Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 5

22 September 2021

CASE I: S1335/17 (JPC 4118308)

Signalment:
4-year-old ewe, Merino sheep, sheep, *Ovis aries*

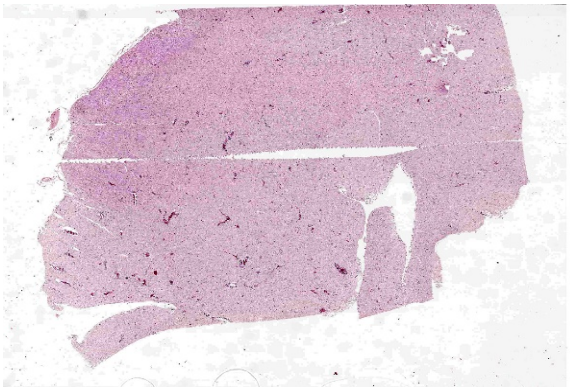
History:
In September 2017, this ewe was presented to a veterinary clinic showing circling and episodes of unconsciousness. Additionally, the shepherd noticed reduced water intake and appetite as well as gnashing of teeth for a few days. A referring veterinarian further diagnosed reduced pupillary reflexes and blindness. Therapy with steroids and antibiotics was ineffective.

At presentation, the animal was mildly emaciated and showed severe ataxia, tachycardia and tachypnea, reduced skin sensibility at the head, blindness and reduced reflexes. Body temperature was 39.5° C. A blood sample was analyzed and showed a mild increase of cells (predominantly monocytes/macrophages). Symptomatic therapy with dexamethasone, penicillin, vitamin B1, and tetanus serum was begun; the animal died suddenly two days later.

Gross Pathology:
Full necropsy was performed. The ewe was pregnant and in good body condition (body mass 86 kg). The carcass was in advanced decomposition. A mild ascites (100 ml), hydrothorax (80ml) and hydropericardium were noticed. Meninges were diffusely reddened with acute congestion of the vessels. There was an abscess, 1.5 cm in diameter, within the left lung lobe and a marked diffuse alveolar edema. Other organs and tissue were unaltered beside marked decomposition.

Laboratory results:
White blood cells: 13.2 G/l (ref. 4.2-6.2 G/l) = mild leukocytosis
Urea: 10.2 mmol/l (ref. 5 mmol/l) = mild azotemia
Transketolase activity: 8% (ref. <50%)
Blue tongue virus (PCR): negative
Pestivirus (PCR): negative

Immunohistochemically bornavirus antigen (BO18) was detected within the cytoplasm of neurons and astrocytes with well discernable immunopositive Joest-Degen inclusion bodies

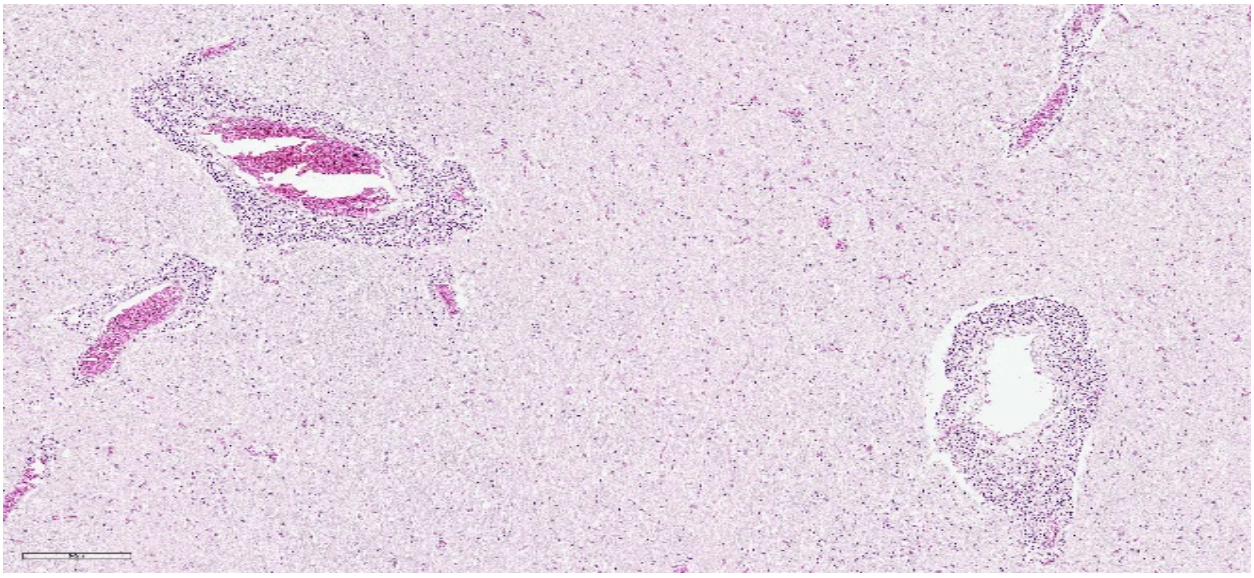


1-1. Cerebrum, sheep. A section of cerebrum is submitted for examination. (HE, 7X)

Microscopic Description:
Brain (cerebrum and brain stem):
Affecting 60% of Virchow-Robin spaces are expanded by perivascular cuffs composed of 1 to 8 layers of lymphocytes, plasma cells and fewer macrophages. Meninges are similarly affected. Multifocally, neurons are shrunken, hypereosinophilic (neuronal necrosis), and surrounded by increased numbers of glial cells (satellitosis). The number of glial cells is increased throughout tissue section sometimes forming nodular aggregates (gliosis). Rarely, neurons contain poorly discernably 4-6 µm, round, eosinophilc Cowdry-type-B inclusion bodies (Joest-Degen bodies)

Contributor's Morphologic Diagnoses:
Brain, meningoencephalitis, lymphoplasmacytic, diffuse, marked with peri-vascular lymphohistiocytic cuffs, multifocal neuronal degeneration and necrosis, gliosis, and neuronal eosinophilic intranuclear viral inclusion bodies, etiology consistent with Borna disease, ovine.

Contributor's Comment:
This ovine case is a classic example of Borna disease, first described in horses in 1813 and named after the town Borna in Saxony/ Germany, due to a local severe outbreak in military horses in 1894.[3] The etiologic agent is borna disease virus-1 (BoDV-1), a single-stranded negative-orientated RNA-virus, order Mononegavirales. After the discovery of several new members of the virus family Bornaviridae in recent years, the International Committee on Taxonomy of Viruses has proposed a new taxonomy in 2018.[1] Now the family Bornaviridae consists of two genera: the genus *Carbovirus* with members infecting snakes (carpet pythons in Australia) and the genus *Orthobornavirus*, including mammalian-1 orthobornavirus with BoDV-1 as the classic prototype, and several other virus species. Mammalian-2 orthobornavirus includes the variegated squirrel Bornavirus-1 (VSBV-1) with a reservoir in certain squirrels (*Sciurus variegatoides*, *Callosciurus sp.*)[3] Infections with VSBV-1 have been linked to fatal cases of human encephalitis in squirrel breeders.[13] Psittaciform-1 orthobornavirus is the etiologic agent of psittacine proventricular dilatation disease.[8]

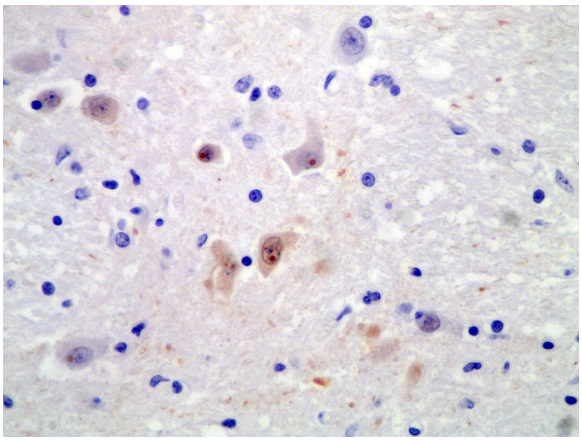


1-2. Cerebrum, sheep. Vessels within all layers of the cortex are surrounded by multiple layers of lymphocytes and fewer histiocytes which expand Virchow-Robins' space. (HE, 144X)

Additionally, several other viral species have been found in different wild birds.[1]

Classical Borna disease mainly occurs in horses and sheep, but can occasionally affect other warm-blooded animals.[14] The first outbreaks at the end of the 19th century were epidemically, but in recent years, only sporadic cases in endemic regions occur. Clinical signs of Borna disease include therapy-resistant fever, hyperesthesia, pharyngeal paralysis, circling, muscular tremors, spasm and blindness, drowsiness and flaccid paresis. Incubation time is about 2-6 months, after onset of clinical signs animal usually die within 4 weeks. Mortality reaches 90%-100%. [3, 14]

BoDV-1 is neurotropic, likely uses a nasal infection route and enters the brain via transaxonal migration along the olfactory bulb. Within the CNS, the virus shows widespread distribution of viral antigens in neurons, astrocytes, ependymal cells and oligodendrocytes. In later stages, highest viral loads can be detected in the grey matter of the limbic system, the hippocampus, in basal ganglia and brainstem. BoDV-1 replicates in the nucleus of the host cells and does not cause any direct cytopathic effect. Brain lesions are induced by immune-mediated mechanisms. Affected animals do not develop obvious gross lesions, but develop a fulminant non-suppurative polioencephalitis with occasional involvement of meninges. Lesions include thick perivascular cuffs composed of lymphocytes, plasma cells and macrophages, neuronal necrosis, satellitosis and gliosis. Rarely, Cowdry type B intranuclear eosinophilic inclusion bodies (Joest-Degen inclusion bodies) can be found.[3] In this case, they were easily visible immunohistochemically but not by using Giemsa stain.



