Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 20

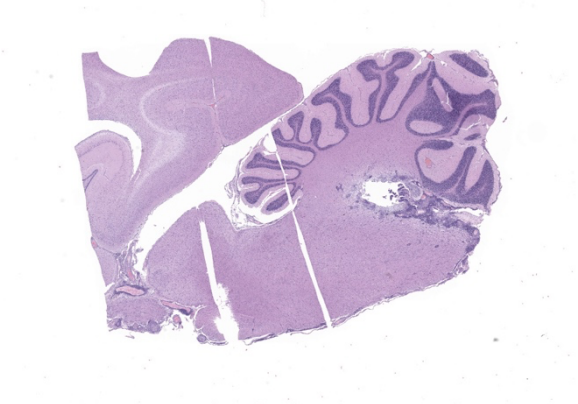
24 March, 2021

Joint Pathology Center
Silver Spring, Maryland

CASE 1: WSC Case 2 (4153750-00)

Signalment:
2-year-old, female spayed, ferret, *Mustela putorius furo*

History:
The ferret presented four months of history of hindlimb weakness with evidence of diarrhea. Blood examination revealed slight anemia and hyperglobulinemia and elevated creatinine. Urine protein electrophoresis was unremarkable, and the blood protein electrophoresis showed a polyclonal spike. CT scan of the spinal cord showed no abnormalities. The animal became acutely paraplegic one night, and euthanasia and post-mortem examination were elected.



Brain, ferret. A sagittal section of diencephalon through myelencephalon, including the cerebellum is submitted for examination. At low magnification, a dense cellular infiltrate is present within the anterior third ventricle and the entirety of the fourth ventricle which extends into tissues surrounding the fourth ventricle. (HE, 6X)

Gross Pathology:
The right adrenal gland was moderately enlarged and had multiple variably sized, discrete, white to tan nodules, near the termination of the right limb of the pancreas with adhesions to the mesentery and duodenum, and a single nodule within the right renal cortex. Significant abdominal lymphadenomegaly and splenomegaly were present.

Laboratory results:
Lesions in the brain and adrenal glands reacted strongly positive for coronavirus gp70 antigen

Fecal PCR for Aleutian Disease Virus and Ferret Coronavirus Genotype 1 and Genotype 2: All were negative

Hematology:

	Result	Reference	Units
WBC	4.40	4.3-10.7	$x10^3$/ul
RBC	7.23	7.01-9.65	$x10^6$/ul
Hemoglobin	11.7	12.2-16.5	gm/dl
Hematocrit	33.0	36-48	%
MCV	45.7	50-54	fl
MCH	16.2	15-18	pg
MCHC	35.4	32-35	gm/dl
RDW	12.8		%
Platelet-Auto	220	200-459	$x10^3$/ul
MPV	7.2		fl

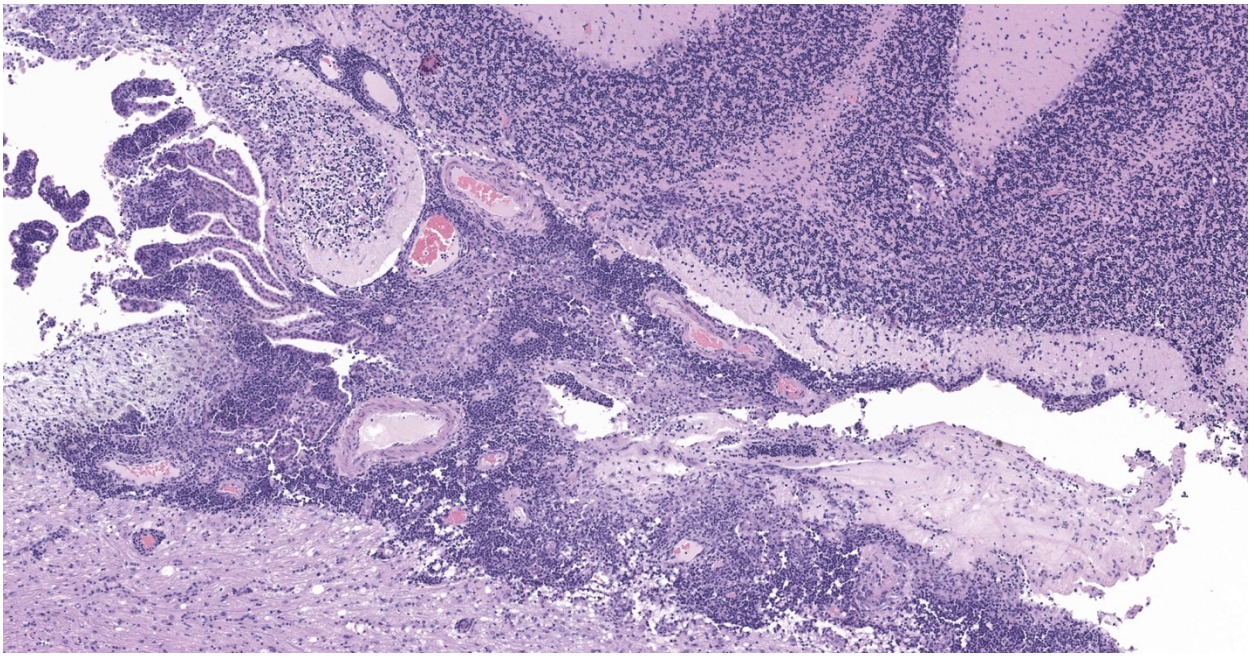
Chemistry:

	Result	Reference	Units
BUN	25	12-43	mg/dl
Creat	0.9	0.2-0.6	mg/dl
Glucose	205	62.5-134	mg/dl
Total Protein	9.1	5.3-7.2	gm/dl
Albumin	2.9	2.5-4.0	gm/dl
Alk Phos	45	25-60	IU/L
ALT	42	14-80	IU/L
Hemolytic Indice	15.0		
Icteric Indice	2.0		
Lipemic Indice	20.0		

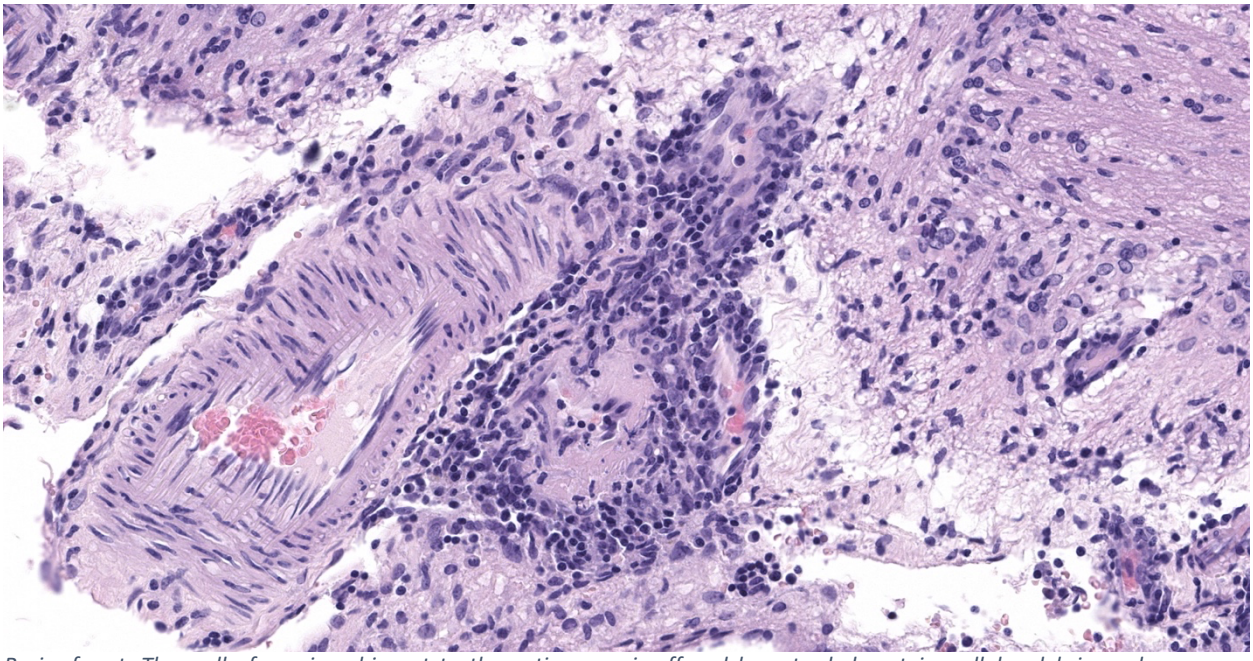
Microscopic description:

Cerebrum, diencephalon, cerebellum, and brainstem: A parasagittal section of the brain is examined. Multifocally and extensively expanding the leptomeninges, Virchow-Robin space, 3rd and 4th ventricle subependyma, choroid plexus, and infiltrating the perivascular and periventricular neuroparenchyma are infiltrates of moderate numbers of lymphocytes, epithelioid macrophages, some plasma cells, and few viable and degenerate neutrophils. The infiltrates usually surround and obscure vessel walls (predominantly veins/phlebitis). Periventricular and meningeal blood vessel walls are often lined by prominent plump, hypertrophic endothelial cells (reactive) and there are circumferential infiltrates of 5-6 layers of lymphocytes and plasma cells expanding Virchow Robbins space. Multifocally, the tunic media and adventitia are expanded and obscured by accumulation of eosinophilic fibrillar material (fibrin). The inflammatory infiltrates multifocally extend beyond Virchow-Robin space into the brainstem and periventricular neuroparenchyma resulting in rarefication and malacia of the neuropil. The adjacent neuropil is expanded by mild to moderate gliosis composed of gemistocytic astrocytes, gitter cells, and microglia.

