Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #18 31 January 2024

CASE I:

Signalment:
Adult female American bison (*Bison bison*)

History:
Owner reports weight loss and sporadic loss of animals of all ages for the past year.

Gross Pathology:
The pleural surface is covered in a thick mat of fibrin. On cut section, there are multifocal 0.1-1.0 cm diameter abscesses containing caseonecrotic exudate.

Laboratory Results:
Aerobic culture: No growth.
PCR for BHV-1, BRSV, BVDV, PI3: Undetected.
PCR for *Histophilus somni*: Undetected.
PCR for *Mycoplasma bovis*: Undetected.

Microscopic Description:
Throughout the section there is multifocal to coalescing necrosis affecting >50% of the lung tissue. Necrotic foci efface bronchioles and are characterized by a hypereosinophilic core surrounded by a peripheral rim of degenerate neutrophils admixed with pyknotic to karyorrhectic debris. There is central mineralization in some necrotic foci. In the adjacent alveoli, there is frequent necrosis of septa with abundant hyalinized material and/or flooding of remnant alveolar spaces by edema, fibrin,

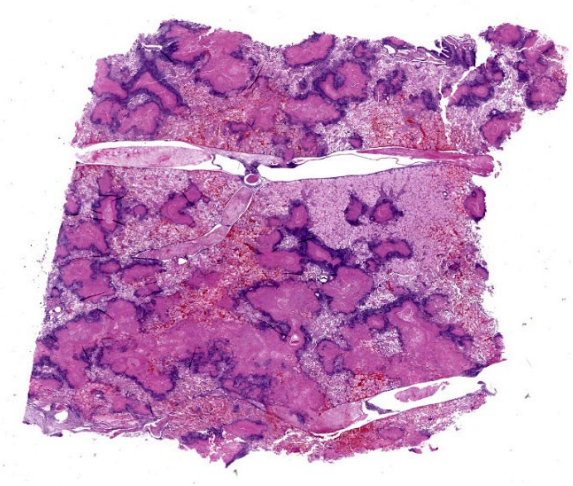

Figure 1-1. Lung, bison. At subgross magnification, there are well-delineated areas of lytic necrosis which correspond to airways. (HE, 5X)

hemorrhage, and many foamy macrophages. Large bronchi have mildly hyperplastic epithelium. Some vessels are occluded by hyalinized fibrin thrombi.

Immunohistochemistry: There is positive immunoreactivity for *Mycoplasma bovis* in the previously described lesions, particularly at the edges of necrotic foci. The lung is diffusely negative for *Histophilus somni* immunoreactivity.

Special stains: No organisms were detected with acid-fast and modified acid-fast stains.

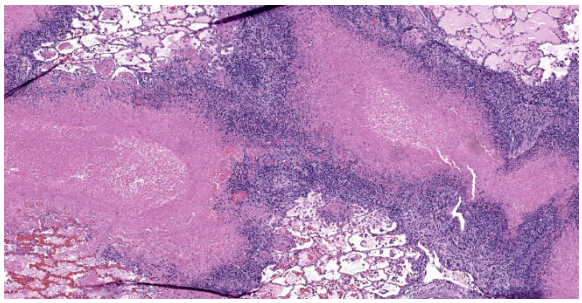

Figure 1-2. Lung, bison. Airway outlines are visible within areas of lytic necrosis. All layers of the airway walls are replaced by cellular debris and neutrophils and bounded by epithelioid macrophages. (HE, 61X)

Contributor's Morphologic Diagnosis:
Bronchopneumonia, severe, diffuse, subacute to chronic, necrosuppurative, with caseonecrotic abscesses and fibrinous pleuritis.

Contributor's Comment:
Mycoplasma is a genus of ubiquitous bacteria that consists of over 100 described species. The organisms are anaerobic, self-replicating, and lack a cell wall. Tissue tropism and host specificity vary between Mycoplasma species, and the manner in which infection leads to disease is not well understood.

Mycoplasma bovis is a globally distributed, economically important bacterial pathogen that contributes to the bovine respiratory disease complex.[4] In addition to pneumonia, *M. bovis* can cause mastitis, polyarthritis, otitis media, keratoconjuncitivitis, and reproductive losses in cattle.[11] Historically, *M. bovis* was rarely detected in other species. In the early 2000's, however, *M. bovis* was documented as the cause of several high morbidity and mortality disease outbreaks in American bison.[19] The emergence of mycoplasmosis in bison has resulted in devastating economic loss to the commercial bison industry and continues to threaten bison production operations throughout North America.[1,5,6,9,18]

Since the initial emergence, numerous clinical manifestations of mycoplasmosis have been dscribed in bison. These include pneumonia, necrotic pharyngitis, polyarthritis, reproductive disorders, and systemic disease.[5,6,9,18] In contrast to cattle in which co-infecting pathogens are common, there is evidence that *M. bovis* acts as a primary pathogen in bison with increased virulence and higher case fatality rates.[9,11,15,19]

Control of *M. bovis* has been challenging.[3] Efforts to protect bison via autogenous vaccines have been met with difficulty due to the remarkable evolutionary capacity of the bacterium.[1,8,12] Treatment is limited due to the absence of a cell wall and associated resistance to many commonly used antibiotics.[4,11] Further, a recent study documented that approximately 3% of bison are asymptomatic carriers of *M. bovis*.[14] Chronic carriers can be difficult to identify and detect, complicating herd assessment and management strategies.[16,17]

Recently, *M. bovis* was detected as the cause of a high mortality outbreak in free-ranging

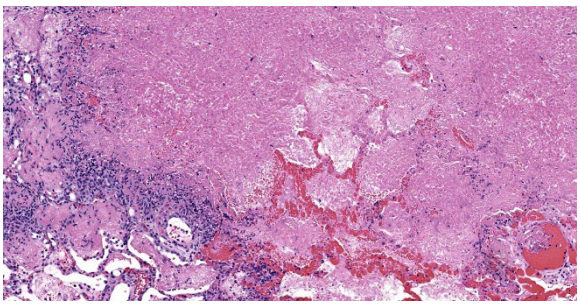

Figure 1-3. Lung, bison. Necrosis extends into the adjacent alveolar parenchyma resulting in septal necrosis and abundant hemorrhage and polymerized fibrin. (HE, 61X)

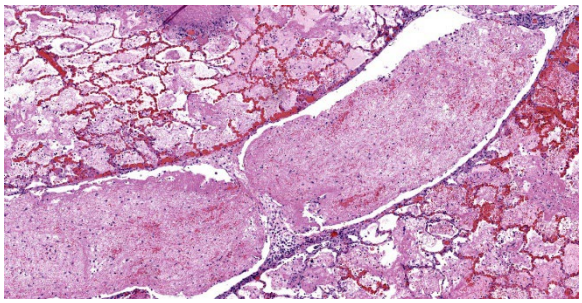

Figure 1-4. Lung, bison. Interlobular lymphatics contain large fibrin thrombi. (HE, 61X)

pronghorn (*Antilocapra americana*) in Wyoming.[10] Mule deer and white-tailed deer are also susceptible to *M. bovis*, meriting concern for impacts to wildlife, including non-commercial bison.[7,13]

Contributing Institution:
University of Wyoming
Wyoming State Veterinary Laboratory
Department of Veterinary Sciences
https://www.uwyo.edu/vetsci/

JPC Diagnosis:
Lung: Bronchopneumonia, necrotizing and fibrinosuppurative, diffuse, severe, with fibrinous pleuritis and bronchiectasis.

JPC Comment:
The rugged American bison is emblematic of the untamed American West and, as of 2016, serves as the national mammal of the United States. Once hunted to near extinction, decades of conservation efforts have restored buffalo numbers to healthy levels in the Western United States and Canada. The emergence of *Mycoplasma bovis* in American bison herds in the last few decades has therefore been met with alarm, particularly due to the severity of disease in this species compared to cattle, and, as the contributor notes, the lack of effective vaccines against or treatments for this wily bacterium.