1-3. Cerebrum, sheep: Immunohistochemical staining for bornavirus demonstrated inclusions within neuronal cytoplasm. (anti-bornavirus, 400X)

The epidemiology of Borna disease, with occurrence in endemic areas in Central Europe (Germany, Switzerland, Austria, Liechtenstein), higher incidence in certain years and seasons (spring and summer) and higher incidence on farms with low hygiene and rodent control, points towards a rodent reservoir.[5] Until now, the only confirmed reservoir of BoDV-1 is the bicolored white-toothed shrew (*Crocidura leucodon*).[2, 6, 7] In shrews, BoDV-1 can be found in the nervous system and additionally in many peripheral organs including excretory organs (skin glands, kidney). In this reservoir species, there is no inflammation associated with the virus and infected shrews do not show clinical signs. [11]

The zoonotic potential of BoDV-1 has been controversial for the last thirty years. An association with neuropsychiatric diseases has been repeatedly suggested.[4] However, in 2018, few cases of encephalitis caused by BoDV-1 infection in human patients receiving donated organs support the hypothesis of a zoonotic potential of BoDV-1.[15]

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JPC Diagnosis:
Cerebrum: Meningoencephalitis, lympho-histiocytic, diffuse, moderate, with rare neuronal necrosis and intranuclear viral inclusions.

JPC Comment:
The contributor provides a concise review of recent Bornaviridae taxonomy updates as well as details of its pathogenesis in its reservoir host, the bicolored white-toothed shrew (*Crocidura leucodon*), and higher mammals dead-end hosts which demonstrate meningoencephalitis. [10]

As noted by the contributor, classical Borna disease is typically associated with horses and sheep, although other warm blooded animals may also be infected. Interestingly, New World camelids have recently been identified as potential sentinels for BoDV-1, with reports of symptomatic cases in these species while other species are lacking in similar geographic regions over the same time periods.[10]

An example of camelid infection was demonstrated on a Swiss farm that maintained a herd of up to 8 breeding llamas in addition to cattle, horses, goats, cats, dogs, rabbits, and chickens. Over a two-decade period, 12 of 30 breeding llamas succumbed to Borna disease, but no other species kept on the farm were affected. Abnormal prehension of food was the first reported clinical sign, followed by separation from the herd, shade-seeking, head rubbing against the ground, and biting inanimate structures. Animals in late stages of the disease ran into fences or walls with signs of visual impairment. In addition, the animals tended to constantly move but did not exhibit aimless circling as seen in horses.[10]

A similar phenomenon occurred in southern Germany, near the Swiss border. Between 2002 and 2008, there were five reported cases of Borna disease, three of which occurred in alpaca herds (the other two cases were a sheep and horse). Surprisingly, this region had been dormant for Borna disease for several decades prior to these cases. In 2011, BoDV-1 was identified via PCR in a bicolored white-toothed shrew in the same region. Complete genome sequencing found the BoDV-1 in the shrew was between 96.6-98.2% identical to those obtained from infected animals.[10]

As previously mentioned by the contributor, BoDV-1 cases are typically seasonal, with most occurring in the in spring and summer months. This is most likely attributed to the biologic behavior of the infected bicolored white-toothed shrews (*Crocidura leucodon*) which are more active during these times. Notably, historical temperature data for the region of the previously discussed llama farm in Switzerland demonstrated increasing temperatures over the last decade with 2011, 2012, 2014, 2015, 2017, 2018, and 2019 being particularly warm. Borna disease outbreaks occurred in six of these seven "hot" years but not in the cooler years of 2013 and 2016. Therefore climate change may present a significant contributing factor in regard to incidence of this disease.[10]

Joest-Degen bodies, also known as Cowdry type B bodies, are named after their discoverer, who found the intranuclear inclusions to be pathognomonic for Borna disease in the horse in 1909.[9,12]

There was significant slide variability associated with this submission. Conference participants noted sections containing hippocampus contained abundant Joest-Degen bodies, which were rarely observed in other sections.

References:

1. Amarasinghe GK, Arechiga Ceballos NG, Banyard AC, et al. Taxonomy of the order Mononegavirales: update 2018. Arch Virol. 2018; doi: 10.1007/s00705-018-3814-x
2. Bourg M, Herzog S, Encarnacao JA, Nobach D, Lange-Herbst H, Eickmann M, Herden C. Bicolored white-toothed shrews as reservoir for borna disease virus, Bavaria, Germany. Emerg Infect Dis. 2013;19:2064-2066.
3. Cantile C, Youssef S. Nervous system. In: Maxie MG, ed. Jubb, Kennedy and Palmers Pathology of Domestic Animals. 6th ed. Vol 1. Elsevier, St. Louis, Missouri; 2015:250-406.
4. Dürrwald R, Kolodziejek J, Herzog S, Nowotny N. Meta-analysis of putative human bornavirus sequences fails to provide evidence implicating Borna disease virus in mental illness. Rev Med Virol. 2007;17:181-203.
5. Dürrwald R, Kolodziejek J, Muluneh A, Herzog S, Nowotny N: Epidemiological pattern of classical Borna disease and regional genetic clustering of Borna disease viruses point towards the existence of to-date unknown endemic reservoir host populations. Microb Infect. 2006;8:917-929.
6. Dürrwald R, Kolodziejek J, Weissenbock H, Nowotny N. The bicolored white-toothed shrew Crocidura leucodon (HERMANN 1780) is an indigenous host of mammalian Borna disease virus. PLoS One 2014;9:e93659.
7. Hilbe M, Herrsche R, Kolodziejek J, Nowotny N, Zlinszky K, Ehrensperger F. Shrews as reservoir hosts of borna disease virus. Emerg Infect Dis. 2006;12:675-677.
8. Hoppes SM, Tizard I, Shivaprasad HL. Avian bornavirus and proventricular dilatation disease: diagnostics, pathology, prevalence, and control. Vet Clin North Am Exot Anim Pract. 2013;16:339-355.
9. Joest E, Degen K. Über eigentümliche Kerneinschlüsse der Ganglienzellen bei der enzootischen Gehirn-Rückenmarksentzündung der Pferde. *Z Inf Krkh Haustiere*. 1909;6:348–356.
10. Malbon AJ, Dürrwald R, Kolodziejek J, et al. New World camelids are sentinels for the presence of Borna disease virus [published online ahead of print, 2021 Jan 27]. *Transbound Emerg Dis*. 2021;10.1111/tbed.14003.
11. Nobach D, Bourg M, Herzog S, Lange-Herbst H, Encarnacao JA, Eickmann M, Herden C. Shedding of Infectious Borna Disease Virus-1 in Living Bicolored White-Toothed Shrews. PLoS One. 2015;10:e0137018.
12. Sasaki S, Ludwig H. In borna disease virus infected rabbit neurons 100 nm particle structures accumulate at areas of Joest-Degen inclusion bodies. *Zentralbl Veterinarmed B*. 1993;40(4):291-297.
13. Schlottau K, Hoffmann B, Homeier-Bachmann T, Fast C, Ulrich RG, Beer M, Hoffmann D. Multiple detection of zoonotic variegated squirrel bornavirus 1 RNA in different squirrel species suggests a possible

unknown origin for the virus. Arch Virol. 2017;162:2747-2754.
14. Vandevelde M, Higgins RJ, Oevermann A. Veterinary Neuropathology. Wiley-Blackwell, Oxford, UK; 2012:57-58.
15. Epidemiol. Bulletin 10/2018, Robert Koch Institute Berlin, Germany, https://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2018/Ausgaben/10_18.pdf?__blob=publicationFile).

