell monolayer),[1] and 4) patient histories[1-3, 7, 9-12, 16, 21]. In addition, ODE has some similarities to subacute, sclerosing panencephalitis (SSPE) seen in children with persistent infections with a replication-defective measles virus.[1, 11, 12] SSPE is slightly different from ODE in that SSPE isolates are constitutively replication defective and therefore never produce viral particles, where in ODE virions can be isolated but with significant difficulty.[1, 12]

Other CNS-only manifestations of CDV are similarly uncommon. Multifocal distemper encephalitis in the mature dog is a rare, chronic progressive disease due to infection in middle-aged to senior dogs (4-8-years-old).[2] Clinical signs include slowly progressive head tremors, hindlimb paresis, and in-

coordination.[2] Seizures are not typical.[2] Histologic changes are restricted to the CNS, where lesions are predominantly distributed to the cerebellum and spinal cord, with sparing of the cerebral cortex.[2] Histologic features include a demyelinating leukoencephalitis, multifocal necrotizing, non-suppurative encephalitis, and rare intranuclear inclusion bodies.[2] Post-vaccinal distemper is uncommon and presents as severe, often lethal neurologic decompensation with aggressive behavior in patient who have been recently vaccinated (within 3 weeks) with an attenuated viral strain.[2, 16] Histologic lesions are not well-characterized, but grey matter changes are similar to those described in natural disease with relative sparing of the white matter.[2]

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JPC Diagnosis: Cerebrum: Encephalitis, lymphohistiocytic, chronic, multifocal, moderate, with spongiosis, gliosis, and neuronal and glial intranuclear and intracytoplasmic viral inclusions.

JPC Comment: The contributor provides an excellent and thorough review of systemic and neurologic manifestations of canine distemper virus infection. This week's moderators, Drs. Eric and Juliana Lee, selected two cases of canine distemper (see Case 2 for CDV-pneumonia in a dog) to demonstrate the spectrum of disease caused by this pancytotropic virus.

CDV is closely related to two other morbilliviruses: rinderpest virus (RPV) and human measles virus (HMV). Rinderpest virus, which was recently eradicated, is the oldest of these viruses, with origins in Asia over three thousand years ago.[11] The virus then spread to the Near East and Europe, and texts from Greece and Rome document rinderpest outbreaks in the fourth century BCE.[15] Human measles virus was first documented around the ninth century CE, and the term morbilli, a diminutive for "morbus" or illness, was coined to differentiate it as a "small" illness compared to smallpox.[11] It is thought that human measles virus originated from RPV or a common morbillivirus precursor of ungulates and phylogenetic analysis suggests that it diverged around 12th century CE.[11,15] Canine distemper virus is much younger than both RPV and HMV, with the first reports in Central and South America in the early 1700s.[11] Two decades years after its first appearance in the New World, CDV was reported in Europe and its initial epizootic spread had a high initial rate of mortality, characteristic of a newly introduced pathogen.[15]

Several hypotheses have been proposed regarding the evolution and first occurrence of CDV in the Americas, including a sylvatic virus that jumped to domestic dogs or a virus of domestic dogs belonging to Paleoindians which spread with their movement.[15] A recent study by Uhl et al evaluated historical records, genetic and epidemiologic characteristics, and paleopathologic data to uncover the origins of CDV, and ultimately, they supported a different theory: that CDV began when HMV jumped from humans to dogs.[15] First, the group evaluated 2335 permanent teeth from 96 dog skeletons from the Weyanoke Old Town (750-1470 CE) site in Virginia and found no evidence of enamel hypoplasia (indicative of in utero CDV infection); additionally, enamel lesions were absent in 42 dogs from South American site dating from 1030-1324 CE.[15] These findings support the fact that CDV arose after the arrival of European explorers and the HMV they carried. Second, the remarkable genetic similarities in sequence, structure, antigenic epitopes, and host receptors they target indicate CDV and HMV likely arose from a common ancestor.[15] Codon usage analysis also indicates that CDV likely evolved from a virus that previously infected humans, as its codon usage bias (which reflects evolutionary host adapta-

tion) favors that of a human over a dog.[15] Finally, the authors used historical records to demonstrate the massive social disruption and upheaval caused by European activities coupled with overwhelming illness and death with the introduction of HMV to naïve populations provided ample opportunity for the virus to jump species and adapt to domestic dogs.[15] While not definitive proof, this interdisciplinary evidence supports the hypothesis that CDV infection in dogs arose from HMV epidemics in native South American populations.[15]

Conference participants debated whether demyelination or encephalitis were the primary lesions. Ultimately, participants felt the inflammation was more significant, and the number of gitter cells and level of myelin debris did not reach a level to support demyelination in the morphologic diagnosis. The term spongiosis was used to describe the white matter vacuolation visible from sub-gross magnification and could be the end-state of either of these two processes.

