Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2022-2023

Conference #6 — 28 September 2022

CASE I:

Signalment:
2-week-old, male castrated, Angus calf (*Bos taurus*)

History:
Ocular opacity, ocular and nasal discharge, foam coming out mouth and nose, dehydration, abdominal distention, dyspnea.

Gross Pathology:
The body is in fair nutritional and postmortem condition. Multiple joints contain small amounts of fibrin. Across the mucosal surface of the larynx and trachea, there are loosely adherent, multifocal to coalescing, slightly raised, moist, tan plaques. The lungs are diffusely dark, wet, and slightly firm. Randomly distributed throughout all lung lobes are 50-100 targetoid, ~0.2 cm diameter nodules that range from tan to dark red. The myocardium heart is slightly mottled and pale. There is mild splenic enlargement and congestion of the renal medullary regions/vessels. The umbilicus is markedly thickened by fibrous tissue (chronic omphalitis). Bilaterally, the umbilical artery lumens persist and are mildly distended with opaque, brown, slightly viscous fluid (interpreted as omphaloateritis).

Laboratory Results:
PCR Results: Lung tissue tested:
Bovine coronavirus - negative
Bovine herpes virus 1 – positive (CT value = 21.77)
Bovine parainfluenza 3 – negative
Bovine syncytial virus - negative
Bovine Viral Diarrhea - negative
Influenza D virus – negative
Malignant Catarrhal Fever – negative
Mycoplasma bovis – negative

Immunohistochemistry Results: Adrenal gland:
BHV-1– positive staining (within foci of necrosis)

Aerobic Culture Results: Lung and liver:

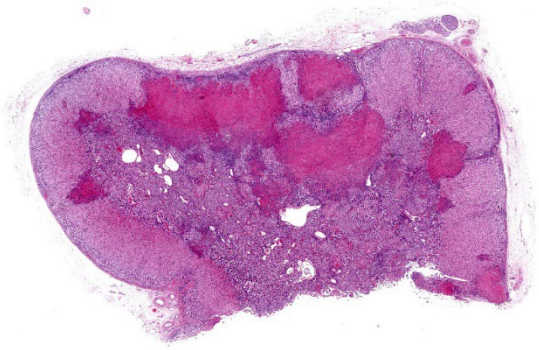

Figure 1-1. Adrenal gland, ox. A section of adrenal gland is submitted for examination. There are extensive areas of necrosis, primarily within the cortex, but which may extend into the medulla. (HE, 5X)

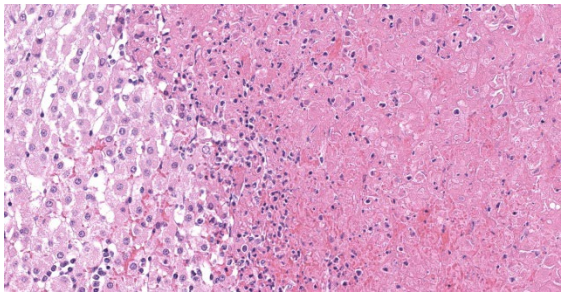

Figure 1-2. Adrenal gland, ox. Areas of necrosis are large coagulative in nature, but are undergoing lytic change, with infiltration of neutrophils admixed with cellular debris. (HE, 304X)

1+ *Pseudomonas aeruginosa*, *Staphylococcus sciur*i and *E. coli* (postmortem overgrowth)

Microscopic Description:
Adrenal gland: Scattered throughout the section, affecting both cortical and medullary regions, there are multifocal, random, variably sized, discrete areas of acute lytic necrosis affecting approximately 40% of the examined section. The foci of necrosis and inflammation are characterized by hypereosinophilic accumulations of necrotic cellular debris, acute hemorrhage, fibrin exudation, and infiltration by degenerating neutrophils and fewer mononuclear cells. Intact adrenal cortical and medullary cells bordering the foci of necrosis frequently show marginalization of nuclear chromatin and variably frequent, 2-4 µm diameter, eosinophilic to amphophilic intranuclear inclusion bodies that are either surrounded by a clear halo or fill the nucleus. Infrequent multinucleate syncytial cells are noted at the margins of necrosis/inflammation. Similar mixtures of degenerate neutrophils and fewer mononuclear cells surround and variably infiltrate the adrenal capsule as well as associated vessels with nerves and ganglia being less affected. Subtle degeneration and lipid-vacuole loss is noted in the surrounding adipose tissue.

Immunohistochemical staining for bovine herpesvirus 1 reveals abundant positive dark brown cytoplasmic and membrane staining associated with the foci of necrosis and virus-infected cells.

Contributor's Morphologic Diagnoses:
Adrenal gland: Adrenalitis, necrotizing, random, acute with eosinophilic intranuclear inclusion bodies (consistent with bovine herpesvirus 1 adrenalitis)

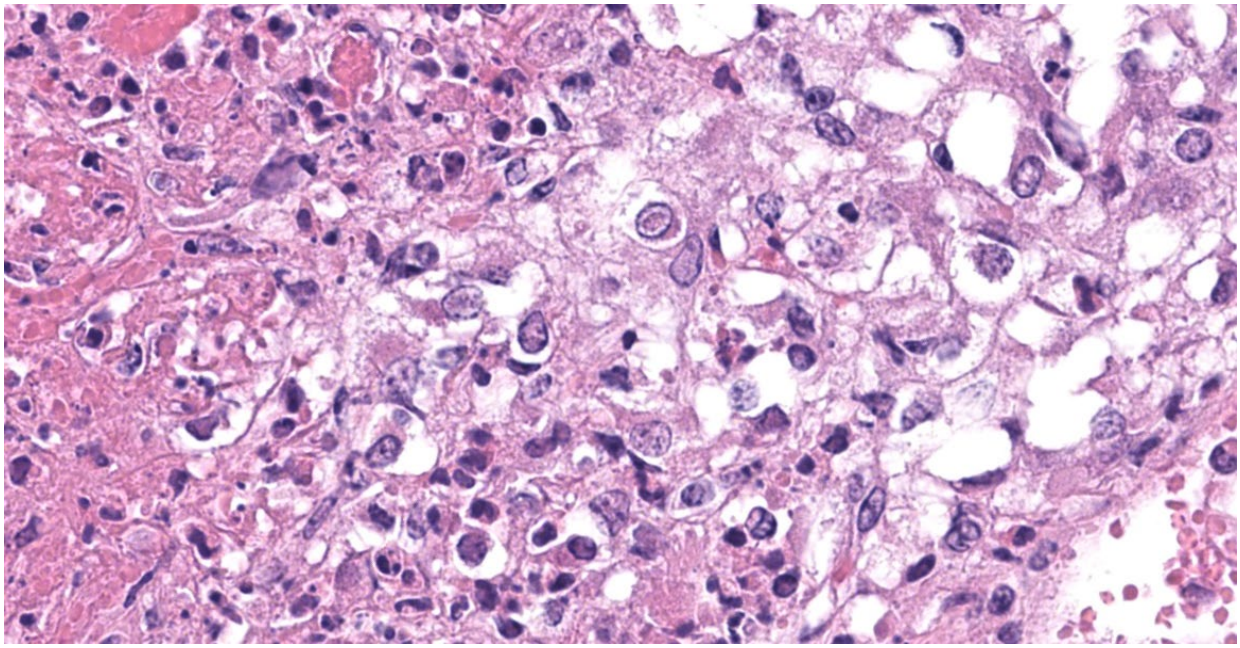

Figure 1-3. Adrenal gland, ox. Adrenocortical cells at the periphery of the necrotic areas contain a single eosinophilic intranuclear viral inclusion that peripheralizes chromatin. (HE, 590X)

Contributor's Comment:
Bovine herpesvirus 1 (BHV-1; Alphaherpesvirinae) infects a wide range of animals, including cattle, sheep, goats, llamas, swine, water buffalo, mustelids, and rabbits.[2] The virus is widespread among cattle populations and is the cause of the following clinical disease patterns: infectious bovine rhinotracheitis (IBR), keratoconjunctivitis, bronchointerstitial pneumonia, abortion, encephalitis, systemic herpesvirus infection in young calves, and pustular vulvovaginitis or balanoposthitis in unvaccinated/naive cattle.[2, 4] Routine vaccination has reduced the incidence of BHV-1 infections in both dairy and beef animals but stress, or steroid treatment, may result in reactivation of latent virus infections.[4]

Respiratory infections usually involve the upper respiratory tract (nasal mucosa and muzzle inflamed) and lungs and can vary from subclinical to severe infections. Young animals may also develop keratoconjunctivitis with or without upper respiratory disease, while young adult animals may also develop pustular vulvovaginitis or balanoposthitis when infected BHV-1.[4]

Disseminated/systemic infections with multiple organ involvement have also been identified and well documented in neonatal calves and fetuses infected with BHV-1.[1, 5, 8, 14, 11] Lesions in affected neonates and fetuses have been reported to involve the upper respiratory tract, lungs, oral cavity, esophagus, rumen, liver, kidney, spleen, as well as adrenal gland,[1, 5, 8, 14] with enteritis and encephalitis being observed in some clinical cases.[1, 5, 14] In this case, the calf age and lesion distribution best fits with the systemic form of BHV-1 infection in young calves.