Adrenal gland: The adrenal architecture of the cortex and medulla is almost completely effaced and severely infiltrated by marked infiltrates of



Brain, ferret. The profound perivascular lymphocytic inflammation expands the choroid plexus, effaces the ependyma and extends into the periventricular white matter, and extends along the cerebellar meninges. (HE, 77X)



Brain, ferret. The wall of a vein subjacent to the optic nerve is effaced by extruded protein, cellular debris, and numerous lymphocytes and histiocytes. (HE 332X)

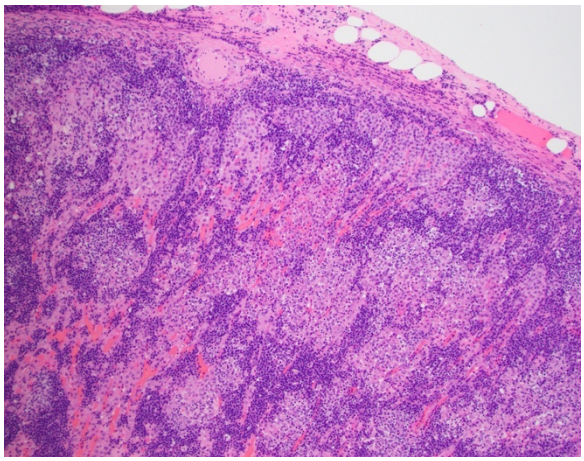
lymphocytes, plasma cells surrounding small aggregates of epithelioid macrophages and occasionally oriented around a central hypereosinophilic and karyorrhectic cellular debris (granuloma). There are similar granulomas with inflammatory infiltrates that extends into the capsule, tunica adventitia and tunica media of the overlying vena cava lined by undulating plump endothelial cells.

(Note: There are slide variation in the submission. A few slides contain section of the adrenal gland)

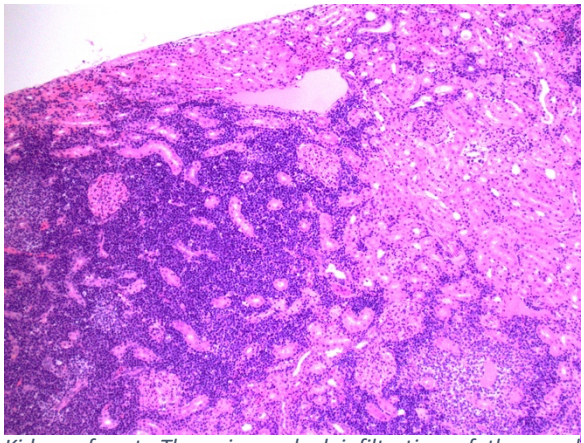
Contributor's morphologic diagnosis:
Cerebrum, cerebellum and brainstem: Meningoencephalitis, perivascular and periventricular, multifocal, pyogranulomatous and lymphoplasmacytic, with phlebitis, periventriculitis, and chorioependymitis, chronic, marked

Adrenal gland: Adrenalitis, multifocal to coalescing, pyogranulomatous and lymphoplasmacytic, chronic, marked

Contributor's comment:
This was a case with morphological findings that were compatible with the infection of ferret systemic coronavirus (FRSCV) and was confirmed by immunohistochemical staining with feline coronavirus anti-gp70 antibody that often cross-reacts with ferret coronavirus. FRSCV-associated disease has been reported in Europe and the USA in the last two decades as a novel and fatal ferret disease remarkably similar to the dry form of feline infectious peritonitis (FIP) in cats.[4,9,10,14] This disease was found associated with an alphacoronavirus that is closely related to ferret enteric coronavirus



Adrenal gland, ferret. There is marked infiltration of the adrenal cortex by a lymphohistiocytic infiltrate. (HE, 100X) (Photo courtesy of Iowa State University, College of Veterinary Medicine Department of Veterinary Pathology, Ames, IA 50010-1250, https://vetmed.iastate.edu/vpath)

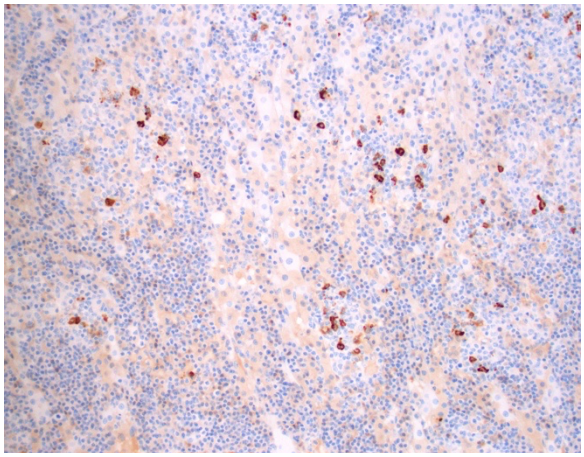


Kidney, ferret. There is marked infiltration of the renal cortex by a lymphohistiocytic infiltrate. (HE, 10X) (Photo courtesy of Iowa State University, College of Veterinary Medicine Department of Veterinary Pathology, Ames, IA 50010-1250, https://vetmed.iastate.edu/vpath)

(FRECV), the cause of epizootic catarrhal enteritis.[10,18] However, sequence data from a limited number of enteric and systemic strains showed that FRSCV differs more from FRECV than feline infectious peritonitis virus (FIPV) does from FCoV.[19] The speculation that FRSCV has similar pathogenesis as FIP has not been confirmed experimentally.[12,19,20] Based on the lower similarity of their S proteins amino acid sequence (79.6%), two ferret coronavirus genotype-specific real-time RT-PCR assays have been developed and remains the gold standard for differentiating between the two viruses.[12,19] Immunohistochemistry using a monoclonal antibody against alphacoronavirus antigen can detect either FRECV or FRSCV but cannot distinguish them.[5,18]

The clinical and pathologic findings in ferrets with FRSCV resemble the non-effusive form of FIPV-induced diseases. FRSCV typically affects ferrets younger than 18 months of age and has common clinical signs of diarrhea, weight loss, lethargy, hyporexia/anorexia, and vomiting.[4,12,14] Ferrets sometimes present primarily neurological symptoms, including hind limb paresis or paraparesis, ataxia, tremors, head tilt, and seizures.[4,12] These clinical signs and hematology findings of nonregenerative anemia, hyperglobulinemia, hypoalbuminemia, and thrombocytopenia are strongly suggestive of the disease but not definite. Serum protein electrophoretograms of FRSCV usually present a polyclonal gammopathy.[4,11-12,14] The histologic evaluation of biopsied or necropsied tissues with demonstrations of intralesional coronaviral nucleic acid remains the definite diagnosis.[1,11]

Predominant gross findings in ferrets with FRSCV include mesenteric lymphadenomegaly and multifocal to coalescing, white to tan irregular nodules of various sizes dispersed along serosal surfaces and mesenteric vessels.[4,12] The inflammation commonly encompasses the small intestines with focal expansion or may completely destroy the muscularis and serosa. Nodules can be found in numerous other organs with the liver, kidneys, spleen, and lung being the commonly affected sites.[4,14] These granulomas are histologically similar to those described in FIP,[2,4] comprising pyogranulomatous to granulomatous inflammation with a central area of necrosis and degenerative neutrophils surrounded by epithelioid macrophages with occasional multinucleated giant cells, lymphoplasmacytic infiltrates, and a variable degree of fibrosis.[1,4,12] Granulomatous inflammation is often centered on vessels and frequently involved the adventitia; thus vasculitis of small arterioles and venules are also common findings.[1,4,12] In ferrets with neurological signs, lesions may be found primarily within the brain with severe pyogranulomatous leptomeningitis, choroiditis, ependymitis, and encephalomyelitis. The inflammation is mainly perivascularly with extension into the underlying parenchyma.[12]



Kidney, ferret. The cytoplasm of macrophages within the cortical inflammatory infiltrate stain strongly immunopositive for coronaviral antigen. (anti- feline coronavirus anti-gp70, 200X)

Gross findings in this case with enlarged adrenal gland, multiple nodules, abdominal lymphadenomegaly, and splenomegaly initially led to the diagnosis of neoplastic diseases. Common primary neoplasms in ferrets include pancreatic islet cell tumors, adrenocortical tumors, and lymphomas.[3] The development of multiple neoplasms, especially islet tumors and adrenal tumors, is not uncommon.[3] Other differential diagnoses of nodules in ferrets include other granulomatous diseases such as mycobacteriosis and infection with *Pseudomonas luteola*.[8,15] It is thus noteworthy that when seeing nodular lesions in ferrets, FRSCV should be considered a potential diagnosis.

