Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2025-2026

Conference #18 — 04 February 2026

CASE I:

Signalment:
Adult, female, breed unspecified sheep (*Ovis aries*)

History:
This sheep displayed progressive clinical signs of neurological disease over a three day period culminating in tetraparesis and lateral recumbency, despite treatment with antibiotics, vitamin B complex and corticosteroids.

Gross Pathology:
At necropsy, the cerebrospinal fluid was cloudy and contained strands of fibrin. No other abnormalities were noted.

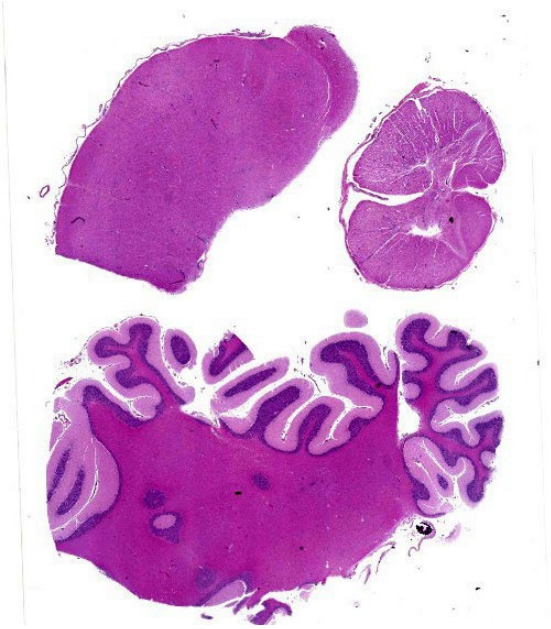

Figure 1-1. Brain, sheep: Sections from the cervical spinal cord, brainstem, and cerebellum are submitted for examination. (HE, 9X)

Laboratory Results:
A replicate section was examined by immunohistochemistry using a rabbit polyclonal anti Listeria monocytogenes antibody (#DF2302-50-0, Fisher Scientific, Houston, TX, USA), at a dilution of 1:2000, using the Dako Envision system (Dako Ag-ilent Pathology Solutions, Carpinteria, CA, USA), via an indirect immunoperoxidase procedure. Within the microabscesses in the brain and cervical spinal cord, there were a low to moderate number of bacteria that bound to the primary antibody.

Microscopic Description:
Brain, cerebellum and brainstem: In the brainstem gray matter, there are random multifocal, mild to moderate, variably discrete, infiltrates of hypersegmented and fragmented neutrophils, admixed with fewer mononuclear cells, consistent with microabscesses. Brainstem neurons within and adjacent to the neutrophilic infiltrates are pale, with central chromatolysis, consistent with neuronal degeneration. There are scattered microglial cells, mild gray matter spongiosis and infrequent microhemorrhages. Scattered blood vessels are lined by plump endothelial cells and have perivascular mild infiltrates of predominantly lymphocytes, with fewer neutrophils (perivascular cuffs). The cerebellar leptomeninges are multifocally, mildly infiltrated by predominantly lymphocytes, with fewer admixed neutrophils.

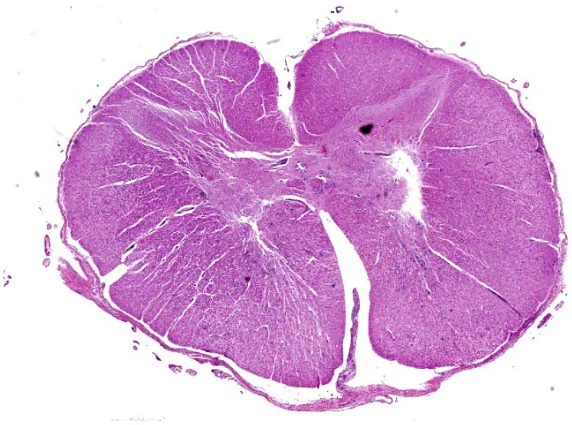

Figure 1-2. Cervical spinal cord, sheep: While inflammatory lesions are present in all sections, they are most severe in the cervical spinal cord. (HE, 30X)

Cervical spinal cord: In the gray matter and white matter, there are scattered, variably discrete, random multifocal, mild to moderate, infiltrates of neutrophils and mononuclear cells, consistent with microabscesses. There is neuronal degeneration, spongiosis, microgliosis, and swollen axons. Blood vessels are lined by plump endothelial cells and have perivascular mild to moderate infiltrates of predominantly lymphocytes, with fewer admixed neutrophils and infrequent plasma cells. The leptomeninges are multifocally expanded by mild to moderate infiltrates of lymphocytes with infrequent admixed neutrophils.

Contributor's Morphologic Diagnoses:

1. Brain, cerebellum and brainstem: Meningoencephalitis, lymphocytic, multifocal, mild to moderate, with multiple brainstem neutrophilic microabscesses.
2. Cervical spinal cord: Meningomyelitis, lymphocytic, multifocal, moderate, with multiple neutrophilic microabscesses.

Contributor's Comment:

A replicate section was examined by tissue Gram stain. There were low numbers of gram-positive bacilli, some intracellular, within areas of inflammation. A replicate section was examined by immunohistochemistry, using a rabbit polyclonal anti *Listeria monocytogenes* antibody (#DF2302-50-0, Fisher Scientific, Houston, TX, USA), at a dilution of 1 :2000, using the Dako Envision system (Dako Agilent Pathology Solutions, Carpinteria, CA, USA), via an indirect immunoperoxidase procedure. Within the microabscesses in the brain and cervical spinal cord, there were a low to moderate number of bacteria that bound to the primary antibody. The microscopic findings in the central nervous system accounted for the clinical signs in this sheep. The microscopic findings were typical of listeric encephalitis in ruminant species and the diagnosis was confirmed by immunohistochemistry.

Listeria monocytogenes is a gram-positive, facultative, anaerobic bacillus common in the environment. The bacteria can multiply within wide temperature and pH ranges. They can tolerate conditions of acidity, salinity and low moisture. They can survive in soil for many months and can be isolated from the feces of healthy cattle. *Listeria monocytogenes* is an intracellular pathogen of macrophages, neutrophils and epithelial cells. The bacterial surface proteins, Type A and Type B internalins, are virulence determinants. These proteins internalize with E-cadherin, an adherens junction transmembrane glycoprotein, to enable bacteria to cross intestinal, placental and blood brain barriers.

In sheep and other ruminants, listeriosis can manifest as three different syndromes, with little or no overlap. They are likely to be separate clinical presentations. The three syndromes are infection of the pregnant uterus resulting in abortion, blood borne infection with septicemia and with disseminated abscesses, and an ascending encephalitis.

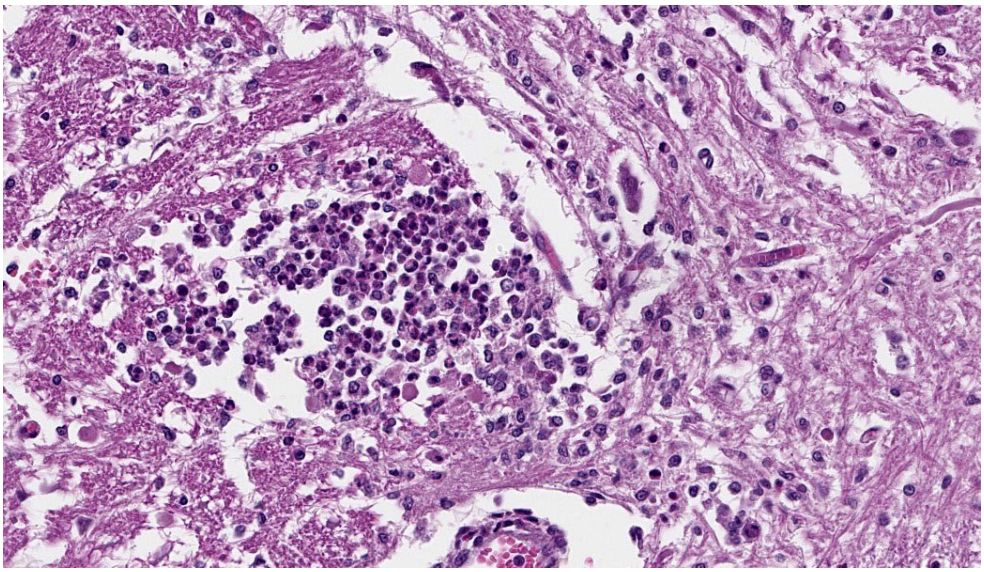

Figure 1-3. Cervical spinal cord: Within the grey matter (and scattered throughout the white matter), there are aggregates of intact and necrotic neutrophils (microabscesses). (HE, 691X)

Although listeric encephalitis has been associated with feeding of spoiled silage, environmental and fecal contamination are more common sources of infection than silage feeding in sheep and goats because many small rumi nants are never fed silage. The onset of the encephalitic form is usually very rapid. Death can ensue 24 to 48 hours after clinical signs appear. Clinical signs of listeric encephalitis in sheep and goats include lethargy, inappetence, loss of coordination, head pressing, circling, blindness, salivation, facial paralysis and culminate in recumbency, convulsions and death. This syndrome is more common in one to three-year-old small ruminants than in older animals.

There are few or no gross lesions in listeric encephalitis. Leptomeningeal opacity and cloudy cerebrospinal fluid may be the only findings seen at necropsy. Typically, microscopic lesions of listeric encephalitis are limited to the brainstem and cervical spinal cord. They include microabscesses, glial nodules, perivascular accumulation of lymphocytes and lymphocytic leptomeningitis. Gram-positive bacilli can be found within the microabscesses.

In listeric encephalitis, the likely route of infection of ingested *Listeria* bacilli is via breaks in the oral mucous membrane, with subsequent invasion of nerve endings of the trigeminal nerve branches. The bacteria travel via axonal flow to the central nervous system to infect neural cells, generally at the level of the brainstem and cervical spinal cord, where these nerve branches terminate. There may be a trigeminal ganglioneuritis. The bacteria multiply in neuronal cell bodies and then move to the inner side of the neuronal cell membrane via actin-filament mediated mobility. Aggregates of bacteria use a bacterial surface protein (surface protein actA) to move by actin polymerization. A pseudopod containing the bacteria invaginates randomly into an adjacent neural cell, forming a double-membrane bound endocytotic vesicle. This vesicle is lysed by listeriolysin 0, aided by a phospholipase and lecithinase, to release the bacteria into the cytoplasm of the newly invaded neural cell, as direct cell-to-cell extension of infection. Neural cell infection and endothelial damage lead to an inflammatory response and disruption of the blood-brain barrier with chemokine production and recruitment and activation of neutrophils.