CASE II: P18-3879 (JPC 4118085)

Signalment:
8 month-old, female, St. Bernard, *Canis lupus familiaris*, dog

History:
The patient was presented due to myoclonus of temporal muscles, melena and purulent discharge from the nostrils. It began with depression and anorexia. It also had a non-regenerative anemia and thrombocytopenia that progressed from mild to severe.

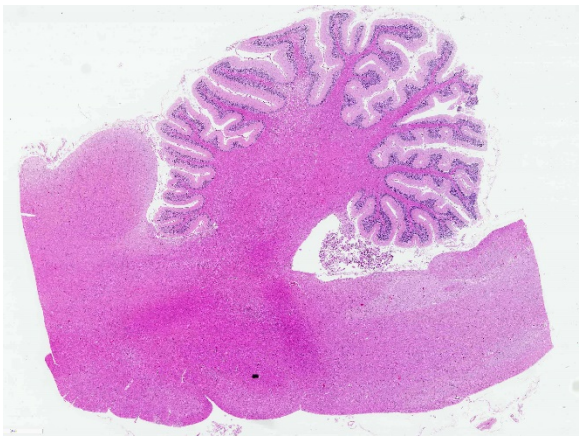
Gross Pathology:
On necropsy, the animal was in good body condition. Lungs were diffusely congested and moderately edematous. There were no gross lesions noted on examination of the brain or spinal cord.

Laboratory results:
Immunohistochemistry (IHC) revealed moderate to strong positive immunoreactivity for canine distemper virus (CDV) in astrocyte nuclei and multifocally in the neuropil next to demyelinated areas.

Microscopic Description:
The cerebellar white matter is severely affected by loss of tissue substance and delineated by numerous reactive astrocytes admixed with large numbers of macrophages that contain abundant, intracytoplasmic, degenerate myelin (Gitter cells) and few lymphocytes. There are multiple foci of gliosis in the adjacent neuropil. There are large numbers of astrocytes with large, swollen nuclei that contain a single, round, amphophilic, glassy inclusion. Blood vessels throughout the lesions are reactive and branched and typically are lined by reactive, hypertrophied endothelium.

Contributor's Morphologic Diagnoses:
Cerebellum, white matter: Demyelination and necrosis, severe, multifocal, with numerous Gitter cells and intranuclear inclusion bodies in astrocytes.

Contributor's Comment:
The clinical history and the distinctive histologic feature of extensive demyelination with intranuclear inclusion bodies in astrocytes are consistent with canine Distemper in this dog. Canine distemper is caused by a morbillivirus *(*family Paramyxoviridae), and affects a wide range of terrestrial carnivores, including domestic dogs, wild canids (e.g. wolves, foxes), mustelids (e.g. ferrets, minks), procyonids (e.g. raccoons), ursids (e.g. black bears, giant pandas), ailurids (e.g. red pandas), viverrids (e.g. civets, genets), hyaenids (e.g. spotted

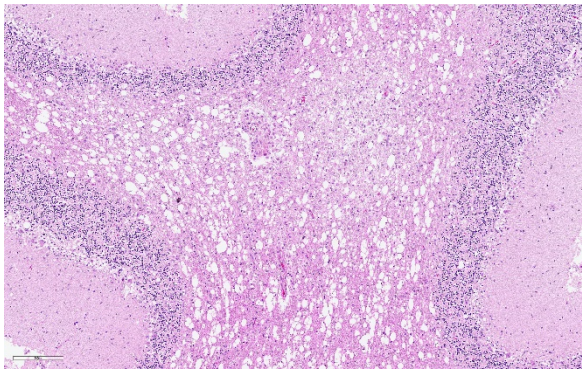


2-1. Cerebellum and brainstem, dog: A tangential section of cerebellum and brainstem is submitted for examination. An area of pallor in the folial white matter on the more cranial folia is barely perceptible at subgross magnification. (HE, 5X)

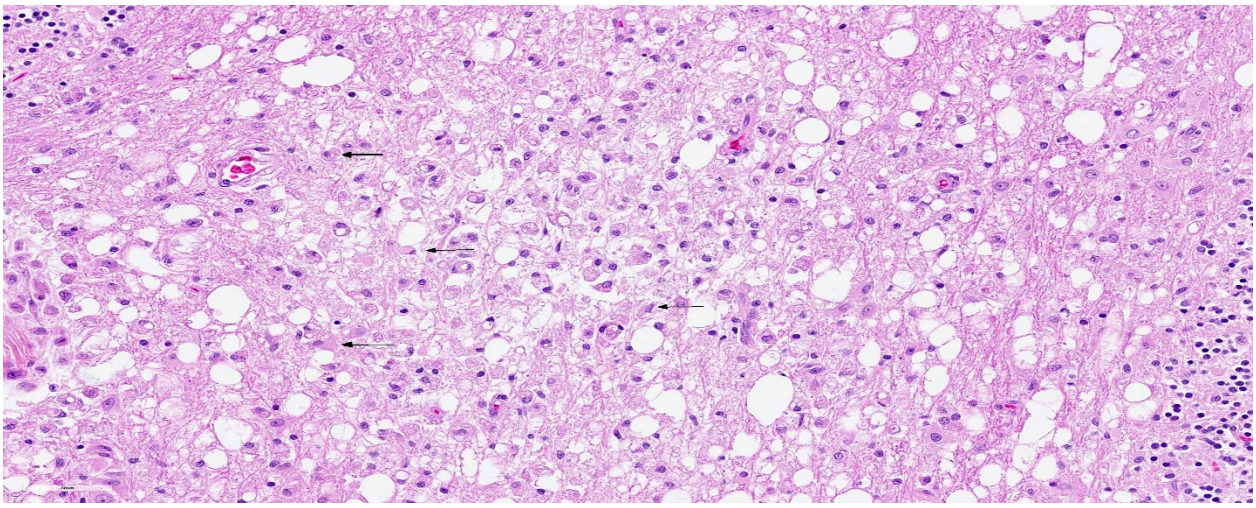
hyenas), large felids (e.g. lions, tigers), and marine mammals, where several epidemics with mass mortalities have been reported. Domestic, feral and even wild canids are considered vectors for this infectious agent in wildlife species.[2]

Canine Distemper Virus (CDV) is shed in secretions mostly from the respiratory tract and infection is usually acquired by inhaling aerosols or by close contact with infected dogs. The virus infects monocytes and macrophages of the upper respiratory tract, which convey it to tonsils and local lymph nodes during the first 24 hours. The virus replicates further in local lymphoid tissues, and by 2-5 days after exposure is present in lymphoid tissues throughout the body, including thymus, spleen, bone marrow, and intestinal lymphoid tissue. During the first viremic phase, generalized infection of lymphoid tissues with lymphoid depletion, lymphopenia and transient fever is observed. Severe immunosuppression is a consequence of leukocyte necrosis, apoptosis and dysfunction. Gross lesions include lymph node swelling, depletion of MALT and reduced thymus size. Lesions are microscopically characterized by a generalized depletion of T and B cell compartments in spleen, lymph nodes, MALT and tonsils as well as hyperplasia of reticular cells in the medullary region of lymph nodes. A second viremia provokes high fever and infection of parenchymal tissues such as the respiratory tract, digestive tract, skin, and CNS. Development of the disease is highly dependent on the immune status of the host, the titer of antibodies to envelope glycoproteins, the age of the host, and the strain of virus. The infection is systemic, and clinical signs usually involve the respiratory, gastrointestinal, and nervous systems.[1,2,3]

The main respiratory lesion is broncho-interstitial pneumonia, with formation of inclusion bodies in the cytoplasm of bronchial and bronchiolar epithelial cells, type II pneumocytes and alveolar macrophages. Alveolar epithelial syncytial cells are a characteristic feature when present. Enteral infection leads to catarrhal enteritis with depletion of Peyer's patches. Often enteric and respiratory lesions are worsened by secondary bacterial infections. Characteristic dermal mani-festations include hyperkeratosis of foodpads and nasal planum (hard pad disease), and pustular dermatitis (distemper exanthema). In young animals, enamel hypoplasia and metaphyseal osteosclerosis (persistence of the primary spongiosa in the metaphyses of long bones), can be seen following CDV infection. Neurologic signs depend on viral distribution in the CNS and include cerebellar and vestibular signs, hyper-esthesia, seizures, cervical rigidity, as well as paraparesis or tetraparesis with sensory ataxia. Histological manifestations include polioencephalitis and demyelinating leukoencephalomyelitis. De-myelination in white matter tracts is most severe in the cerebellum, rostral medullary velum, optic tracts, spinal cord, and surrounding the fourth ventricle, and probably arises from distribution of virus through the cerebrospinal fluid. Astrocytes