Despite being a well-documented member of the bovine respiratory disease complex, the molecular mechanisms that underly the virulence and pathogenicity of *M. bovis* remain incompletely understood.[2] A key contributor to the bacterium's host immune system evasion is a fairly complex system of antigenic variation. This variation is accomplished via several prominent, interchangeable transmembrane proteins that act as major immunogens and are switched out when effective host antibodies are encountered.[2] These proteins belong to a family of 13 variable membrane surface lipoproteins (Vsps), encoded by 13 corresponding genes, only two of which are expressed at any one time.[2] These Vsps can be expressed in different combinations, and their co-expression creates "*M. bovis* surface mosaics" that each have different antigenic properties.[2] Additional antigenic variability can be introduced through insertions and deletions in sequence repeats within these genes, resulting in the production of differently sized protein variants, presumably with different antigenic properties, from a limited number of Vsp genes. Variation in Vsp expression and size has been demonstrated among individual bacterial subpopulations in affected animals, and this subpopulation diversity thwarts the host's immune system and contributes to the chronic, intractable nature of disease caused by *M. bovis*.[2]

M. bovis, like all mycoplasma species, is a streamlined organism with a small genome, no cell wall, and a lifestyle that depends heavily on host cells for life-sustaining nutrients and cellular processes. This co-dependence requires close association between the bacterium and host cells; however, little is definitively known about how *M. bovis* adheres to and survives inside host cells. The previously discussed Vsps are thought to also facilitate

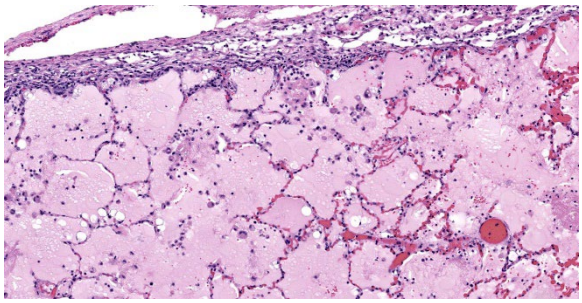

Figure 1-5. Lung, bison. Alveoli throughout the lung have hypercellular septa and are filled with edema. (HE, 128X)

host cell adhesion, though the variability in surface antigens, so useful in immune system evasion, can lead to more or less successful adhesion depending on the efficacy of the expressed bacterial ligands.[2] A bacterial subpopulation's particular Vsp complement can also enhance or degrade its ability to form biofilms, likely a critical component of bacterial persisteance. *M. bovis* is also able to survive intracellulary within phagosomes and to modulate host immune responses by inducing apoptosis of host T lymphocytes, among other immunomodulatory actions; however, the mechanisms behind these antics have not been fully elucidated. The end result of all of this bacterial subterfuge is chronic infection, and the hallmark of *M. bovis* disease in bison is an initially promising response to antibiotic therapy followed by repeated relapses, wasting, and eventual death or human euthanasia.[19]

This week's conference was moderated by LTC Chris Schellhase, Chief of Diagnostic Services at the Joint Pathology Center, who began discussion by noting the massive amount of fibrin, edema, and necrosis appreciable on subgross evaluation. Some participants were intrigued by the multifocal vasculitis present throughout the section and wondered if the vasculitis was a primary lesion, which would be unusual with *M. bovis* infection, or if the vessels were simply bystanders being taken along for a very inflammatory ride. Conference participants also discussed whether some of the large fibrin aggregates, such as those within the interlobular septa, were present within blood or lymphatic vessels. Participants noted the large, dilated bronchi which were multifocally effaced by inflammatory cells, fibrin, and edema and felt that these changes likely represent bronchiectasis, a common finding in *Mycoplasma* pneumonias generally. Finally, participants reviewed special stains, including a Fite-Faraco, which unexpectedly contained many acid fast bacteria. The significance of this finding is unclear.

Discussion of the morphologic diagnosis revisited the vasculitis debate. Participants felt that the vasculitis was likely a bystander rather than a primary process and it was, for this reason, omitted from the final morphologic diagnosis.

References:

1. Bras AL, Barkema HW, Woodbury MR, Ribble CS, Perez-Casal J, Windeyer MC. Clinical presentation, prevalence, and risk factors associated with Mycoplasma bovis-associated disease in farmed bison (Bison bison) herds in western Canada. *J Am Vet Med Assoc*. 2017;250(10):1167-1175.
2. Burki S, Frey J, Pilo P. Virulence, persistence and dissemination of *Mycoplasma bovis*. *Vet Microbiol*. 2015;179(1-2):15-22.
3. Calcutt MJ, Lysnyansky I, Sachse K, Fox LK, Nicholas RAJ, Ayling RD. Gap analysis of Mycoplasma bovis disease, diagnosis and control: An aid to identify future development requirements. *Transbound Emerg Dis*. 2018;65 Suppl 1:91-109.

4. Caswell JL, Archambault M. Mycoplasma bovis pneumonia in cattle. *Anim Health Res Rev*. 2007;8(2):161-186.
5. Dyer N, Hansen-Lardy L, Krogh D, Schaan L, Schamber E. An outbreak of chronic pneumonia and polyarthritis syndrome caused by Mycoplasma bovis in feedlot bison (Bison bison). *J Vet Diagn Invest*. 2008;20(3):369-371.
6. Dyer N, Register KB, Miskimins D, Newell T. Necrotic pharyngitis associated with Mycoplasma bovis infections in American bison (Bison bison). *J Vet Diagn Invest*. 2013;25(2):301-303.
7. Dyer NW, Krogh DF, Schaan LP. Pulmonary mycoplasmosis in farmed white-tailed deer (Odocoileus virginianus). *J Wildl Dis*. 2004;40(2):366-370.
8. Kumar R, Register K, Christopher-Hennings J, et al. Population genomic analysis of Mycoplasma bovis elucidates geographical variations and genes associated with host-types. *Microorganisms*. 2020;8 (10):1561.
9. Kyathanahalli S. Janardhan MH, Neil Dyer, Richard D. Oberst, Brad M. DeBey. Mycoplasma bovis outbreak in a herd of North American bison (Bison bison). *J Vet*
10. Malmberg JL, O'Toole D, Creekmore T, et al. Mycoplasma bovis Infections in Free-Ranging Pronghorn, Wyoming, USA. *Emerg Infect Dis*. 2020;26(10): 2807-2814.
11. Maunsell FP, Woolums AR, Francoz D, et al. Mycoplasma bovis infections in cattle. *J Vet Intern Med*. 2011;25: 772-783.
12. Perez-Casal J, Prysliak T, Maina T, Suleman M, Jimbo S. Status of the development of a vaccine against Mycoplasma bovis. *Vaccine*. 2017;35(22):2902-2907.
13. Register KB, Jelinski MD, Waldner M, et al. Comparison of multilocus sequence types found among North American isolates of Mycoplasma bovis from cattle, bison, and deer, 2007-2017. *J Vet Diagn Invest*. 2019;31(6):899-904.
14. Register KB, Jones LC, Boatwright WD, et al. Prevalence of Mycoplasma spp. in the Respiratory Tract of Healthy North American Bison (Bison bison) and Comparison with Serum Antibody Status. *J Wildlife Dis*. 2021;57(3):683-688.
15. Register KB, Olsen SC, Sacco RE, et al. Relative virulence in bison and cattle of bison-associated genotypes of Mycoplasma bovis. *Vet Microbiol*. 2018;222:55-63.
16. Register KB, Parker M, Patyk KA, et al. Serological evidence for historical and present-day exposure of North American bison to Mycoplasma bovis. *BMC Vet Res*. 2021;17(1):1-10.
17. Register KB, Sacco RE, Olsen SC. Evaluation of enzyme-linked immunosorbent assays for detection of Mycoplasma bovis-specific antibody in bison sera. *Clin Vaccine Immunol*. 2013;20(9):1405-1409.
18. Register KB, Woodbury MR, Davies JL, et al. Systemic mycoplasmosis with dystocia and abortion in a North American bison (Bison bison) herd. *J Vet Diagn Invest*. 2013;25(4):541-545.
19. Sweeney S, Jones R, Patyk K, LoSapio C. Mycoplasma bovis-an emerging pathogen in ranched bison. 2013. Available at https://www.aphis.usda.gov/animal_health/nahms/bison/downloads/bison14/

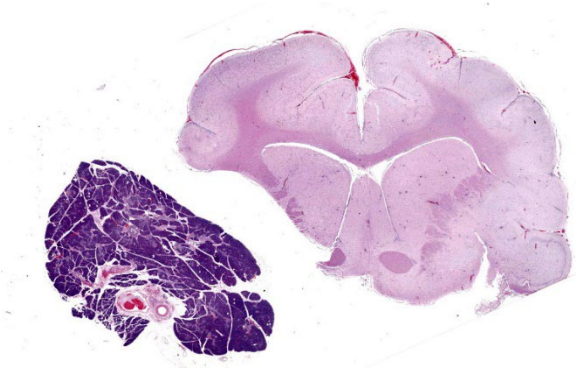

Figure 2-1. Pancreas and cerebrum, skunk. Aside from a small amount of meningeal hemorrhage, no lesions are visible at subgross magnification. (HE, 5X)

CASE II:

Signalment:
Adult male striped skunk (*Mephitis mephitis*)

History:
This striped skunk was found alive but twitching in a residential area of Charlottetown, Prince Edward Island. The skunk was euthanized and submitted for post mortem examination.

Gross Pathology:
The skunk was in good body condition. The caudodorsal lung lobes were bright red and slightly firm with numerous flat, white, 2 mm in greatest diameter, white foci randomly scattered throughout all lung lobes. The trachea and mainstem bronchi contained pink-tinged froth. The liver was diffusely and mildly enlarged, dark red and friable and all liver lobes contained numerous pinpoint, white, flat, round foci. There were multiple skull fractures with clotted blood multifocally within the temporal muscles and meninges.

Laboratory Results:
Real-time RT-PCR for Influenza A matrix gene on oropharyngeal swab: Positive.

Real-time RT-PCR for Avian Influenza Virus H5 subtype on oropharyngeal swab: Positive.