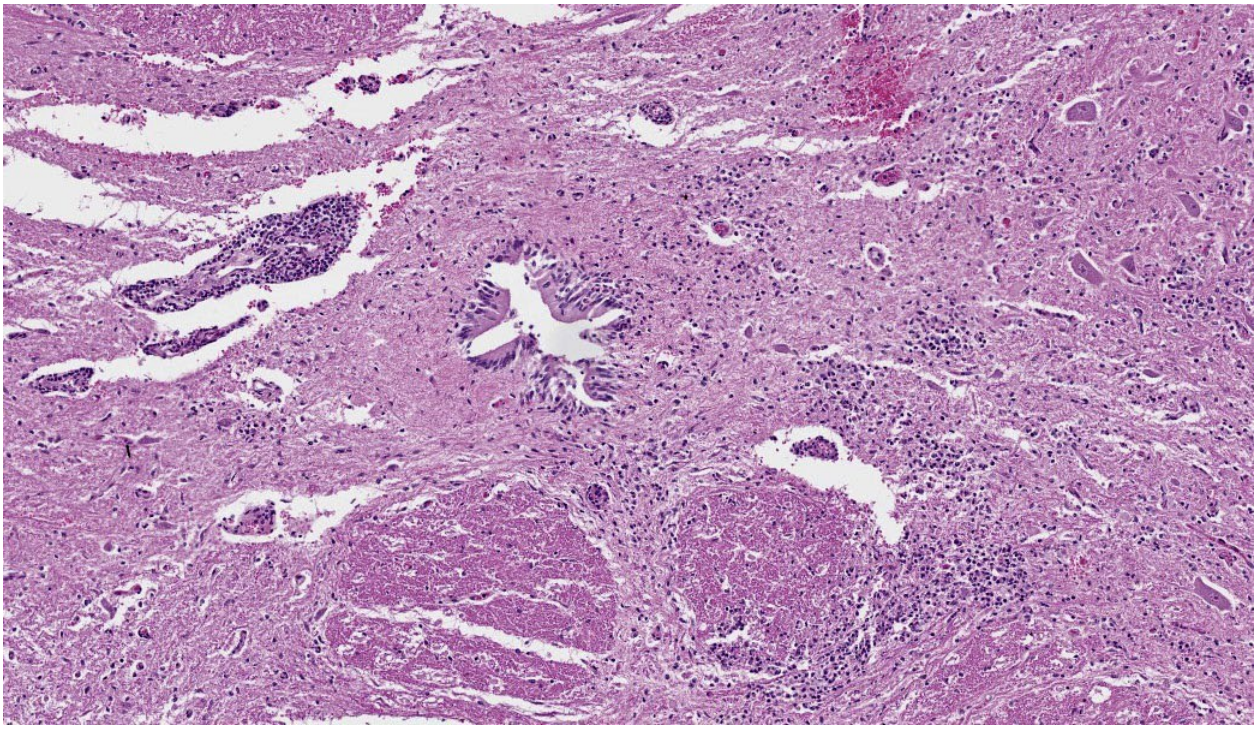

Figure 1-4. Cervical spinal cord: Throughout the section, in rarefied areas, there are microabscesses, perivascular cuffs of lymphocytes, plama cells, transmigrating neutrophils in Virchow-Robin spaces, and focal areas of hemorrhage. (HE, 171X)

Listeria monocytogenes is a zoonotic agent. Listeriosis in humans is a rare disease with high mortality (20-30%). Generally, it is a self-limiting foodborne illness. Certain risk groups (pregnant women, neonates, adults 65 years of age and over and individuals with compromised immune systems) may develop invasive disease one to four weeks following an episode of food borne illness. Invasive disease may include pregnancy complications (miscarriage, stillbirth, premature delivery due to *in utero* infection), life-threatening infection of the newborn, septicemia, meningitis and meningoencephalitis. Occasionally, it is an occupational disease of veterinarians and farmers, usually contracted from direct contact with infected livestock or their products of conception. To prevent contamination by *Listeria monocytogenes* in food processing establishments, cleaning and disinfection of facilities must be emphasized, with attention paid to proper maintenance and temperature control of food refrigeration equipment.

Contributing Institution:
United States Army Medical Research Institute of Infectious Diseases
https://www.usamriid.army.mil

JPC Diagnoses:
1. Cervical spinal cord: Meningomyelitis, suppurative, subacute, multifocal, moderate, with microabscesses.
2. Brainstem and cerebellar white matter: Leukoencephalitis, suppurative, subacute, multifocal, mild to moderate, with microabscesses and lymphohistiocytic meningitis.

JPC Comment:
This week's conference was moderated by MAJ Sarah Sulkosky, a former WSC Coordinator! *Listeria monocytogenes* has already

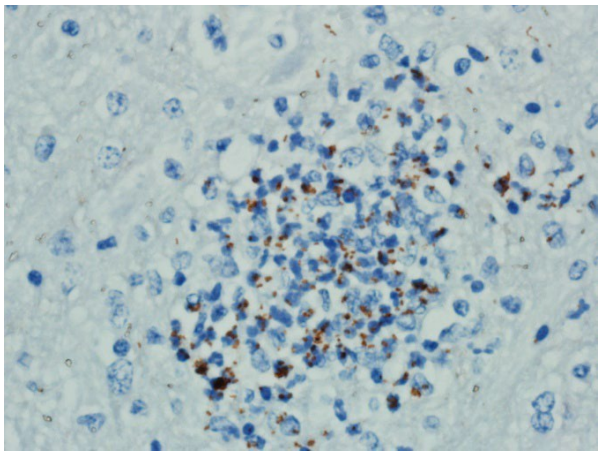

Figure 1-5. Brain: Within the microabscesses in the brain and cervical spinal cord, there were low to moderate numbers of bacteria that bound to anti-*Listeria monocytogenes* antibody. (Courtesy of United States Army Medical Research Insti-tute of Infectious Diseases; https://www.usamriid.army.mil)

been seen this year in a case of sepsis in a non-human primate (Conference 10, Case 3), but the CNS presentation of Listeriosis, particularly in a small ruminant, is worth its own conversation. The contributor provided a wonderful write-up on this entity, which contains most of what was discussed in conference. There was a quick discussion on the minimum histologic features of vasculitis, which include inflammatory cells (particularly neutrophils) AND cellular debris in the vessel wall, and extrusion of protein (which often polymerizes into fibrin) within the vessel wall.

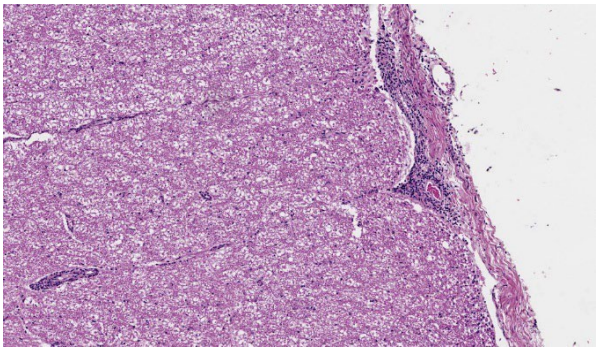

Figure 1-6. Cervical spinal cord: There are scattered aggregates of lymphocytes, plasma cells and fewer macrophages within the meninges and extending down along Virchow-Robin spaces. (HE, 189X)

Listeria monocytogenes was first described in 1926 by Murray as *Bacterium monocytogene*s after isolating it from the livers of diseased rabbits and guinea pigs.[1] A year later in 1927, Pirie isolated a similar organism from gerbils and proposed the genus name *Listerella* in 1930 to honor British surgeon and antiseptic technique frontman, Dr. Joseph Lister.[1] Dr. Lister discovered that sterilizing surgical instruments prior to use resulted in fewer post-operative infections. He is also the inspiration behind the name for one of our most popular antiseptic brands, “Listerine.” "Listerella", however, was a name previously used for a slime mold, so Pirie changed the name to *Listeria* in 1940.[1]

In cases of listeric encephalitis, *Listeria monocytogenes* is most classically associated with a rhombencephalitis, which is inflammation of the brainstem and cerebellum. These areas are most often affected due to the retrograde axonal transport *Listeria* makes along the trigeminal nerve. The sensory root for the trgimenal nerve emerges from the ventrolateral aspect of the pons, specifically the middle cerebellar peduncle, which connects the pons to the cerebellum.[3]

References:

1. Etymologia: *Listeria*. *Emerg Infect Dis*. 2016;22(4):633.
2. Maxie MG, Youssef S. Nervous System. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals.* Vol I. 5th ed. Elsevier; 2007:405-408.
3. Ogino MH, Tadi P. Neuroanatomy, Trigeminal Reflexes. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025.
4. Zachary J. Mechanisms of Microbial Infections. In: Zachary J, McGavin MD, ed.

Pathologic Basis of Veterinary Disease. 5th ed. Elsevier, 2012:192-195.
5. Zachary J. Nervous System. In: Zachary J, McGavin MD, ed. *Pathologic Basis of Veterinary Disease.* 5th ed. Elsevier, 2012:845-846.
6. https://www.fda.gov/.../get-facts-about-listeria
7. https://www.cdc.gov/listeria/index.html.
8. https://www.canr.msu.edu/news/listeriosis-in-sheep-and-goats.

CASE II:

Signalment:
7-week-old, intact male, mixed breed dog (*Canis lupus familiaris*)

History:
This puppy came from a litter of 14 born to a rescued, unvaccinated dam. Half of the litter failed to thrive and was reportedly unable to latch; affected puppies were subsequently tube fed. There was concern for aspiration pneumonia and antibiotic treatment was initiated. The puppies had been dewormed and received the first of a vaccine series. This puppy was acutely anorexic, and was bottle fed prior to death.

Gross Pathology:
The liver was diffusely mottled yellow to red and had tens of pinpoint, flat, red discoloration randomly scattered across the capsular surface. The lungs were diffusely mottled tan to pink, slightly rubbery, and exuded a moderate amount of clear to yellow, watery to slightly viscous fluid on cut section. On cut surface of the brain, primarily within the thalamus, was a fairly well demarcated region of red to brown discoloration and there were pinpoint regions of red-brown discoloration throughout the adjacent parenchyma.