***2-2.** Cerebellum dog: The area of pallor corresponds to a large area of spongiosis, with a central more several affected area of demyelination. (HE, 144X)*



2-3. Cerebellum, dog: Higher magnification of the area of rarefaction demonstrates axonal loss with swelling and coalescence of myelin sheaths, numerous infiltrating foamy Gitter cells, and low number of gemistocytic astrocytes (arrows). (HE, 400X)

usually contain nuclear and, rarely, cytoplasmic inclusions.[2,3,4]

CDV may enter the brain via infected mononuclear cells trafficking through the blood-brain-barrier, which results in local virus release and subsequent infection of resident epithelial and endothelial cells. Choroid plexus cells and brain vessels cells are firstly infected. Once inside the brain, the virus spreads via the cerebrospinal fluid (CSF), where it may infect ependymal lining cells of the ventricles and ultimately glial cells and neurons. Astrocytes are the main cell population infected by CDV, whereas neuronal infection is more prominent during the early phase and most prominent in distemper polioencephalitis. Oligodendroglial infection by CDV has been inconsistently documented.[4]

There are two recognized stages in the development of CDV-induced demyelination. In acute demyelinating lesions, there is massive down-regulation of myelin transcription and metabolic impairment of the myelin-producing cells, but there is no evidence that oligodendrocytes are undergoing apoptosis or necrosis. It has been suggested that CDV induced microglial cell activation could contribute to oligodendrocyte/myelin damage. Although perivascular inflammation is entirely lacking, some CD8+ cells are found in acute demyelinating lesions. The invasion of these cells might be triggered by microglial cell activation. Metalloproteinases and their inhibitors also appear to be strongly upregulated in the acute stage. Six to seven weeks post-infection, perivascular inflammation with lymphocytes, plasma cells and monocytes is detected. This inflammation, together with the demyelinating lesions leads to progression of tissue damage. Pro-inflammatory cytokines are markedly upregulated, whereas anti-inflammatory cytokines remain at normal levels. Chronic inflammatory demyelination is due to a bystander mechanism resulting from interactions between macrophages and antiviral antibodies.[6]

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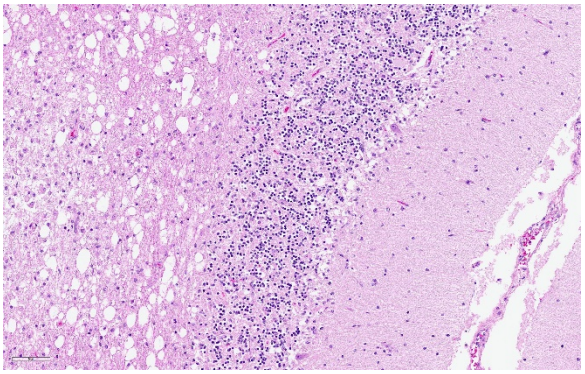


2-4. Cerebellum, dog. Adjacent to the area of demyelination and rarefaction, Purkinje cells are segmentally lost and there is a significant decrease in nuclei in the granular cell layer.

JPC Diagnosis:
Cerebellum and brainstem: Demyelination, multifocal to coalescing, moderate, with gliosis and astrocytic intranuclear inclusions.

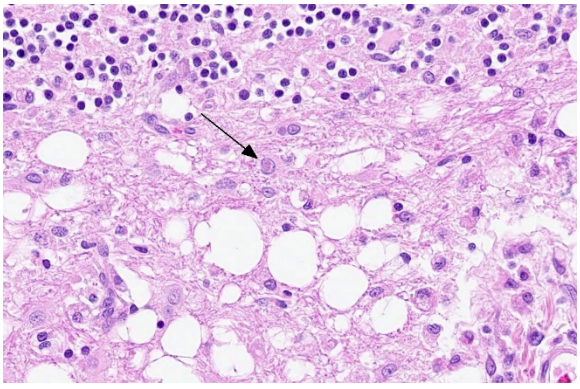
JPC Comment:
The contributor provides an excellent thorough explanation of the pathogenesis and variety of lesions associated with canine distemper virus (CDV), a morbillivirus that infects numerous domestic and wildlife species.

A novel strain of CDV named "American-4" was recently identified following an increased number of cases of CDV that were seen at the University of Tennessee, including vaccinated dogs. A subsequent study found 77% of CDV positive raccoons and gray foxes in Tennessee to be infected with this strain.[8] Another study found serum antibody titer responses to this strain did not increase following vaccination in dogs despite increased responses to the vaccine strain. Cross-protection was titer-dependent with higher titers required for adequate protection, suggesting decreased cross-protection against this emergent strain, likely as a result of antigenic strain differences.[7]

Domestic species are important reservoirs of CDV throughout the world and can be a source of wildlife exposure to CDV. Wildlife species in turn are also frequently implicated as the source of CDV outbreaks in both domestic and non-domestic species, and often serve as intermediate hosts of CDV.[8]

One such outbreak occurred in 2016 at a private east Tennessee zoo, resulting in the first pathologic description of the virus in sloths. Five of eight wild caught 2-toed sloths (*Choloepus didactylus*) died while completing a 15-month quarantine following a 2-3 week history of hyporexia, lethargy, and oronasal discharge. Common gross findings included multifocal crusts and ulcers within the oral cavity, lips, and nares, which corresponded to discrete eosinophilic intranuclear and intracytoplasmic inclusion bodies in epithelial cells observed during microscopic examination. In addition, neutronphilic, necrotizing, bronchointerstitial pneumonia was present in all cases with syncytial cells seen within the bronchiolar epithelium.[8]

A striking feature noted in this brief report was that each animal was also affected by hepatic necrosis with numerous viral inclusions observed within hepatocytes, which is not typically seen in domestic canines and nondomestic carnivores.[8]



2-5. Cerebellum, dog. Occasional astrocytes contain a single 2-3 µm eosinophilic intranuclear viral inclusion. (HE, 400X).

No sloths had gross or histologic lesions in the central nervous system (CNS); however, CDV antigen was found to be present within meningeal vessels, choroid plexus, and ependyma. It is possible no CNS lesions were observed given that all affected animals had severe systemic disease and insufficient time may have elapsed prior to death for their development.[8]

Virus isolation and sequencing of tissue from one affected sloth determined it was infected with the American-4 strain of CDV. Although the sloths were housed indoors, it is possible transmission occurred as the result of exposure to organic material or other fomites exposed to wildlife.[8]

Interestingly, three of four adult kinkajous (*Potos flavus)* housed in the same building died after developing similar clinical signs. CDV was confirmed via polymerase chain reaction (PCR) in one animal. Similar to the sloths, none had CNS lesions and one had hepatic necrosis and intranuclear inclusions in hepatocytes. Virus isolation sequencing was not performed although the most likely etiology in this case was also the American-4 strain of CDV. [8]

Conference participants observed and discussed the loss of Purkinje cells and up to 50% of granular cells in areas adjacent to folial demyelination. This finding is not classically described in regard to CDV. It is possible these cells were lost due to the virus' tropism for astrocytes and subsequent changes in the neuropil microenvironment. Astrocytes perform many roles within the CNS, including regulation of ionic exchanges between cells and the formation of functional connections by production of molecules that are tropic for other specialized cells of the CNS.[1]

References:

1. Cantile C, Youssef S. Nervous system. In: Maxie MG, ed. Jubb, *Kennedy and Palmers Pathology of Domestic Animals*. 6th ed. Vol 1. Elsevier, St. Louis, Missouri; 2016:260-262.
2. Caswell JL, Williams KJ. Respiratory system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol 2. 6th ed. St. Louis, MO: Elsevier limited; 2016:574-576.
3. Beineke A, Baumgärtner W, Wohlsein P. Cross-species transmission of canine distemper virus—an update. One Health. 2015; 1:49–59
4. Beineke A, Puff C, Seehusen F, Baumgärtner W. Review paper: Pathogenesis and immunopathology of systemic and nervous canine Distemper. Veterinary Immunology and Immunopathology. 2009; 127: 1–18
5. Lempp C, Spitzbarth I, Puff C, Cana A, Kegler K, Techangamsuwan S, Baumgärtner W, Seehusen F. Review: New Aspects of the Pathogenesis of Canine Distemper Leukoencephalitis. Viruses. 2014; 6:2571-2601.
6. Stilwell JM, Anis E, Wilkes RP, Rissi DR. Dual infection with an emergent strain of canine distemper virus and canine parvovirus in an Arctic wolf under managed care. *J Vet Diagn Invest*. 2019;31(4):594-597.
7. Vandevelde M, Zurbriggen A. Demyelination in canine distemper virus infection: a review. Acta Neuropathol. 2005; 109: 56–68.
8. Watson AM, Cushing AC, Sheldon JD, et al. Natural Canine Distemper Virus Infection in Linnaeus's 2-Toed Sloths (*Choloepus didactylus*). *Vet Pathol*. 2020;57(2):311-315.