Microscopic Description:
Brain: Surrounding numerous blood vessels in the cerebral grey matter and meninges are small numbers of degenerate neutrophils, lymphocytes, plasma cells, microglial cells and pyknotic cellular debris. Adjacent to these areas in the cerebral cortex, neurons are occasionally hypereosinophilic and shrunken with pyknotic nuclei (acute degeneration/necrosis) and are surrounded by small numbers of microglial cells. The Virchow Robin space is diffusely expanded by small to moderate numbers of lymphocytes and plasma cells. The grey matter neuropil is moderately hypercellular with an increased number of microglial and glial cells. Meningeal blood vessels are diffusely cuffed by lymphocytes and plasma cells and there are multiple areas where the meninges contain moderate numbers of erythrocytes (hemorrhage).

Pancreas: Randomly scattered throughout the exocrine pancreas, replacing pancreatic acini and occasionally surrounding blood vessels are numerous, variably-sized nodular aggregates that are composed of moderate numbers

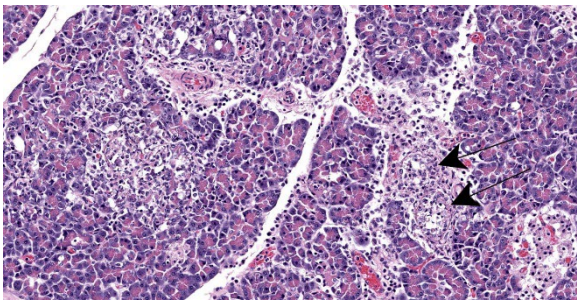

Figure 2-2. Pancreas, skunk. There are randomly scattered areas of necrosis affecting both acinar tissue (left) and ducts (right, arrows). (HE, 61X)

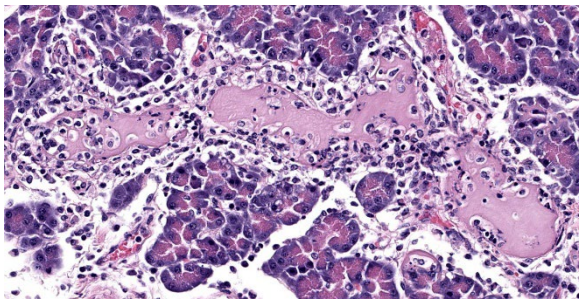

Figure 2-3. Pancreas, skunk. Numerous vessel contain inflammatory cells within their walls where they are admixed with cellular debris (vasculitis). (HE, 387)

of degenerate neutrophils, macrophages, eosinophilic granular material, and pyknotic cellular debris . These areas often contain moderate amounts of pale eosinophilic, amorphous, fibrillar to flocculent, extracellular matrix (amyloid). Pancreatic acini in the periphery of these areas are often fragmented with singular acinar cells remaining. The interstitium contains moderate numbers of neutrophils, macrophages, eosinophilic fluid, and fewer lymphocytes and plasma cells and pyknotic cellular debris. The lumen of pancreatic ducts often contains small numbers of neutrophils

Contributor's Morphologic Diagnosis:

1. Brain: Necrotizing meningoencephalitis, multifocal, acute, severe, with multifocal acute neuronal necrosis, gliosis and satellitosis
2. Brain: Multifocal, acute, severe, meningeal hemorrhage
3. Pancreas: Necrotizing pancreatitis, multifocal, subacute, severe with multifocal acinar loss

Contributor's Comment:

Influenza viruses are enveloped RNA viruses that have the potential to undergo gene reassortment in a host when they are infected with multiple viruses.[1,3] Gene reassortment can lead to the development of highly pathogenic strains that infect multiple species and can result in pandemics.[3]

The A/Goose/Guangdong/1/96 lineage of H5 highly pathogenic avian influenza virus (HPAI), which first emerged in Southeast Asia in 1996, was detected in wild and domestic birds in Atlantic Canada in late 2021.[2] H5N1 viruses in Newfoundland, Canada, in late 2021 were most closely related to HPAI viruses circulating in northwestern Europe in spring 2021, and were most likely carried across the Atlantic via migratory birds.[2] Since that time, H5N1 viruses have spread throughout North America, resulting in neurologic signs and widespread mortality in a wide variety of wild birds and mammals, as well as significant impacts on the poultry industry.[1] In early 2023, fully Eurasian H5N5 viruses began to be detected in wild birds in Atlantic Canada, presumably representing a new incursion to the region via migratory birds. Genetic analysis of samples from the skunk in this submission revealed H5N5 subtype of the 2.3.4.4b Eurasian lineage of HPAI.

In Atlantic Canada, H5N1 HPAI has been detected in red foxes (*Vulpes vulpes*), striped skunks (*Mephitis mephitis*), and raccoons (*Procyon lotor*), with additional diagnoses in

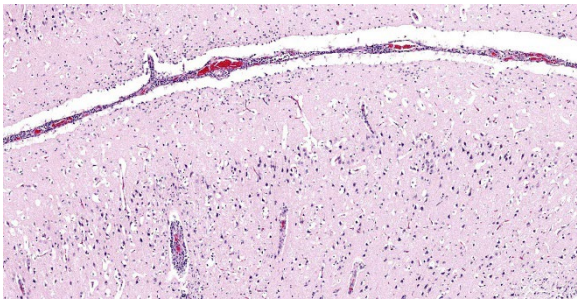

Figure 2-4. Cerebrum, skunk. The meninges and Virchow Robins spaces are expanded by a moderate mixed cell infiltrate. (HE, 89X)

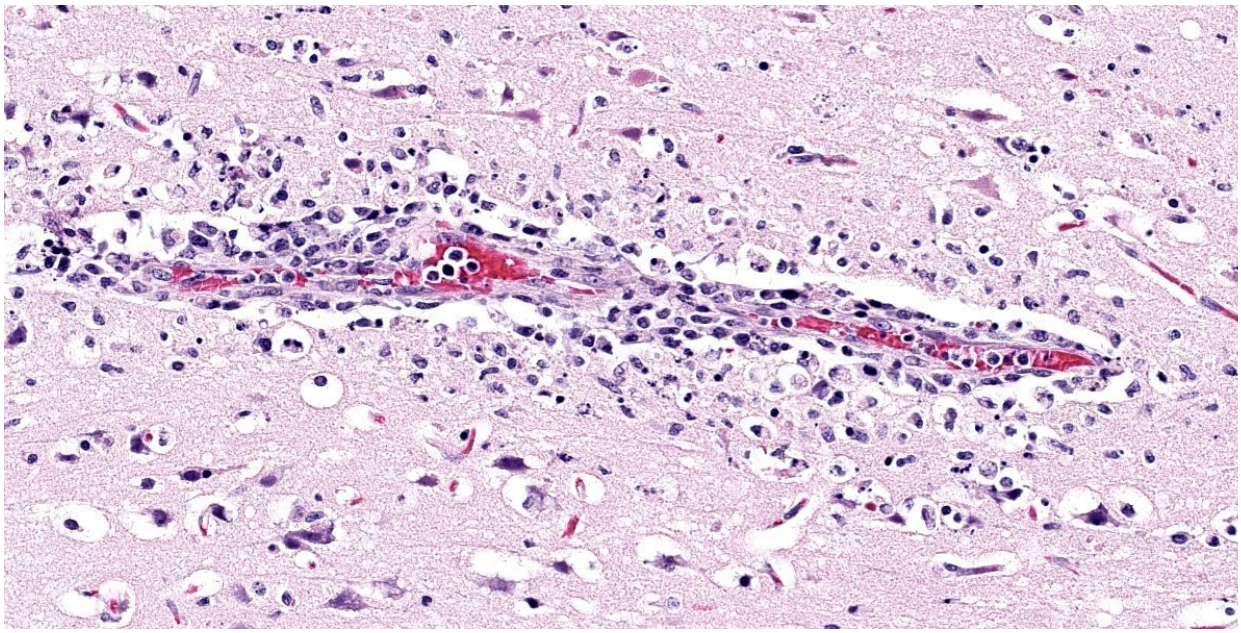

Figure 2-5. Cerebrum, skunk. Inflammatory cells emigrate into the surrounding neuropil, resulting in necrosis. (HE, 314X)

these species and other mammals throughout North America.[1] H5N5 HPAI has been detected in raccoons, one striped skunk, and multiple American crows in the region. Mammals infected with H5N1 HPAI typically show neurologic signs, including seizures, twitching, foaming at the mouth, circling, or are found dead.[1] Gross post mortem lesions include pulmonary edema, bronchointerstitial pneumonia, nasal turbinate edema, multifocal (2 to 3 mm in greatest diameter) areas of necrosis throughout the pulmonary and hepatic parenchyma, and meningeal congestion. The most common microscopic lesions include meningoencephalitis, gliosis, bronchointerstitial pneumonia, pulmonary edema and congestion, necrotizing hepatitis and cerebral and brainstem neuronal necrosis.[1] Although the lesions in H5N5 HPAI positive cases are not yet described, the gross and microscopic lesions in this case are consistent with those described in H5N1 HPAI positive skunks and other North American mammals in the current outbreak.