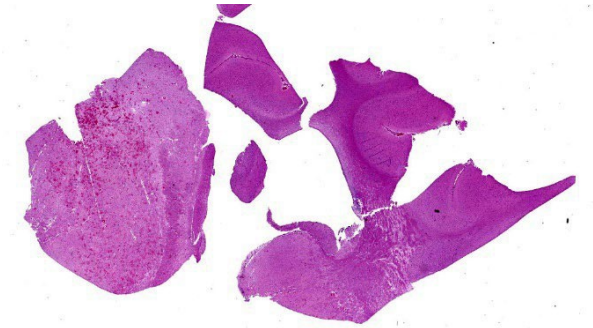

Figure 2-1. Cerebrum, dog: Multiple sections of cerebrum are submitted for examination. At subgross magnification, multiple hemorrhagic foci may be seen in the section at left. (HE, 9X)

Laboratory Results:
- Canine adenovirus-2 PCR: Positive
- Aerobic culture, lung: No growth
- Aerobic culture, liver: No growth
- Canine herpesvirus PCR: Negative

Microscopic Description:
Throughout the cerebral gray matter, small caliber vessel walls were frequently obscured by mild to moderate amounts of eosinophilic, fibrillar, proteinaceous material and hemorrhage. Endothelial cells frequently contained large, eosinophilic, intranuclear inclusions which peripheralize chromatin. Affected vessels were frequently cuffed by moderate numbers of macrophages and lymphocytes, and there were frequent, small areas of hemorrhage spilling into the adjacent tissue. The meninges overlying the cerebellum were moderately expanded by lymphocytes and macrophages, and endothelial cells frequently contained intranuclear inclusions similar to those found in the cerebral vessels.

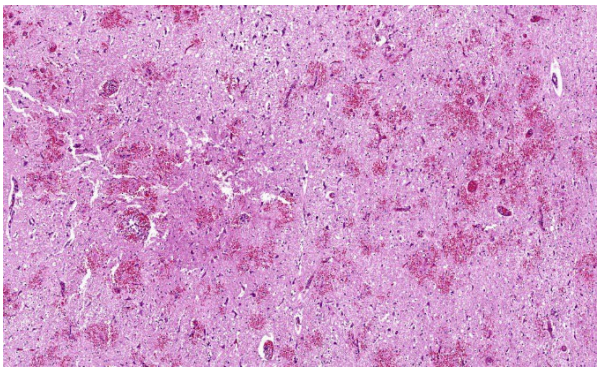

Figure 2-2. Cerebrum, dog: Higher magnification of the numerous foci of hemorrhage within the cerebrum (HE, 102X)

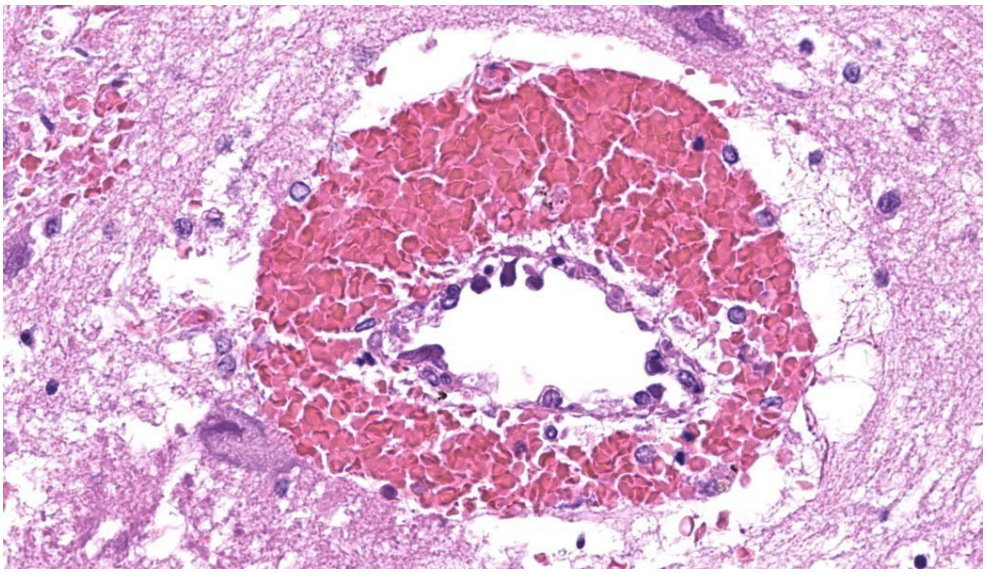

Figure 2-3. Cerebrum, dog: High magnification of an affected vessel with perivascular hemorrhage. (HE, 990X)

Contributor's Morphologic Diagnoses:
Severe, multifocal, acute, histiocytic vasculitis and meningitis with cerebral hemorrhage and intranuclear viral inclusions.

Contributor's Comment:
Adenoviruses are linear, double-stranded DNA, icosahedral viruses which are subdivided into four genera – *Mastadenovirus*, *Aviadenovirus*, *Atadenovirus*, and *Siadenovirus*. Mammalian adenoviruses generally belong to the *Mastadenovirus* genus and are categorized based on neutralization assays. This viral family replicates within nuclei, leading to the formation of characteristic intranuclear inclusions seen in a variety of cell types depending on the species and site of infection. Canine adenovirus-1 infection, colloquially known as infectious canine hepatitis, most often infects hepatocytes leading to acute hepatitis, but can infect the endothelium, causing disseminated intravascular coagulation and hemorrhage. Infection with canine adenovirus-2 is typically localized to the respiratory system, causing mild, non-specific signs of acute respiratory disease which eventually resolve.[8]

Although unusual, systemic infection with canine adenovirus-2 has previously been reported.[1] In one report, neurologic signs were noted in 3 of 4 puppies out of a litter of 9 who were diagnosed with CAV-2 systemic infection. Two of these puppies displayed concurrent, mild respiratory signs. Interestingly, as in the submitted case, these puppies were born to an unvaccinated dam. In contrast to the submitted case, despite the clinical presence of neurologic abnormalities prior to death, nervous system lesions were not present histologically, although virus was detected via PCR in the brain in one affected animal.[1]

While reports of CAV2-induced vasculitis are sparse, other species of adenovirus-associated vasculitis are more common. Perhaps the most similar syndrome of adenovirus-associated vasculitis is seen in adenovirus hemorrhagic disease of cervids. Clinically, this disease presents similarly to epizootic hemorrhagic disease, with histologic lesions including systemic vasculitis and petechiae, along with intranuclear inclusions which separate this disease from epizootic hemorrhagic disease. Affected animals have numerous intranuclear inclusion bodies found in the endothelial cells of a variety of organs, including the brain, which are occasionally associated with fibrin thrombi or fibrinoid necrosis, vasculitis, and/or perivascular cuffs.[2,9] In affected animals, lesions are most frequent in the lungs

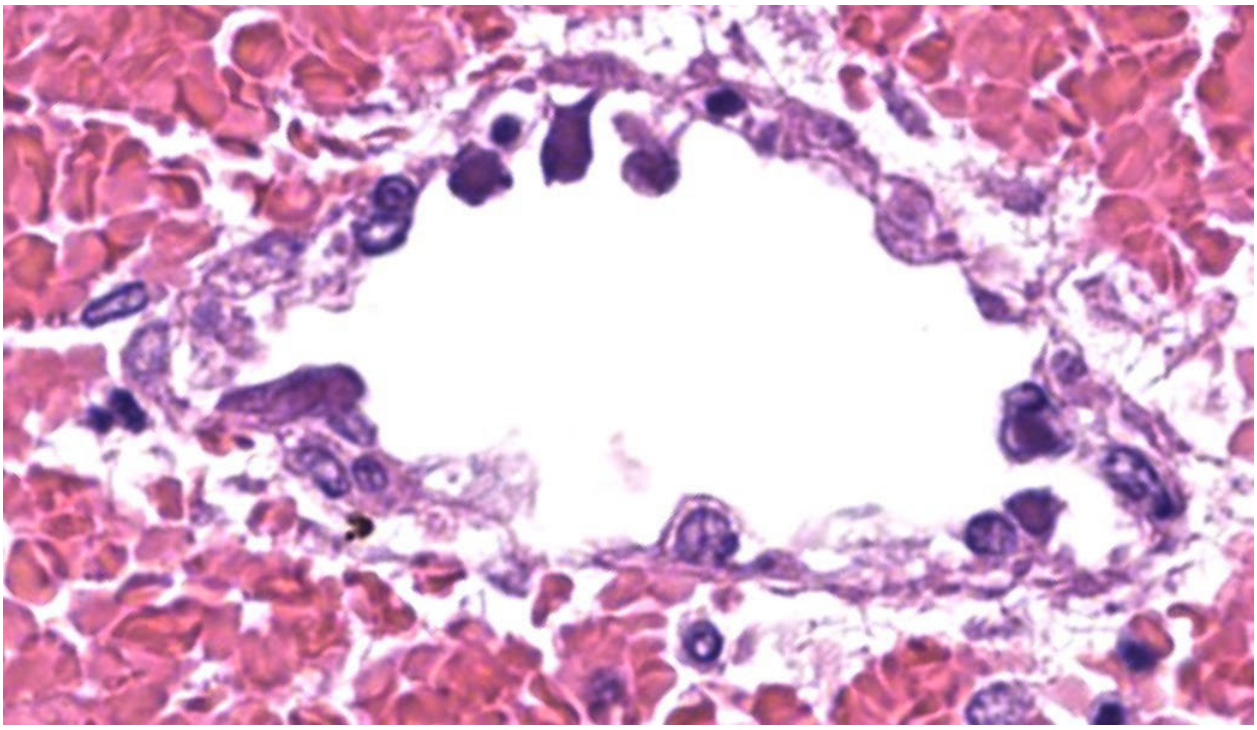

Figure 2-4. Cerebrum, dog: High magnification of endothelial cells which are swollen by karyomegalic intranuclear viral inclusions consistent with adenoviral inclusions. (HE, 2500X)

and brain, although hepatic lesions may also be seen, as in this case.[9]

Human adenovirus of an undetermined serotype has been reported as a cause of acute retinal necrosis due to occlusive vasculitis.[7] One study examining the presence of vasculitis associated with severe respiratory infections found uncommon reports of thrombosis or vasculitis associated with human respiratory adenovirus infections.[5] Following a variety of immunosuppressive therapies and stem cell transplant, adenovirus-associated vasculitis with microscopic regions of cortical necrosis and hemorrhage were seen in a human multiple myeloma patient. In this case, abundant viral inclusions were found in the endothelial cells, but not epithelial cells within the examined organs.[4]

In the current case, CAV-2 was detected via PCR and immunohistochemistry within the histologically observed cerebral and meningeal lesions associated with vasculitis. Previously reported cases of disseminated CAV-2 infection with vasculitis have been proposed to be associated with other infectious agents or diseases causing immunosuppression. There was not evidence of an underlying cause for immunosuppression in the current case, and there was no evidence of concurrent systemic disease. Thus, the vascular lesions associated with CAV-2 appear to be the primary cause of disease in this puppy. As in the above discussed previously reported case of CAV-2 infections associated with neurologic signs in a litter of puppies, it appears likely that the lack of maternal vaccination may have contributed to disease in this case.[1]

Contributing Institution:
National Institute of Health Comparative Biomedical Scientist Training Program

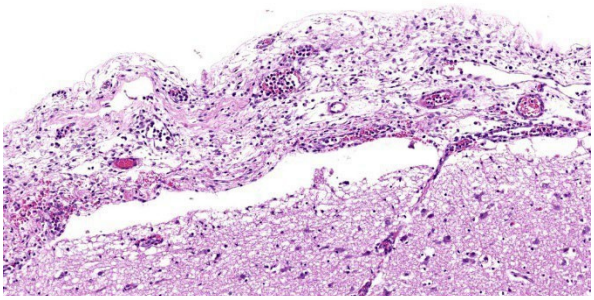

Figure 2-5. Cerebrum, dog: There is mild lymphoplasmacytic meningitis with edema. Vascular changes are present, but far less severe than in the underlying neuropil. (HE, 246X)

JPC Diagnosis:
Cerebrum: Vasculitis, neutrophilic and lymphocytic, subacute, multifocal to coalescing, marked, with fibrin thrombi, lymphohistiocytic meningitis, and endothelial intranuclear viral inclusions.