CASE III: 31712 (JPC 4153553)

Signalment:
Male, howler monkey, *Alouatta* spp. (age unknown)

History:
Multiple free ranging neotropical primates were found dead in the state of Rio de Janeiro, Brazil during the last outbreak of yellow fever (YF). All primates found dead were submitted to necropsy and YF diagnosis as a part of the YF epizootic surveillance program of the Brazilian Ministry of Health. From 1,304 primates, 56 were considered positive for YF by real time RT-PCR from the official diagnostic laboratory. Several non-human primate species tested positive for YF, including *Alouatta* sp., *Callithrix* sp., *Sapajus* sp., *Callicebus* sp. and *Leontho-pitecus* sp.

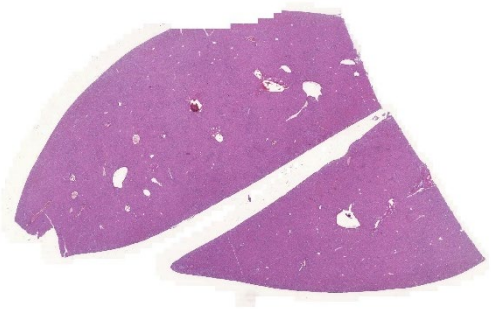
The slide presented here is from a howler monkey (*Alouatta* sp.) found dead in a rural area in the State of Rio de Janeiro, Brazil.

Gross Pathology:
No data available.

Laboratory results:
Yellow Fever RNA was detected in tissue samples by Real Time RT-PCR performed by the official diagnostic laboratory.

Microscopic Description:
Liver: diffuse severe hepatocyte coagulative necrosis with a few remaining hepatocytes in the portal and central zones with mild intracytoplasmatic vacuoles (compatible with lipidosis). Multifocal mild lympho-histioplasmacytic portal infiltrate with a few neutrophils.

Lungs: mild multifocal areas of alveolar septum thickening with a lymphohistiocytic infiltrate. Mild multifocal edema and hemorrhage within the alveolar lumen. Mild multifocal antracosis.



3-1. Liver, howler monkey. Two sections of liver are submitted for examination. At subgross magnification, a retiform pattern of pallor sparing portal areas is present in both sections. (HE, 5X)

Kidney: mild multifocal lympho-histioplasmacytic interstitial infiltrate. Few glomeruli with mesangial thickening and mesangial cells proliferation.

Heart: mild focal lymphohistiocytic infiltrate in the myocardium.

Spleen and brain with no significant histological lesion.

Contributor's Morphologic Diagnoses:
Liver: diffuse severe acute necrotizing hepatitis with mild lipidosis.

Lungs: mild multifocal lymphohistiocytic interstitial pneumonia with mild multifocal edema and hemorrhage.

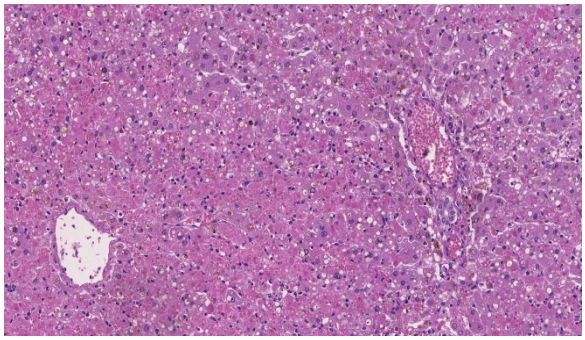
Kidney: mild multifocal lympho-histioplasmacytic intersticial nefritis with mild multifocal membranoproliferative glomerulopathy.

Heart: mild focal lymphohistiocytic myocarditis.

Contributor's Comment:

Yellow fever is an important zoonotic disease related to death of both human and non-human primates. YF is a mosquito-borne hemorrhagic disease transmitted to a susceptible host by *Haemagogus* sp. and *Sabethes* sp., or *Aedes aegypti*, in the sylvatic or urban environments, respectively.[9] The disease is considered endemic in the Brazilian Amazon region; however, outbreaks occur in non-endemic areas, as the most recent one which happened mainly in the southeastern region from 2016 to 2018, with more than one thousand human cases and 35.1% case fatality rate.[4,6]

Neotropical primates are susceptible to YF infection and this disease became an important concern for conservation of free-ranging populations.[1,5,19] Monitoring the occurrence of YF in non-human primates is part of the Epizootic Surveillance Program of the Brazilian Ministry of Health acting as an early warning to viral circulation because death of non-human primates usually precedes YV human cases.[3,11,16,19] The official diagnosis is made by reference laboratories and it is based on real time RT-PCR and/or immunohistochemistry from samples of liver, spleen, kidney, heart, lungs, and brain.[16]



3-2. Liver, howler monkey. At higher magnification, there is coagulative necrosis of centrilobular and midzonal hepatocytes. Necrotic hepatocytes are eosinophilic and swollen with loss of nuclei, and their cytoplasm retains lipid droplets and well-defined cytoplasmic cytosegrosomes (Councilman bodies). (HE, 186X)

Information about microscopic changes associated with YF in non-human primates are limited, including both natural and experimental infection.[7,8,11] Even with scarce information, there is evidence of differences in susceptibility for different neotropical primates. Briefly, *Alouatta* sp. and *Callithrix* sp. are considered susceptible to YF while *Sapajus* sp. are resistant.[1,9,11,19] Recently, these differences of susceptibility were associated with histopathological patterns of changes, demonstrating that *Alouatta* sp. develop a severe necrotizing hepatitis while *Callithrix* sp., a reported susceptible specie, showed minimal hepatic injuries.[15] Another recent study demonstrated that *Callithrix* sp. had less viral load than other primates, such as *Alouatta* sp. and *Sapajus* sp. [4]

Yellow fever virus affects mainly the liver of infected hosts, causing necrosis/apoptosis of hepatocytes as the most relevant lesion typically affecting primarily midzonal hepatocytes.[14] This pattern of distribution is associated with viral tropism and was described in human and non-human primates, including free-ranging naturally infected neotropical primates and experimentally infected Rhesus monkeys.[1,14,15,17]

Another hepatic injury observed in these cases was lipidosis, which has been reported in both human and neotropical primates.[6,14,15,20] Inflammatory infiltrate is usually mild during YF infection, mostly because of the transforming growth factor beta (TGF-β), an inductor of apoptosis that also acts as an anti-inflammatory cytokine.[11,14,19] Acute tubular necrosis causing renal failure is a major complication associated with human fatal cases of yellow fever, however this is not an important lesion associated with primate infection.[10,11,15]

The case reported here was part of a recent study[15] that demonstrated histopathological patters in different species of neotropical primates naturally infected with the yellow fever virus. The pattern observed in the liver of *Alouatta* sp. is associated with severe hepatic failure caused by loss of most of the hepatocytes. Histologically, primates of this specie showed severe hepatocyte necrosis, mainly of the midzonal region expanding to the portal and central regions of the hepatic lobule.

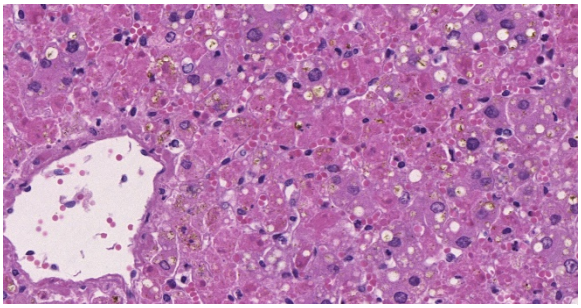
Contributing Institution:
Escola de Veterinária, Universidade Federal de Minas Gerais – www.vet.ufmg.br

JPC Diagnosis:

Liver: Hepatocellular degeneration and necrosis, centrilobular and midzonal, diffuse, severe, with Councilman bodies and intracytoplasmic lipid.