Coronaviruses are large, enveloped, positive-stranded RNA viruses constitute the subfamily Orthocoronavirinae of the Coronaviridae family, in the order Nirdovirales (http://www.ictvonline.org).[7] The subfamily can be classified into four genera: Alphacoronavirus (alphaCoV), Betacoronavirus (betaCoV), Deltacoronavirus (deltaCoV), and Gammacoronavirus (gammaCoV).[7] The former two genera are known to infect mammals, while deltaCoV and gammaCoV mainly infect wild birds.[7] Numerous important enteric diseases in animals are caused by alphacoronavirus, formerly classified as group 1 coronaviruses, including porcine transmissible gastroenteritis virus, canine coronavirus, porcine epidemic diarrhea virus, feline coronavirus (FCoV), and ferret enteric coronavirus (FRECV).[7,20] The recent outbreaks of viral pneumonia in human, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were all caused by betaCoV.[6,7,21] Coronaviruses of veterinary importance are summarized in the chart.[4,7]

Genus	Virus(es)	Reported Disease/Syndrome
Alphacoronavirus	Feline coronavirus (feline enteric coronavirus; feline infectious peritonitis virus)	Feline enteric coronavirus: mild gastroenteritis and diarrhea Feline infectious peritonitis virus: peritonitis, pneumonia, CNS signs etc
	Canine coronavirus	Mild gastroenteritis and diarrhea Possible severe enteritis and systemic signs (leucopenia)
	Transmissible gastroenteritis (TGE) virus of swine	Gastroenteritis. Watery diarrhea, vomiting, dehydration
	Porcine respiratory coronavirus	Mild respiratory disease or subclinical
	Porcine epidemic diarrhea virus (PEDV)	Gastroenteritis. Watery diarrhea, vomiting, dehydration
	Ferret coronavirus (ferret enteric coronavirus; ferret systemic coronavirus)	Ferret enteric coronavirus: Gastroenteritis. Watery diarrhea Ferret systemic coronavirus: Peritonitis, CNS signs etc
Betacoronavirus Group A	Porcine hemagglutinating encephalomyelitis virus	Vomiting, wasting disease, encephalomyelitis. Anorexia, hyperesthesia, muscle tremors, emaciation
	Mouse hepatitis virus	Enteritis, hepatitis, demyelinating encephalomyelitis
	Rat sialodacryoadenitis virus	Rhinitis, epiphora, pneumonia
	Bovine coronavirus	Gastroenteritis with profuse or bloody diarrhea, dehydration, decreased milk, or respiratory disease
	Equine coronavirus	Gastroenteritis
	Canine respiratory coronavirus	Respiratory disease
Betacoronavirus Group B	Severe acute respiratory syndrome (SARS) coronavirus	Respiratory disease; zoonotic with bats as natural reservoir
Betacoronavirus Group C	Middle East respiratory syndrome (MERS) coronavirus	Respiratory disease; zoonotic with camels and bats as a likely reservoir
Gammacoronavirus	Avian infectious bronchitis virus	Tracheobronchitis, nephritis Rales, decreased egg production
	Turkey coronavirus, Bluecomb virus	Enteritis Diarrhea, depression, cyanotic skin
Deltacoronavirus	Porcine deltacoronavirus	Gastroenteritis in sows and nursing pigs; low mortality in nursing pigs; clinically indistinguishable from TGE and PEDV

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JPC diagnosis:
Brain: Phlebitis, histiocytic and lymphocytic, multifocal, severe, with histiocytic and lymphocytic meningoencephalitis, choroiditis, and ventriculitis.

JPC comment:
The contributor provides an excellent review of ferret systemic coronavirus, as well as a concise summary of coronaviruses of veterinary importance. As previously mentioned, the clinical signs of ferret systemic coronavirus infection are non-specific, and often include lethargy, diarrhea, vomiting, hyporexia or anorexia, and weight loss. These patients may have non-regenerative anemia, neutrophilia, thrombocytopenia, and lymphopenia. In addition to these signs, a recent case report may expand the list of pathologic changes due to infection. A 1 year old spayed female ferret initially presented for lethargy, inappetence, sneezing, and vomiting, but ultimately progressed to a state with non-regenerative anemia, severe pancytopenia, and hyperglobulinemia. This case detected the virus in bone marrow, which had abundant hemorrhage and necrosis throughout the medullary cavity, novel findings to date for FRSCV.[17]

With recent world events bringing attention to mink farms and the spread of SARS-CoV-2 within farmed populations, attention turned to the ferret as a potential animal model of the COVID-19. The ferret remains susceptible to SARS-CoV, SARS-CoV-2, but is not susceptible to MERS-CoV through experimental infection. Ferrets are also capable of efficient transmission of the SARS viruses to naïve animals, making them candidates for pathogenicity and transmission studies.[16]

Recent therapeutic interventions for feline infectious peritonitis (FIP) have included protease inhibitors, which have met with moderate success. Coronaviruses encode two viral proteases, 3C-like protease and papain-like protease. Previous research has shown antiviral therapeutics targeting 3C-like protease has had efficacy against SARS coronavirus, MERS coronavirus, and murine and feline coronaviruses, and now ferret and mink coronaviruses as well. Challenges remain in the development of these antiviral therapeutics, but this may represent a future option for ferrets and minks in the future.[13]

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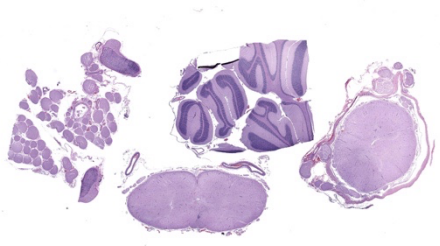
CASE 2: 20V-12504-37 (4152969-00)

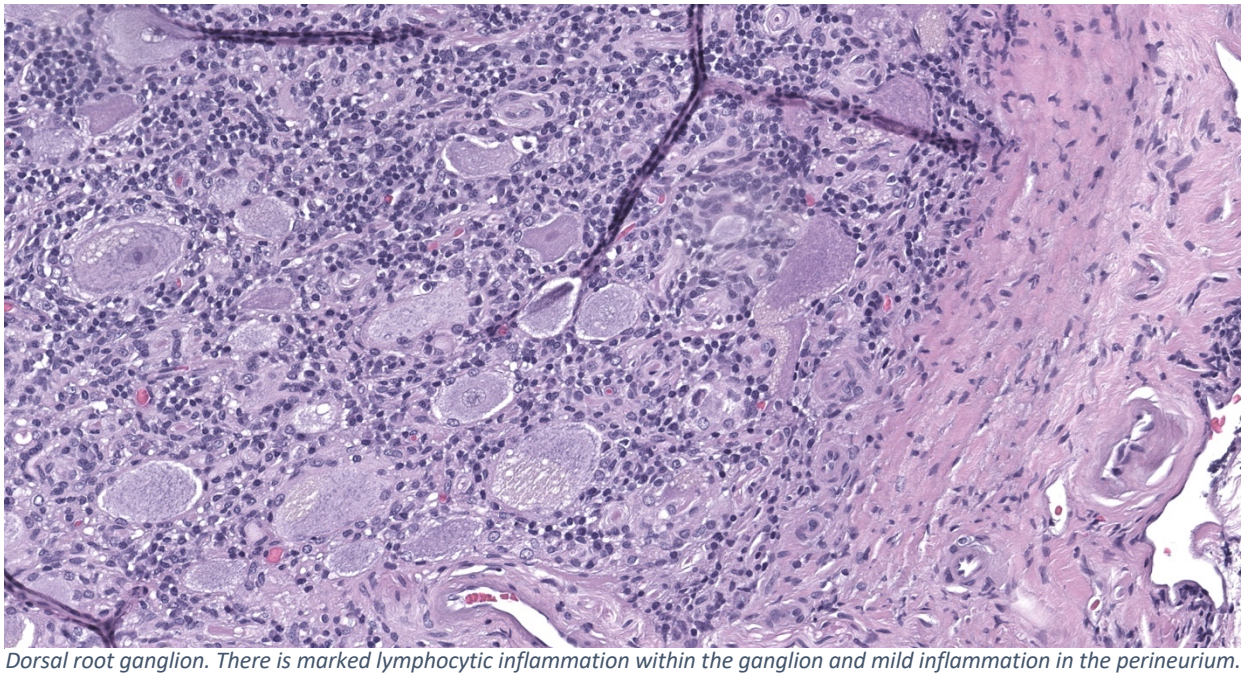
Signalment:
30-year-old, intact female, 72.4kg Harbor Seal (*Phoca vitulina*)