JPC Comment:
This case stimulated great discussion during conference and the WSC team would like to thank this contributor for sending in such an interesting submission and a fantastic write-ip! MAJ Sulkosky stated that, although the causative agent was diagnosed as CAV-2 by the contributor, this case should be considered classic for a CAV-1 infection in a puppy. CAV-1 has been seen a couple of times recently in the WSC in 2022, Conference 22, Case 3, and in 2019, Conference 16, Case 1.

Conference participants speculated as to why CAV-2 was isolated in this case, as there was no mention of testing for CAV-1 in the contributor's writeup, and wondered if there had potentially been cross-reactivity on the test. The papers that describe CAV-2 encephalitic disease in dogs lack supporting evidence to demonstrate that the lesions were caused exclusively by CAV-2, so attendees remained unconvinced.[1,3] Most wondered if this case was instead a CAV-1 encephalitis and the CAV-2 positivity was either a false positive, was showing as positive due to the recent history of a distemper combo vaccine in this puppy, or was due to a co-infection with CAV-1 that was just not tested for. It is still unclear if CAV-2 can truly cause vasculitis in the brains of puppies. CAV-2 is considered primarily a mild respiratory pathogen, and there are very rare reports of other organs affected.

There is one paper that describes 2 cases of fatal encephalitic CAV-1 infection in puppies, but the sequence of the pVII gene in that CAV-1 was more closely related to that seen in CAV-2, suggesting a possible recombinant strain.[10] The pVII gene in canine adenoviruses encodes an arginine-rich core protein (pre-VII) which is cleaved to form pVII. The pVII protein is responsible for condensing the viral DNA within the capsid, acting like a histone protein.[6] pVII plays a critical role in the virus's structure and, due to its nuclear localization, serves as a target for labeling in research studies.[6]

Wrapping up this spirited case discussion was a review of the viruses that form paracrystalline arrays that can be seen with electron microscopy. This list of viruses can be remembered using the acronym "PPPICCA," which stands for paramyxovirus, picornavirus, polyomavirus, iridovirus, circovirus, calicivirus, and adenovirus.

References:

1. Benetka V, Weissenböck H, Kudielka I, Pallan C, Rothmüller G, Möstl K. Canine adenovirus type 2 infection in four puppies with neurological signs. 2006;158(3):91–94.
2. Burek-Huntington K, Miller MM, Beckmen K. Adenovirus hemorrhagic disease in Moose (*Alces americanus gigas*) in Alaska, USA. *J Wildl Dis*. 2021;57(2):418–422.
3. Chander V, et al. Isolation and genetic characterization of canine adenovirus type

2 from a domestic dog showing neurological symptoms. *Braz J Microbiol*. 2021 Dec;52(4):2521-2528.
4. Claveau JS, LeBlanc R, Ahmad I, et al. Cerebral adenovirus endotheliitis presenting as posterior reversible encephalopathy syndrome after allogeneic stem cell transplantation. *Bone Marrow Transplantation*. 2017:52;1457–1459.
5. Dolby HW, Potey P, Wilder-Smith AB, et al. Histological evidence of pulmonary microthrombosis and vasculitis in life-threatening respiratory virus diseases. *Open Forum Infect Dis*. 2021;8(2).
6. Morrison MD, Onions DE, Nicolson L. Complete DNA sequence of canine adenovirus type 1. *J Gen Virol*. 1997;78(4):873-878.
7. Özdemir HB, Özdal PÇ. Human adenovirus: An unusual causative agent for acute retinal necrosis presented with central retinal vascular occlusion. *Ocul Immunol Inflamm*. 2020;28(5):749–753.
8. Quinn PJ, Markey BK, Leonard FC, Fitz-Patrick ES, Fanning S, Hartigan PJ. Adenoviridae. In: *Veterinary Microbiology and Microbial Disease, 2nd ed*. Blackwell Publishing; 2011:588-592.
9. Woods LW, Swift PK, Barr BC, et al. Systemic Adenovirus Infection Associated with High Mortality in Mule Deer (*Odocoileus hemionus*) in California. *Vet Pathol*. 1996;33.
10. Wong M, Woolford L, Hasan NH, Hemmatzadeh F. A Novel Recombinant Canine Adenovirus Type 1 Detected from Acute Lethal Cases of Infectious Canine Hepatitis. *Viral Immunol*. 2017;30(4):258-263.

CASE III:

Signalment:
1-year-old, intact female, pit bull dog (*Canis familiaris*)

History:
History of weight loss despite appetite and diarrhea. Animal became neurologic and blind for approximately one week until developing seizures. No reported bloodwork abnormalities (complete blood count, blood serum chemistry, and bile acids). Dog was euthanized and submitted for necropsy.

Gross Pathology:
The animal was emaciated with generalized muscle atrophy and scant visceral adipose. The pancreas was reduced to small, thin, red-grey lobules. In the small intestine, there was mild ancylostomiasis with segmental intraluminal hemorrhage. The feces in the distal colon were pale green and soft. The lateral ventricles of the brain were mildly dilated.

Laboratory Results:
- *Toxoplasma gondii* PCR: positive (CT brain: 25.4, heart: 21.4)

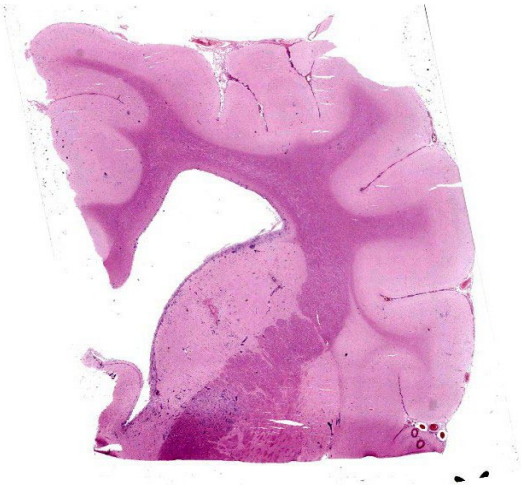

Figure 3-1. Cerebrum, dog: A section of cerebrum is submitted for examination. At subgross magnification, there is mild dilation of the lateral ventricle, and hypercellularity of the periventricular white matter is visible. (HE, 9X)

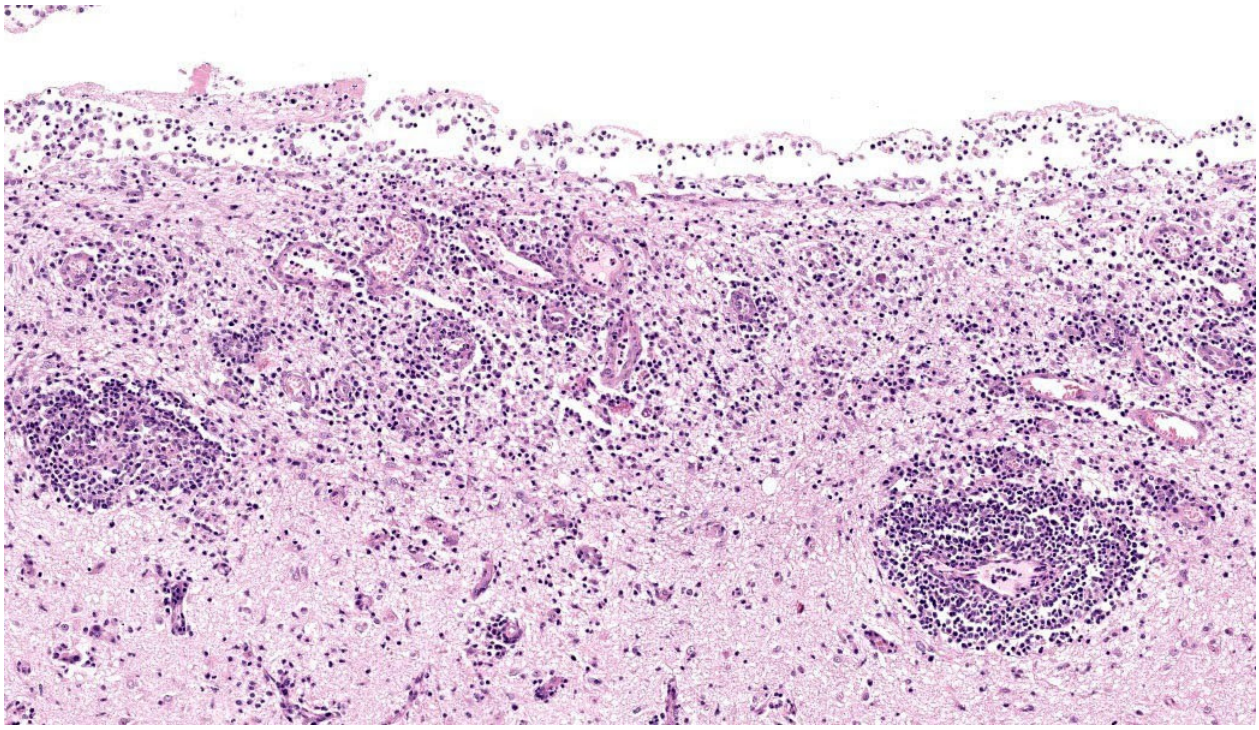

Figure 3-2. Cerebrum, dog: While there is inflammation throughout all layers of the cerebrum, to including both gray and white matter, inflammation is most prominent in the edematous periventricular white matter. This area is infiltrated by large numbers of of lymphocytes, macrophages, and fewer neutrophils and plasma cells. Cuffs of numerous lymphocytes and plasma cells, and fewer macrophages are present around blood vessels. (HE, 194X)

- *Neospora caninum* PCR: not detected
- Fecal testing: *Ancylostoma* spp. ova detected

Microscopic Description:
Brain: The meninges are moderately expanded by macrophages, plasma cells, and lymphocytes that surround and partially obscure reactive meningeal vessels. Inflammatory cells extend around vessels into the subtending parenchyma, filling and expanding Virchow Robin spaces up to 12-15 cell layers thick. There are focal areas of infiltration of macrophages, lymphocytes, and plasma cells into the surrounding parenchyma with associated vacuolation and necrosis of the parenchyma, neuronal satellitosis and necrosis, infiltration of gitter cells, gliosis, and astrocytosis. Isolated within the parenchyma and associated with areas of inflammation and necrosis are numerous 12 to 45-µm diameter protozoal cysts containing closely packed clusters of 1 to 2-µm wide, crescent-shaped bradyzoites. At the level of the corpus striatum, there is vacuolation of white matter tracts with occasional spheroids, digestion chambers, and numerous protozoa. Vessels adjacent to the lateral ventricle are obscured by macrophages, lymphocytes, plasma cells, and neutrophils with indistinct, hypereosinophilic vascular walls (necrosis) and perivascular exudation of fibrin and edema. The ependyma spanning the lateral ventricle is segmentally discontinuous with disruption by inflammatory cells and necrotic material that extend into the ventricular lumen.