The adrenal lesions are consistent with those previously reported in systemic herpesvirus infection and are characterized as multifocal random foci of necrosis range from coagulative to lytic) accompanied by infiltration with scattered degenerate neutrophils.[1,12] Like other reports, occasional eosinophilic intranuclear viral inclusion bodies and syncytial cells are observed amongst the necrotic cells as well as within intact virus-infected cells bordering foci of necrosis.[1, 2, 12] As Moeller Jr. and others (2013) noted, microscopic le-

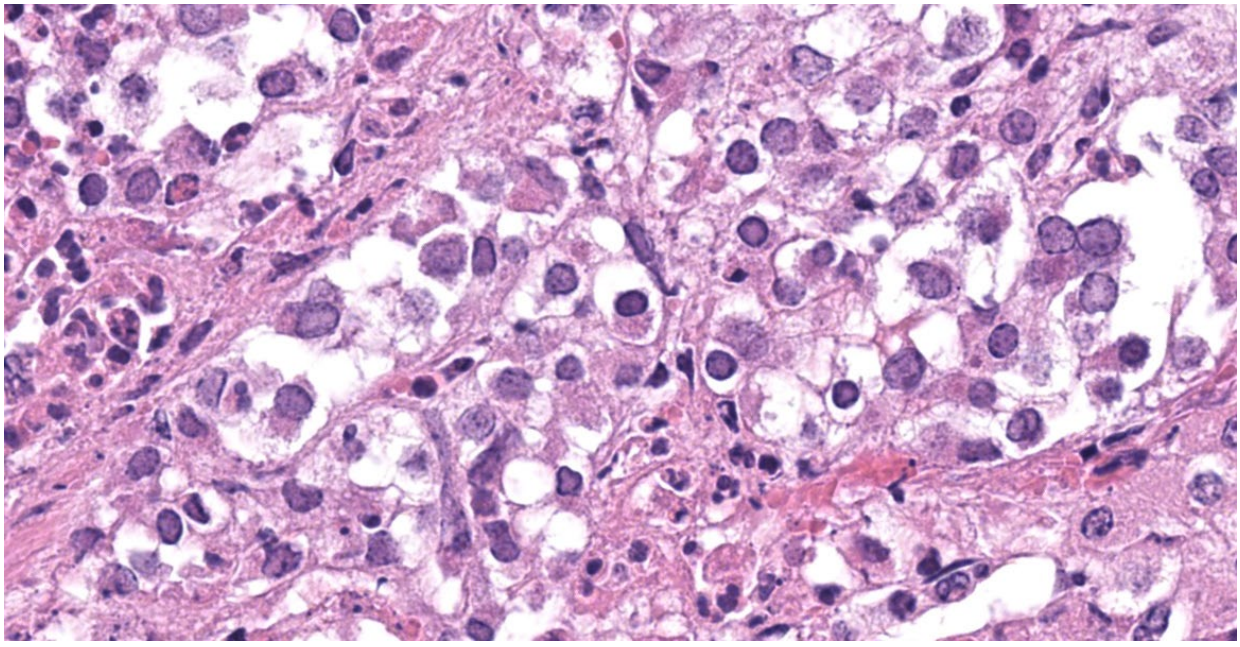

Figure 1-4. Adrenal gland, ox. In some fields, almost every cortical cell contains an intranuclear viral inclusion (HE, 759X)

sions associated with neonatal BHV-1 infection may be confined to the adrenal gland only (29/62 animals examined had adrenal lesions only) and the diagnosis may be missed if adrenal gland is not sampled during postmortem examination.[12]

The source of BHV-1 transmission from infected dams to calves may occur in utero (late gestation) or during parturition and can result in fetal abortion or systemic disease in neonatal or suckling calves (up to 1 month of age; clinical disease following a 2–6-day incubation period).[2, 11, 12] Reactivation of latent infections or activation of modified live vaccine strain virus may also be linked to environmental stressors and/or immunosuppression (e.g., inadequate colostrum intact) have been shown to contribute to the expression of clinical disease in calves and cattle infected BHV-1.[1, 9, 10] A limited vaccination history of dams and potential inadequate colostrum intact may account for the development of systemic disease in this calf.

Contributing Institution:
Department of Veterinary Clinical and Diagnostic Sciences with the Faculty of Veterinary Medicine, University of Calgary.

JPC Diagnosis:
Adrenal gland: Adrenalitis, necrohemorrhagic, multifocal to coalescing, moderate, with intranuclear viral inclusions and viral syncytia.

JPC Comment:
Bovine herpesvirus-1 is a double-stranded DNA virus which was first isolated in 1956.[3] There are three subtypes, BHV 1.1, 1.2a, and 1.2b, which have slight variations in clinical presentation, pathogenicity, and geographic distribution.[6] All subtypes can cause infectious bovine rhinotracheitis which is the most common presentation in feedlot cattle. BHV-1 is similar to *Mycoplasma bovis*, as seen in Conference 1 Case 3 earlier this year, in that it can damage host respiratory defense mechanisms and predispose to secondary bacterial invaders, culminating in bovine respiratory disease complex (shipping fever), an economically important disease which costs at least $1 billion annually in the United States alone.[7]

BHV lacks tissue tropism and can infect a wide range of cells, as illustrated in this case of systemic disease. BHV-1 attaches to the cell surface using envelope proteins gB and gC, interacts with the intercellular adhesion molecule Nectin-1 using envelope protein gD, and enters the cell after viral and cell membrane fusion.[6] The virus can spread directly between cells without cell lysis using a variety of other glycoproteins and can spread systemically through a cell-associated viremia.[3,6,13] BHV-1 can also spread through rupture of infected cells. The virus induces production of "virus host shut off" (vhs) protein, so named because it disables cellular protein synthesis, compromises membrane stability, and ultimately results in cell necrosis.[3] Virus particles liberated from ruptured cells can subsequently infect adjacent cells.[3] In the airways, necrosis of epithelial cells disrupts the mucociliary apparatus, preventing clearance of inhaled particles and allowing bacterial deposition within the lungs.[3] Additional, the virus can replicate in endothelial cells, causing vascular damage and ischemia. Confer-

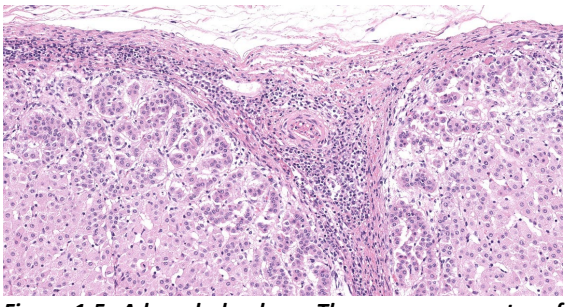

Figure 1-5. Adrenal gland, ox. There are aggregates of lymphocytes and macrophages in the capsule, often overlying areas of necrosis deeper in the cortex. (HE, 150X)

ence participants remarked on the lack of viral inclusions in the endothelium in this case, which was surprising given the degree of necrosis in the section.

BHV-1 also induces a transient immunosuppression through a variety of mechanisms. Viral infection induces apoptosis of CD4+ T cells and impairs CD8+ CTL function by inhibiting antigen processing and repressing MCHI expression.[6] The vhs protein inhibits MHCII expression. Finally, viral bICP0 gene activity inhibits interferon gamma production by inhibiting interferon regulatory factor (IRF) 7 and stimulating proteosomic destruction of IRF3.[6]

Latency and recrudescence are a hallmark of herpesviral infection and make control and eradication of BHV-1 difficult.[3,13] After initial infection, BHV-1 spreads between cells and ultimately enters the peripheral nervous system.[3] In respiratory infections, the virus becomes latent in the trigeminal ganglion, while genital infections result in latency in the sciatic nerve.[13] Latency in other organs, including the tonsils, lymph nodes, blood, and spleen, has also been documented.[6] Latent infection and recrudescence are controlled by a few viral proteins and the host immune system.[6] The latency-related (LR) gene is active early in infection of neurons and helps inhibits neuronal apoptosis, productive infection, and bICP0 expression.[6] Active cell-mediated immunity also enforces latency, and CD8+ T cells which persist in the infected ganglia produce IFN-gamma and may induce cytotoxicity to reduce viral spread.[6] Stress (i.e. from shipping or introduction into a new herd) or administration of corticosteroids is associated with reduced LR expression, active viral replication, and recrudescence. [6]

References:

1. Bryan LA, Fenton RA, Misra V, Haines DM. Fatal, generalized bovine herpesvirus type-1 infection associated with a modified-live infectious bovine rhinotracheitis parainfluenza-3 vaccine administered to neonatal calves. *Can Vet J.* 1994;35: 223-228.
2. Caswell JL, Williams KJ. Chapter 5 - Respiratory System. In: Maxie MG, ed. *Jubb, Kennedy & Palmer's Pathology of Domestic Animals:* Volume 2. 6th ed. Philadelphia, PA: Elsevier Saunders; 2016:465-591, e464.
3. Ellis JA. Update on viral pathogenesis in BRD. *Animal Health Research Reviews.* 2010; 10(2):149-153.
4. Graham DA. Bovine herpes virus-1 (BoHV-1) in cattle-a review with emphasis on reproductive impacts and the emergence of infection in Ireland and the United Kingdom. *Ir Vet J.* 2013;66: 15.
5. Hill BD, Hill MW, Chung YS, Whittle RJ. Meningoencephalitis in calves due to bovine herpesvirus type 1 infection. *Aust Vet J.* 1984;61: 242-243.
6. Jones C, Chowdhury S. A review of the biology of bovine herpesvirus type 1 (BHV-1), its role as a cofactor in the bovine respiratory disease complex and development of improved vaccines. *Animal Health Research Reviews*. 2008; 8(2):187-205.
7. Jones C, Chowdhury. Bovine Herpesvirus Type 1 (BHV-1) is an Important cofactor in the Bovine Respiratory Disease Complex. *Vet Clin Food Anim.* 2010; 26:303-321.
8. Kennedy PC, Richards WPC. The Pathology of Abortion Caused by the Virus of Infectious Bovine Rhinotracheitis. *Pathologia veterinaria*. 1964;1: 7-17.