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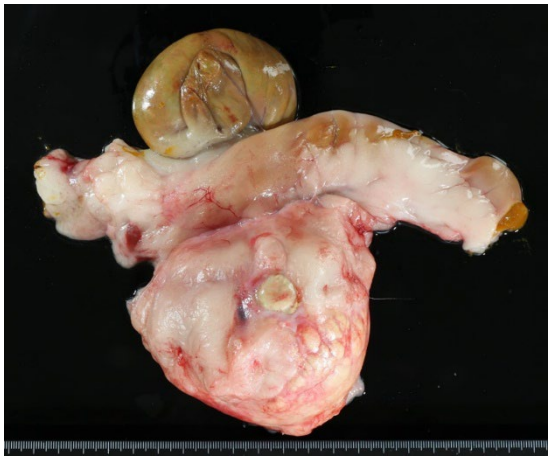
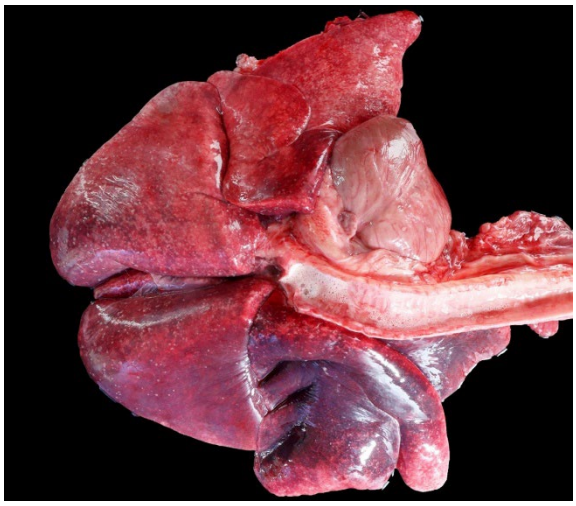
CASE IV:

Signalment:
7-year-old male Alaskan Malamute dog

History:
A 7-year-old male Alaskan Malamute dog was presented with non-specific clinical



Mesenteric lymph node, dog. The mesenteric lymph node is effaced by encapsulated mass of about 15 cm. in diameter, with a central area of caseous necrosis. (*Photo courtesy of:* Servei de Diagnostic de Patologia Veterinària, Facultat de Veterinària UAB, Bellaterra (Barcelona).)



Lung, dog. Multifocal to coalescing, whitish and dense nodules up to 0.5 cm in diameter are randomly scattered through all pulmonary lobules. Moderate amount of white foam in the trachea and bronchial lumen. (*Photo courtesy of:* Servei de Diagnostic de Patologia Veterinària, Facultat de Veterinària UAB, Bellaterra (Barcelona).)

signs (weight loss, apathy and anorexia). Ultrasonography and CT scan revealed the presence of a large mass in the abdominal cavity and multiple smaller masses in the liver. Biopsies of the masses were taken, and a pyogranulomatous hepatitis and lymphadenitis was diagnosed histologically. GRAM, Ziehl-Neelsen (ZN), Grocott and PAS stains did not reveal bacterial or fungal aetiology. Negative results were obtained by PCR for *Leishmania* spp., *Bartonella* spp., atypical Mycobacteria, *Neospora caninum*, *Toxoplasma gondii* and *Cryptosporidium* spp. Because of the poor prognosis the dog was euthanatized.