History:
This seal had an on/off history of head tremors for more than 6 months starting on May 2019. These head-tremors/bobbling apparently resolved in November 2019; the head twitching was partially attributed to stress and presumptive West Nile virus (WNV) infection based on remarkably elevated titers (>6000). In March 2020 the abnormal head movement returned, on physical examination increased respiratory effort was noted; animal started on antibiotics, antifungals and anxiolytics (diazepam); this animal has had a chronic (since 2010) dental disease with previous extractions at former institution. The affected seal was Immobilized on 03/03/2020 for exam, bronchoscopy, CT scan. Bronchoscopy only revealed moderate to severe congestion of proximal tracheal mucosa. CT scan of head and thorax obtained; Iohexol (+/- 200mls) passed through a jugular catheter for thoracic CT scan but no enhancement noted and there seems likely that there was extravasation on this area, however during necropsy no evidence of SC Fluid was observed. Euthanasia was ultimately elected at this time.

Gross Pathology:
There are appropriate subcutaneous and visceral fat stores, there are no signs of dehydration, and the body is in fresh postmortem condition. The cribriform plate is grossly unremarkable. Peripheral lymph nodes, including the axillary, subscapular, and mesenteric lymph nodes, are all prominent. The mesenteric lymph node chain is approximately 1.5 cm in diameter and over 15 cm long. The ventral 50% of the lungs in all fields are atelectatic. There is mild thickening of the hepatic capsule with an irregular margin. There is similar



Central nervous system, harbor seal. Sections of cerebellum, brainstem and spinal cord with dorsal root ganglion are submitted for examination. (HE, 5X).



Dorsal root ganglion. There is marked lymphocytic inflammation within the ganglion and mild inflammation in the perineurium. Multifocally, occasional neurons are swollen with clear vacuoles at their periphery. (HE, 400X).

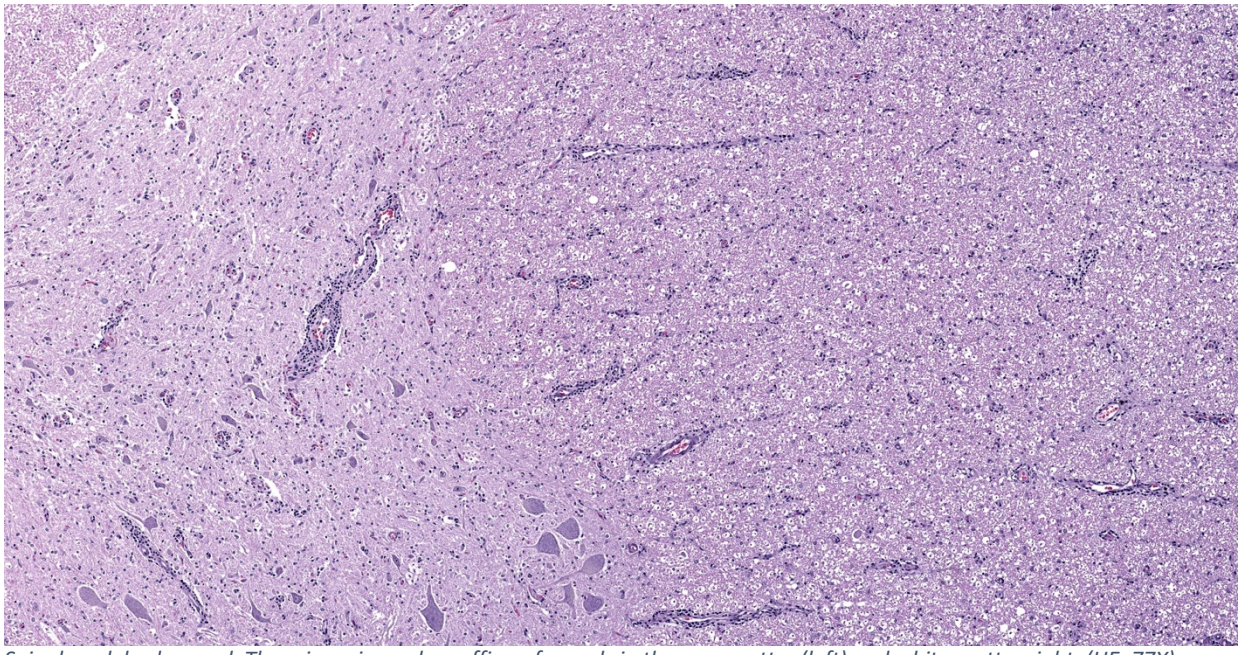
thickening of the renal capsules bilaterally. The gingiva is mildly reddened and inflamed. There is scant, mucoid digesta present within the stomach and small intestines; there are well-formed feces in the descending colon. The brain and spinal cords are unremarkable, grossly.

Laboratory results:

Test	Pathogens	Result
Serum neutralization titers	*Toxoplasma* spp.	1:160
	Sarcocystis spp.	<1:40
	Neospora spp.	<1:40
	West Nile Virus	**>1:6144**
	Eastern equine encephalitis virus	Negative
	Western equine encephalitis virus	Negative
	Venezuelan encephalitis virus	Negative
	Canine distemper virus	>1:512
	Phocine distemper virus	1:128
PCR	*Toxoplasma* spp.	Negative
	Sarcocystis spp.	Negative
	Neospora spp.	Negative
	Morbillivirus	Negative
	West Nile virus	**Positive**
Agar gel immunodiffusion test	*Leptospira spp.*	1:100
	Blastomyces spp.	Negative

Microscopic description:

Multifocally in the white and gray matter, there are perivascular infiltrates of moderate numbers of lymphocytes, plasma cells and occasional macrophages. There are multifocal areas of rarefaction in the white matter neuropil creating discrete cavitary spaces in some of which is cellular debris (digestion chamber). There are dilated myelin sheaths with swollen hypereosinophilic axons (spheroids) scattered throughout the neuropil. These are minimal/mild multifocal areas of proliferation of glial cells. The spinal meninges are diffusely expanded by extravasated erythrocytes and frequently contain moderate numbers of scattered interstitial and perivascular infiltrates of lymphocytes, plasma cells and histiocytes. The gray matter contains multifocal glial nodules and frequent neurons contain small to moderate amounts of intracytoplasmic perinuclear yellowish granular material (ceroid-lipofuscin). Within the ganglion, there is locally extensive area of lymphoplasmacytic infiltrates and neuronal ganglion degeneration characterized by shrunken and infrequently dispersed Nissl substances (central chromatolysis). In the same area, adjacent neuronal ganglion is hypercellular with increased number and prominence of satellite cells dissecting perineurium and endoneurium



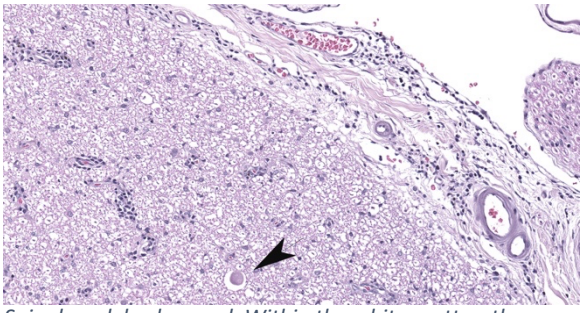
Spinal cord, harbor seal. There is perivascular cuffing of vessels in the grey matter (left) and white matter right. (HE, 77X)

(satellitosis). There are also multifocal areas of mild swollen axons. The neuronal ganglions also contain variable amounts of ceroid-lipofuscin pigments and intracytoplasmic vacuoles that represent incidental aging changes.