JPC Comment:
The contributor provides an excellent overview of yellow fever, the most severe of five arboviruses that have emerged or continually reemerge over recent decades in the Americas. The other four arboviruses include dengue, West Nile, Chikungunya, and Zika.[13]

Yellow fever is flavivirus that likely originated in Africa and was imported to the Americas during the 1600s along trans-Atlantic trade routes.[13,14,7] The virus was responsible for hundreds of thousands of deaths throughout the 18th and 19th centuries, including 10% of the population of Philadelphia in 1793, the United States capital at the time. Nearly a century later, Cuban epidemiologist Carlos Finlay first proposed the disease was mosquito-borne, which was verified by US Army physician Walter Reed and the Yellow Fever



3-3 Liver, howler monkey. Higher magnification of necrotic centrilobular and midzonal hepatocytes. (HE, 452X)

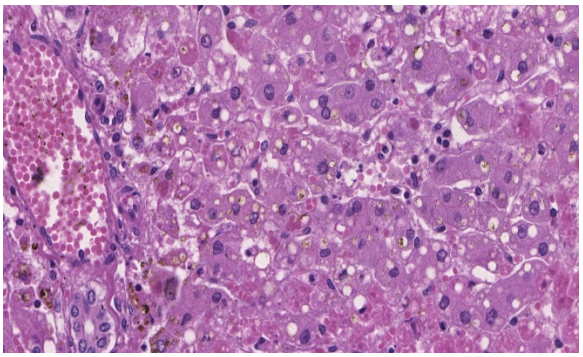
Commission in 1900. Subsequent mosquito control and sanitation efforts essentially eliminated yellow fever from the United States and non-endemic areas of the Americas.[13]

In 1937, virologist Max Theiler developed a vaccine still in use today that provides lifetime immunity in up to 99% of vaccinated individuals.[13,19] Theiler was awarded the 1951 Nobel Prize in Physiology or Medicine, the first and only Nobel Prize given for the development of a vaccine. Interestingly, Max Theiler's father was Sir Arthur Theiler, a Swiss veterinarian and researcher who also made significant contributions in the realm of scientific discovery.[19]

Yellow fever was substantially suppressed though the combination of vector control, sanitation, and vaccination campaigns; however, the virus continues to persist due to a sylvatic transmission cycle maintained between forest mosquitos (*Haemagogus sp.* and *Sabethes sp.*) and susceptible non-human primates. Periodically, the virus becomes re-introduced into the human population (i.e. urban cycle) and is readily transmitted by the vector *Aedes aegypti,* resulting in outbreaks. These outbreaks occur in parts of Africa and Central and South America, resulting in an estimated 84,000 to 170,000 severe cases and 29,000 to 60,000 related human deaths per year.[13]

In South America, outbreaks occur every 5 to 10 years. Human cases are preceded by a rise in nonhuman primate cases, which is due to an increased susceptibility of the nonhuman primate population.[14] Both old and new world primates are susceptible to yellow fever; however, new world primates are particularly susceptible, especially howler monkeys (*Alouatta sp.*).[14] In this context, highly susceptible nonhuman primate species likely play an important role in amplifying the virus and therefore infecting large numbers of invertebrate vectors. Therefore, monitoring the occurrence of yellow fever in nonhuman primates serves as an early warning to viral circulation in a given area, which facilitates identification of exposed human populations and use of targeted vaccination programs, as a preventive measure.[14]

In contrast, animals that develop a mild yellow fever virus infection and long-lasting immunity, including resistant new world primates, probably suppresses circulation of yellow fever virus between outbreaks.[14] As noted by the contributor, yellow fever is classified as a viral hemorrhagic fever. Although much attention has been paid to the hepatic pathology of viral hemorrhagic fevers, and abnormal liver function tests are common, clinically significant liver disease and death from liver failure are rare except in yellow fever. Disseminated intravascular coagulation and depletion of vitamin-K dependent clotting factors due to decreased production as the result of hepatic necrosis contribute to the recognized bleeding diathesis.[22]



3-4. Liver, howler monkey. Periportal hepatocytes are largely still vital, although rounded up, hypereosinophilic apoptotic hepatocytes are scattered throughout. Lipid vacuoles and Councilman bodies are present. Abundant brownish acid hematin is present. (HE, 450X)

Apoptotic hepatocytes were first clearly described in yellow fever by William Thomas Councilman and therefore have often been referred to as "Councilman bodies". Although apoptosis occurs in many forms of liver disease, by convention this eponym is restricted to use in regard to yellow fever.[9]

References:

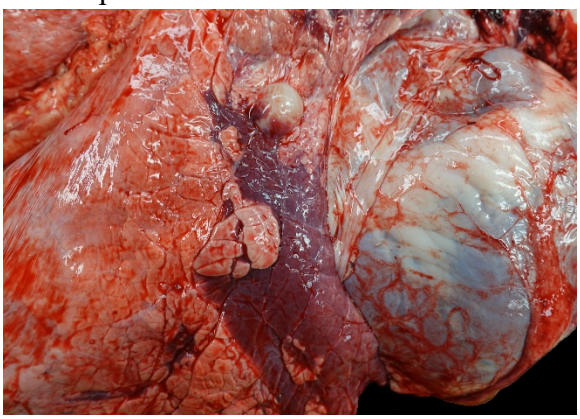
1. Almeida MAB. Yellow fever. In: Fuentes A, The international encyclopedia of primatology. 1th ed. Hoboken, NJ: John Wiley & Sons, Inc; 2017:1-2.
2. Almeida MAB, Santos E, Cardoso JC, et al. Yellow fever outbreak affecting *Alouatta* populations in southern Brazil (Rio Grande do Sul State), 2008–2009. Am J Promatol. 2012;74(1):68-76.
3. Bacha HA, Johanson GH. Yellow fever. Rev Assoc Med Bras. 2017;63(4):291-292.
4. Cunha MS, Costa AC, Fernandes NCCA, et al. Epizootics due to Yellow Fever Virus in São Paulo State, Brazil: viral dissemination to new areas (2016–2017). *Scientific Reports*. 2019;9(1):1-13.
5. Dietz JM, Hankerson SJ, Alexandre BR, et al. Yellow fever in Brazil threatens successful recovery of endangered golden lion tamarins. Sci Rep. 2019;9:12926.
6. Duarte-Neto AN, Cunha MP, Marcilio I, et al. Yellow fever and orthotopic liver transplantation: new insights from the autopsy room for an old but re-emerging disease. Histopathology. 2019;75:638-648.

7. Engelmann F, Josset L, Girke T, et al. Pathophysiologic and trancriptomic analyses of viscerotropic yellow fever in a rhesus macaque model. PLOS Negl Trop Dis. 2014;8(11):e3295.
8. Fernandes NCCA, Cunha MS, Guerra JM, et al. Outbreak of yellow fever among nonhuman primates, Espírito Santo, Brazil, 2017. Emerg Infect Dis. 2017; 23(12):2038-2041.
9. Gill RM, Kakar S. Liver and gallbladder. In: Kumar V, Abbas AK, Aster JC, eds. *Robbins and Cotran Pathologic Basis of Disease*. 9th ed. Philadelphia, PA: Elsevier; 2021:825.
10. Litvoc MN, Novaes CTG, Lopes MIB. Yellow fever. Rev Assoc Med Bras. 2018;64(2):106-113.
11. Monath TP. Yellow fever: an update. Lancet Infect Dis. 2001;1(1):11-20.
12. Monath TP, Vasconcelos PFC. Yellow fever. J Clin Virol. 2015;64:160-173.
13. Norrby E. Yellow fever and Max Theiler: the only Nobel Prize for a virus vaccine. *J Exp Med*. 2007;204(12):2779-2784.
14. Paules CI, Fauci AS. Yellow Fever - Once Again on the Radar Screen in the Americas. *N Engl J Med*. 2017;376(15):1397-1399.
15. Quaresma JAS, Pagliari C, Medeiros DBA, et al. Immunity and immune response, pathology and pathologic changes: progress and challenges in immunopathology of yellow fever. Rev Med Virol. 2013;23(5):305-318.
16. Santos DO, Oliveira AR, Lucena FP et al. Histopathologic patterns and susceptibility of neotropical primates naturally infected with yellow fever virus. Vet Pathol, 2020. In press.
17. Silva NIO, Sacchetto L, Rezende IM, et al. Recent sylvatic yellow fever virus transmission in Brazil: the news from an old disease. Virol J. 2020;17(9):1-12.
18. Stokes A, Bauer JH, Hudson NP. The transmission of yellow fever to macacus rhesus: preliminary note. J Am Med Assoc, 1928;90(4):253-254.
19. Tan SY, Pettigrew K. Max Theiler (1899-1972): Creator of the yellow fever vaccine. *Singapore Med J*. 2017;58(4):223-224.
20. Vasconcelos PFC. Febre amarela. Rev Soc Bras Med Trop. 2003;36(2):275-293.
21. Vieira V, Pacheco L, Demetrio L, et al. Liver transplantation for acute liver failure due to yellow fever: a case report. Transplant Proc. 2019;51:1625-1628.
22. Wachtman L, Mansfield J. Viral diseases of nonhuman primates. In. Abee CR, Mansfield K, Tardif S, Morris T. eds. *Nonhuman primates in biomedical research*. Vol 2. 2nd ed. Oxford, UK: Elsevier; 2012:50-51.