Gross Pathology:

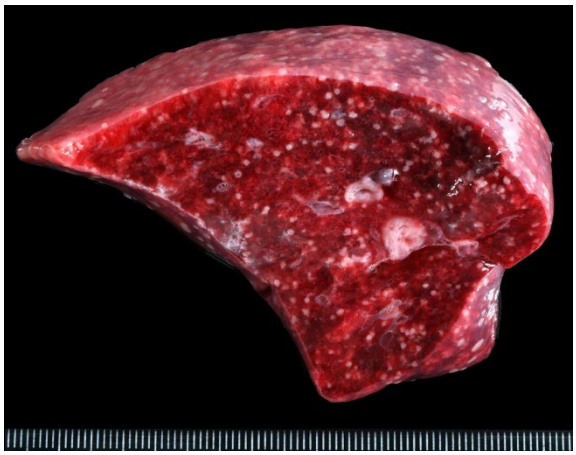
At postmortem examination, the dog showed loss of body condition. The mesenteric lymph node was replaced by a soft, encapsulated mass of about 15 cm. in diameter, with a central area of caseous necrosis. Also, multiple, randomly distributed granulomas of 0.2 to 0.5 cm in diameter were seen along the serosa and in the parenchyma of lung, heart, liver, kidneys, omentum and brain.

Laboratory Results:

Postmortem: Additional ZN staining were performed on several tissues taken at necropsy. ZN stain showed acid-fast bacilli in the necrotic center of granulomas and also within macrophages. Frozen samples preserved at necropsy were submitted for mycobacterial investigation. Direct PCR for the *Mycobacterium tuberculosis* complex (MTC) yielded a positive result, and a mycobacteria belonging to the MTC was cultured. The isolated mycobacteria was identified as *Mycobacterium tuberculosis* by spoligotyping.

Microscopic Description:

Kidney: Multifocal to coalescing, variably size (0.2 to 0.5 cm in diameter) granulomatous-necrotizing nodular lesions scattered throughout the cortex, medulla and to a lesser extent pelvis, are observed replacing approximately 20-25% of the evaluated parenchyma. The center of some of these nodules is composed of moderate amount of acellular and amorphous hypereosinophilic necrotic material admixed with cellular debris, karyorexis and karyolysis (lytic necrosis). Depending on the section assessed, occasional necrotic glomeruli and tubules are trapped

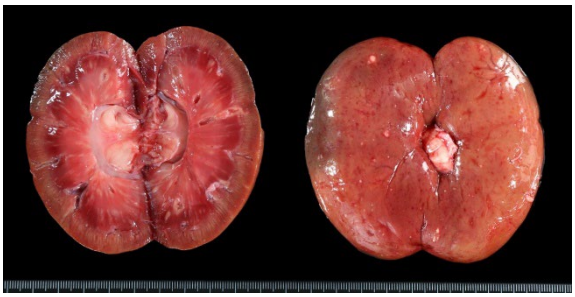


Lung, dog. Transverse section showing the intraparenchymatous and subpleural location of the nodules. (*Photo courtesy of:* Servei de Diagnostic de Patologia Veterinària, Facultat de Veterinària UAB, Bellaterra (Barcelona).)

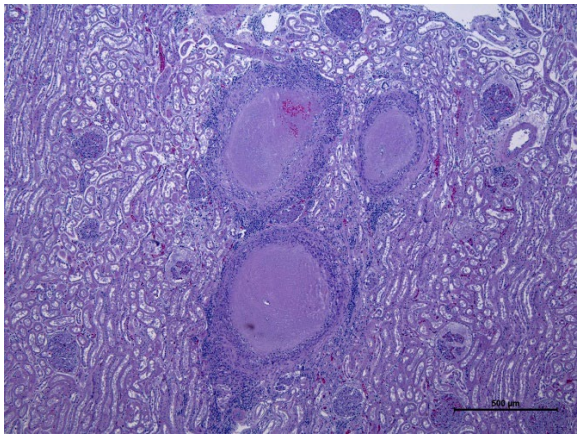
within the necrotic debris also with mild deposition of granular and basophilic mineral material (dystrophic mineralization). These nodules are demarcated by mild to moderate macrophagic inflammation, mainly epithelioid cells, with scarce multinucleated giant cells (Langhans type), surrounded by a thin capsule of mature connective tissue (fibrosis) at the periphery (Granuloma). Multifocally, other non-encapsulated granulomas are also observed and are composed of moderate amount of viable and degenerated neutrophils and macrophages without obvious areas of lytic necrosis in the center. Acid-fast bacilli were observed in the cytoplasm of macrophages and free within the necrotic material with ZN staining. Diffusely, the glomeruli of non-affected parenchyma showed moderate thickening of the glomerular Bowman capsule and basement membranes due to the presence of acellular and amorphous eosinophilic material.