9. Mechor GD, Rousseaux CG, Radostits OM, Babiuk LA, Petrie L. Protection of newborn calves against fatal multisystemic infectious bovine rhinotracheitis by feeding colostrum from vaccinated cows. *Can J Vet Res*. 1987;51: 452-459.
10. Meyer G, Lemaire M, Ros C, et al. Comparative pathogenesis of acute and latent infections of calves with bovine herpesvirus types 1 and 5. *Arch Virol.* 2001;146: 633-652.
11. Miller RB, Smith MW, Lawson KF. Some lesions observed in calves born to cows exposed to the virus of infectious bovine rhinotracheitis in the last trimester of gestation. *Can J Comp Med.* 1978;42: 438-445.
12. Moeller RB, Adaska J, Reynolds J, Blanchard PC. Systemic Bovine herpesvirus 1 infections in neonatal dairy calves. *Jour Vet Diag Invest*. 2013;25: 136-141.
13. Osterrieder K. Herpesvirales. In: MacLachlan NJ, Dubovi EJ, eds. *Fenner's Veterinary Virology.* 5th ed. Cambridge, MA: Elsevier. 2017; 194-195, 199-201.
14. Penny CD, Sargison ND, Howie F, Nettleton PF, Schock A. Upper respiratory disease and encephalitis in neonatal beef calves caused by bovine herpesvirus type 1. *Veterinary Record.* 2002;151: 89-91.

CASE II:

Signalment:
0.5 years old, male, golden retriever (*Canis familiaris*)

History:
This dog was purchased through a breeder at 7 weeks of age. At around 4 months of age, the dog started having uncontrolled watery−mucoid diarrhea that was large volume and frequent. He was placed on metronidazole and Proviable and needed to stay on the medication, as diarrhea would return when the drugs were not used. The dog was diagnosed with carpal flexure deformity at 3 months of age. Radiographs of the cervical spine and hips revealed mild hip dysplasia and shortened distal cervical vertebral bodies. The dog developed urinary accidents (normal volume) at home. On presentation to Iowa State University Teaching Hospital, the dog had lateral strabismus, vertical nystagmus, ataxia that was worse in hind limbs with normal reflexes and placement and hopping, head tremor, and rectal mucosa felt very irregular on rectal palpation. The owner did not allow many additional tests; only creatine kinase, which was mildly increased at 378, and blood smear which showed possible granules in lymphocytes. The dog's condition deteriorated over a week and was euthanized.

Gross Pathology:
Small intestine: A small number (approximately 10) of 2−5 cm in length and 2 mm in

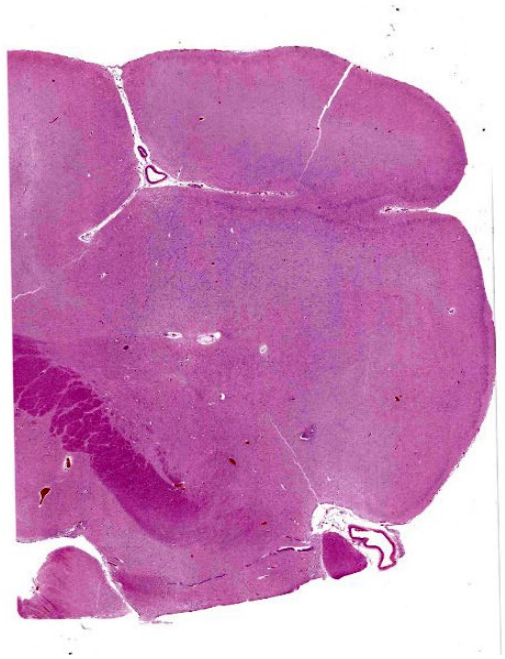

Figure 2-1. Cerebrum, dog. A single section of cerebrum is submitted for examination; there is no visible lesion at subgross magnification. (HE, 7X)

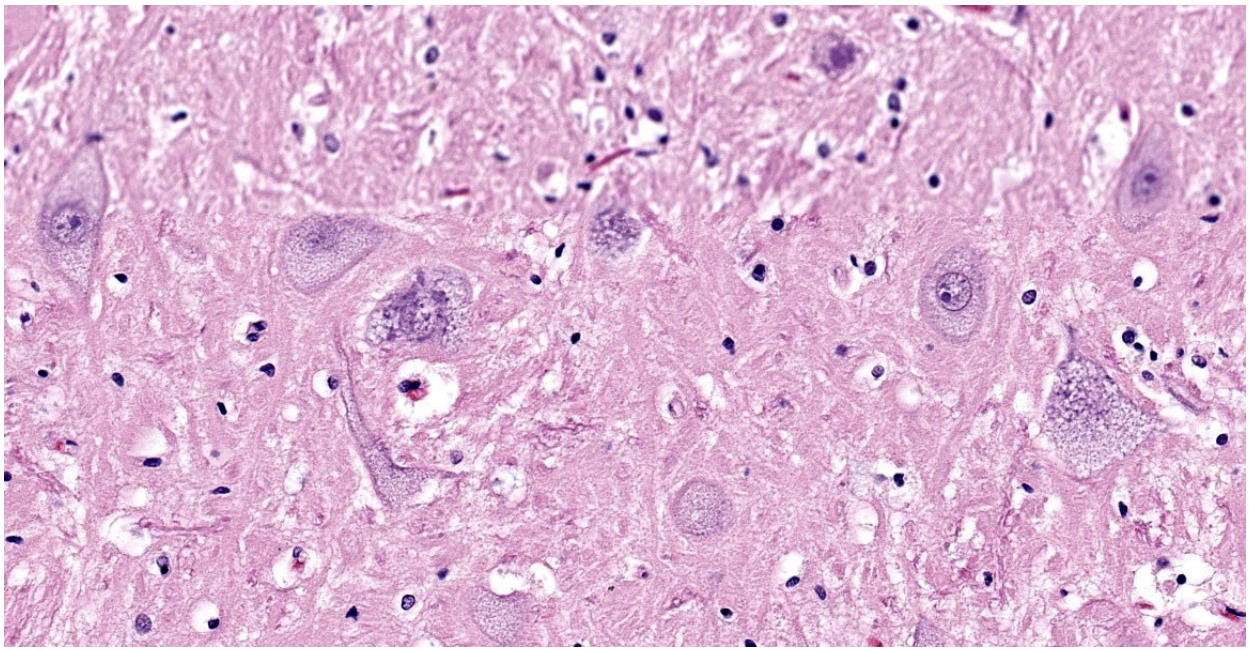

Figure 2-2. Cerebrum, dog. Diffusely, neurons are swollen by an accumulation of cytoplasm vacuoles which disperses Nissl substance. (HE, 381X)

diameter, white, elongate nematodes with tapered ends (roundworms) were present multifocally within the small intestinal lumen.

Laboratory Results:
Hepatic beta-hexosaminidase (*Hex*) An enzyme activity in the liver was elevated.

Microscopic Description:
Brain (cerebral cortex at the level of internal capsule): Diffusely, neurons are moderate to markedly expanded by microvacuolated cytoplasm, which often peripheralizes the nucleus and occasionally extends into and expands proximal processes. Infrequently, neurons also contain larger vacuoles up to 8 microns in diameter. Microglia are subjectively increased in number. There is rare neuronal necrosis, and rare blood vessels are cuffed by few lymphocytes. White matter is mildly vacuolated with occasional spheroids and few vacuolated cells (small neuron or astrocyte morphology). The caudate nucleus is regionally hypercellular with vascular proliferation and gliosis − extant neurons are similarly vacuolated.

Other findings:
Spinal cord (Slides not submitted): All spinal cord segments are similarly affected. Diffusely, neurons are variably expanded by abundant finely vacuolated cytoplasm, which often peripheralizes the nucleus and Nissl substance. Multifocal neurons contain larger vacuoles, up to 8 microns in diameter, containing pale eosinophilic material. Some neurons contain both foamy microvacuolated cytoplasm and larger vacuoles. Glia appears unaffected. White matter and nerve rootlets contain scattered dilated and often empty axon sheaths. Meninges, blood vessels − no specific pathologic findings.