Contributing Institution:
Atlantic Veterinary College, University of Prince Edward Island
Department of Pathology and Microbiology
https://www.upei.ca/avc/pathology-and-microbiology

JPC Diagnosis:
1. Cerebrum: Meningoencephalitis, necrotizing and lymphohistiocytic, diffuse, mild to moderate.
2. Pancreas: Pancreatitis, necrotizing and lymphoplasmacytic, diffuse, moderate.
3. Pancreas: Amyloidosis, multifocal, mild.

JPC Comment:
As noted by the contributor, Influenza A viruses exchange genetic information through reassortment of their segmented RNA genomes. Reassortment, a method to supercharge viral evolution, is a strategy available only to RNA viruses with segemented genomes and requires concurrent co-infection with more than one Influenza A strain. Reassortment occurs when RNA segments from

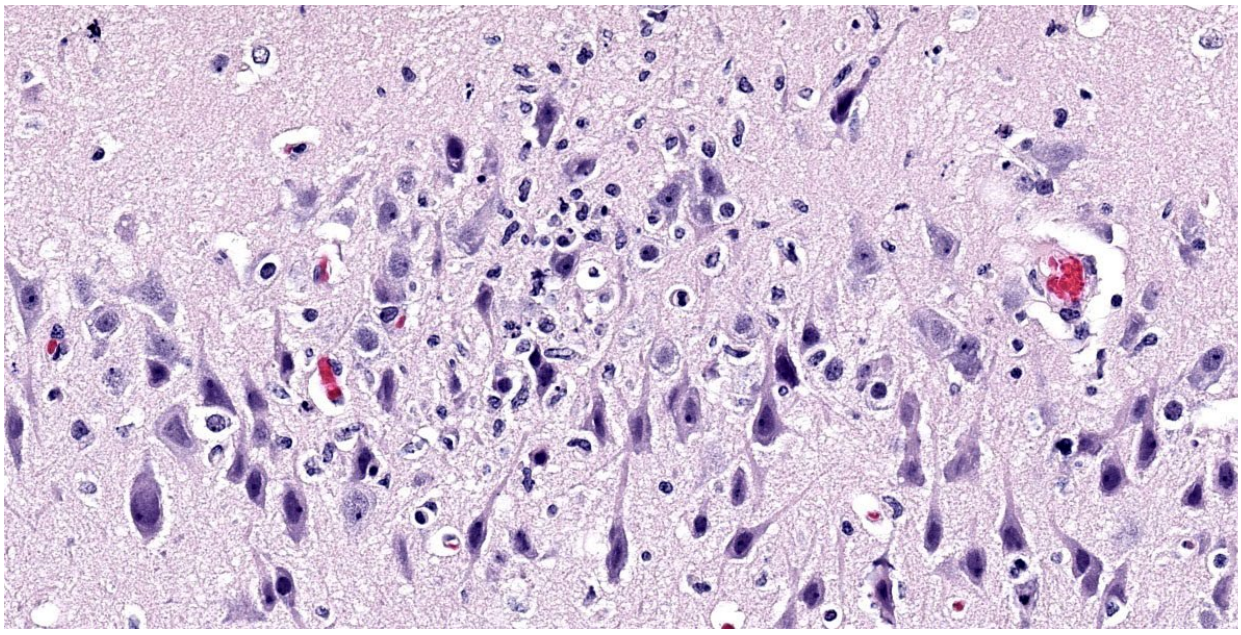

Figure 2-6. Cerebrum, skunk. There is scattered neuronal necrosis adjacent to areas of meningeal inflammation within the submeningeal cortex. (HE, 381X)

different influenza viruses are intermixed during the virion assembly process, resulting in a large genomic change and the development of a new virus subtype, a process known as genetic shift. This is in contrast to the emergence of new virus types due to random point mutations, a slower, more conservative change known as antigenic drift.

Influenza A viruses have a broad host range, with established lineages circulating in poultry, pigs, humans, and other mammals, and a particularly rich tapestry of viral diversity circulating in wild birds.[3] Particular influenza viral lineages tend to be restricted to their preferred mammalian hosts; however, reassortment occasionally produces novel influenza viruses that are able to cross species barriers and cause viral pandemics.[3] Research has shown that such reassortment occurs frequently in vivo, leading to viral genotypic diversity both between animals and among the various levels of the respiratory tract in individual animals.[3]

Pigs are of particular interest in influenza ecology as they are susceptible to swine, human, and avian influenzas, and thus provide an accommodating microbial mixing vessel that can give rise to recombinant viruses.[3] These novel viral subtypes have been behind most major historical influenza pandemics to date, and the emergence of a new viral influenza subtype with pandemic potential is an ever-present threat.[4]

This case generated robust discussion, particulary around changes within the neuroparenchyma. Multiple conference participants were convinced that many neurons contained intranuclear eosinophilic viral inclusions, which would not be expected with influenza infection. The discussion quickly turned to the age-old "inclusion body or nucleoli" debate without satisfactory resolution. Several participants also believed there to be neuronal loss, though while neuronal necrosis was evident,

most participants felt that the substantial gliosis made the neuroparenchyma appeared hypercellular, not hypocellular.

Despite the nuances in the neuroparenchymal pathology, the moderator emphasized that the nature and distribution of the inflammation in section are highly suggestive of a viral etiology. Participants discussed various differential diagnoses before noting that the presence of necrotizing pancreatitis considerably narrowed the differential list. Differentials for pancreatic necrosis in a bird include West Nile Virus, Avian Adenovirus, HPAI, *Sarcocystis* sp., and zinc toxicity; however, the most likely cause of necrotizing pancreatitis and meningoencephalitis in a bird is HPAI.

The ensuring morphologic diagnosis discussion was scentillating. Participants were skeptical that the meningeal hemorrhage was replated to the HPAI and wondered if this could be euthanasia artifact. Participants were similarly unconvinced that the amyloid noted in the pancreatic acini and islets was due to HPAI infection. The agreed-upon morphologic diagnosis highlights the necrosis that characterizes the disease and separates out the amyloidosis as a discrete disease process.

References:

1. Alkie TN, Cox, S, Embury-Hyatt C, et al. Characteriziation of neurotropic HPAI H5N1 viruses with novel genome constellations and mammalian adaptive mutations in free-living mesocarnivores in Canada. *Emerg Microbes Infect*. 2023;12(1):2186608.
2. Caliendo V, Lewis NS, Pohlmann A, et al. Transatlantic spread of highly pathogenic avian influenza H5N1 by wild birds from Europe to North America in 2021. *Sci Rep*. 2022;12(1):1-18.
3. Ganti K, Bagga A, Caranccini S, et al. Influenza A virus reassortment in mammals gives rise to genetically distinct within-host subpopulations. *Nat Commun*. 2022;13(1): 6846.
4. Quinn PJ, Markey BK, Leonard FC, Fitz-Patrick ES, Fanning S, Hartigan PJ. *Veterinary Microbiology and Microbial Diseases*. Blackwell Publishing Ltd;2011: 647-659.

CASE III:

Signalment:
Adult male black-tailed jackrabbit (*Lepus californicus*)

History:
A dead black-tailed jackrabbit without signs of predation was found by a private citizen that was falcon hunting in southern California.

Gross Pathology:
The carcass was in a mild to moderate state of postmortem decomposition and poor nutritional condition. There was a moderate amount of serosanguineous fluid in the thorax. The lungs were pink to red, swollen, and wet. The liver was dark-red, congested, and oozed dark-red fluid on cut surface.

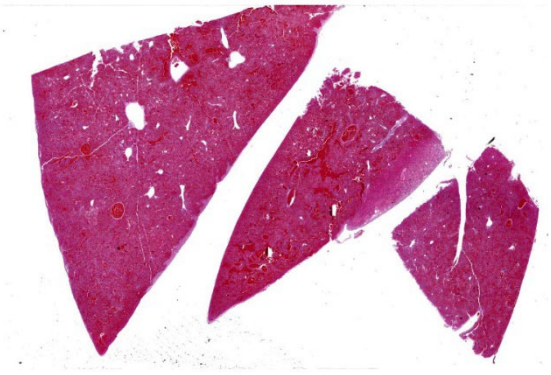

Figure 3-1. Liver, jackrabbit. Three sections of liver are presented for examination. (HE, 3X)

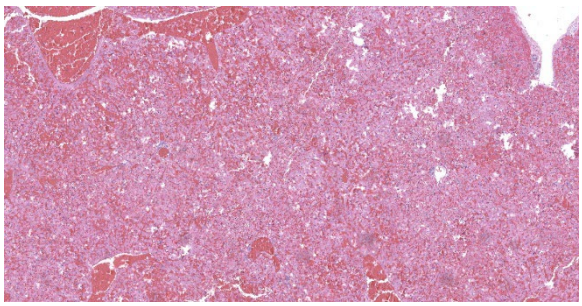

Figure 3-2. Liver, jackrabbit. There is diffuse loss of sinusoidal architecture with numerous areas of hemorrhage. (HE, 60X)

Laboratory Results:
RHDV2 RT-qPCR: Positive (Ct 11.8).
Pan-lagovirus IHC in liver (antibodies provided by Drs. Lavazza and Capucci, IZSLER, Italy): Positive.