Contributor's morphologic diagnosis:
Spinal cord & Ganglia**:** Moderate to severe, subacute to chronic, multifocal lymphoplasmacytic and histiocytic meningomyelitis and moderate to severe, subacute, locally extensive lymphoplasmacytic ganglioneuritis with satellitosis

Contributor's comment:
West Nile Virus (WNV) is one of the arthropod-borne encephalitides (arboviruses), and is a positive-sense, single-stranded, RNA virus that belongs to genus Flavivirus, family Flaviviridae.[9] The virus is distributed worldwide with a wild host range. A variety of *Culex sp.* mosquitos are the primary vector, but ticks are also capable of harboring the virus. Several bird species are highly susceptible to infection, while domestic poultry and psittacines are generally resistant to fatal infections. A member of Covidae (crow family) and American Robin play a key role in bird-mosquito cycle and act as amplifying hosts.[1] A disease transmission through prey or contaminated water have been reported in additional to horizontal transmission.[4] WNV is composed of a virus envelope from the host cell membrane with two integral transmembrane glycoprotein E which play a crucial role in a viral pathogenesis. The E2 glycoprotein is involved with attachment to target cells while the E1 glycoprotein has a role in entering host target cells via endocytosis. Within host cells, after inoculation by mosquitoes, the virus propagates in cutaneous dendritic cells, Langerhans cells, fibroblasts, endothelial cells, and keratinocytes, and spreads to regional lymph nodes.[1] The virus can cross the blood brain barrier and enter the brain hematogenously, and possibly also through a retrograde axonal transportation.[1] Neurons and microglial cells are a vulnerable target for the virus and contribute to encephalitis while mononuclear cells are important targets and contribute to the systemic spread.

In marine mammals, WNV infection has been only reported in a harbor seal (*Phoca vitulina*)[2] and a killer whale (*Orcinus orca*).[8] In this case, the histopathological findings and gross findings are somewhat similar to those previously described in the harbor seal[2] and the killer whale,[8] which includes non-suppurative encephalomyelitis, gliosis, with occasional neuronal degeneration and necrosis. In addition to previous reports, our case demonstrates intense



Spinal cord, harbor seal. Within the white matter, there are lymphocytes and plasma cells within Virchow Robin's spaces, and within the meninges (at right). Swollen axons (spheroid) arrow (HE, 240X)

lymphoplasmacytic ganglioneuritis. In terms of serum neutralization titer, according to Cornell WNV diagnostic laboratory a titer of >6144 is confirmatory of WNV infection and disease based on what is seen in other mammals. Additionally, a serological screening test has been reported in non-fatal WNV infection in a seal at the Detroit zoo with signs of neurologic dysfunction of 4 days' duration[2] and bottle nose dolphins (*Tursiops truncatus*) from the Indian River Lagoon even though WNV-associated disease in these animals has not been reported.[7] Other differential diagnoses for encephalomyelitis and/or meningoencephalitis in seals are well summarized in Del Piero et al. (2006)[2] including phocine morbillivirus, influenza A8 and B11 orthomyxoviruses, *Toxoplasma gondii*, and *Sarcocystis neurona.* Therefore, the combination of routine histopathological examination (multiple sections of brain, brain stem, and spinal cord) and ancillary laboratory tests such as immunohistochemistry, viral isolation, serum neutralization test and qPCR increase the specificity and sensitivity for WNV identification.[2] This report also implicates the WNV infection in marine mammals that can carry a potential risk for zoonotic transmission to zookeepers and veterinarians.

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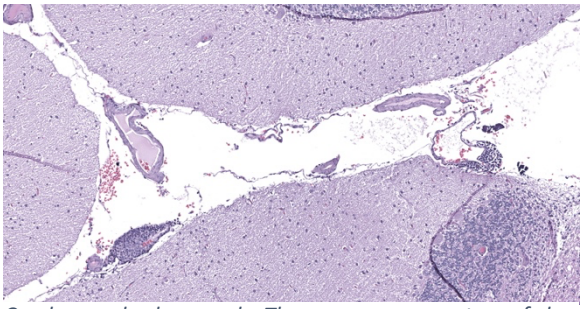
JPC diagnosis:

1. Ganglion: Ganglioneuritis, lymphoplasmacytic, multifocal, moderate with mild neuronal degeneration and satellite cell hyperplasia.
2. Spinal cord: Meningomyelitis, lymphoplasmacytic, diffuse, mild to moderate.
3. Cerebellum: Leptomeningitis, lymphoplasmacytic, multifocal, mild.

JPC comment:

In the decades since its introduction to the New World in 1999, the geographic distribution of West Nile Virus has expanded tremendously. The accepted virus cycle is between birds and mosquitos, but as noted by the contributor, mammalian species may be infected as well. Beyond the discussion of infection and the development of disease, research questions remain as to which species may serve as competent hosts for the virus. Some species, such as the fox squirrel (*Sciurus niger*) develop a viremia at titers suggesting it may be a moderately competent host for mosquito infection, but a great many others' capacity for viral propagation are currently unknown.[5]

Tracking the geographic spread of the virus and being able to quickly identify small variations in the virus provides additional information for public health professionals, researchers, educators, and students. A program that tracks the evolution of West Nile Virus in the Americas is



Cerebrum, harbor seal. There are aggregates of low numbers of lymphocytes and plasma cells within the cerebellar folial meninges and extending downward along Virchow-Robin's spaces. (HE, 112X).

called Nextstrain (nextstrain.org/WNV/NA), which implements a GIS system and phylogenetic analyses with user tools for display variable modifications. This tool is potentially useful for demonstrating the need to dedicate resources in affected areas to prepare for and combat this virus.[3]

Research in animal models has shown that WNV infection provokes both innate and adaptive immune response in the host. TLR3 and TLR7 (endosomal) and RLR (RIG like receptors) ultimately induce the synthesis of type 1 interferons (IFN) and proinflammatory cytokines. Type III interferons (IFN-γ) promote the integrity of the blood brain barrier, limiting entry of WNV to the CNS. γ-δ T cells play a role in early disease response by secreting IFN-γ, promoting dendritic cell maturation, and priming of T lymphocytes. B lymphocytes and a good humoral response helps control the spread of the infection but is not sufficient alone. A combination of complement, α-β T lymphocytes, CD4+ T lymphocytes, and CD8+ lymphocytes are required to eliminate the infection and prevent viral persistence.[6]

During conference discussion, the moderator indicated that a more specific name for ganglionic satellite cells is the term amphicytes. Additionally, an aggregate of satellite cells with a likely loss of ganglionic neuronal body is a Nageotte body. In order to help differentiate satellite cells from inflammatory cells, immunohistochemistry may be performed, with satellite cells demonstrate positive immunoreactivity to CD204, CD18, IBA1, GFAP, SOX2, nestin, and a few others.

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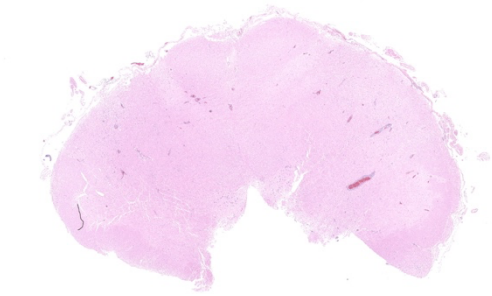
CASE 3: O240/19.6 (4152972-00)

Signalment:
Species: Dog (*Canis familiaris*)
Breed: Mixed breed (Pointer x Labrador)
Gender: Male, castrated
Age: 4 years old

History:
The dog presented with depression, acute-onset ataxia with mild bilateral forelimb hypermetria and mild pain on palpation of the thoracic spine. Rectal temperature was 39.1 °C and C-reactive protein (CRP) 14.3 mg/L (reference range 0-10 mg/L). The dog was treated with steroids, antimicrobials and sedation, but during the next 24 hours it deteriorated to non-ambulatory recumbency with seizures. The dog was subsequently euthanized and submitted for necropsy.



Brainstem, dog. A fragmented section (minus the ventral white tracts) is submitted for examination. At low magnification, large vessels are outlined by an inflammatory infiltrate. (HE, 6X).

Gross Pathology:
Two approximately 0.5 cm in diameter areas of light red discoloration was present on the surface of the cerebral frontal lobes, one in each hemisphere. Meningeal blood vessels were prominently dilated and the brain tissue diffusely slightly edematous.