Eye (Slides not submitted): Retinal ganglion cells are similarly enlarged and vacuolated with margination of the nucleus.

Duodenum and colon (Slides not submitted): Myenteric and submucosal plexus neurons occasionally appear enlarged with microvacuolated clear to eosinophilic cytoplasm with marginated nuclei.

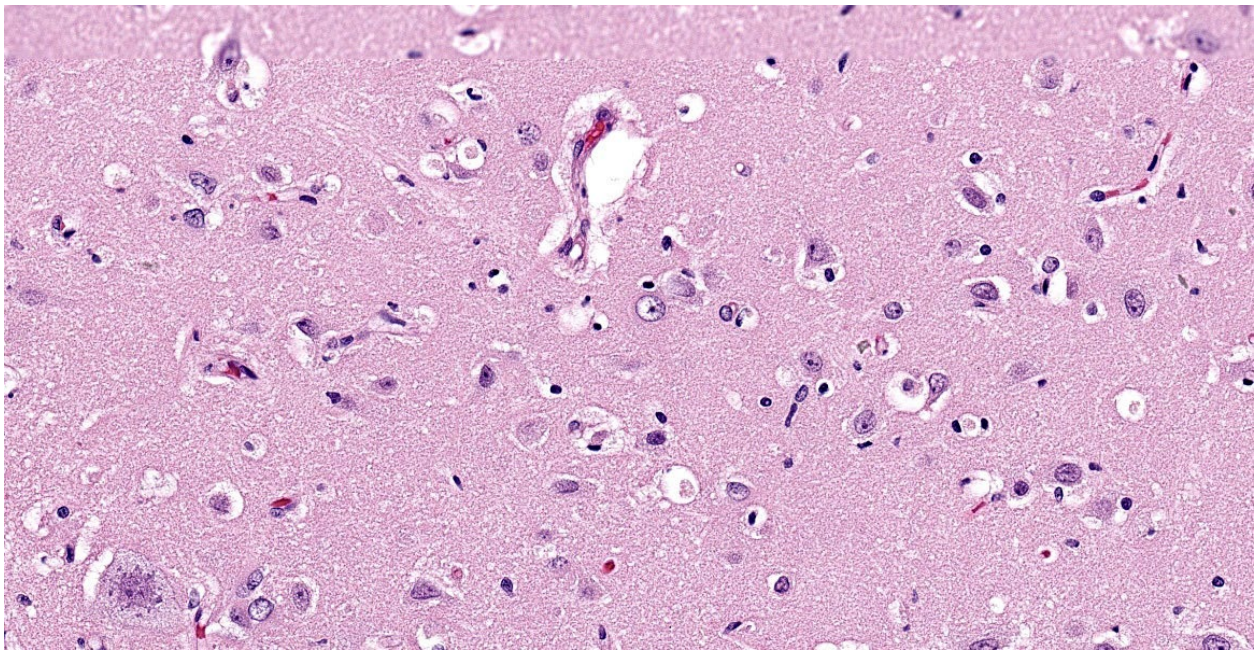

Figure 2-3. Cerebrum, dog. Neurons of all sizes are swollen with cytoplasmic vacuoles

Urinary bladder (Slides not submitted): Ganglion cells within the bladder wall are similarly enlarged and vacuolated.

Mesenteric ganglion (Slides not submitted): Ganglion cells are markedly distended by similar microvaculated cytoplasm as described above.

Contributor's Morphologic Diagnoses:
Cerebral cortex: Neuronal cytoplasmic vacuolation, diffuse, moderate to severe.

Table 2-1: Select sphingolipidoses

Name		Protein defect		Ultrastructural feature of vacuole	Histological features
GM1 gangliosidosis		beta-galactosidase		Lamellar	Vacuoles in neurons, astrocytes, endothelial cells, retinal cells, hepatocytes, and Kupffer cells
GM2 gangliosidosis	B variant (Tay-Sachs disease)	beta-hexosaminidase (Hex)	Alpha subunit of Hex A	Lamellar	Vacuoles in neurons and retinal ganglion cells
	O variant (Sandhoff disease)		Beta subunit of Hex A and B		
	AB variant	Activator protein			
Sphingomyelinosis (Niemann-Pick disease)		Sphingomyelinase		Lamellar	Vacuoles in macrophages and parenchymal cells in many tissues
		Cholesterol transporter			
Galactocerebrosidosis (galactosylceramide lipidosis, globoid cell leukodystrophy, Krabbe disease)		Beta-galactocerebrosidase		Tubular	Vacuoles in oligodendrocytes, Schwann cells, macrophages, globoid cells, with demyelination
Glucocerebrosidosis (glucosylceramidosis, Gaucher disease)		Glucocerebrosidase		Tubular	Vacuoles in neurons, macrophages, Gaucher cells in the spleen, lymph nodes, liver, and bone marrow

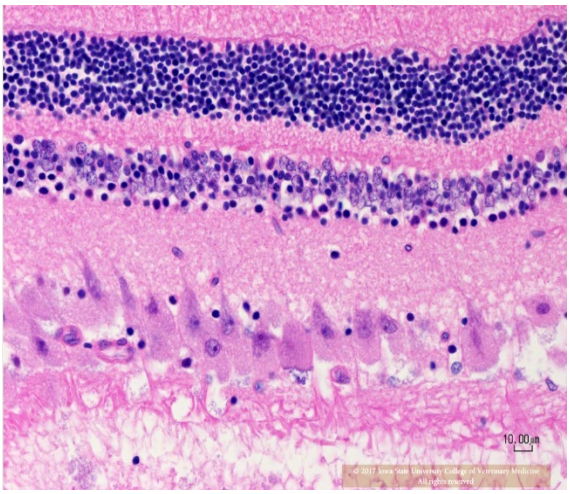

Figure 2-4. Retina, dog. Ganglion cells of the retina are markedly dilated due to an accumulation of clear vacuoles within the cytoplasm. (HE, 400X) (Photo courtesy of: Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, https://vetmed.iastate.edu/vpath)

Spinal cord, mesenteric ganglia: Neuronal cytoplasmic vacuolation, diffuse, moderate to severe.

Retina: Retinal ganglion cell vacuolation, cytoplasmic, diffuse, moderate.

Contributor's Comment:

Microscopic changes in this dog were consistent with a lysosomal storage disease (LSD). Vacuolation was predominantly, if not solely, intraneuronal affecting the central, autonomic nervous systems, and retina. The vacuoles were positive for luxol fast blue (myelin stain) but negative for PAS stain, indicating the inclusions are composed predominately of lipid. Ultrastructural examination of the affected neurons revealed that the vacuoles contain concentric lamellated inclusions which are most consistent with the GM1 and GM2 gangliosidoses or sphingomyelinosis.

Sphingolipidoses are characterized by genetic defects in catabolism of glycosphingolipids which are normal components of cell membranes and extracellular matrix. In animals, based on protein defects, there are at least five important types, including GM1 gangliosidosis, GM2 gangliosidosis, sphingomyelinosis, galactocerebrosidosis, and glucocerebrosidosis (table 2-1)[5–7]. These protein defects lead to impaired function of lysosomal enzymes themselves or proteins assisting lysosomal enzymes in substrate processing. Ultrastructurally, the inclusions in GM1 and GM2 gangliosidoses and sphingomyelinosis reveal whorls and laminar arrangements, while the inclusions in galactocerebrosidosis and glucocerebrosidosis are characterized by long tortuous and twisted tubules.

GM1 gangliosidosis results from the deficiency of beta-galactosidase and shows accumulation of glycolipids and oligosaccharides in many cell types and tissues, including neurons, astrocytes, retinal cells, hepatocytes, Kupffer cells, and endothelial cells in different tissues. Canine GM1 gangliosidosis has an autosomal recessive pattern of inheritance.[25] Different breeds reveal variable defects in the *GLB1* (Galactosidase, beta 1) gene. In Portuguese Water Dogs, a G→A mutation in exon 2 results in an Arg→His amino acid substitution.[22] In Shiba Inu dogs,

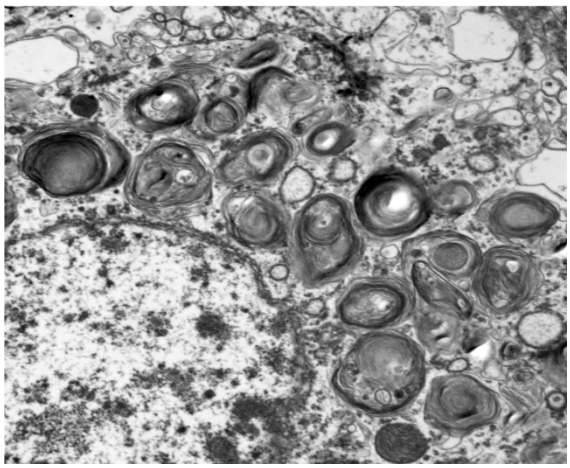

Figure 2-5. Thalamus, dog. Numerous lysosomes with lamellated contents populate the cytoplasm of a thalamic neuron. (TEM, 15,000X). (Photo courtesy of: Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, https://vetmed.iastate.edu/vpath)