Microscopic Description:
Liver: There is diffuse, periportal to panlobular hyperosinophilia with disorganization of hepatic cords (necrosis) and abundant hemorrhage and congestion. Hepatocytes show one or more of the following changes: membrane and cytoplasmic fragmentation, pyknosis, karyorrhexis, and occasional individual mineralization. There are rare, scattered heteorophils, and mild infiltrates of lymphocytes, histiocytes, and plasma cells in the portal spaces, as well as some subscapular follicular aggregates of lymphocytes.

Contributor's Morphologic Diagnosis:
Liver, necrosis, panlobular, severe, acute with hemorrhage

Contributor's Comment:
Rabbit hemorrhagic disease virus type 2 (RHDV2 or GI.2) belongs to the genus *Lagovirus*, a group of viruses within the family *Caliciviridae* that affects species in the order Lagomorpha.[5] This virus was detected for the first time in France in 2010 and has been spreading among domestic and wild leporid species in North America since 2020.[7,11] A distinctive feature of RHDV2 is its host range, which is wider than that of its close relative, classic RHDV (or GI.1); the latter affects principally domestic and wild European rabbits (*Oryctolagus cuniculus*), whereas RHDV2 affects also several hare and jackrabbit species (*Lepus* spp.), as well as cottontails and other wild rabbits native to the American continent (*Sylvilagus* spp.).[11] RHDV2 is broadening its host range as it spreads and new leporid species are exposed to it for the first time.

In domestic rabbits, classic RHD is associated with high mortality; animals generally succumb after a short period of fever, lethargy, seizures and bleeding through body orifices.[1] Hepatomegaly with pallor and reticular pattern, pulmonary congestion with hemorrhages, splenomegaly, hemorrhages in multiple tissues, and icterus are the most common gross findings. Histologically, there is periportal to panlobular hepatic necrosis, splenic red pulp necrosis and lymphoid depletion, and systemic thrombosis in small capillaries (e.g., in renal glomeruli and pulmonary septae).

RHDV2 seems to cause similar clinical signs and lesions, although possibly with more variable mortality according to some reports.[7,10] Specifically in hares (*Lepus* spp.), RHDV2 causes a disease process similar to RHD in rabbits or to the European brown hare syndrome (caused by another *Lagovirus*, EBHSV or GII) in several hare species from Europe.[4] Clinicopathologic data of RHDV2 infection in hares is limited to just a few studies that would suggest that mortality is lower and more variable than in rabbits.[4,12]

Data on the pathology of RHDV2 on North American leporid species is very limited. An experimental study confirmed for the first

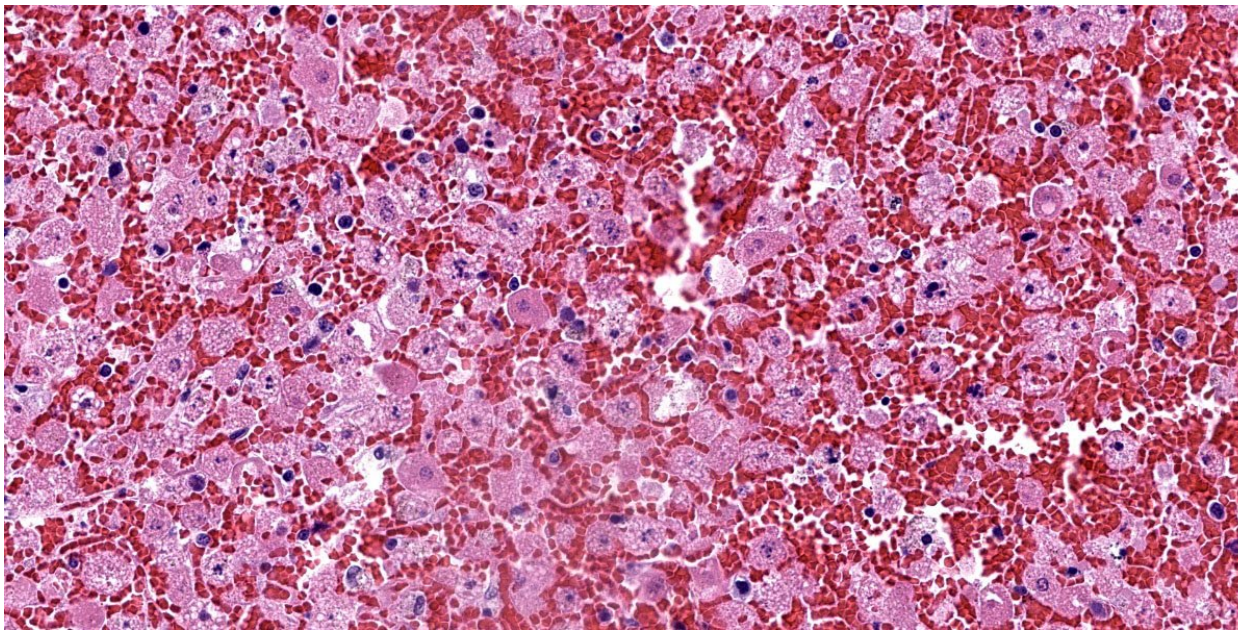

Figure 3-3. Liver, jackrabbit. Hepatocytes are disassociated and necrotic throughout the entire lobule. (HE, 381X)

time that that the eastern cottontail (*Sylvilagus floridanus*) was susceptible to RHDV2 and developed a similar disease process as observed in New Zealand white rabbits (*Oryctolagus cuniculus*).[9] According to one of the first reports of natural disease in cases from early 2020 in the USA, the pathology of RHDV2 in desert cottontails (*Sylvilagus audubonii*) and black-tailed jackrabbits (*Lepus californicus*) is similar to previous descriptions in wild and domestic European rabbits and other hare species, with hepatic necrosis as the most diagnostically relevant finding; however, glomerular or pulmonary fibrin thrombi and splenic necrosis were not identified in the animals of the mentioned report, which differs with previous descriptions of RHDV2 in rabbits of European origin.[6]

In California, RHDV2 was confirmed for the first time in a black-tailed jackrabbit on May 2020 as part of an outbreak that was spreading throughout the southwest at that time.[2] The disease spread quickly among multiple southern counties of the state between 2020 and 2021, and was later detected in northern areas as well. Domestic rabbits (including pets, backyard rabbitries, and a few commercial farms), black-tailed jackrabbits, desert cottontails, Western brush rabbits (*Sylvilagus bachmani*) and its endangered subspecies, the Riparian brush rabbit (*Sylvilagus bachmani riparius*), have been affected by RHDV2 in California. Some of the early circulating viruses in the south of the state were phylogenetically similar to other detections in Arizona, Texas, and New Mexico earlier in 2020. Among all the detections of RHVD2 in North America since the first cases in Quebec, Canada, in 2016, the early California sequences were more similar to a virus collected in British Columbia, Canada, in 2018.[3]

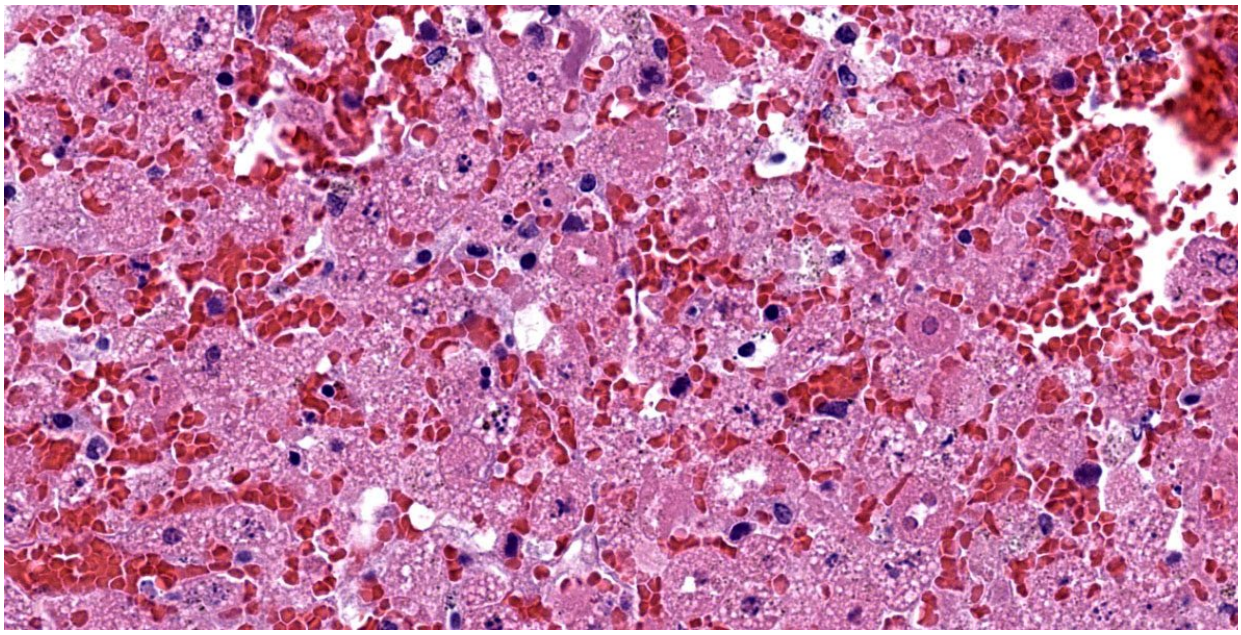

Figure 3-4. Liver, jackrabbit. Degenerating and necrotic hepatocytes contain numerous cytoplasmic vacuoles which may be the result of apoptosis or dilation of smooth endoplasmic reticulum. (HE, 578X)

Contributing Institution:
California Animal Health and Food Safety Laboratory System (CAHFS)
University of California-Davis
San Bernardino, California
https://cahfs.vetmed.ucdavis.edu/locations/san-bernardino-lab

JPC Diagnosis:
Liver: Hepatitis, necrotizing, acute, massive, diffuse, severe, with hemorrhage.