Laboratory results:
RT-qPCR of tissue from the brain: positive for tick-borne encephalitis virus (TBEV)

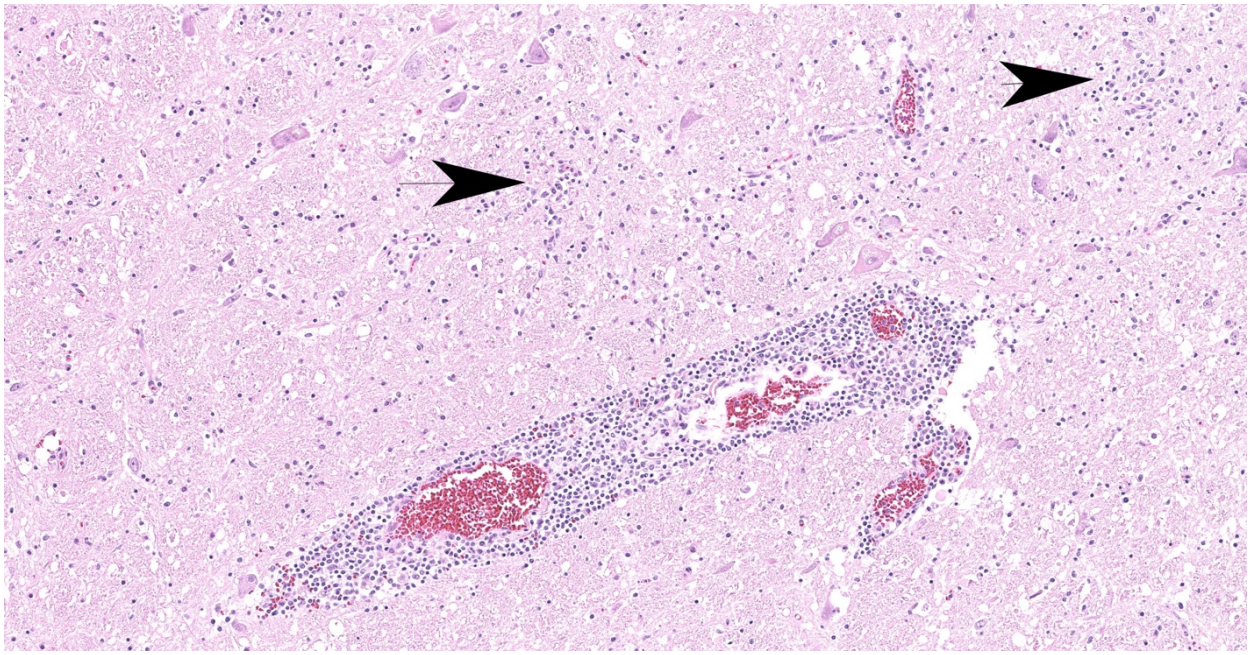
Microscopic description:
Brainstem, level of medulla oblongata. Both white and grey matter show multifocal to coalescing severe inflammatory infiltrates comprising lymphocytes, plasma cells and histiocytes, located within the neuroparenchyma and surrounding vessels as up to 10 layer thick perivascular cuffs. Affected vessels are outlined by hypertrophic endothelium, often blood filled and occasionally show mild separation by clear spaces between vessel walls and surrounding parenchyma (perivascular edema). Multifocally, there is mild to moderate vacuolation of the neuroparenchyma (rarefaction). The neuroparenchyma shows multifocal nodular to diffuse gliosis, as well as satellitosis and neuronal degeneration and loss. Multifocally, cells display hypereosinophilic shrunken cytoplasm with pycnotic or karryorhectic nuclei (necrosis). Occasionally, microglia are seen surrounding neuronal debris (neuronophagia). In addition, multifocal to coalescent infiltrates comprising moderate numbers of lymphocytes and plasma cells, and occasional histiocytes are present within the leptomeningeal subarachnoid space and surround leptomeningeal vessels as perivascular cuffs.

Contributor's morphologic diagnosis:
Meningoencephalitis, lymphoplasmacytic and histiocytic, necrotizing, multifocal to coalescing, severe, with gliosis and glial nodule formation

Contributor's comment:
Tick-borne encephalitis virus (TBEV), belonging to the genus flavivirus and family *Flaviviridae*[5], is the causative agent of the zoonotic disease tick-borne encephalitis (TBE).[5,13] TBEV is a positive-sense, single-stranded RNA (+ssRNA) enveloped virus with a viral genome of approximately 11kb in length.[5,10] There are three subtypes of TBEV; the Western European subtype, the Siberian subtype and the Far Eastern subtype.[5,13] The tick vector for the European subtype is *Ixodes ricinus*, whereas *Ixodes persulcatus* is the main vector for the Siberian and Far Eastern subtypes.[5,10] Small mammals, mainly rodents, serve as virus reservoirs and play a major role in the spread of TBEV.[7,13]

TBEV is of major health concern in humans, with thousands of human cases reported annually in endemic areas of Europe and Asia.[1,5] Humans are typically infected after being bitten by ticks, but in approximately 1% of human cases, the source of infection is unpasteurized dairy milk and milk products containing TBEV.[10] Several wild and domestic animals are also susceptible to TBEV[1], including sheep, dogs and horses.[13] In contrast to humans, TBEV infection in these species commonly causes seroconversion, but rarely causes clinical disease.[1,4,7,13] Only a few clinical cases of TBEV infection in dogs have been previously reported in Europe.[7,14] However, the clinical cases seen in dogs present as very severe, with a high fatality rate.[1,7] In acute cases, death commonly occurs within a week.[4]

After a bite from a tick infested with TBEV, the virus replicates in Langerhans cells and neutrophils in the hosts' skin, upon which dendritic cells migrate to the site of infection. Dendritic cells are subsequently activated and bring the virus to regional lymph nodes.[10,13] After viral replication in the lymph nodes, spread into



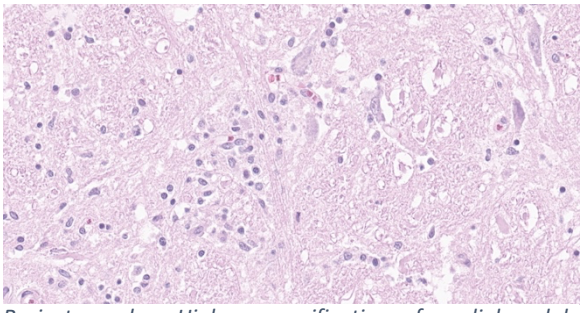
Brainstem, dog. Large vessels throughout the section are cuffed by multiple layers of lymphocytes and histiocytes. There are small glial nodules scattered randomly within the grey matter (arrowhead).

the bloodstream and primary viremia occurs. The virus is then able to infect various organs, especially spleen, liver and thymus, thereby causing a secondary viremia.[10,13] In this stage of the disease, the infection may be cleared, and seroconversion may occur without development of clinical signs.[10,13] In patients with high viral titers or insufficient neutralizing TBEV specific antibodies, TBEV may however infect the brain during the secondary viremia by crossing the blood brain barrier (BBB).[10,13]

The exact mechanism behind the viral crossing of BBB is not known, but probably includes a combination of several proposed ways; infection of microvascular endothelial cells of the BBB, cytokine/chemokine mediated BBB disruption, direct axonal retrograde transport from infected peripheral neurons into somatic motor neurons in the spinal cord, infection of olfactory neurons or transportation of virus by infected immune cells that traffic to the CNS.[10] Inside the brain, the virus replicates in large neurons of the anterior horns, medulla oblongata, pons, dentate nucleus, Purkinje cells and striatum.[10]

Both humoral and cellular immunity seem to be required to clear TBEV infection from a vertebrate host. Antibody responses include TBEV-specific IgM and IgG antibodies in serum and cerebrospinal fluid and are critically important in controlling and clearing the infection, primarily by targeting the E and NS1 protein of TBEV.[10] Both neutralizing antibodies and in part non-neutralizing antibodies play a role in prevention of the disease. Neutralization occurs by several mechanisms, including direct neutralization of receptor binding, post-binding or pre-fusion neutralization inside endosomes, and Fc receptor-mediated clearance by cells of the reticuloendothelial system.[10]

TBEV-infected neurons in the CNS are also targets for cytotoxic T-cells (CD8+). Whether or not T-cell responses are protective or pathological during TBE seems to depend on several factors, for example the pathogenicity of a particular TBEV strain, infectious dose and immunological status of host.[9,10] In transfer of CD8+ T-cells into SCID mice infected with TBEV, mean survival time was decreased, meanwhile after infection with low-pathogenic TBEV strain, CD8+ T-cells seemed to increase survival.[9] Possibly, CD4+ T-cells also prevent lethal TBE through CD4+-mediated secretion of IFN-$_{\gamma}$ and other pro-inflammatory cytokines, and/or stimulation of macrophage-like cells.[9]



Brainstem. dog. Higher magnification of a glial nodule within the gray matter. (HE, 459X)

The incubation period in dogs is estimated to be between 5-9 days.[4] The clinical signs reflect multifocal neurological lesions in the cerebrum and brain stem, including elevated body temperature and behavioral changes such as depression, altered consciousness, proprioceptive deficits, hyporeflexia in front and/or hind legs, paresis, generalized ataxia, tetraplegia, tremor, convulsions, cervical hyperalgesia, vestibular syndrome, and cranial and/or spinal nerve function deficits.[1,4,7]

The histopathological changes seen in TBEV infection include necrotizing lymphoplasmacytic and histiocytic meningoencephalomyelitis with severe neuronal necrosis and neuronophagia, diffuse gliosis and glial nodule formation, and non-purulent perivascular cuffing in both grey and white matter.[1,14] Almost the entire brain may be affected but the most severe changes are typically seen in basal ganglia, thalamus, mesencephalon, neuroparenchyma surrounding fourth ventricle and medulla oblongata.[1,7,14] Focal microgliosis (so called "glial shrubbery") in the molecular layer of cerebellum has been reported.[14]

A possible histopathological differential diagnosis to TBE in dogs comprise encephalitis caused by another flavivirus; the West Nile (WN) virus. Equids are most susceptible to WN disease, but subclinical disease may occur in canids. The histologic lesions in WN infection resemble those caused by TBEV, but in WN neuronal degeneration and necrosis is not as severe, and lesions are more confined to the grey matter. In addition, glial nodules may contain a few neutrophils. Furthermore, in WN infection lesions are overall often milder and restricted to the brain stem and thoracolumbar spinal cord, where axonal swelling and spheroid formation may be seen.[1]

Confirmation of TBEV infection include RT-PCR analysis of serum or cerebrospinal fluid[7,12], or post-mortem detection of viral antigen in brain tissue by RT-PCR or immunohistochemistry.[7,14] Serum IgM or IgG TBEV-specific antibody titers may be measured by immunofluorescence assays (IFA) or ELISA, but possible cross reaction with other flaviviral antibodies (such as WN virus-specific antibodies) may interfere with the results.[2,3,7] Under such circumstances, specific immunity against TBE virus may be assessed in virus neutralization assays.[2]

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JPC diagnosis:
Brainstem: Meningoencephalitis, lymphohistiocytic, diffuse, moderate, with astrogliosis, glial nodules, and rare spheroids.