CASE IV: 19-355 (JPC 4161131)

Signalment:
3-month-old, castrated male, Holstein-Friesian ox (*Bos taurus*)

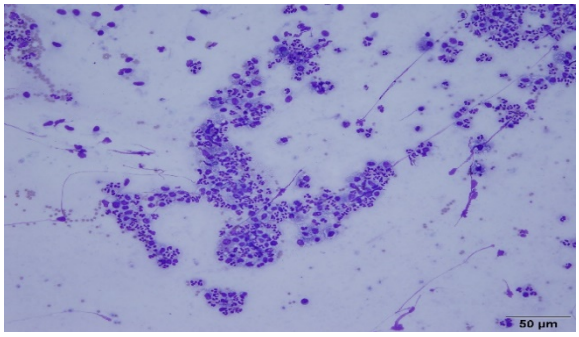
History:
The calf arrived at the barn late November as part of an artificial heart preclinical trial. On arrival, it had a dry cough and wheezes in the right cranioventral lung, but no ocular or nasal discharge. The calf had received a dose of tildipirosin antibiotic from the vendor.



4-1. Lung, calf. A sharply demarcated 2.5x3x1.5 cm region in the cranial aspect of the caudal left lung lobe is distinctly dark red and depressed (atelectasis). (Photo courtesy of: Penn State College of Medicine, https://med.psu.edu/comparative-medicine)

Later that afternoon, the staff noticed the calf appeared to have a weird breathing pattern and was using abdominal effort, so the calf was placed on report, and a veterinarian checked on it once a day. It did have an abdominal effort to its breathing, but the calf's respiratory rate was within normal limits, with a normal temperature. There was no change in the severity of the wheezes. One week after arrival, the calf received a dose of ceftiofur antibiotic to the base of its left ear and was moved from the receiving barn to the calf room. Two days later, the calf was put under isoflurane anesthesia to receive bilateral jugular catheters and remove the growth hormone implant in its right ear in preparation for the longer artificial heart implantation surgery. Induction and anesthesia went smoothly. However, it took a while for the calf to breathe on its own so that it could be extubated. When the calf was extubated, there was a brief period where it seemed to be in respiratory distress, despite pulse oximeter oxygen levels within normal limits. When the calf was standing on its own, it was taken back to the calf room, where it ate grain. About an hour later, the calf began to show signs of respiratory distress (open mouth breathing, increased abdominal effort). The calf was given supplemental oxygen through a nasal cannula, and blood gas showed severe respiratory acidosis. It was administered intravenous furosemide and steroids. The calf did not show any significant improvement with treatment and was a poor surgical candidate. The laboratory elected humane euthanasia.

Gross Pathology:
Upon opening the chest, a 2.5x3x1.5 cm region in the cranial aspect of the caudal left lung lobe is sharply and distinctly dark red and depressed (atelectasis). Up to 20% of the right cranial and middle lung lobes are also dark red and consolidated. When bisected, the consolidated tissue has a faint tan-lobulated pattern. A tracheobronchial lymph node near the region of lung consolidation on the right side is 1.7 mm-diameter and dense. The trachea diffusely contains adherent and free strands of tan fibrillary material and has a roughened surface.



4-2. Lung, calf. An impression smear of the cut section of lung demonstrates numerous intact neutrophils admixed with foamy macrophages. (Lung, calf. A sharply demarcated 2.5x3x1.5 cm region in the cranial aspect of the caudal left lung lobe is distinctly dark red and depressed (atelectasis). (HE, 7X) (Photo courtesy of: Penn State College of Medicine, https://med.psu.edu/comparative-medicine)

The cranial pole of the right kidney contains two multiloculated, yellow translucent fluid-filled, thin-walled spaces (renal cysts) concentrated on the renal pelvis/collecting ducts of the affected lobules. The first is 5x5x4.5 cm, and the second is 2x1x1 cm. The left kidney and remaining organs are grossly within normal limits. The rumen contains hay and less abundant grain. The intestines are filled with green-brown digesta.

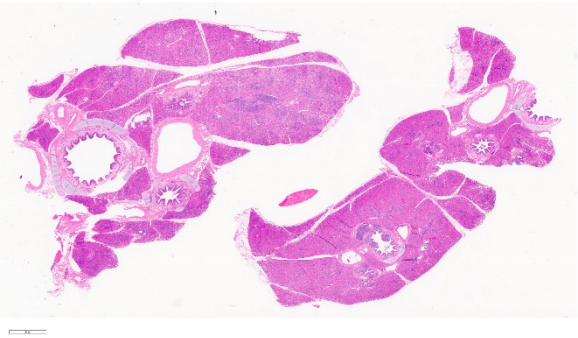
Laboratory results:
Bacterial culture:
Aerobic bacterial culture of the tracheal mucosa failed to grow bacteria.

Virus isolation:
The lung was positive for bovine coronavirus (BCoV) but negative for bovine parainfluenza 3 (PI3), bovine respiratory syncytial virus (BRSV), and infectious bovine rhinotracheitis virus (IBR)/bovine herpes virus (BHV1).

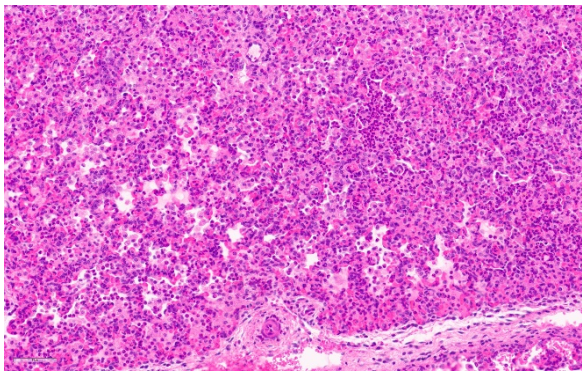
Cytology:
The highly cellular slide was stained with Diff-Quik. It contained myriads of viable and degenerate neutrophils, eosinophilic cellular debris, abundant light blue streaming material (mucous), and many alveolar macrophages on a background of numerous red blood cells (peripheral blood). The macrophages often had basophilic, foamy cytoplasm and contained cellular debris. There were rare, sloughed, ciliated bronchiolar epithelial cells.

Interpretation: Suppurative pneumonia

Microscopic Description:
Lung. 100% of the alveolar spaces are completely or partially collapsed, with the most intense regions surrounding bronchi or bronchioles (atelectasis). Airways contain abundant neutrophils and fewer macrophages along with eosinophilic cellular debris and degenerate epithelial cells that lack cytoplasmic and nuclear detail. The bronchiolar and bronchial epithelium contains dozens of scattered neutrophils and fewer macrophages that extend into the surrounding alveolar walls and alveolar spaces (bronchopneumonia). Peribronchiolar lymphoid tissue is prominent (lymphoid hyperplasia) throughout the section with additional clear space (edema), and abundant apoptotic lymphocytes and tingible body macrophages.



4-3. Lung, calf. Two sections of lung display diffuse hypercellularity with loss of alveolar architecture (atelectasis) as well as prominent BALT hyperplasia and widening of interlobular septa.



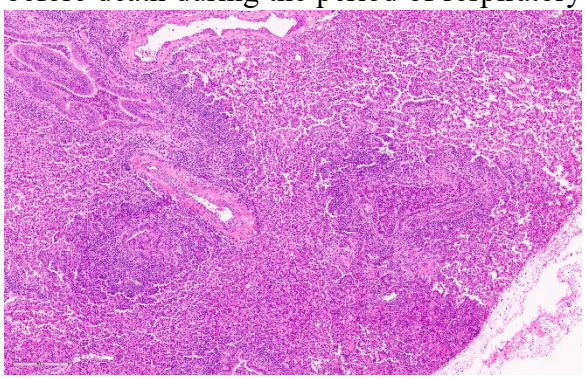
4-4 Lung, calf. There is diffuse filling of alveoli by innumerable intact neutrophils and foamy alveolar macrophages (left). At right, alveolar septa are discernable – they are congested and contain numerous circulating neutrophils which help in their identification (in many areas of the section, it is a solid sheet of inflammatory cells.) (HE, 400X)

Contributor's Morphologic Diagnoses:
Lung: Bronchointerstitial pneumonia, regionally extensive, subacute, moderate-severe, suppurative with mild lymphoid hyperplasia.