JPC Comment:
The contributor provides an excellent overview of the biology, natural history, and unfortunate spread of RHDV2. The spread of RHDV2 is concerning not only for native susceptible lagomorph species, but also for the predators which depend on them for a food source. RHDV2 can profoundly alter the ecosystems in which it is introduced as evidenced by the emergence of RHDV2 on the Iberian Peninsula in 2013. In that outbreak, the wild rabbit population of northern Spain declined precipitously; unfortunately, population numbers for the Iberian lynx and the Spanish eagle, both endanged species that rely heavily on rabbit-based diets, declined in tandem.[3] This scenario has repeated with each introduction of RHDV2 into a new ecosystem and is particularly instructive for the current outbreak in California, as the state has established populations of mountain lions, bobcats, coyotes, and golden eagles that also make rabbit a staple of their diets.[3]

Until recently, prevention measures have been confined to biosecurity and husbandry practices such as handwashing, quarantining animals, disinfectants, and limiting contact with animals. These efforts have been generally unsuccessful in slowing the unrelenting spread of the virus due to RHDV's multiple routes of transmission; the virus can be spread mechanically by flies and other vectors, by fomites, and by infected carcasses which can harbor communicable virus for up to three months.[4] The primary route of transmission is oral, though

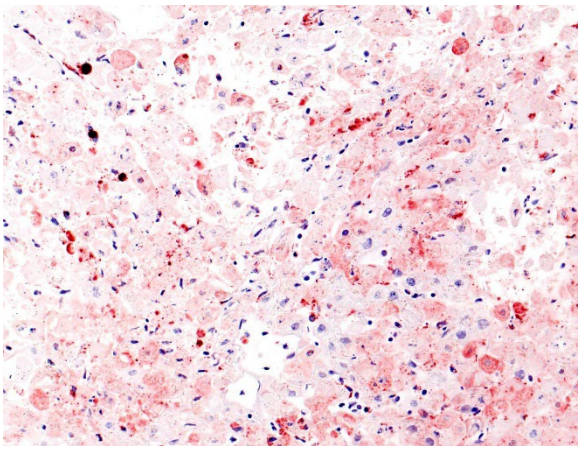

Figure 3-5. Liver, jackrabbit. A pan-fungal RHDV IHC demonstrates abundant antigen within degenerating hepatocytes. (*Photo courtesy of:* California Animal Health and Food Safety, San Bernardino Branch).

nasal, subcutaneous, intravenous, and intramuscular transmission has been identified.[4] Wildlife researchers and rabbit hobbyists alike were cheered in 2021, when the USDA gave Emergency Use Authorization to a US-based RHDV2 vaccine; the vaccine was given a Conditional Use license in November 2023, providing a new weapon in the fight against this fatal, emerging disease.[8]

The hallmark of RHDV2 is massive hepatocellular necrosis as abundantly illustrated by this case. Conference participants remarked on the incredible degree of hepatocellular destruction, the abundant hemorrhage that separated and surrounded the remnant hepatocytes, and the general lack of associated inflammation. Conference participants also considered a reticulin stain which showed complete loss of sinusoidal architecture, confirming the tissue destruction evident on H&E. The moderator noted that it would be easy to focus on the astonishing amount of hemorrhage and destruction and overlook the finer descriptive points of nuclear features (karyorrhexis, karyolysis), type and extent of necrosis, and the mineralization noted occasionally throughout the section.

Conference participants noted the vacuolar changes in the hepatocytes and debated whether these were lipid-type or glycogen-type changes. Participants decided that the vacuoles were likely neither, but instead represented swelling of the endoplasmic reticulum during cellular necrosis. The moderator called attention to the eosinophilic globules present throughout the section, called acidophilic or "Councilman bodies." Councilman bodies are aggregated apoptotic hepatocyte fragments that are observed in viral hepatitides and are named after American pathologist William Thomas Councilman who discovered them during his work on yellow fever. Finally, participants discussed lung lobe torsion, a differential common in rabbits, which would appear as a whole-lobe coagulative necrosis with retention of hepatocellular architecture, quite different from the impressive lytic necrosis present in this slide.

Discussion of the morphologic diagnosis was fairly straightforward in this case, though not without minor drama. One particularly insistent conference participant argued for the inclusion of hemorrhage in the morphologic diagnosis due to the prominence of this feature in the disease itself and on the examined slide. Dissenting voices argued that necrosis implies hemorrhage, making its inclusion redudant; however, persistence carried the day and hemorrhage won by a hare.

References:

1. Abrantes J, van der Loo W, Le Pendu J, et al. Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): a review. *Vet Res*. 2012;43(1): 12.

2. Asin J, Nyaoke AC, Moore JD, et al. Outbreak of rabbit hemorrhagic disease virus 2 in the southwestern United States: first detections in southern California. *J Vet Diagn Invest*. 2021;33(4):728-731.
3. Asin J, Rejmanek D, Clifford DL, et al. Early circulation of rabbit haemorrhagic disease virus type 2 in domestic and wild lagomorphs in southern California, USA (2020-2021). *Transbound Emerg Dis*. 2022;69(4):e394-e405.
4. Byrne AW, Marnell F, Barrett D, et al. Rabbit Haemorrhagic Disease Virus 2 (RHDV2; GI.2) in Ireland Focusing on Wild Irish Hares (Lepus timidus hibernicus): An Overview of the First Outbreaks and Contextual Review. *Pathogens*. 2022; 11(3):288.
5. Capucci L, Cavadini P, Lavazza A. Rabbit hemorrhagic disease virus and European brown hare syndrome virus. In: Bamford DH, Zuckerman M, eds. *Encyclopedia of Virology*. 4th ed. Vol. 2. pp. Academic Press, Elsevier; 2021:724-729.
6. Lankton JS, Knowles S, Keller S, Shearn-Bochsler VI, Ip HS. Pathology of Lagovirus europaeus GI.2/RHDV2/b (Rabbit Hemorrhagic Disease Virus 2) in Native North American Lagomorphs. *J Wildl Dis*. 2021;57(3):694-700.
7. Le Gall-Reculé G, Lavazza A, Marchandeau S, et al. Emergence of a new lagovirus related to Rabbit Haemorrhagic Disease Virus. *Vet Res*. 2013;44(1):81.
8. "Medgene's RHDV2 vaccine granted critical license of USDA's center for veterinary biologics." Nov 2, 2023. Available at: https://www.prnewswire.com/news-releases/medgenes-rhdv2-vaccine-granted-critical-license-by-usdas-center-for-veterinary-biologics-301974936.html.
9. Mohamed F, Gidlewski T, Berninger ML, et al. Comparative susceptibility of eastern cottontails and New Zealand white rabbits to classical rabbit haemorrhagic disease virus (RHDV) and RHDV2. *Transbound Emerg Dis*. 2022;69(4):e968-978.
10. Neimanis A, Larsson Pettersson U, Huang N, et al. Elucidation of the pathology and tissue distribution of Lagovirus europaeus GI.2/RHDV2 (rabbit haemorrhagic disease virus 2) in young and adult rabbits (Oryctolagus cuniculus). *Vet Res*. 2018;49(1):46.
11. Shapiro HG, Pienaar EF, Kohl MT. Barriers to management of a foreign animal disease at the wildlife-domestic animal interface: the case of rabbit hemorrhagic disease in the United States. *Front Conserv Sci*. 2022;3:857678.
12. Velarde R, Cavadini P, Neimanis A, et al. Spillover events of infection of brown hares (Lepus europaeus) with Rabbit Haemorrhagic Disease Type 2 Virus (RHDV2) caused sporadic cases of an European brown hare syndrome-like disease in Italy and Spain. *Transbound Emerg Dis*. 2017;64(6):1750-1761.