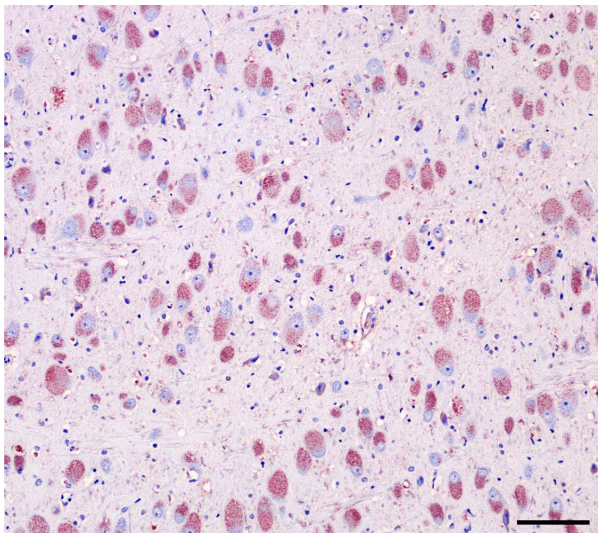

Figure 2-6. Cerebrum, dog. Vacuolated neurons stain strongly immunopositive with cholera toxin subunit B. (anti CTSB, 200X) (Photo courtesy of: Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, https://vetmed.iastate.edu/vpath)

the mutation is a deletion of C nucleotide 1668 in exon 15.[23] Alaskan huskies reveal 19-bp duplication in exon 15 of *GLB1*. [12, 13] Due to similar genetic defects, clinical signs, and lesions, dogs are considered as a suitable animal model of human GM1 gangliosidosis. [1, 8, 11, 22] In humans, GM1 gangliosidosis is divided into three types: type 1 (infantile), type 2 (late infantile/juvenile), and type 3 (adult). Except for the English springer spaniel, other canine breeds are comparable with the late infantile/juvenile form (Table 2-2).[24] In this form, these dogs show progressive clinical nervous signs accompanied by possible skeletal abnormality and hepatosplenomegaly.

In this dog, elevated *Hex*A activity ruled out B and O variants of GM2 gangliosidosis. Strong signal of cholera toxin subunit B in the cytoplasm of vacuolated neurons demonstrated the presences of GM1 ganglioside, potentially implicating GM1 gangliosidosis as the disease entity affecting the patient.[9] However, GM1 ganglioside can also accumulate secondarily in other lysosomal storage diseases.[19] Assay for enzyme deficiency or genetic analysis of the *GLB1* gene is necessary for a definitive diagnosis of GM1 gangliosidosis. In this case, whole genome sequencing revealed a missense mutation in *GLB1* resulting in a C→A substitution at exon 3. Additionally, beta galactosidase activity was significantly decreased in brain white matter, gray matter and liver. Activity levels of neuraminidase and protective protein/cathepsin A (PPCA) were normal. Taken together, these results are consistent with GM1 gangliosidosis, novel in the Golden Retriever breed, and most comparable to the late infantile/juvenile form in humans.

Table 2-2：Comparison of human and canine GM1 gangliosidosis

Human	Features	Dog
Type 1 (infantile)	Dwarfism, facial distortion, bone deformities, hepatosplenomegaly, seizures, vision loss (early), startled response to sound	English Springer Spaniel[3]
Type 2 (late infantile / juvenile)	Bone deformities+/-, hepatosplenomegaly+/-, seizures, ataxia, spasticity, vision loss (late), startled response to sound	German Shorthair Pointer[9], Beagle/Mixed-breed[14,15], Portuguese Water Dog[2], Shiba Inu[18], Alaskan husky[13]
Type 3 (adult)	Ataxia, spasticity	

Contributing Institution:
Department of Veterinary Pathology
College of Veterinary Medicine
Iowa State University
https://vetmed.iastate.edu/vpath

JPC Diagnosis:
Cerebrum, neurons: Vacuolation, cytoplasmic, diffuse, moderate, with mild gliosis.

JPC Comment:
Gangliosides have been studied extensively due to their role in gangliosidosis and the altered levels found in various neurodegenerative disorders such as Alzheimer's disease.[21] Gangliosides are a diverse group of molecules synthesized in the endoplasmic reticulum and Golgi apparatus. While found throughout

the body, they are concentrated in the nervous system, residing in lipid rafts on the external plasma membrane surface of neuronal cells and synapses.[21] Ganglioside prevalence changes with age; in humans, for instance, GM1 concentration increases until adulthood and gradually decreases in old age.[21] While elevated concentration of gangliosides can cause severe disease, in certain conditions, gangliosides can have neurotrophic or neuroprotective effects.[21] Human trials, for instance, have demonstrated that exogenous GM1 helped alleviate neurotoxicity associated with chemotherapy.[21]

In addition to various breeds of dogs, gangliosidoses have been reported in cats, sheep, cattle, pigs American black bears, and emus.[4,15] In sheep, GM1 gangliosidosis has been reported in the Suffolk (type 1) and Romney (types 1, 2, and 3) breeds; in cats, it has been documented in Siamese, Korat, and mixed breed cats.[18,20]

Muthupalani et al. described seven young free-ranging American black bears in New England with poor body condition and variable neurologic symptoms including tremors, ataxia, and hypermetria. The animals had few vacuolated mononuclear cells and neutrophils on blood smear and cerebral atrophy with enlarged ventricles and prominent sulci on necropsy. [15] Eosinophilic vacuoles were present in neurons, retinal ganglion cells, renal proximal tubular epithelium, chondrocytes, and hepatocytes; the vacuoles in the neurons and retina were blue on Luxol fast blue staining. [15] Enzymatic testing revealed drastically decreased beta galactosidase activity compared to the control, and gene sequencing identified a missense mutation Y348H on exon 10 in the GLB1 gene. [15] These findings were consistent with GM1-gangliosidosis. [15]

Bermudez et al described two adolescent emu hatchmates with progressive neurologic signs had similar vacuoles reported in neurons of the cerebrum, brainstem, cerebellum, spinal and autonomic ganglia, and retina. [4] GM1 and GM3 levels were up to 25 times higher than controls, and decreased beta-galactosidase activity; these findings were all consistent with gangliosidosis.[4] This was the first documented report of a neuronal storage disease in avian species outside of Lafora body neuropathy.

References:

1. Ahern-RindellAJ, KretzKA, O'BrienJS. Comparison of the canine and human acid beta-galactosidase gene. *Am J Med Genet*. 1996 May 17 [cited 2018 May 27];63:340–345.
2. AlroyJ, OrgadU, DeGasperiR, et al. Canine GM1-gangliosidosis. A clinical, morphologic, histochemical, and biochemical comparison of two different models. *Am J Pathol*. 1992;140:675–689.
3. AlroyJ, OrgadU, UcciAA, et al. Neurovisceral and skeletal GM1-gangliosidosis in dogs with beta-galactosidase deficiency. *Science*. 1985 Aug 2 [cited 2018 May 27];229:470–472.
4. Bermudez AJ, Johnson GC, Vanier MT, et al. Gangliosidosis in Emus (Dromaius novaehollandiae). *Avian Diseases*. 1995; 39:292-303.
5. CantileC, Youssef S. Nervous System. In: Maxi MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals.* Vol 1. 6th ed. Philadelphia, PA: Elsevier Saunders. 2016:287–288.
6. Cheville NF. Metabolic and Storage Diseases. In: *Ultrastructural Pathology The Comparative Cellular Basis of Disease*. Wiley-Blackwell; 2009:856–861.