Contributor's Comment:
Bovine coronavirus (BCoV) was isolated from the affected areas of lung. BCoV is a single-stranded, positive-sense RNA *Betacoronavirus* in the family Coronaviridae.[5] It is an important cause of enteric disease in young calves that can also induce respiratory disease.[1,4] BoCV is shed in feces and nasal secretions. Wildlife such as deer, waterbuck, elk, and water buffalo are possible reservoirs since they harbor coronaviruses that are closely related to BCoV.[4] BCoV respiratory infections are exacerbated by stress or respiratory coinfections and may be observed with diarrhea.[4] Respiratory disease outbreaks caused by BoCV are relatively common and mostly occur during winter months. A Belgium study found BoCV was one of the most frequently isolated respiratory viruses in young calves (38.4%), followed by bovine

respiratory syncytial virus, and parainfluenza virus type 3.[2] Coinfection of BCoV with bovine viral diarrhea virus or *Histophilus somni* may play an essential role in the pathogenesis of bovine respiratory disease.[3,6] Therefore, the presence of BCoV likely increased the calf's susceptibility to developing suppurative pneumonia by damaging the tracheal epithelium and mucociliary apparatus. No bacteria were cultured from the tracheal swab. However, the calf had a dose of antibiotics three days before necropsy that may have decreased the bacterial load in the trachea. The enlarged lymph node near the condensed section of the right lung is suggestive of lymph node activation secondary to pneumonia. Additional differential diagnoses for BoCV respiratory infections in young calves include bovine parainfluenza 3 (PI3), bovine respiratory syncytial virus (BRSV), and infectious bovine rhinotracheitis virus (IBR)/bovine herpes virus (BHV1).

The renal cyst isolated to one pole of the kidney is considered an incidental finding. The cyst appeared to originate from the collecting duct or distal nephron based on its epithelial lining. No evidence of collecting duct obstruction was identified.[1] The lymphocyte apoptosis is consistent with the dose of exogenous steroids administered before death during the period of respiratory distress. The localized infection of the skin and subcutaneous tissue at the base of the scrotum is common in calves castrated using elastic bands and would have healed with time and supportive care.



4-5 Lung, calf: Airways are surrounded by marked BALT hyperplasia, and contain refluxed exudate from the surrounding alveoli. Lining epithelium in intact and in excellent shape. (HE, 152X)

Contributing Institution:
Penn State College of Medicine, https://med.psu.edu/comparative-medicine

JPC Diagnosis:
Lung: Pneumonia, bronchointerstitial, suppurative, and histiocytic diffuse, severe, with marked BALT hyperplasia.

JPC Comment:
The contributor provides a concise review of bovine coronavirus (BoCV), systems affected, and predisposing factors of this entity.

In addition to calf diarrhea and calf respiratory disease, BoCV is also associated with a third clinical syndrome known as "winter dysentery". Calf diarrhea caused by BoCV typically affects animals less than one month of age and is associated with declining maternal antibodies. Epithelial cells of the distal small and large intestines and colon are infected, resulting in villous atrophy and crypt hyperplasia. Following an incubation period of 3-4 days, calves develop severe malabsorptive diarrhea that persists for 2-8 days and associated with progressive dehydration, acidosis, hyperkalemia, hypoglycemia, and may progress to circulatory collapse and death. BoCV is interestingly found in both the intestinal and upper respiratory tract of most infected diarrheic calves at necropsy, consistent with concurrent fecal and nasal shedding. The disease is more prevalent in winter and tends to recur annually on the same farms, indicating

reservoirs for infection within a herd may include subclinical infected calves or cows.[4]

Winter dysentery, in contrast to calf diarrhea, affects adult dairy and beef cattle as well as captive wild ruminants.[4] This acute disease is characterized by hemorrhagic diarrhea, anorexia, and is predominantly seen in young postpartum dairy cows, resulting in significantly decreased milk production. [1,4] Similar to calf diarrhea, winter dysentery is also frequently associated with respiratory signs. Intestinal lesions are similar to those of calf diarrhea with high morbidity and low mortality rates of 20-100% and 1-2%, respectively.[1,4] Despite the name "winter dysentery", this condition occurs in both cold and warm seasons.[1]

Respiratory disease caused by BoCV affects both calves (2-6mo) as well as young adult feedlot cattle (6-10mo). Uncomplicated cases typically exhibit mild respiratory disease, such as coughing and rhinitis. However, BoCV is recognized as one of many potential inciting factors in the development of bovine respiratory disease complex (BRDC), a multifactorial disease that predominately affects 6 to10-month-old feedlot cattle, also known as “shipping fever”. The disease complex consists of interactions between viral, environmental, and host stress factors that create a permissive environment for bacterial infection, resulting in severe respiratory disease characterized by fever, dyspnea, and inflammatory and necrotizing pulmonary lesions leading to broncho(interstitial) pneumonia, weight loss, and often death.[4] Etiologies associated with BRDC include, but are not limited to, viruses such as bovine respiratory syncytial virus, parainfluenza-3, bovine herpes virus-1 (infectious bovine rhinotracheitis), reovirus, rhinovirus, and BoCV, as well as the bacterial etiologies *Histophilus somni*, *Mannheimia haemolytica*, *Pasteurella multocida*, *Bibersteinia trehalosi*, *Mycoplasma mycoides* spp *mycoides* small colony type (contagious bovine pleuropneumonia), and *Mycoplasma bovis*.[1]

Since the first human coronavirus (HCoV) was discovered in the 1960s within the nares of patients with the common cold, seven species have since been discovered in humans that lead to either mild or lethal respiratory disease depending on the strain type and patient's condition. HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1 are typically associated with mild respiratory disease while SARS-CoV, MERS-CoV, and SARS-CoV-2 are associated with higher mortality rates (9.2%, 34%, and 2.9-12.6% respectively).[6]

These viruses typically originate in a natural host, such as a bat. However, an intermediate host is the typical source of zoonotic transmission. An intermediate host of SARS-CoV-2 has not yet been identified; however, analysis of samples obtained from Malytan pangolins in China indicate they are potential intermediate hosts for SARS-CoV-2.[6]

Although coronaviruses predominately cause respiratory disease in humans, different host receptors are targeted. As an example, SARS-CoV-2 first binds to ACE2 on the host cell surface through the S1 subunit and then fuses viral and host membranes through the S2 subunit, whereas MERS-CoV recognizes dipeptidyl peptidase 4 (DPP4; also known as CD26).[6]

It is unlikely SARS-CoV-2 will be the last novel coronavirus to infect humans given the trend observed since the 1960s. However, the robust research and scientific discovery

associated with the current pandemic will contribute toward better understanding of novel species encountered in the future.[6]

References:

1. Caswell JL, Williams KJ. Respiratory system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol 2. 6th ed. St. Louis, MO: Elsevier limited; 2016:148-150,537-546.
2. Pardon B, Callens J, Maris J, et al. Pathogen-specific risk factors in acute outbreaks of respiratory disease in calves. *Journal of dairy science*. 2020;103**:** 2556-2566.
3. Ridpath JF, Fulton RW, Bauermann FV, Falkenberg SM, Welch J, Confer AW. Sequential exposure to bovine viral diarrhea virus and bovine coronavirus results in increased respiratory disease lesions: clinical, immunologic, pathologic, and immunohistochemical findings. *Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc*. 2020;32**:** 513-526.
4. Saif LJ, Jung K. Comparative Pathogenesis of Bovine and Porcine Respiratory Coronaviruses in the Animal Host Species and SARS-CoV-2 in Humans. *Journal of clinical microbiology*. 2020;58.
5. Suzuki T, Otake Y, Uchimoto S, Hasebe A, Goto Y. Genomic Characterization and Phylogenetic Classification of Bovine Coronaviruses Through Whole Genome Sequence Analysis. *Viruses*. 2020;12.
6. Tang D, Comish P, Kang R. The hallmarks of COVID-19 disease. *PLoS Pathog*. 2020;16(5):e1008536. Published 2020 May 22.
7. Workman AM, Kuehn LA, McDaneld TG, Clawson ML, Loy JD. Longitudinal study of humoral immunity to bovine coronavirus, virus shedding, and treatment for bovine respiratory disease in pre-weaned beef calves. *BMC veterinary research*. 2019;15**:** 161.