CASE IV:

Signalment:
Immature (presumed yearling) female white-tailed deer (*Odocolieus virginianus*)

History:
In the Canadian province of Nova Scotia, Department of Natural Resources officers were called by a concerned citizen because a young deer was acting strangely in a residential area. The deer would walk in tight circles, had a prominent head tilt, lacked fear of people and appeared to be blind as it would walk into obstacles, fall down, and struggle back to its feet. The deer appeared to be breathing heavily and was drooling excessively. The officer euthanized the deer by shooting her in the head

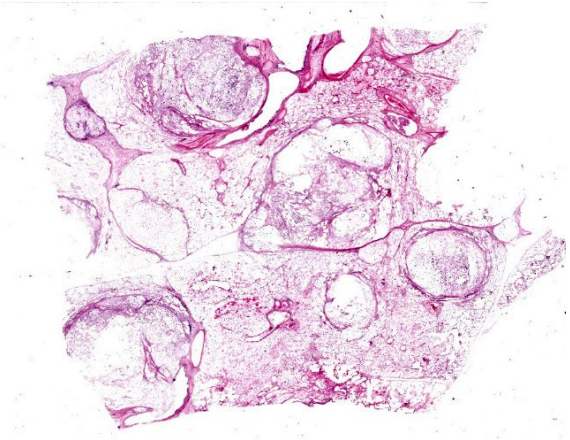

Figure 4-1. Lung, deer. A section of lung with multiple expansile, poorly cellular nodules is submitted. (HE, 5X)

several times with a 22 caliber gun. The head, lungs, heart, gastrointestinal tract, liver, and kidneys were harvested, frozen, and submitted to the Canadian Wildlife Health Cooperative, Atlantic Region, for examination.

Gross Pathology:
Abundant fat stores were noted in the epicardial groove, in the connective tissue surrounding the kidneys, and within the mesentery indicating that this animal appeared to be in good body condition. There were several skin abrasions on the head (attributed to trauma associated with falling) and traumatic lesions due to mode of euthanasia (i.e., gunshot to the head) were noted.

An approximately 4.5 cm x 3.5 cm x 3.5 cm, round, expansile, pale tan mass focally replaced the parenchyma in the left cranioventral lung. This mass was soft and when sectioned, was white-tan with fine fibrous septa dissecting through it. Multiple similar, smaller masses, ranging from 0.5 cm - 1.0 cm in greatest diameter, were randomly scattered throughout the remaining left lung. The right lung was enlarged and largely effaced by multifocal to coalescing similar soft masses, the largest of which measured approximately 8 cm in greatest diameter. The tracheobronchial lymph nodes were moderately to severely enlarged; the largest was approximately 10 cm in greatest diameter and had a necrotic center filled with viscous yellow material. When sectioned, these lymph nodes were otherwise similar in appearance to the grossly affected lung.

Prominent cerebellar coning was noted when the calvarium was removed. When the brain was sectioned sagitally along the midline, several, small, 1-2 mm in greatest diameter, pitted lesions with dark rims were noted in the neuropil of the thalamus and superior colliculus. Following fixation, similar tiny lesions were also noted in the caudal nasal cavity. This exudate largely replaced the right ethmoturbinates and disrupted the adjacent cribiform plate. The inner and middle ears were grossly unremarkable. No significant abnormalities were noted in the liver, kidney, or gastrointestinal tract.

Laboratory Results:
Light growth of *Cryptococcus* sp. was identified from lymph node culture swabs.

Multilocus sequence typing identified this fungus as *Cryptococcus gattii* (type VGII).

Microscopic Description:
Tissues exhibit moderate autolysis. Sections of the mass-like lesions in the lung are all similar. These masses are poorly defined and are composed of areas where alveoli are markedly expanded and are often ruptured forming variably sized spaces filled with myriads of yeast. Small to moderate infiltrates of large, pale macrophages are also present within these air spaces and the interstitium. A mild increase in

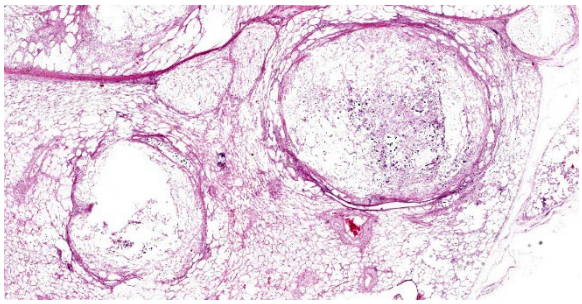

Figure 4-2. Lung, deer. Higher magnification of several of the pulmonary nodules. (HE, 16X)

fine fibrous stroma multifocally expands the interstitium forming thin septa and sometimes thicker fibrous bands. Yeast are round to ovoid, 10-20 microns in diameter, and have a very pale pink, often faintly visible, thick capsule that is highlighted with mucicarmine staining. In some areas, the capsule appears basophilic. Rarely, very narrow based budding is noted. Many scattered yeasts contain coarse black melanin pigment which is highlighted with Fontana-Masson staining. Large bronchioles are sometimes partially distended with basophilic debris. Occasional dense clusters of bacilli are scattered within the tissue (interpreted as postmortem bacterial overgrowth).

Sections of the ethmoturbinates, brain, and tracheobronchial lymph nodes contain similar lesions, which often appear cystic, and consist of myriads of the described yeast associated with mild to moderate granulomatous inflammation.

Contributor's Morphologic Diagnosis:
Lung: Pneumonia, granulomatous, multifocal, mild to moderate, chronic, with myriads of intralesional yeast (compatible with *Cryptococcus* sp.).

Contributor's Comment:
The neurologic and respiratory clinical signs reported in this young deer are attributed to severe, systemic infection due to *Cryptococcus* sp. Multilocus sequence typing identified this fungus as *Cryptococcus gattii* type VGII. To our knowledge, this is the first laboratory confirmed case of *C. gattii* infection in the Atlantic provinces in eastern Canada, and the first confirmed case in a deer.

Disease in humans and susceptible animals due to cryptococcosis is generally restricted to several species, grouped into the *C. neoformans*-*C. gattii* complex, which include *C. neoformans* var. *neoformans*, *C. neoformans* var. *grubii* and *C. gattii*.[1,2] *C. gattii* is further classified via genotyping into 4 groups: VGI, VGII, VGIII, and VGIV.

Until the 1990's, *C. gattii* was generally considered restricted to several tropical and subtropical regions of the world, including Australia, South America, southeast Asia, parts of Africa, and an area of California. Since 1999, human hospitals on Vancouver Island, Canada, have reported a marked increase in laboratory confirmed cases of *C. gattii* (mainly types VGI and VGII) infection and the Pacific Northwest is now considered an endemic area.[1] In this same time period, laboratory confirmed cases of *C. gattii* infections (again, mainly type VGI and VGII) in pet dogs and cats from the area were also common.[5] In general, veterinary cases were diagnosed 2-3 times more frequently than human cases.[5]

C. gattii is considered an emerging pathogen in a broad range of animals and infection has been reported in domestic cats, dogs, horses, sheep, cattle, koalas, dolphins, gray squirrels, ferrets, birds, and marsupials.[1] The organism lives in the environment and can be isolated

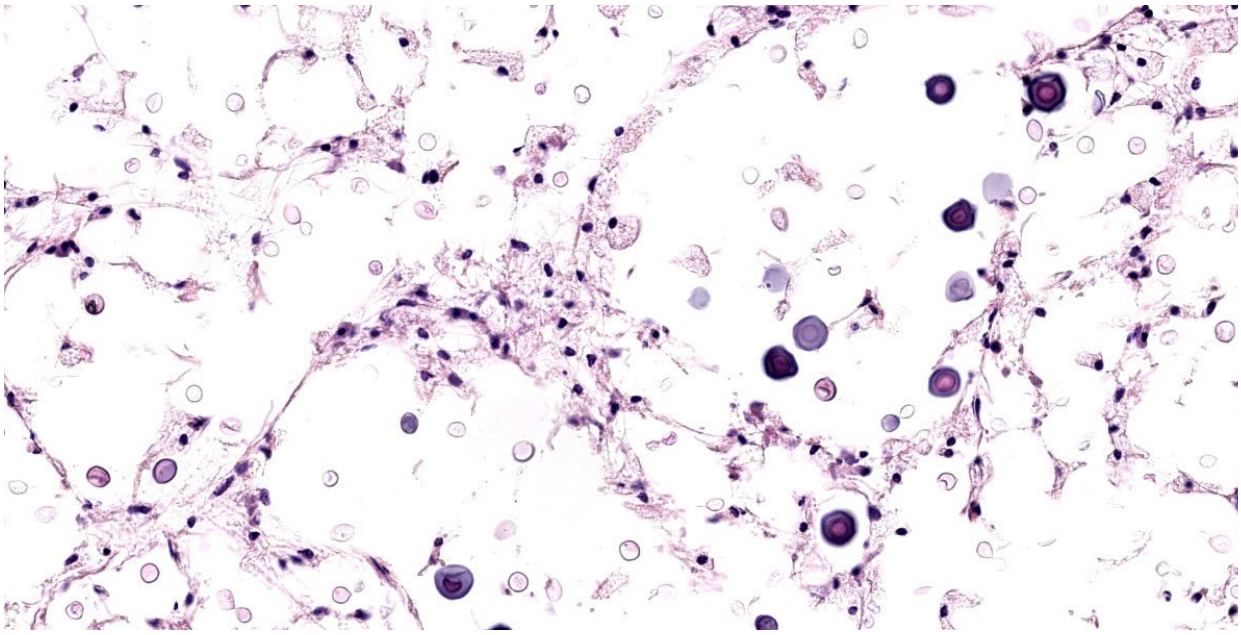

Figure 4-3. Lung, deer. Alveolar nodules contain low numbers of widely spaced, occasionally concave yeasts consistent with *Cryptococcus* sp. (HE, 379X)

from the soil, bark from a wide variety of trees, and decaying organic material. In endemic areas, the fungus has also been isolated from the air, freshwater, and sea water, and has been recovered from car wheel wells and on footwear from high traffic areas.[2] Because of some of the latter findings, disruptive environmental activities (like logging and construction), traffic from endemic areas, and movement of bark mulch have all been implicated in the gradual spread of this organism to new areas.[5]