7. GilmanS. Lysosomal disorders of the nervous system. In: *Neurobiology of Disease*. Elsevier; 2011:1–9.
8. HubertJJ, O 'brienJS. Dog and human acid beta-D-galactosidases are structurally similar. *Biochem J*. 1983;213:473–478.
9. IwamasaT, OhshitaT, NashiroK, IwanagaM. Demonstration of GM1-ganglioside in nervous system in generalized GM1-gangliosidosis using cholera toxin B subunit. *Acta Neuropathol*. 1987 [cited 2018 May 27];73:357–360.
10. Karbe E, Schiefer B. Familial Amaurotic Idiocy in Male German Shorthair Pointers. *Pathol Vet.* 1967; 4(3):223-32. [cited 2018 May 27].
11. Kaye EM, Alroy J, Raghavan SS, et al. Dysmyelinogenesis in animal model of GM1 gangliosidosis. *Pediatr Neurol*. 1992 [cited 2018 May 27];8:255–261.
12. KreutzerR, KreutzerM, PröpstingMJ, et al. Insights into post-translational processing of β-galactosidase in an animal model resembling late infantile human GM1-gangliosidosis. *J Cell Mol Med.* 2008;12:1661–1671.
13. Kreutzer R, Leeb T, Müller G, Moritz A, Baumgärtner W. A duplication in the canine β-galactosidase gene GLB1 causes exon skipping and GM1-gangliosidosis in Alaskan huskies. *Genetics*. 2005;170:1857–1861.
14. Lldinger SA, Oritz AM, Urbriggen AZ, Irchhof NK, Ewell AS, Aumga WB. GM 1 -gangliosidosis in Alaskan Huskies : Clinical and Pathologic Findings. *Vet Pathol.* 2001; 38(3); 281–290.
15. Muthupalani S, Torres PA, Wang BC, et al. GM1-gangliosidosis in American black bears: Clinical, pathological biochemical, and molecular genetic characterization.*Mol Genet Metab.* 2014; 111(4): 513-521.
16. Read DH, Harrington DD, Keenana TW, Hinsman EJ. Neuronal-visceral GM1 gangliosidosis in a dog with beta-galactosidase deficiency. *Science*. 1976 Oct 22 [cited 2018 May 27];194:442–445.
17. RodriguezM, O'BrienJS, GarrettRS, PowellHC. Canine GM1 gangliosidosis. An ultrastructural and biochemical study. *J Neuropathol Exp Neurol*. 1982 Nov [cited 2018 May 27];41:618–629.
18. Ryder SJ, Simmons MM. A lysosomal storage disease of Romney sheep that resembles human type 3 GM1 gangliosidosis. *Acta Neuropathol*. 2001; 101:225-228. Mol Genet Metab. 2014; 111:513-521.
19. Satoh H, Yamato O, Asano T, Yamasaki M, Maede Y. Increased concentration of G M1 -ganglioside in cerebrospinal fluid in dogs with GM1- and GM2-gangliosidoses and its clinical application for diagnosis. *J Vet Diagn Invest.* 2004;16:223–226.
20. Uddin MM, Hossain MA, Rahman MM, et a. Identification of Bagladeshi Cats with GM1 Gangliosidosis caused by the c.1448>C Mutation of the Feline GLB1 Gene: Case Study. *J Vet Med Sci.* 2013; 75(3):395-397.
21. Vasques JF, de Jesus Goncalves RG, da Silva-Junior AJ, Martins RS, Gubert F, Mendez-Otero R. Gangliosides in nervous system development, regeneration, and pathologies. *Neural Regeneration Research*. 2023: 18(1): 81-86.
22. WangZH, ZengB, ShibuyaH, et al. Isolation and characterization of the normal canine beta-galactosidase gene and its mutation in a dog model

of GM1-gangliosidosis. *J Inherit Metab Dis.* 2000;23:593–606.
23. Yamato O, Endoh D, Kobayashi A, et al. A novel mutation in the gene for canine acid beta-galactosidase that causes GM1-gangliosidosis in Shiba dogs. *J Inherit Metab Dis.* 2002 Oct [cited 2018 May 27];25:525–526.
24. YamatoO, MasuokaY, YonemuraM, et al. Clinical and clinico-pathologic characteristics of Shiba dogs with a deficiency of lysosomal acid beta-galactosidase: a canine model of human GM1 gangliosidosis. *J Vet Med Sci.* 2003;65:213–217.
25. Yamato O, Ochiai K, Masuoka Y, et al. GM1 gangliosidosis in shiba dogs. *Vet Rec.* 2000;146:493–496.

CASE III:

Signalment:
8-month old, male, Birman cat (*Felis catus*)

History:
Animal referred to the Neurology Unit of the Small Animal Hospital (School of Veterinary Medicine, University of Glasgow) with a 3 month history of hind limb weakness and hyporexia.

On clinical examination, the animal showed weakness in all limbs and urinary incontinence.

Clinical chemistry Hyperglobulinaemia, hyperproteinaemia.

Neurological examination revealed mild right sided head tilt, tremors of the head, CP severely delayed right side, tetra-ataxia and tetraparesis. Spinal and cranial nerve reflexes were normal.

MRI investigation identified ventriculomegaly, perivascular and white matter extensive oedema.

The owners elected euthanasia and authorised postmortem collection of the brain for histological evaluation.

Gross Pathology:
Upon dissection of the fixed brain, there is moderate bilateral dilation of the ventricles, with accumulation of moderate amount of pale translucent gelatinous fluid.

Laboratory Results:
Immunohistochemistry for Feline Coronavirus (FCoV) confirmed the presence of FCoV antigen within the cytoplasm of macrophages in the inflammatory infiltrates.

Microscopic Description:
Section of brain at the level of the thalamus. Bilaterally affecting the lateral ventricles there are diffuse inflammatory changes effac-

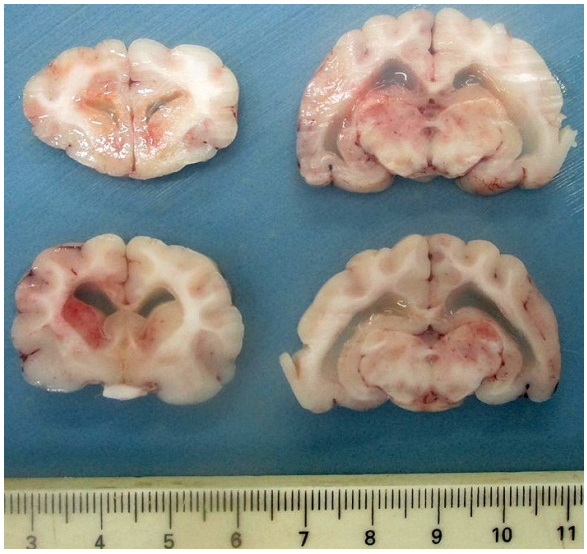

Figure 3-1. Cerebrum, cat. There is marked dilation of ventricles which contain a gelatinous exudate. (Photo courtesy of: Division of Pathology, Public Health and Disease Investigation, Veterinary Diagnostic Services, School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow (Garscube Campus), 464 Bearsden Road, Glasgow G61 1QH, Scotland, https://www.gla.ac.uk/schools/vet/cad/)

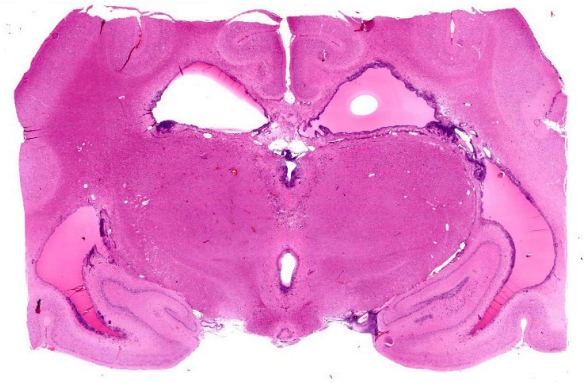

Figure 3-2. Diencephalon, cat. A section of cerebrum at the level of the diencephalon is submitted for examination. There is marked periventricular inflammation and the dilated ventricles contain a protein rich exudate. (HE, 5X)

ing the ependymal lining and variably extending into the subependymal neuropil. Inflammation is dominated by macrophages admixed with scattered neutrophils, and variable numbers of plasma cells, and lymphocytes. Multifocal perivascular (cuffs up to 5 layers) and in some instances mural inflammatory infiltrates dominated by lymphocytes and plasma cells and variable numbers of macrophages affect small to medium sized vessels (venules) in the periventricular neuroparenchyma. Bilaterally the periventricular neuroparenchyma medial to the upper portion of the ventricles is partially effaced by accumulation of eosinophilic proteinaceous fluid with associated sparse infiltration of foamy macrophages admixed with lymphocytes and plasma cells. Multifocally, the periventricular grey and white matter is irregularly vacuolated (spongy change) with few scattered spheroids.

Bilaterally, the lateral ventricles are distended by moderate to large amounts of eosinophilic proteinaceous fluid.

Multifocally, within the hippocampus, in both the pyramidal and molecular layers, there are numerous deposits of coarse globular to laminar basophilic material (mineralisation) variably obliterating the wall and lumen of small capillary vessels and in some instances apparently associated with neurones.