Contributor's Morphologic Diagnoses:
Nephritis, granulomatous and necrotizing, multifocal to coalescing, severe, chronic.

Contributor's Comment:
Tuberculosis (TB) is a zoonotic disease caused by mycobacteria of the *Mycobacterium tuberculosis* complex (MTC) in a broad range of mammalian hosts. MTC comprises *M. tuberculosis, M. bovis, M. africanum, M. microti, M. canetti, M. caprae, and M. pinnipedii* ([3][16]). Though a significant progress has been made toward the elimination of tuberculosis from humans, this disease remains an important health, social and economic problem ([1][14]).



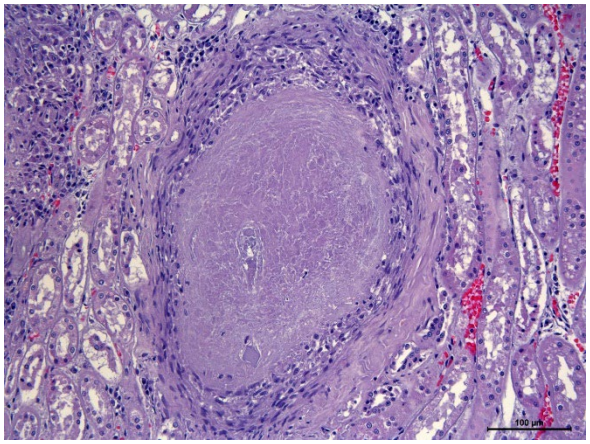
Kidneys, dog. Multifocal to coalescing, prominent white nodules up to 0,3 cm in diameter surrounded by a red halo are randomly distributed through the cortex, affecting both cortex and medulla in the opened section. (*Photo courtesy of:* Servei de Diagnostic de Patologia Veterinària, Facultat de Veterinària UAB, Bellaterra (Barcelona).)



Kidney, dog. Multifocal to coalescing, randomly distributed 500 µm to 600 µm in diameter granulomatous and necrotizing nodules scattered through renal cortex, composed of moderate amounts of central lytic necrosis .. (HE, 100X) (*Photo courtesy of:* Servei de Diagnostic de Patologia Veterinària, Facultat de Veterinària UAB, Bellaterra (Barcelona).)

M. tuberculosis and *M. bovis* occur most frequently in their respective hosts, but may infect many other species. Humans are the only reservoir host for *M. tuberculosis*; however, this bacterium has a wide host range, including dogs, cats, pigs, cattle, captive monkeys, psittacine birds, fish, reptiles and marine animals ([2][7][16][17]).

Dogs and cats have been pointed as potential carriers and as a possible source of tuberculous infection to other species ([1]). The susceptibility of dogs to infection with *M. tuberculosis* is still a matter of debate. Malin (1940) was the first who reported tuberculosis in dogs and their owners. Later on, tuberculosis in dogs was linked to the disease in humans and cattle ([1][13]). Studies on dogs being in contact with people suffering from



Kidney, dog. Granulomas are surrounded by moderate amounts of inflammatory cells and thin fibrous capsule. (HE, 200X) (*Photo courtesy of:* Servei de Diagnostic de Patologia Veterinària, Facultat de Veterinària UAB, Bellaterra (Barcelona).)

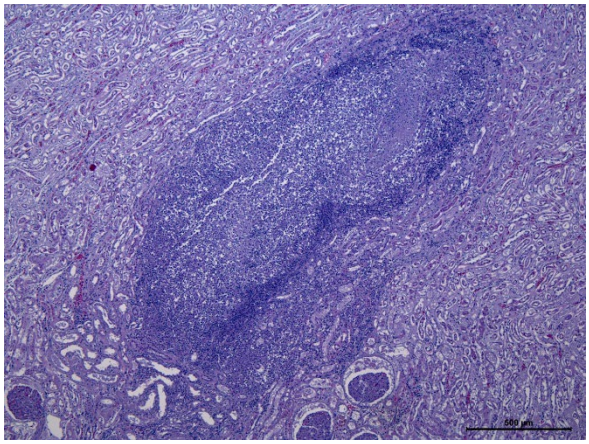
tuberculosis show high levels of *M. tuberculosis* isolated from otherwise healthy-looking animals and, in some cases, dogs infected with *M. tuberculosis* demonstrated the full clinical picture of the disease ([1]).