Contributor's Morphologic Diagnoses:
Brain: Meningoencephalitis and ventriculitis, necrotizing and histiocytic to lymphoplasmacytic, subacute, multifocal, moderate,

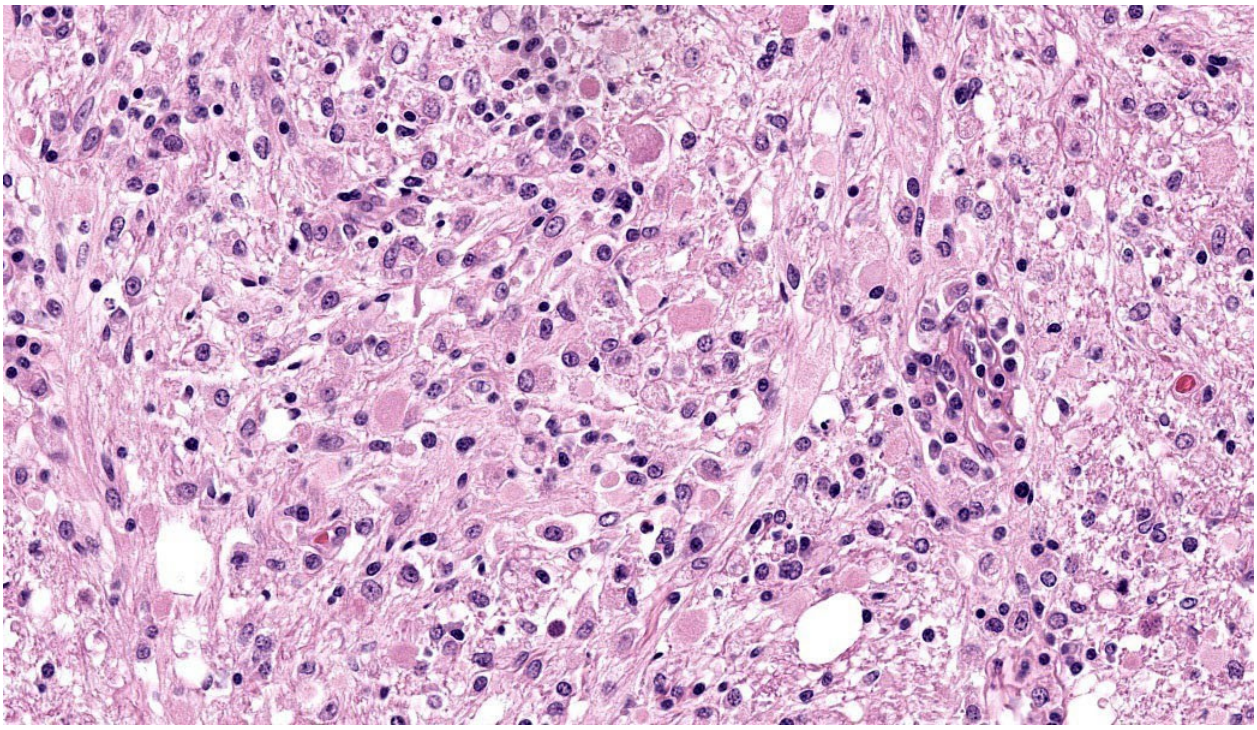

Figure 3-3. Cerebrum, dog: Within the area of rarefaction and inflammation within the internal capsule, there is infiltration of large number of lymphocytes, macrophages, and fewer plasma cells and numerous dilated axon sheaths and spheroids. (HE, 582X)

with protozoal cysts and zoites, vasculitis and vascular necrosis.

Contributor's Comment:

Toxoplasmosis is caused by the apicomplexan protozoan *Toxoplasma gondii*.[4] It can infect a variety of hosts including mammals and birds and causes a wide spectrum of disease.[3,4] Toxoplasmosis is an important zoonotic pathogen and cause of abortion in primates, prosimians, and ruminants.[6,7]

Infected felines may serve as definitive or intermediate hosts, while all other non-felids act as intermediate hosts.[3,4,7] In felines, enteroepithelial replication culminates in the release of unsporulated oocysts in the feces, which sporulate in the environment and become a source of infection for both definitive and intermediate hosts.[4,7] Oocysts are only produced in the definitive felid host.[3,4] Infection of intermediate hosts follows an extraintestinal replication cycle with rapid division of tachyzoites spread widely throughout multiple tissues.[6] A third infective form involves the deposition of latent tissue cysts in tissues containing slowly replicating bradyzoites.[3,4] Infection occurs following ingestion of sporulated oocysts shed from definitive host in the environment or via consumption of zoites or tissue cysts from infected prey. Alternatively, infection can occur transplacentally.[3]

Manifestation of clinical disease may vary given the wide distribution of protozoa within tissues. Systemic disease is classically characterized by interstitial pneumonia, hepatic necrosis, lymphadenitis, myocarditis, and meningoencephalitis.[7] Other lesions may include uveitis, adrenalitis, nephritis, myositis, and placental necrosis/placentitis.[3]

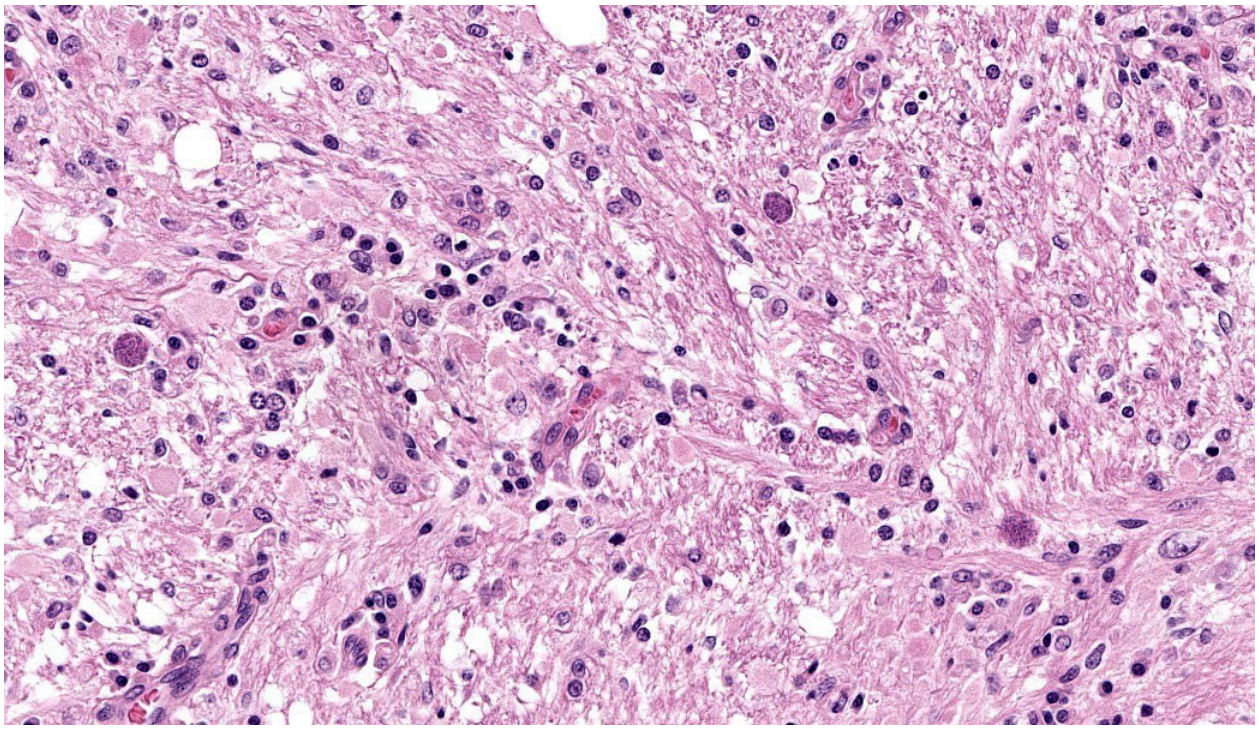

Figure 3-4. Cerebrum, dog: In areas of inflammation (including the internal capsule, pictured here), there are scattered 15-20um apicomplexan cysts containing numerous tachyzoites. (HE, 582X)

Central nervous system disease is more common in young or immunosuppressed individuals and is difficult to differentiate histologically from *Neospora caninum*, *Sarcocystis* spp., or *Encephalitozoon cuniculi*, thus requiring additional testing modalities (immunohistochemistry, PCR, and/or special histochemical staining) to differentiate.[4] However, observing necrosis in multiple organ systems associated with the presence of protozoal infective stages is most suggestive of toxoplasmosis.[7]

In dogs, toxoplasmosis is rarely a primary disease and often seen in conjunction with immunosuppression or comorbidities.[2] Immunosuppression was suspected in this dog. Although clinical data (i.e. serum trypsin-line immunereactivity) supportive of exocrine pancreatic insufficiency (EPI) was not available, the reported clinical signs of diarrhea and weight loss despite sufficient appetite are suggestive of EPI. This is further supported by postmortem lesions of pancreatic atrophy, pale stools, and emaciation. It is possible that EPI resulted in immunosuppression from chronic malnutrition, nutrient deficiency, and possible cobalamin deficiency from small intestinal bacterial overgrowth (SIBO). These factors may have predisposed this dog to the development of systemic toxoplasmosis.