JPC comment:
The contributor provides an excellent review of this Flaviviral disease in dogs. As noted above this virus is of more paramount concern in human populations, can affect some domestic species, but does not often cause clinical disease. However, canine cases have reported pyrexia, changes in behavior and mentation, and apathy. Affected animals have exhibited forelimb or hindlimb paresis, quadriplegia, seizures, convulsions, ataxia, proprioceptive deficits, neck hyperesthesia, facial nerve paralysis, strabismus, anisocoria, nystagmus, miosis, loss of corneal reflex, and optic neuritis. Using seroprevalence studies, canine infection rates may be as high as 11.6% in endemic areas, but clinical manifestations are seen in rare instances. In endemic areas of Austria and German, seroprevalence rates in horses is as high as 26.1% and 20-30%, respectively, with only rarely reported cases of clinical disease. Infections in

ruminants are largely asymptomatic, but experimental infection has been demonstrated in lambs. A small number of captive exotic species have been reportedly infected, including a macaque (*Macaca sylvanus*), a markhor (*Capra falconeri*) and a reindeer (*Rangifer tarandus*).[11]

Infection by ingestion of milk represents an interesting transmission mechanism. Human infection through this method often results in family clusters, as opposed to the less organized infection patterns through ticks. TBEV is sensitive to its environment, but milk allows it to survive exposure to acidic gastric contents for up to two hours. Within that timeframe, most of the ingested contents are found in the lower gastrointestinal tract, allowing for infection by the intact virus through M cells in Peyer's patches.[11]

In human TBEV infections, rare cases of hepatic compromise have been reported. One study found that 22.2% of patients had abnormal liver function tests. A separate study found approximately 22% of patients had elevated liver enzymes, with the most often affected values in AST and ALT. While most cases of TBEV in animals present with advanced neurologic symptoms, in animals presenting with non-specific signs, a complete blood chemistry panel may help identify an etiology.[6]

Because humans are more severely affected by TBEV than dogs, and humans and dogs often live in close proximity, dogs are a potential sentinel species. Studies in Scandinavian dogs illustrated geographic areas where TBE was more common than originally expected, new foci of TBEV, and showed the need for better and more extensive TBEV surveillance. In Belgium, a serologic screening of 880 dogs revealed only two ELISA-positive cases, and 8 borderline positive cases. While Belgium does not consider this disease to be endemic, this provides a baseline for subsequent seroprevalence studies which might inform future policy, screening, and treatment.[8]

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CASE 4: 18-081 (4132734-00)

Signalment:
22-month-old Holstein steer, *Bos taurus*, bovine.

History:
In June 2018, a Holstein steer in a group of 37 steers in a ~300-hectare farm in Colonia, Uruguay, developed progressive neurological sings including unusual behavior, aimless walking, circling, ataxia, repetitive and uncoordinated tongue movements, and recumbency. The herd grazed on an annual oat pasture and was supplemented with corn silage. A presumptive clinical diagnosis of cerebral listeriosis by the veterinary practitioner prompted treatment with penicillin and streptomycin, however the animal died spontaneously after a clinical course lasting 3 days.

Gross Pathology:
No gross findings.

Laboratory results:
Aerobic and anaerobic bacterial cultures, selective culture for *Listeria monocytogenes*, PCR for bovine alphaherpesvirus 1 and 5 (BoHV-1 and -5, *Varicellovirus*), immunohistochemistry for rabies virus (*Lyssavirus*), West Nile virus (WNV, *Flavivirus*), and *Chlamydia* spp. were all negative/unremarkable.

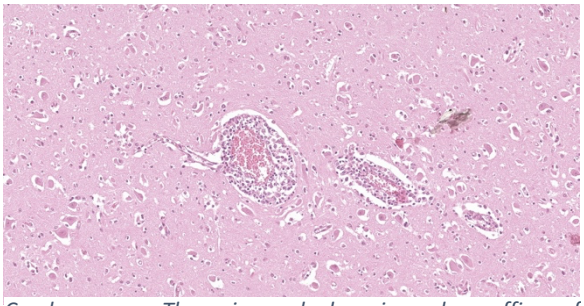
Microscopic description:
Examined is a section of brain (some slides contain telencephalon and other brainstem). Multifocally and predominantly in the gray matter, the perivascular spaces are expanded by numerous lymphocytes, histiocytes and fewer plasma cells, forming layers up to 20 cells thick (variable in different sections). The inflammatory infiltrate extends into the neuropil in the gray matter, and occasionally limiting areas of the white matter. There are multifocal aggregates of glial cells (microglia, astrocytes) sometimes associated with areas of neuropil rarefaction. Scattered neurons show perikaryal hypereosinophilia, shrinkage, angular borders, and nuclear fading, pyknosis or karyorrhexis (necrosis). Necrotic neurons are sometimes surrounded by inflammatory and glial cells (satellitosis) or invaded by them (neuronophagia, not present in all sections). Some axons are swollen and hypereosinophilic (spheroids/torpedoes). The leptomeninges are multifocally infiltrated by small numbers of lymphocytes, histiocytes and fewer plasma cells (not present in all sections).

Cerebrum, ox. A section of cerebrum with some overlying meningeal tissue is submitted for examination (HE, 6X)

Contributor's morphologic diagnosis:
Brain (telencephalon or brainstem): meningoencephalitis, lymphocytic, histiocytic and plasmacytic with neuronal necrosis, multifocal, subacute, moderate to severe, bovine.

Contributor's comment:
The diagnosis of bovine astrovirus-associated encephalitis in this case was based on the microscopic lesions, typical of viral encephalitis, coupled with intralesional identification of BoAstV RNA within neurons by *in situ* hybridization, and molecular identification of BoAstV by RT-PCR followed by whole genome sequencing. Other viral and bacterial causes of encephalitis in cattle were ruled out by specific testing.

The *Astroviridae* family contains non-enveloped, positive-sense, single-stranded RNA viruses within two genera, *Mamastrovirus* and *Avastrovirus*, which infect mammals and birds, respectively. Currently, the International Committee on Taxonomy of Viruses[11] recognizes



Cerebrum, ox. There is marked perivascular cuffing of vessels in the neocortex by a mixed cell population of large numbers of lymphocytes and histiocytes, and fewer plasma cells and neutrophils which are not migrating into the adjacent parenchyma. (HE, 156X)

19 species, namely *Mamastrovirus-1* through -*19*, within the *Mamastrovirus* genus; however, there are numerous strains awaiting classification, some of which are tentatively considered new species.[8]