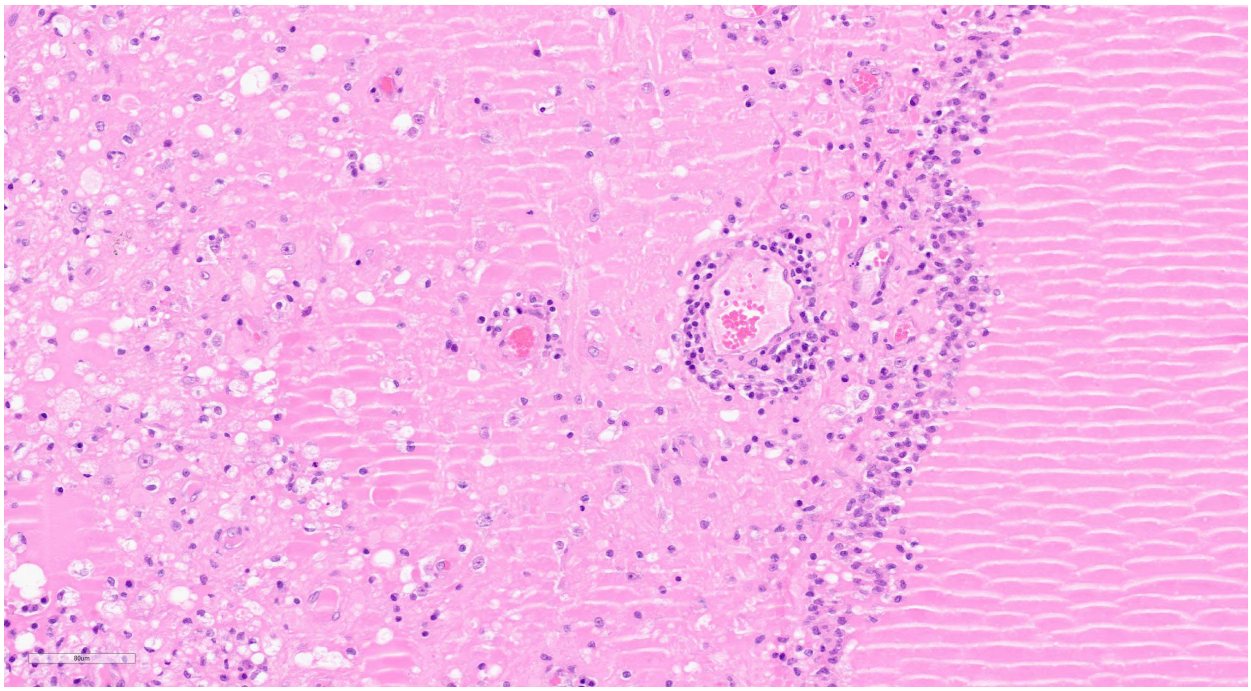

Figure 3-3. Diencephalon, cat. The ventricle contains a protein rich exudate. Numerous lymphocytes, macrophages, and neutrophils infiltrate the ventricle, ependyma, and periventricular white matter. Similar inflammatory cells are present within the walls and perivascularly within the periventricular veins. The periventricular white matter is spongiotic. (HE, 284X)

Table 3-1: coronaviruses affecting different species and their associated disease/lesions

Animal species	Virus	Associated disease/lesions	References
Feline	Feline infectious peritonitis virus (FIPV)	Multisystemic granulomatous/pyogranulomatous disease with vasculitis, serositis, meningoencephalitis, uveitis/ophthalmitis	12,14,22,23
	Feline enteric coronavirus (FECV)	Enteritis with diarrhoea in kittens	19
Canine	Canine Coronavirus (CCV)	Nonfatal enteritis in puppies	2,4
Ferret	Ferret enteric coronavirus (FRECV)	Epizootic catarrhal enteritis	24
	Ferret systemic coronavirus (FRSCV)	Systemic pyogranulomatous inflammation similar to FIP in cats	5,7
Bovine	Bovine coronavirus (BCV)	Severe diarrhoea and respiratory disease in calves and diarrhoea (winter dysentery) in adults	1,15
Mouse	Mouse hepatitis virus (MHV)	Enteritis, hepatitis and demyelinating encephalomyelitis	3
Porcine	Porcine endemic diarrhoea virus (PEDV)	Atrophic enteritis in neonatal piglets	10
	Porcine hemagglutinating encephalomyelitis virus (PHEV)	Lymphoplasmacytic perivascular cuffing in the brain, and stomach muscularis and submucosa	13
	Transmissible gastroenteritis virus (TGEV)	Enteritis with diarrhoea	11
Rat	Rat coronavirus (RCV)	Rhinitis, tracheitis, pneumonitis in young rats	17,21
	Rat sialodacryoadenitis virus (SDAV)	Sialoadenitis, dacryoadenitis, rhinitis, tracheitis, bronchitis/bronchiolitis and alveolitis	21,25
Chickens	Avian infectious bronchitis virus (IBV)	Tracheobronchitis, nephritis	9
Turkeys	Turkey coronavirus (TCV)/Bluecomb virus	Enteritis, cyanosis, severe growth depression	8
Humans	Middle East respiratory syndrome coronavirus (MERS-CoV)	Mild respiratory illness to severe pneumonia and multi-organ failure	16
	Severe acute respiratory syndrome coronavirus (SARS-CoV)	Severe acute respiratory syndrome	20
	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	Severe acute respiratory syndrome / COVID-19	26,27

In the leptomeninges on the ventral aspect of the thalamus there is variable mild to moderate infiltration of lymphocytes, macrophages, neutrophils plasma cells with sparse and perivascular distribution and moderate necrosis. Immunohistochemistry for Feline Coronavirus (FCoV) confirmed the presence of FCoV antigen within the cytoplasm of macrophages in the inflammatory infiltrates.

Note:
Due to availability of material in the original samples, the slides have been prepared from two different tissue blocks. Inflammatory changes in the leptomeninges ventral to the thalamus are mild in one section and more prominent in the other section. In the latter section, mineralisation is also variably present in the periventricular neuroparenchyma in the thalamus.

Contributor's Morphologic Diagnoses:

Brain: periventricular encephalitis, granulomatous/pyogranulomatous and lymphoplasmacytic, chronic extensive marked.
Meningitis, pyogranulomatous necrotising and lymphoplasmacytic, chronic multifocal moderate.
Hippocampus, capillary vessels and neurones, mineralisation, multifocal moderate.

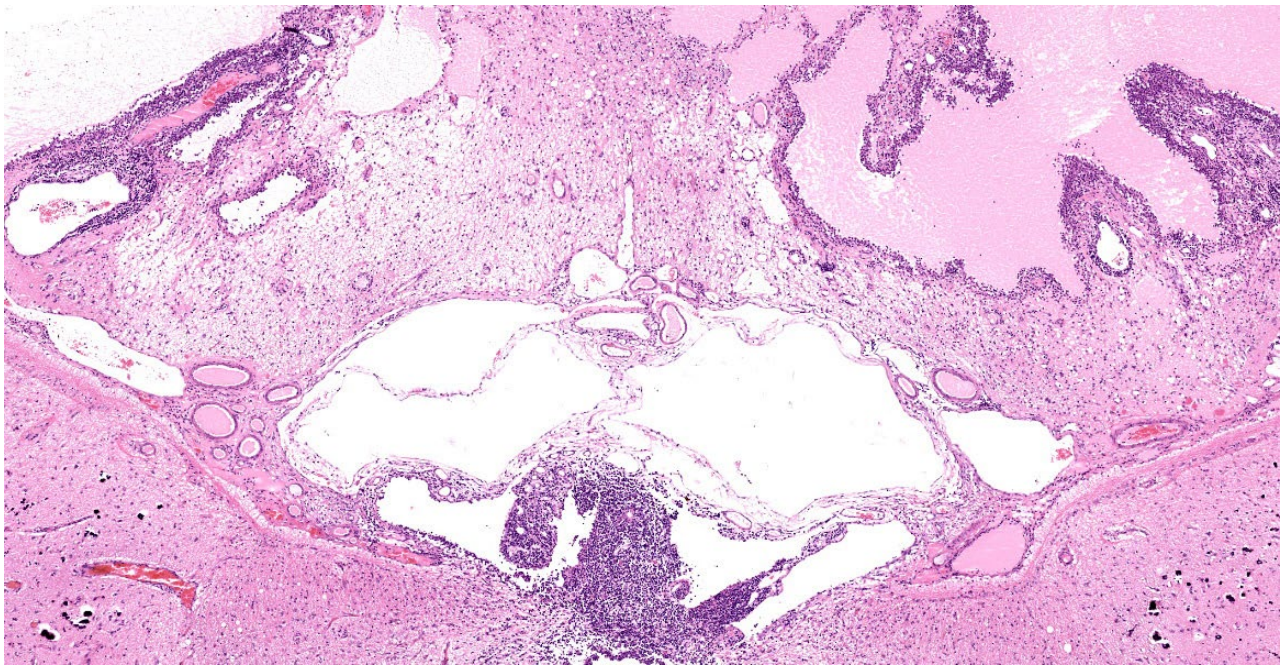

Figure 3-4. Diencephalon, cat. There is marked edema of the periventricular white matter and tela choroidea. The choroid plexus of the third ventricle is expanded by lymphocytes, neutrophils and macrophages and similar inflammation bordering the ventricular ependyma and veins. (HE, 43X)

Contributor's Comment:

Feline coronaviruses (FCoVs) are pleomorphic, enveloped, single-stranded positive sense RNA viruses that belong to the family *Coronaviridae*, order Nidovirales, and genus Alphacoronavirus, species Alphacoronavirus 1.[13] FCoVs occur as 2 biological pathotypes: feline enteric coronavirus (FECV), defined as the ''ubiquitous enteric biotype,'' and feline infectious peritonitis virus (FIPV), the ''virulent biotype" that causes FIP in individual cats.[13, 25] FCoVs can also be divided into two antigenically distinct serotypes (I and II) based on cell culture cytopathic effect and other features, and type I FCoV are more frequently associated with FIP than type II FCoV.[25] Given their close genetic relationship, the viral strains are serologically indistinguishable and difficult to differentiate by routine laboratory testing, making an accurate clinical diagnosis of FIP often difficult.[24] FCoVs are ubiquitous in cats, but the disease FIP is sporadic, and purebred, young, intact male cats appear to be more susceptible.[25] It is transmitted via the faecal-oral route and primarily infect enterocytes.[13] Whereas FECVs replicate mainly in intestinal epithelium and are shed in faeces, FIPVs replicate efficiently in monocytes and induce systemic disease. The host's genetics and immune system also play important roles.[13, 25]