Contributing Institution:
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JPC Diagnoses:
Cerebrum: Meningoencephalitis, necrotizing and lymphohistiocytic, subacute, multifocal to

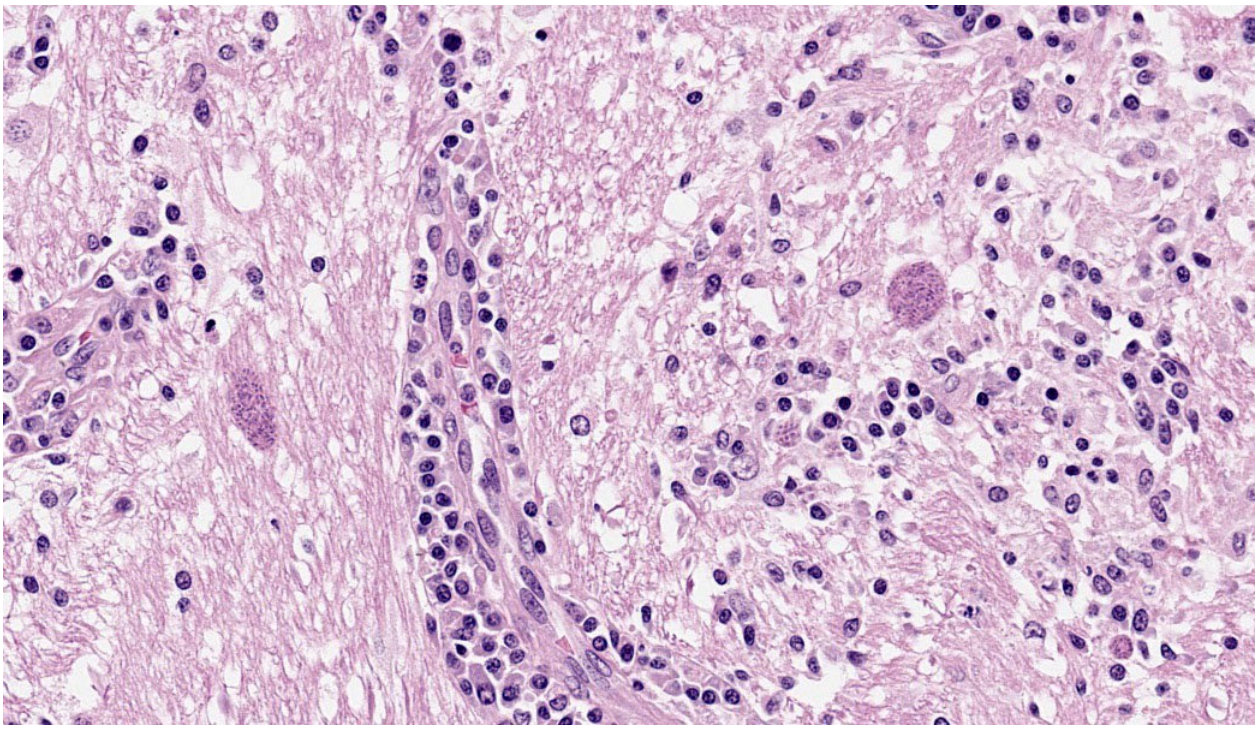

Figure 3-5. Cerebrum, dog: In areas of inflammation, there are scattered 15-20um apicomplexan cysts containing numerous zoites. (HE, 691X)

coalescing, marked, with vasculitis and apicomplexan cysts.

JPC Comment:
A classic and oft-seen entity in the WSC, *Toxoplasma gondii* makes another appearance this year in a fantastic canine case. Organisms were readily apparent within the neuropil of the periventricular grey matter on the H&E slide, prompting an excellent discussion of *T. gondii* and the zoonotic risk these infections pose. The contributor provided an excellent comment in this case and, once again, many of their points were mentioned in conference discussion.

Toxoplasma gondii is an obligate intracellular apicomplexan protozoal parasite that was named in 1908 by researchers Nicolle and Manceaux.[1] They the names comes from the Greek word "toxon*"* (meaning "bow" or "arc"), referring to the crescentic shape of the tachyzoite stage, and "gondii", chosen in honor of the North African gundi (*Ctenodactylus gundi*). This rodent is the host animal in which the parasite was first identified.[1] Since its initial discovery in the gundi, *T. gondii* has been identified in numerous mammalian species and in a wide range of tissue types.

The pathogenesis of *T. gondii* starts with penetration of the intestinal mucosa by a tachyzoite, sporozoite, or bradyzoite either from ingestion of an infected host, from transplacental transmission, or by consumption of oocysts from the environment. The zoites can then either be trafficked in leukocytes to other tissues (i.e., lymph nodes and liver) or remain free in the bloodstream. The tachyzoites replicate within host cells until the cell lyses, at which point the tachyzoites are free to infect other cells and cause additional tissue damage. Cell-mediated immunity eventually can control, but not eliminate, the infection, and the parasites form dormant tissue cysts filled with

bradyzoites within the heart, skeletal muscle, and brain in the chronic stages of infection.

Systemic toxoplasmosis is seen predominantly in neonates that were infected transplacentally or in immunocompromised animals that have low levels of interferon gamma and an inability to activate macrophages. In systemic "toxo", the hallmarks of infection include necrosis and the presence of zoites. These most often manifest as either interstitial pneumonia (most common), meningoencephalitis (with necrosis, malacia, vasculitis, and vascular necrosis), focal hepatic necrosis, lymphadenitis, and/or myocarditis.

In cases of meningoencephalitis, the parasites can enter the brain by breaching the blood–brain barrier (BBB) using three possible mechanisms: 1) infection of monocytes that then traverse the endothelium, 2) direct penetration and migration across the BBB, or 3) infection of and growth within endothelial cells and eventual exit into the neuropil.[5] Once inside the brain, *T. gondii* can infect multiple cell types, including astrocytes, microglia, and neurons, and form tissue cysts.[5]

The contributor mentioned that this dog also had histologic and gross evidence of pancreatic atrophy and atrophic lymphocytic pancreatitis. In dogs, juvenile pancreatic atrophy is an immune-mediated condition characterized by infiltration of T-cells (predominantly CD8+) into the pancreas.[2] Around 6-12mo of age, affected dogs start to demonstrate clinical signs of exocrine pancreatic insufficiency. Histologically, pancreatic ducts and connective tissue structures predominate in the later stages of this condition with fatty replacement and minimal exocrine tissue, little inflammation, and fibrosis.[2] Participants speculated on and agree with the contributor that the exocrine pancreatic insufficiency suspected in this dog may have led to immunocompromise and subsequent systemic toxoplasmosis infection.

References:

1. Black MW, Boothroyd JC. Lytic cycle of Toxoplasma gondii. *Microbiol Mol Biol Rev*. 2000;64(3):607-623.
2. Brenner K, Harkin KR, Andrews GA, Kennedy G. Juvenile pancreatic atrophy in Greyhounds: 12 cases (1995-2000). *J Vet Intern Med*. 2009;23(1):67-71.
3. Calero-Bernal R, Gennari SM. Clinical toxoplasmosis in dogs and cats: an update. *Front Vet Sci.* 2019;6:54.
4. Cantile C, Youssef S. Nervous System. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. Vol. 1.* 6th ed. St. Louise, MO: Elsevier; 2016:387-389.
5. Courret N, Darche S, Sonigo P, Milon G, Buzoni-Gâtel D, Tardieux I. CD11c- and CD11b-expressing mouse leukocytes transport single Toxoplasma gondii tachyzoites to the brain. *Blood*. 2006;107(1):309-316.
6. McAloose D, Stalis IH. Prosimians. In: Terio KA, McAloose D, St. Leger J, eds. *Pathology of Wildlife and Zoo Animals*. San Diego, CA: Elsevier; 2018: 338.
7. Uzal FA, Plattner BL, Hostetter JM. Alimentary system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. Vol. 2.* 6th ed. St. Louise, MO: Elsevier; 2016:236-238.

CASE IV:

Signalment:
4-month-old, female, Angus cow (*Bos taurus*)

History:
Calf presented with history of ongoing generalised ataxia, wide-based stance, incoordination and intermittent head tremor. The calf was otherwise in good body condition. One other calf was similarly affected and there were 74 other unaffected calves in the herd.

Gross Pathology:
There was subjectively increased amount of CSF present upon removing the brain. A unilateral, focal, kidney cyst was also present.

Laboratory Results:

Test	Result
Blood lead	Negative
Chlamydia CFT	Negative
Pestivirus antigen capture ELISA	Negative
CBC & Biochemistry	Mild Elevation GLDH – 15.09 (6.14-13.34 U/L) Moderate Elevation CK – 2401 (43-580 U/L) Mild Elevation AST – 133 (58-125 U/L) Mild elevation Leukocytes – 13.46 (4.8-13.0 U/L) Otherwise unremarkable
CSF Analysis	Colour: Opaque white Transparency: cloudy Flocculation: none Microprotein: 0.515 g/L Total nucleated cell count (TNCC): 0.725 x 10^9/L Red cell count: 0.005 x 10^{12}/L Neutrophils: 22% Small mononuclear cells: 45% Large mononuclear cells: 33% Interpretation: Pleocytosis with elevated protein
Neimann-Pick Type C genotyping PCR	Non-carrier
Alpha-mannosidosis genotyping PCR	Affected

Microscopic Description:
Cerebellum: Multifocally, numerous Purkinje cells at the junction between the molecular and granule cell layer, as well as occasional neurons within the granular cell layer and cerebellar deep nuclei are moderately swollen, have pale granular eosinophilic to clear, finely vacuolated or foamy cytoplasm that often dis-places the nuclei towards the periphery. Neurons are multifocally lost within the Purkinje (empty baskets) and granular layers. Through-out the white matter tracts, there are numerous variably sized (up to 60µm in diameter) hypereosinophilic axonal swellings (spheroids) as well as less frequent dilation of peri-axonal spaces containing fragmented myelin debris (ellipsoids) and foamy macrophages (digestion chambers). Multifocally, few axonal swellings are also present within the granule cell layer (torpedoes).

Contributor's Morphologic Diagnoses:
Cerebellum: Neuronal vacuolation and loss, diffuse, severe with occasional neuronal intracellular granular eosinophilic material accumulation, torpedoes, spheroids and digestion chambers (Wallerian degeneration).