Canine TB has been caused by *M. tuberculosis, M. bovis*, and *M. microti* ([3]). Although dogs are less susceptible to *M. tuberculosis* than to the other tuberculous mycobacteria, they can become infected after prolonged exposition to infected human respiratory secretions ([1][3][14][16]). Thus, *M. tuberculosis* infection in dogs is considered an anthropozoonosis ([7][11]). Therefore, the incidence of canine TB is closely related to the incidence of human TB, being higher in urban areas, where the human patients are concentrated, or in developing countries ([1][7][16]). Middle Africa and Southern Asia seem to be the parts of the world where human TB is most prevalent ([15]). Evidence indicates that many companion animals infected with *M. tuberculosis* had a history of living in an environment with humans infected by TB, or that the animals had intimate contact with owners affected by TB ([11]). Genotypic comparison of mycobacteria recovered from owners and dogs may confirm the similarity of strains that infected household members and/or their pet ([5][11]).

The inhalation of aerosolized droplets from humans appears to be the primary route of canine TB infections ([12]). In addition, cutaneous and ingestion of contaminated food or sputum are well-recognized sources of *M. tuberculosis* transmission from humans to dogs ([1][11][12]).

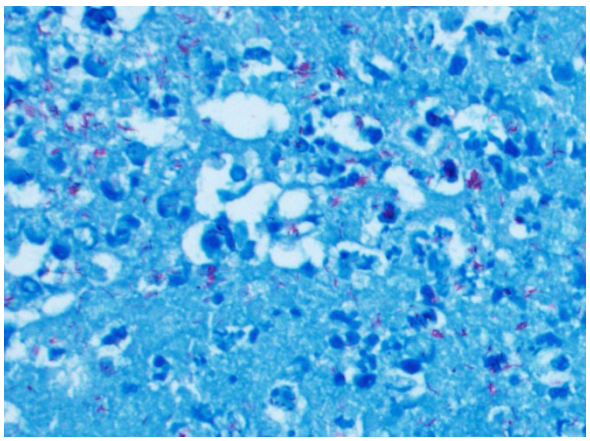
Although no clear canine-to-human *M. tuberculosis* transmission has been documented, dogs can discharge organism in the sputum leading to elimination of *M. tuberculosis* into the home environment ([7][11][16]). In addition, experimental infection results led to the assumption that the transmission of *M. tuberculosis* between infected and healthy dogs kept in close contact is possible, suggesting that naturally infected dogs may be a continuous source of infection for humans and other animals ([1][11]).

Natural TB in companion animals is most commonly a subclinical disease. TB-infected dogs with less than two years old rarely exhibit clinical manifestations except when ex-



Kidney, dog. Deeper in the cortex and within the medulla, granulomas are forming in areas of tubular and vascular necrosis. (HE, 100X) (*Photo courtesy of:* Servei de Diagnostic de Patologia Veterinària, Facultat de Veterinària UAB, Bellaterra (Barcelona).)

posed to a high number of pathogenic mycobacteria ([10]). In dogs that develop clinical signs, the most common primary sites of TB infection are the lungs and the pulmonary lymph nodes, being pneumonia the main clinical manifestation of *M. tuberculosis* ([1][4][11]). Nevertheless, occasional cases of canine mycobacterial infection involving primarily the digestive tract have been described worldwide, although some of them caused by non-tuberculous mycobacteria, especially *M. avium* ([8][9]). A generalized form of TB is considered rare in dogs infected with *M. tuberculosis* ([11]). These cases probably result from hematogenous dissemination of bacilli following erosion of the wall of a blood vessel by an expanding tubercle. In some instances, presumably following substantial release of bacilli into the blood, the presence of innumerable tiny white foci justifies the term "miliary tuberculosis." Embolic lesions are most common in lung, and may involve lymph nodes, bone, liver, kidney, mammary gland, uterus, pleura, peritoneum, pericardium, and meninges. Lesions are rare in salivary gland, pancreas, spleen, brain, myocardium, or muscle ([2][3]). Unlike other species, such as bovine, TB in dogs often appears as granulation tissue in which macrophages are scattered at random and giant cells are rare. Small and generalized granulomas are uncommon, and are composed