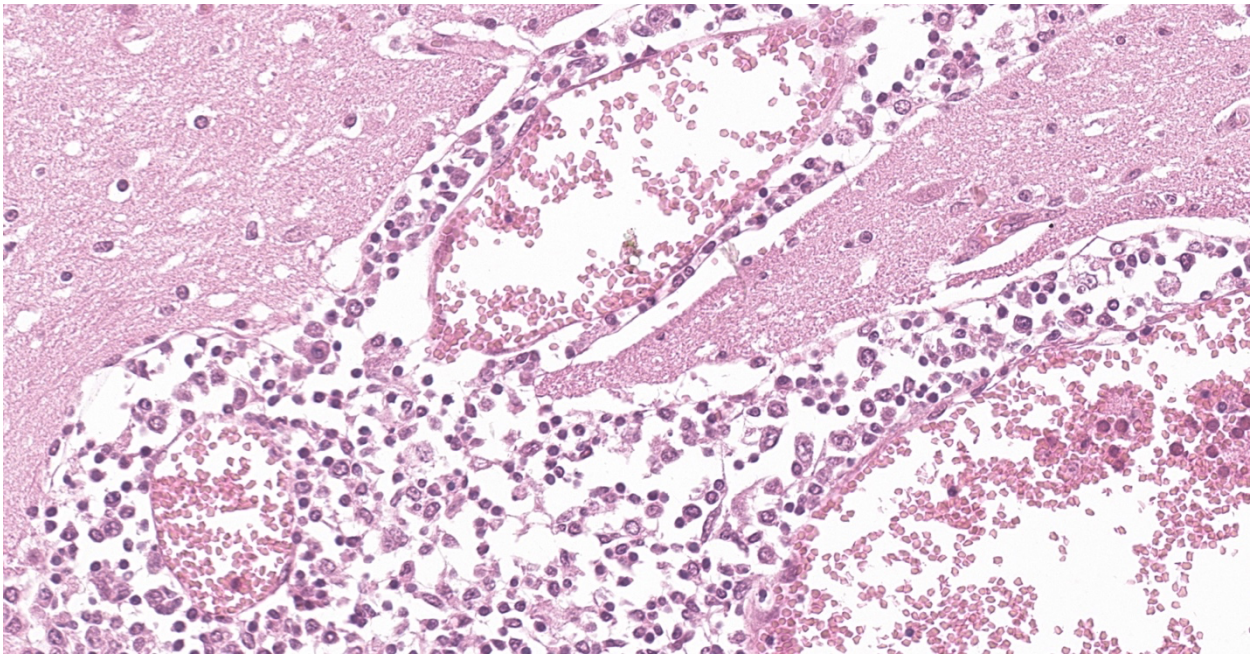
Since 2010, several astroviruses have increasingly been recognized as neuroinvasive in various mammalian species, including humans,[16,18] mink,[1] cattle,[14] sheep,[17] pigs,[2] and muskox.[3] After initial recognition of bovine astrovirus-associated encephalitis in United States cattle,[14] a retrospective study in cases of sporadic bovine encephalitis of undetermined etiology from Switzerland revealed that this neuroinvasive astrovirus had gone undetected for decades.[23] Although the epidemiology and transmission routes of these astroviruses are unknown, cross-species transmission has been suggested based on the high level of identity (>98%), shared between bovine and ovine neuroinvasive astroviruses at the nucleotide and amino acid levels.[4]

Bovine astroviruses (BoAstVs), named BoAstV-NeuroS1[14] and BoAstV-CH13,[6] were initially found in the brain of cattle with non-suppurative encephalitis in the United States and Switzerland, respectively. Despite the different nomenclature, both viruses represent the same genotype species[5,21] that is still awaiting official classification by the ICTV. In 2015, a previously unknown BoAstV strain, named BoAstV-CH15, was identified in the brain of cows with encephalitis in Switzerland. Full genome phylogenetic comparison revealed a closer relationship of BoAstV-CH15 with an ovine astrovirus (OvAstV) than with BoAstV-CH13.[24] Coinfection with BoAstV-CH13 and BoAstV-CH15 was also documented in one case.[24] The same year in Germany, Schlottau et al. (2016)[20] reported a novel astrovirus, namely BoAstV-BH89/14, in a cow with encephalitis, that was most closely related to OvAstV and BoAstV-CH15. Subsequently, BoAstV-CH13/NeuroS1 was identified in 2017 in cases of bovine encephalitis in eastern and western Canada.[22,25] In 2018, a novel neuroinvasive BoAstV closely related with North American and European BoAstV-NeuroS1/BoAstV-CH13, was identified in a steer with non-suppurative encephalomyelitis in Japan, and the occurrence of intra-genotypic recombination between the North American and European strains was suggested.[10]

While in the northern hemisphere cases of BoAstV-associated encephalitis have been reported in North America, Europe, and Asia, in the southern hemisphere the disease so far has only been documented in Uruguay[9] and is probably underdiagnosed. A Bayesian phylogeographic analysis including North American, European, Asian and the Uruguayan strain suggested that the common viral ancestor circulated in Europe between 1794-1940, and was introduced in Uruguay between 1849-1976, to later spread to North America and Japan.[9]

The clinical signs and epidemiological findings in the case described herein, albeit non-specific, were similar to those described in other cases of bovine astrovirus-associated encephalitis, which is usually described as sporadic,[23] with a variety of neurological deficits,[7] with a duration of clinical signs that typically ranges from 1 day to 3 weeks.[7,10,20,25]

Macroscopic examination of the brain and the C1 segment of the spinal cord did not reveal significant gross anatomic lesions. Histologically, there was moderate to severe, lymphocytic, histiocytic and plasmacytic meningoencephalomyelitis affecting the telencephalon (including the cerebral hemisphere and hippocampus), brainstem, and the only examined segment of spinal cord. Lesions were predominantly distributed in the gray matter and



Cerebrum, ox. The inflammatory infiltrate of lymphocytes, macrophages and fewer plasma cells and neutrophils are confined to the wall of the neocortical vessels. (HE, 458X).

limiting areas of white matter. In affected areas there was perivascular cuffing and lymphoplasmacytic and histiocytic inflammation and neuronal necrosis/neuronophagia with gliosis in the adjacent neuropil. There was satellitosis of affected, necrotic neurons. The lesions were much less frequent and severe in the cerebellar parenchyma, although there was multifocal moderate cerebellar leptomeningitis. No intralesional bacteria were found with H&E and Gram stains, and no viral inclusions were identified. *In situ* hybridization was performed using a probe generated from BoAstV-NeuroS1, and there was probe hybridization abundant within, and limited to the cytoplasm of neurons in the cerebral hemisphere and hippocampus.

Astrovirus was detected in brain by RT-PCR. Nearly complete genome sequence analysis revealed a *Mamastrovirus* strain within the CH13/NeuroS1 clade, we named BoAstV-Neuro-Uy (GenBank under accession number MK386569). Phylogenetic analysis suggests that BoAstV-Neuro-Uy and other members of this clade should be classified within one same species; *Mamastrovirus-13*, as proposed by others,[8,10] although definite species assignation by the ICTV is pending.

Additional details and figures of the case presented here were published elsewhere.[9] A detailed review on neurotropic astroviruses in humans and animals is also available in the literature.[19]

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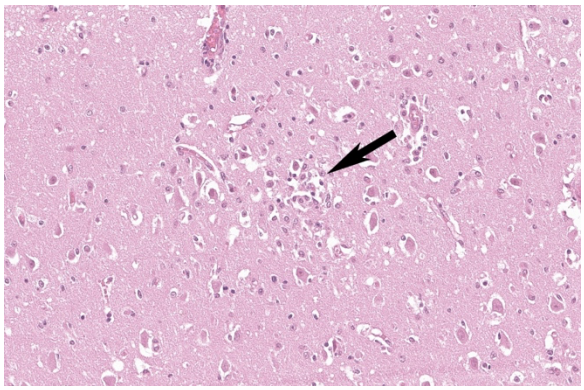
JPC diagnosis:
Cerebrum: Meningoencephalitis, lymphohistiocytic, diffuse, moderate, with gliosis and rare glial nodules.

JPC comment:
The contributor provides an excellent and well researched summary of bovine astrovirus, with important knowledge gaps identified, but still remaining. Since their first description in 1975, astroviruses have become an important cause of gastroenteritis in human children and some domestic species, but also encephalitis in cattle, mink, sheep, pigs, and humans, and hepatitis and nephritis in avians. Astroviruses are so named because when visualized on TEM, the surface of some virus particles has a distinct star-like shape.[15]



Cerebrum, ox. There are rare glial nodules in the external neuronal layers of the cortex. (HE, 400X).

It was recently hypothesized that the respiratory or gastrointestinal tract may serve as a reservoir for encephalitis causing astroviruses in cattle in Switzerland. Nasal and fecal swabbing of study veal calves resulted in two positive animals using RT-qPCR protocols, infected with BoAstV-CH13/NeuroS1. Additional research is required to determine whether the hypothesis is correct, but fecal samples may be a relatively simple method to determine infection status of specific animals.[12]

A recent case of neurotropic astrovirus was reported in an alpaca in Switzerland. The patient presented for anorexia, trembling, two day duration of colic, and general ill thrift. After several days with progressive neurologic signs, the animal was euthanized. Histology identified non-suppurative polioencephalomyelitis, with prominent lymphohistiocytic perivascular cuffs, multifocal neuronal degeneration and necrosis, and multifocal glial nodules. RT-qPCR, Next Generation Sequencing, IHC, and *in situ* hybridization determined that the causative agent was a virus almost identical to BoAstV-CH13/NeuroS1. In endemic areas, this virus should remain on the differential diagnosis list in cattle and other domestic species.[13]

During the conference, the moderator emphasized the different between astrocytosis and astrogliosis. Astrocytosis indicates a raw increase in number of astrocytes, while astrogliosis indicates an increased in reactive astrocytes. The two terms are easily confused, but are often consistent with different pathologic processes.

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