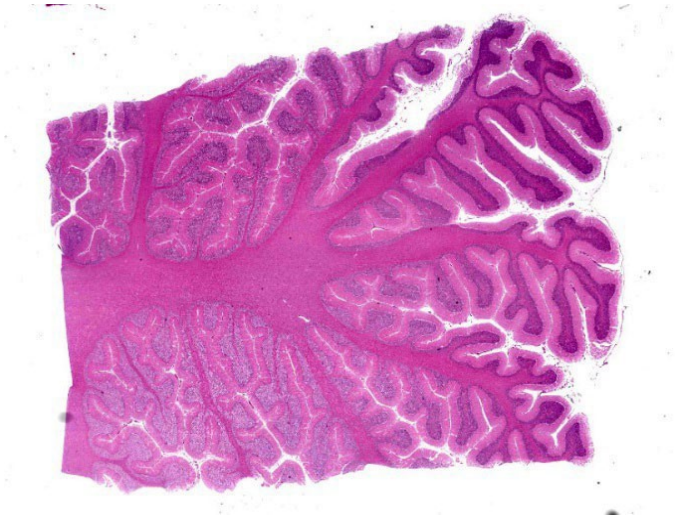

Figure 4-1. Cerebellum, calf: One section of cerebellum is submitted for examination. The cerebellar folia appears thinned. (HE, 9X)

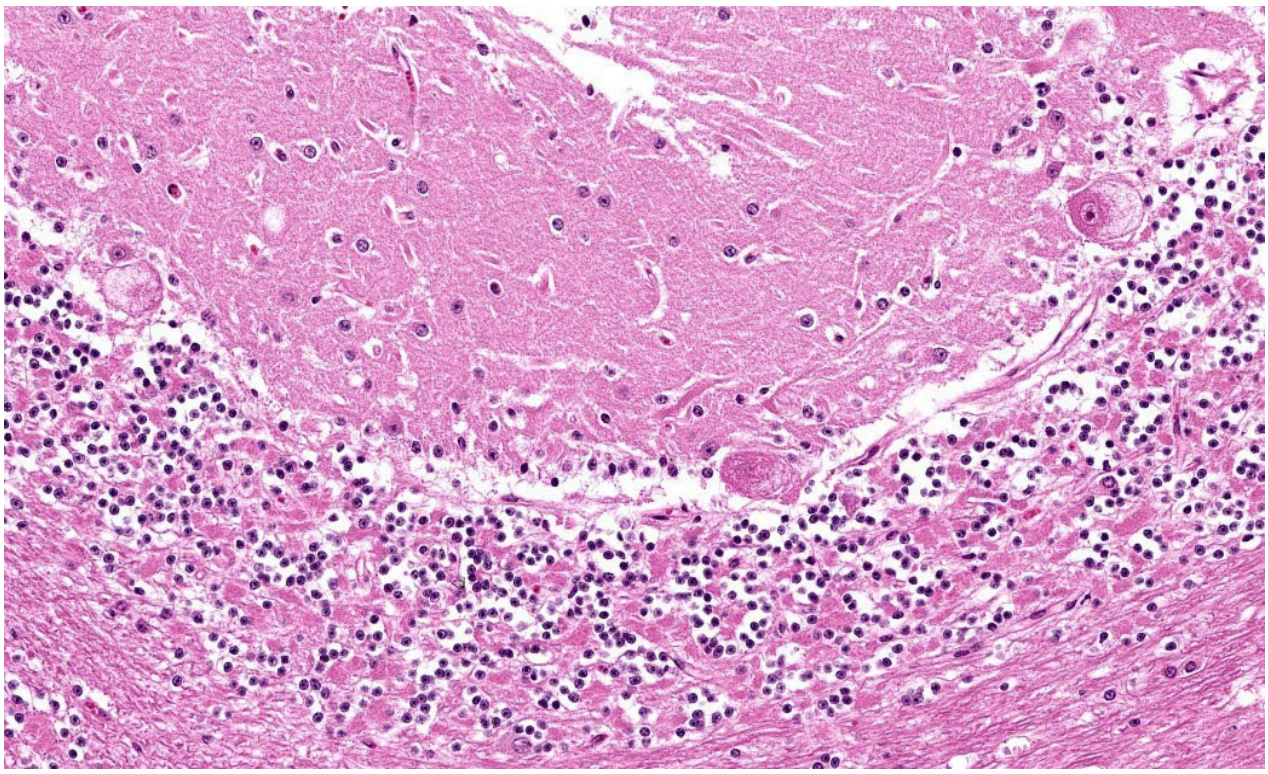

Figure 4-2. Cerebellum, calf: There is segmental loss of Purkinje cells with formation of "empty baskets." Remnant Purkinje cells are swollen due to the accumulation of numerous clear vacuoles within their cytoplasm. (HE, 381X)

Contributor's Comment:

Alpha-mannosidosis is a type of inherited lysosomal storage disease predominantly seen in cattle.[3] The condition is best characterized in Angus and Murray Grey cattle.[3] It has also been reported in Galloway cattle as well as Persian, domestic longhair, and shorthair cats.[2,7,12,15] The condition is rare in humans.

This storage disease was first described in Angus calves in Australia in 1957 and later extensively studied in New Zealand, becoming one of the earliest major investigations into lysosomal storage diseases in animals.[18,24] Due to high economic impact, characterization of the disease and detection of both affected and carrier animals became a priority within the livestock industry. Clinically, affected animals develop ill-thrift, ataxia, incoordination, head and intention tremors, often display a wide-based stance and can be abnormally aggressive.[16,24] These signs can be seen as young as 4 weeks of age, with some animals able to survive up to 18 months of age.[3,24]

Individuals affected with alpha-mannosidosis are deficient in lysosomal alpha-mannosidase activity, leading to accumulation and storage of mannose within vacuoles of affected cells.[3] Under light microscopy, these vacuoles are empty and expand the cytoplasm.[16] The condition affects all cells within the body except hepatocytes, though is generally most notice-able microscopically within the nervous system.[3] In this case, neuronal vacuolation was most prominent within the thalamus and in Purkinje cells of the cerebellum. Few renal tubular epithelial cells were also affected. In addition to neuronal vacuolation, dysfunction and death of neurons has led to other secondary changes such as Wallerian degeneration with prominent spheroid formation, as well as torpedoes.

Histologically, alpha-mannosidosis is typically indistinguishable from other forms of storage disease, however differentials can generally be

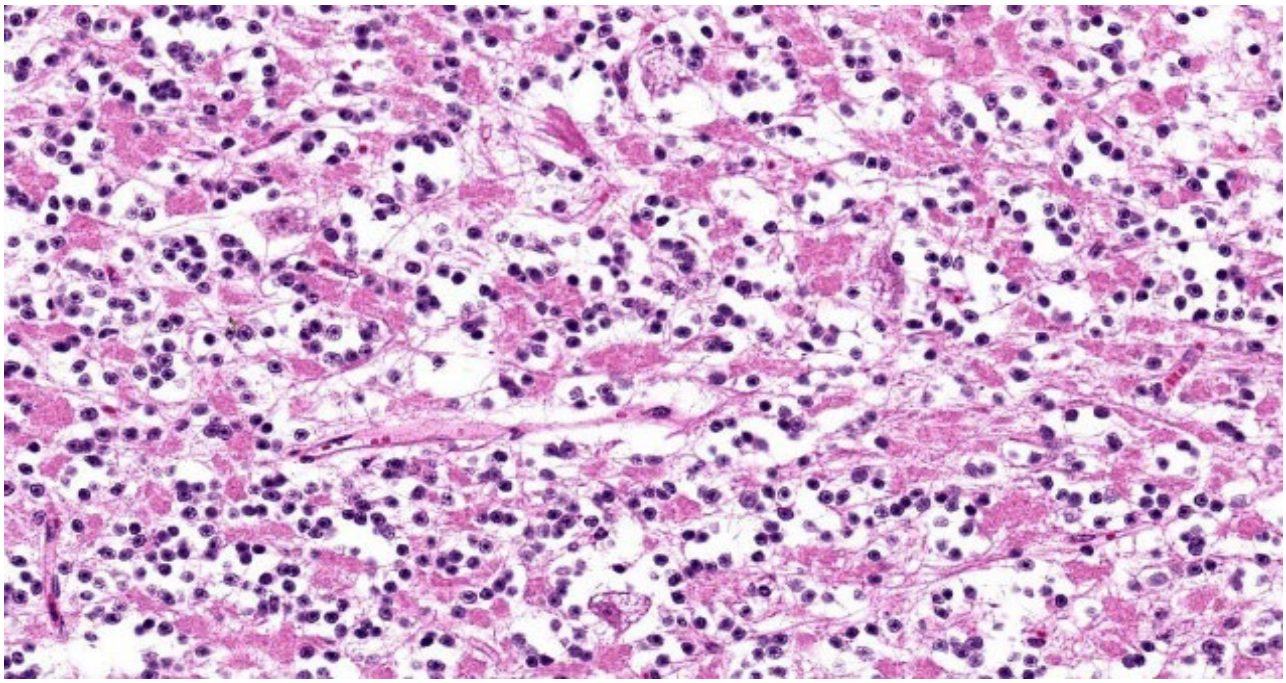

Figure 4-3. Cerebellum, calf: There is diffuse and mild loss of neurons within the granular layer and several neurons are also swollen due to cytoplasmic vacuolation. (HE, 580X)

narrowed down based on the breed affected. In this case, Neimann Pick Type C was also considered as a differential, given this lysosomal storage disease also affects Angus cattle.[25] However, this condition has only been reported in one herd in Australia between 2002 and 2005.[25] Other inherited storage diseases identified in cattle is summarized below (Table 1). While this case was confirmed to be inherited, acquired mannosidosis is also possible in herbivorous species, particularly livestock. Ingestion of plants containing the indolizidine alkaloid swainsonine can lead to inhibition of alpha-mannosidase, resulting in similar effects to the inherited con-dition.[5,6] Common swainsonine containing plants include *Swainsonia* spp. (Darling Pea and Swainson pea) mostly seen in Australia, as well as *Oxytropis* spp. and *Astragalus* spp. (locoweeds) found in areas of Africa, China and North America.[9,14]

Table 1: Inherited storage diseases reported in cattle. [4,8,12,1313,20,21,22,23,24,27]

Disorder	Breed/s affected
Alpha-mannosidosis	Angus, Galloway, Murray Grey
Beta-mannosidosis	Salers
GM, gangliosidosis	Holstein-Friesian
Glycogen storage disease type II (Pompe's disease)	Brahman, Droughtmaster, Shorthorn, Blanco Orejinegro
Sphingomyelinosis (Neimann Pick type C)	Angus
Neuronal ceroid lipofuscinosis*	Beefmaster, Devon, Holstein

*This condition is generally histologically distinct from other storage diseases, as the accumulation of storage material within neurons is pigmented (yellow-brown).