Kidney, dog. Acid fast bacilli are scattered in low numbers throughout areas of granulomatous inflammation. (Fite-Faraco, 400X)

principally of epithelioid cells surrounded by narrow zones of fibrous tissue in which there are scattered small collections of lymphocytes and plasma cells. Thus, the lack of Langhans cells and demarcation of the granulomas is considered as an essential feature of TB in dogs. In addition, the necrosis is not a feature in these kind of granulomas but is often present in the centers of larger and more chronic ones ([2]). In dogs, intrabronchial dissemination within the lungs occurs quite rapidly and can lead to tuberculous bronchitis and bronchiolitis. Pleuritis or peritonitis often accompanies primary infections in the lungs or intestine, respectively, with diffuse or finely nodular pleural thickening by granulation tissue containing few macrophages and bacilli ([2]).

Dogs can also be infected by non-tuberculous mycobacteria which, in contrast, are ubiquitous and potentially pathogenic. Within the nontuberculous mycobacteria group, the Mycobacterium avium complex (MAC) encompasses mycobacterial species considered to be the most likely causative agent of disseminated disease in humans and dogs. Zoonotic transmission of MAC is as likely as environmental acquisition ([3]). Dogs are relatively less susceptible to infection with MAC organisms than to MTC pathogens, and despite the ubiquitous and widespread nature of MAC organisms, infections in dogs are rare, owing to their innate resistance ([6]).

Intradermal tuberculin testing and serological tests in dogs are considered inconsistent and unreliable. The pathomorphological and bacteriological analyses are still the most reliable tests and constitute mainly post-mortem diagnosis. Although PCR techniques are becoming very valuable, mycobacterial culture is considered the reference standard for TB diagnosis ([1][3][11]). The use of ZN staining in microscopic examination, cytology, and/or histology samples for the identification of

acid-fast organisms is a valuable method for routine mycobacterial diagnosis. In this case, the presence of acid-fast bacteria in the granulomas observed was not homogeneous with ZN stain. Very few or absence of bacteria were observed in the most chronic granulomas in which areas of central necrosis are seen but higher amounts of them were seen in the more incipient granulomas, in which there are not areas of evident central necrosis. This fact can justify that bacteria were not observed in biopsies taken antemortem with Zn stain. These results demonstrate the possibility of obtaining false negative results using ZN and show the need for combining methods in the diagnosis of canine TB ([11]).

Contributing Institution: Servei de Diagnostic de Patologia Veterinària, Facultat de Veterinària UAB, Bellaterra (Barcelona).

JPC Diagnosis: Kidney: Nephritis, granulomatous, multifocal to coalescing, marked.

JPC Comment: The hallmark of *Mycobacterium tuberculosis* (MTB) infection is the pulmonary granuloma; this case illustrates these classical caseous granulomas in an extrapulmonary location, and the contributor does an excellent job summarizing this disease in dogs.

A recent article by Pereira et al in the *Journal of Comparative Pathology* described the histologic appearance and distribution of MTB lesions in naturally infected fifteen nonhuman primates, and the authors demonstrated that the infection did not always manifest as pulmonary granuloma.[12] In all nine infected Old World Monkeys (OWMs), granulomas were present in at least one organ, with six having typical pulmonary granulomas.[12] These granulomas were either caseous (with a necrotic core), non-necrotizing (solidly-cellular), or suppurative.[12]

In New World Monkeys (NWMs), on the other hand, pulmonary granulomas were only observed in one of six infected animals.[12] Four other infected NWMs had diffuse interstitial pneumonia with foamy macrophages expanding alveolar septae but without distinct granulomas or necrosis.[12] This macrophage morphology may be due to accumulation of MTB lipids in vesicles, and foam cell formation may be induced by oxygenated forms of mycolic acid. Only one NWM, an Uta Hick's bearded saki, had typical pulmonary granulomas.

In this case, conference participants discussed the variable appearance of the lesions in the kidney: some are mature granulomas, while others are poorly organized and likely reflect an earlier change. Conference participants also discussed the spread of these lesions: while arrival at the kidney was likely hematogenous given the multisystemic distribution, spread within the kidney may have occurred through blood vessels or within affected tubules. Ultimately, participants decided the route of spread could not be determined in this histologic section.

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