Infection is typically via inhalation of small basidiospores or desiccated yeast forms present in the environment. Rarely direct infections through skin wounds have been reported, most frequently in cats. A variety of virulence factors have been identified. The fungus can multiply at body temperatures. The large gelatinous capsule, which gradually enlarges in chronic infections, serves to prevent phagocytosis by macrophages and neutrophils. The capsule traps and depletes complement and circulating capsular antigens aid in the removal of selectins from endothelial surfaces, thus inhibiting migration of neutrophils into tissues. Melanin production helps to protect the yeast from host-induced oxidative injury and when present, appears to assist the organism in invading the central nervous system.[7] It is interesting that although *C. neoformans* and *C. gattii* share many of the same virulence factors, *C. gattii* (especially types VGI, VGII, and possibly VGIII) most commonly causes infection in non-immunocompromised people and animals while *C. neoformans* primarily infects immune-compromised individuals.[2] *C. gattii* is considered a primary pathogen, while *C. neoformans* is generally an opportunistic infection. The cause of this observation is unknown. It may be related to the level of environmental exposure, that is, immunocompromised individuals may have more exposure to *C. neoformans* than *C. gattii*. Host genetic factors and inherent resistances or susceptibility to cryptococcal infection may also play a role.[2]

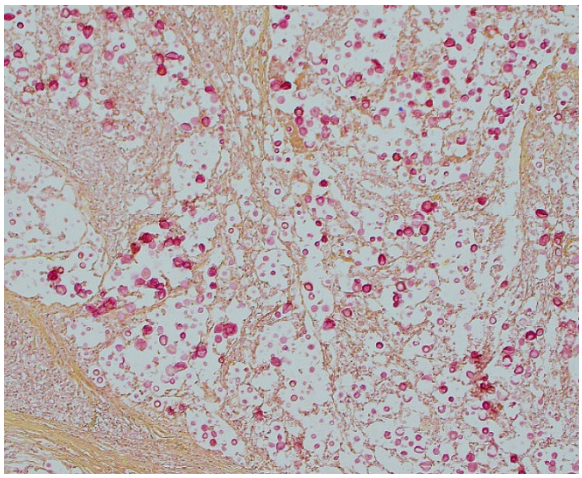

Figure 4-4. Lung, deer. Yeasts are strongly positive for mucicarmine. (Mucicarmine, 400X)

Given that infection is typically via inhalation, in animals, clinical disease due to *C. gattii* infection most commonly presents as chronic rhinitis and pneumonia. Encephalitis, via direct invasion from the nasal cavity, or sometimes via hematogenous routes, may result in a wide range of neurologic clinical signs. Widespread systemic infection has been reported in affected animals. As mentioned, cutaneous granulomatous lesions typically resulting from wound contamination may also occur, most commonly in cats.

In other areas, wild and domestic animals have acted as sentinels in the early detection of *C. gattii* infection and clinical disease is often detected first in animals before human cases are identified. Because of this finding, Nova Scotia public health officials have been notified to include *C. gattii* infection on their list of differential diagnoses, and testing protocols for suspect cases have been established.

Contributing Institution:
Department of Pathology/Microbiology
Atlantic Veterinary College
University of Prince Edward Island
www.avc.upei.ca

JPC Diagnosis:
Lung: Pneumonia, granulomatous, multifocal to coalescing, mild, with numerous yeast consistent with *Cryptococcus* sp.

JPC Comment:
Cryptococcus neoformans and *C. gattii* are both stunners, notable among the dimorphic fungi for their arresting, large capsule. While *C. neoformans* has been known and admired since it was first isolated from fermented peach juice in the 1890s, *C. gattii*, perhaps a bit more shy, waited until 1970 to be discovered in a leukemic patient and has, until its recent incursion in to the Northwest of Canada and the United States, stayed respectfully confined to a fairly narrow subtropical niche.[3]

Despite their differences in temperament, however, they both share, as a primary virulence factor, a carbohydrate-rich outer capsule composed primarily of glucuronoxylomannan with lesser amounts of galactoxylomannan.[3] Far from being just a pretty adornment, the capsule is a dynamic and defensive structure. It can suppress the host immune response by downregulating cytokine and chemokine secretion in dendritic cells, inhibiting binding of complement component C3, frustrating phagocytosis, and preventing dessication in the environment.[3,8]

One of the more interesting attributes of the *Cryptococcus* capsule is its ability to switch phenotypes. Phenotype switching is an adaptive mechanism characterized by structural modifications to the capsule and the cell wall.[3] Phenotype switching in *C. gattii* is a reversible change between two forms – a mucoid form and a smooth form – that occurs infrequently in vitro and in chronic infections.[3] Typically, infection begins with mucoid form, but subsequent phenotype switching to the smooth

form, which is characterized by reduced capsular polysaccharide, allows for penetration of the blood-brain barrier and dissemination to the brain; consequently, while both smooth and mucoid forms are found in pulmonary infection, only smooth variants are typically found in the brain.[3]

The importance of the capsule to *Cryptococcus* pathogenicity is best illustrated by the effects of its absence. Acapsular *C. gattii* strains are far less virulent, and are quickly recognized and killed by macrophages and dendritic cells.[6] If, however, these acapsular strains have *C. gattii* capsular polysaccharide deposited on their surface, phagocytosis is hindered, the secretion of pro-inflammatory cytokines is not triggered, and the fungi are not cleared, confirming the key role that the capsule plays in immune evasion.[8]

Conference participants appreciated the straight-forward nature of this slide, which provides an embarrassment of visual fungal riches. Participants noted the minimal amount of granulomatous inflammation in section, which is much less than would be expected with other dimorphic fungal infections of this severity. Partipants hypothesized that this rather languid inflammatory response might be due to the cloaking of the *Cryptococcus* capsule. Participants also admired a mucicarmine stain which, while beautiful, also provoked some discussion on whether mucicarine was staining the capsule, the organism, or both. Participants noted that mucicarmine stains the capsule, while silver stains such as GMS can be used to highlight the organism and to evaluate the broad or narrow-based nature of the budding.

Participants discussed the size of the yeast which, in some cases, appeared to exceed the standard 5-10 um expected size for *Cryptococcus gattii*. This lead to a discussion of Titan cells, which are a unique adaptation of *Cryptococcus* species where they grow to unusually large size as another mechanism to evade phagocytosis. Finally, the efforts of some industrious participants were rewarded by the discovery of several unheralded nematode larvae within the examined section.

Discussion of the morphologic diagnosis centered around whether the diagnosis should refer to fungal organisms generally or *Cryptococcus* sp. specifically. The majority of participants felt strongly that, given the unique morphology, budding, and staining characteristics exhibited in section, this was a diagnosis that could confidently be made on H&E examination alone.

References:

1. Chen S, Meyer W, Sorrell T. *Cryptococcus gattii* infections. *Clin Microbiol Rev*. 2014;27(4):980-1024.
2. Diti A, Carroll SF, Qureshi ST. *Cryptococcus gattii*: an emerging cause of fungal disease in North America. *Interdiscip Perspect Infect Dis.* 2009;840452.
3. Dixit A, Carroll SF, Qureshi ST. *Cryptococcus gattii*: an emerging cause of fungal disease in North America. *Interdiscip Perspect Infect Dis*. 2009;840452.
4. Dylag M, Colon-Reyes RJ, Kozubowski L. Titan cell formation is unique to *Cryptococcus* species complex. *Virulence*. 2020;11(1):719-729.
5. Epsinel-Ingroff A, Kidd SE. Current trends in the prevalence of *Cryptococcus gattii* in the United States and Canada. *Infect Drug Resist*. 2015;8:89-97.
6. Frietas GJC, Santos DA. *Cryptococcus gattii* polysaccharide capsule: an insight on fungal-host interactions and vaccine

studies. *Eur J Immunol*. 2021;51(9):2206-2209.
7. Lester SJ, Malik R, Bartlett KH, Duncan CG. Cryptococcosis: update and emergence of *Cryptococcus gattii*. *Vet Clin Pathol*. 2011;40(1):4-17.
8. Saidykhan L, Onyishi CU, May RC. The *Cryptococcus gattii* species complex: unique pathogenic yeasts with understudied virulence mechanisms. *PLoS Negl Trop Dis*. 2022;16(12):e0010916.