Contributing Institution:

Elizabeth Macarthur Agricultural Institute (EMAi), NSW, DPIRD

https://www.dpi.nsw.gov.au/about-us/science-and-research_old/centres/emai

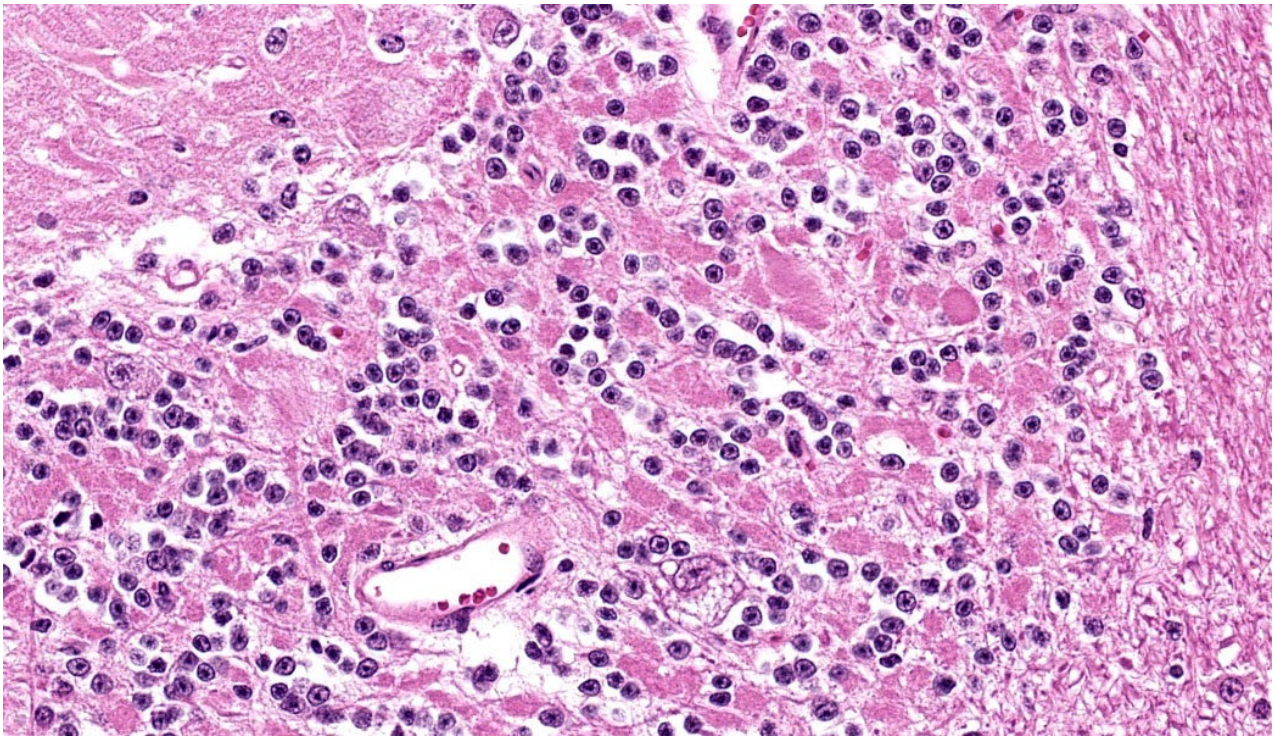

Figure 4-4. Cerebellum: There are numerous spheroids and torpedoes within the depleted granular cell layer. (HE, 590X)

JPC Diagnoses:
Cerebellum: Neuronal vacuolation and loss, chronic, diffuse, severe, with axonal swelling.

JPC Comment:
This case provided a wonderful example of a lysosomal storage disease (LSD) in a bovine. Cattle are the poster children for many different LSDs amongst our domestic species, as mentioned by the contributor in their excellent comment. Other species with known genetic predispositions for certain LSDs include: Nu-bian goats and German shepherd dogs (beta-mannosidosis), English Springer spaniel dogs (alpha-L-fucosidosis), and Persian cats (alpha-mannosidosis). Participants also learned a fun new term to describe the proliferation of microglia around Purkinje cells: "microglial shrubbery."

Cattle affected with alpha-mannosidosis typically are slow to grow, develop progressive ataxia, exhibit behavioral changes, and do not live longer than 18-mo.[3] Most of their cells are affected by this condition except for hepatocytes. However, there is a case report in Galloway calves that reported consistent, severe hepatocyte vacuolation in calves diagnosed with alpha-mannosidosis, so it is possible that a spectrum of severity exists.[11] In other species affected by alpha-mannosidosis, including cats, humans, and certain strains of mice, there is hepatocyte involvement. However, in bovine alpha-mannosidosis, the oligosaccharides that accumulate are similar to those seen in a partial deficiency of normal lysosomal alpha-mannosidase, meaning that they correspond to intermediates in the catabolic pathway.[10] This suggests that there might be some residual activity of alpha-mannosidase in affected cattle. In comparison, the oligosaccharides that accumulate in cats, who are usually more severely affected by alpha-mannosidosis than cattle, are representative of intact moieties rather than intermediates, indicating that there is no residual activity.[10]

This may explain why the liver is generally less affected in cattle compared to other species.

Top differentials in this case discussed during conference included other LSDs, swainsonine toxicosis, and perennial ryegrass toxicosis ("ryegrass staggers"). Of these, perennial ryegrass is the only one not discussed by the contributor in their comment. "Ryegrass staggers" is caused by ingestion of ryegrass containing the endophytic fungus *Epichloë festucae* var. *lolii* (formerly known as *Neotyphodium lolii*). This fungus, while it has been demonstrated to work symbiotically with the ryegrass to increase its hardiness and survivability, produces four indolic lolitrems that are tremorigenic neurotoxins. The most common of these neurotoxins is lolitrem B. These toxins bind and inhibit calcium-activated potassium channels that are responsible for stoppage of action potentials in depolarizing neurons.[3] Inhibition of these channels leads to prolonged cellular depolarization and sustained impulse transmission at the motor endplate, causing sustained tremors and spasms in affected animals. Histologically, there are limited findings, but these can be similar to some of the lesions seen in an LSD. These include proximal axonal swelling ("torpedo formation") in the granular and Purkinje cell layers of the cerebellum and vacuolar degeneration and loss of Purkinje cells.[3] However, there will not be accumulation of material within cytoplasmic vacuoles, as seen with LSDs.

The JPC's morphologic diagnosis for this case made use of the term "axonal swelling" to encompass the torpedoes, spheroids, and digestion chambers seen histologically, as all three of these are variations of axonal swelling at different portions of the neuron. Torpedoes are proximal swellings seen within the Purkinje and granular cell layers of the cerebellum; spheroids are swellings at the distal portions of the axons. Digestion chambers are the result of these swellings, where the axons are undergoing Wallerian degeneration and are fragmenting. The JPC acknowledged that it is not wrong to list these things out individually. Participants simply chose to group them for the sake of brevity.

References:

1. Bryan L, Schmutz S, Hodges SD, et al. Bovine β-mannosidosis: pathologic and genetic findings in Salers calves. *Veteri-nary Pathology.* 1993;30(2):130-9.
2. Burditt LJ, Chotai Kand Hirani Set al. Biochemical studies on a case of feline mannosidosis. *Biochemical Journal.* 1980;189(3):467-73.

1. Cantile C and Youssef S. Nervous System. In: *Jubb, Kennedy and Palmer's Pathology of Domestic Animals.* Vol 2. 6th Ed. St. Louis, MO. Elsevier; 2016: 286-293.
2. Caivio-Nasner S, Lopez-Herrera A and Gonzalez-Herrera LG et al. Frequency of genotypic markers for genetic disorders, colour, polledness, and major genes in Blanco Orejinegro cattle. *Tropical Animal Health and Production.* 2021;53:1-1.
3. Cholich LA, Martinez A and Micheloud JF et al. Alpha-mannosidosis caused by toxic plants in ruminants of Argentina. *Anais da Academia Brasileira de Ciencias.* 2021;93(suppl 3):e20191496.
4. Cook D, Gardner DR and Welch KD et al. A survey of swainsonine content in Swainsona species. *The Rangeland Journal.* 2017;39(2):213-8.
5. Cummings JF, Wood PA, De Lahunta A et al. The clinical and pathologic heterogeneity of feline alpha-mannosidosis. *Journal of veterinary internal medicine.* 1988;2(4):163-70.
6. Donnelly WJ and Sheahan BJ. GM1 gan-

gliosidosis of Friesian calves: a review. *Irish Veterinary Journal.* 1981; 35(3):45-48, 51-55.
7. Darling PR, Huxtable CR and Colegate SM. Inhibition of lysosomal alpha-mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens*. *Biochemical Journal.* 1980;191(2):649.
8. DeGasperi R, al Daher S, Daniel PF, Winchester BG, Jeanloz RW, Warren CD. The substrate specificity of bovine and feline lysosomal alpha-D-mannosidases in relation to alpha-mannosidosis. *J Biol Chem*. 1991;266(25):16556-16563.
9. Embury DH and Jerrett IV. Mannosidosis in Galloway calves. *Veterinary pathology.* 1985; 22(6): 548-551.
10. Hafner S, Flynn Sand Harmon BG et al. Neuronal ceroid-lipofuscinosis in a Holstein steer. *Journal of veterinary diagnostic investigation.* 2005;17(2):194-7.
11. James LF, Kampen KR and Hartley WJ. Comparative pathology of Astragalus (locoweed) and *Swainsona* poisoning in sheep. *Pathologia veterinaria.* 1970;7(2):116-24.
12. Jezyk PF, Haskins ME and Newman LR. Alpha-mannosidosis in a Persian cat. *Journal of the American Veterinary Medical Association.* 1986;189(11):1483-5.
13. Jolly RD. The pathology of the central nervous system in pseudolipidosis of Angus calves. *Journal of Pathology.* 1971; 103:113-121.
14. Jolly RD. Mannosidosis and its control in Angus and Murray Grey cattle. *New Zealand Veterinary Journal.* 1978;26(8):194-8.
15. Jolly RD, Dittmer KE and Jones BR et al. Animal medical genetics: a historical perspective on more than 50 years of research into genetic disorders of animals at Mas-sey University. *New Zealand Veterinary Journal.* 2021;69(5):255-66.
16. Jolly RD, Martinus RD and Palmer ON. Sheep and other animals with ceroid-lipofuscinoses: Their relevance to Batten disease. *American journal of medical genetics.* 1992;42(4):609-14.
17. Jolly RD, Van-De-Water NS and Richards RB et al. Generalized glycogenosis in beef shorthorn cattle-heterozygote detection. *Australian Journal of Experimental Biology and Medical Science.* 1977;55(2):141-50.

    Lyons RE, Johnston DJ and McGowan MR et al. E7 (1057LHA) mutation of the acidic a-glucosidase gene causes Pompe's disease in Droughtmaster cattle. *Australian Veterinary Journal.* 2017;95(5):138-42.
18. O'Sullivan BM, Healy PJ and Fraser IR et al. Generalised glycogenosis in Brahman cattle. *Australian Veterinary Journal.* 1981;57(5):227-9.
19. Read WK and Bridges CH. Neuronal Lipodystrophy. Occurrence in an lmbred Strain of Cattle. *Pathologia veterinaria.* 1969;6(3):235-43.
20. Whittem JH, Walker D. "Neuronopathy" and" Pseudolipidosis" in Aberdeen-Angus calves. *Journal of Pathology and Bacteriology.* 1957; 74:281-288.
21. Woolley SA, Tsimnadis ER and Lenghaus C et al. Molecular basis for a new bovine model of Niemann-Pick type C disease. *Plos One.* 2020;15(9):e0238697.