FIP is characterized by fibrinous serositis, with protein-rich effusions in the body cavities of affected cats (effusive or "wet" FIP), as well as granulomatous-necrotising lesions, periphlebitis, and granulomatous and pyogranulomatous inflammatory lesions in several organs, especially, liver, kidney, spleen, leptomeninges, and eyes (non-effusive or "dry" FIP)[16] However, mixed forms are probably common.[25]

Lesions in the CNS are typically oriented toward the surface and target the leptomeninges, ependyma, choroid plexus, and neuroparenchyma.[24] The presence of eosinophilic proteinaceous material within the lateral ventricles observed in this case is interpreted as hydrocephalus, which is a common secondary gross finding described in cases of FIP.[20,

[24, 25] Inflammation within or around the ventricular system may lead to obstruction of cerebrospinal fluid and cause secondary hydrocephalus.[24]

It can be speculated that the presence of multifocal mineral deposits within the hippocampus is a consequence of hypercalcaemia in this cat, which has been reported in association with a few infectious diseases featuring granulomatous inflammation (including FIP). Macrophages can synthesize calcitriol from calcidiol without any negative feedback regulation, thus leading to hypercalcaemia and subsequently metastatic mineralisation.[6]

Contributing Institution:
Division of Pathology, Public Health and Disease Investigation
Veterinary Diagnostic Services
School of Veterinary Medicine
College of Medical, Veterinary and Life Sciences
University of Glasgow (Garscube Campus)
464 Bearsden Road
Glasgow G61 1QH, Scotland
https://www.gla.ac.uk/schools/vet/cad/

JPC Diagnosis:
Diencephalon: Ventriculitis, pyogranulomatous and lymphoplasmacytic, diffuse, severe, with hydrocephalus, phlebitis, choroid plexitis, periventricular necrosis and edema, meningitis, and mineralization.

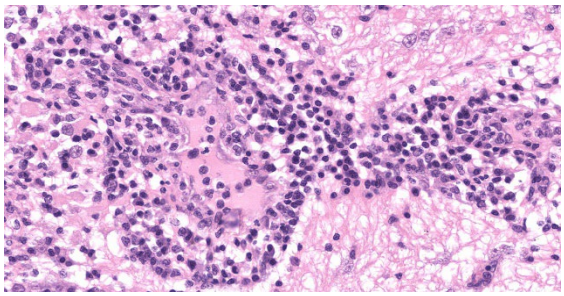

Figure 3-5. Diencephalon, cat. There are lymphocytes, macrophages, and fewer plasma cells within walls off veins which infiltrate the surrounding perivascular parenchyma. (HE, 505X)

JPC Comment:
Neurologic manifestations of feline infectious peritonitis have three general distribution patterns as described by Rissi: diffuse leptomeningitis; rhombencephalitis (or inflammation of the brainstem/cerebellum); and periventricular encephalitis, which is demonstrated in this case and was the most prevalent form in Rissi's study of 26 cats affected by FIP in the central nervous system.

Feline coronavirus (FCoV) is extremely common in domestic cats, and it's estimated that 25% of cats in single cat households and over 75% of cats within multi-cat households are infected.[9] Of those infected, anywhere from 1 to 12% may develop FIP.[9,14] As an RNA-virus, FCoV is prone to replication errors, and a mutation within the spike protein likely accounts for the ability of FIP to infect monocytes and macrophages and cause systemic infection.[14] The spike protein has two subunits, S1 (receptor binding) and S2 (fusion), and a proteolytic cleavage site which, when cleaved, results in activation of the spike protein.[14] In a study of 11 cats with FIP, unique mutations were consistently found in the S1/S2 cleavage site, which the authors speculate may make the protein more susceptible to cleavage by other enzymes.[14]

The internal mutation theory is the most widely accepted model of FIP pathogenesis and asserts that, in each patient, FCoV spontaneously mutates into the pathogenic non-contagious FIP.[9] A variation of the internal mutation theory called the circulating virulent and avirulent theory asserts that certain strains are more likely to induce FIP, which may explain periodic clusters of FIP cases.[9,14] Such a case series was documented in four cats from a single household who succumbed to FIP after being displaced due to a house fire and surrendered to a shelter.[9] The three cats which had viral genetic sequencing each

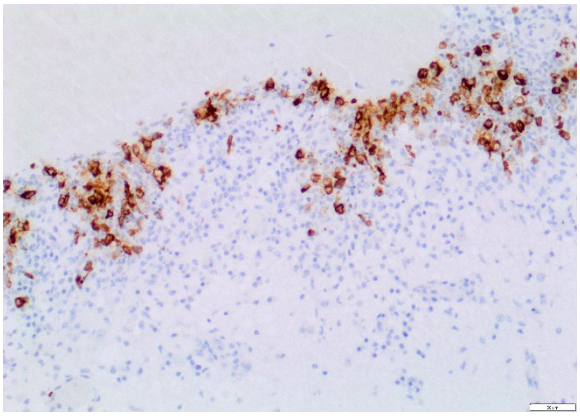

Figure 3-6. Diencephalon, cat. Large numbers of macrophages in the ependymal and periventricular inflammatory infiltrates demonstrate strong cytoplasmic staining for feline coronaviurs (FCoV) antigen. (anti-feline coronavirus, 400X)

had different mutation profiles within the *S1/S2* gene. [9] The authors concluded that these cats likely had a unique FCoV strain prone to FIP-mutation, and the mutations in each cat may have been precipitated by significant physiologic stress. [9]

Finally, rare cases of horizontal transmission have also been reported. At one Taiwanese shelter, 13 cats (28% of the population) succumbed to FIP over a one year time period.[26] All cats were infected with type II FCoV, and the viruses all had identical *S* gene mutations.[26] Those infected later in the year had additional unique mutations in the *3c* gene indicating ongoing mutation.[26] Additionally, the type II FCoV associated with FIP was isolated from both the feces and oronasal and conjunctival samples from infected cats, indicating horizontal transmission was likely responsible for the spread of the virus within the shelter.[26]

References:

1. Bok M, Alassia M, Frank F, Vega CG, Wigdorovitz A, Parreño V. Passive immunity to control Bovine coronavirus diarrhea in a dairy herd in Argentina. *Rev Arg Microb*. 2018;50:23–30.
2. Buonavoglia C, Decaro N, Martella V, et al. Canine Coronavirus Highly Pathogenic for Dogs. *Emerg Infect Dis.* 2006;12:492–494.
3. Compton SR, Ball-Goodrich LJ, Johnson LK, Johnson EA, Paturzo FX, Macy JD. Pathogenesis of Enterotropic Mouse Hepatitis Virus in Immunocompetent and Immunodeficient Mice. *Comp Med*. 2004;54:681-689.
4. Decaro N, Buonavoglia C. An update on canine coronaviruses: Viral evolution and pathobiology. *Vet Microb*. 2008;132:221–234.
5. Doria-Torra G, Vidaña B, Ramis A, Amarilla SP, Martínez J. Coronavirus Infection in Ferrets: Antigen Distribution and Inflammatory Response. *Vet Pathol.* 2016;53:1180–1186.
6. Finch NC. Hypercalcaemia in cats: The complexities of calcium regulation and associated clinical challenges. *J Feline Med Surg.* 2016;18:387–399.
7. Garner MM, Ramsell K, Morera N, et al. Clinicopathologic Features of a Systemic Coronavirus-Associated Disease Resembling Feline Infectious Peritonitis in the Domestic Ferret (Mustela putorius). *Vet Pathol*. 2008;45:236–246.
8. Guy JS. Turkey coronavirus is more closely related to avian infectious bronchitis virus than to mammalian coronaviruses: A review. *Avian Pathol.* 2000;29:207–212.
9. Healey EA, Andre NM, Miller AD, Whitaker GR, Berliner EA. Outbreak of feline infectious peritonitis (FIP) in shelter-housed cats: molecular analysis of the feline coronavirus S1/S2 cleavage site consistent with a 'circulating virulent-avirulent theory' of FIP pathogenesis. *JFMS Open Rep.* 2022. 8(1):1-8.

10. Ignjatovic J, Sapats S. Avian infectious bronchitis virus. *Rev Sci Tech*. 2000;19:493-508.
11. Jung K, Saif LJ. Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunoprophylaxis. *Vet J.* 2015;204:134–143.
12. Kim SH, Kim IJ, Pyo HM, Tark D-S, Song JY, Hyun BH. Multiplex real-time RT-PCR for the simultaneous detection and quantification of transmissible gastroenteritis virus and porcine epidemic diarrhea virus. *J Virol Methods.* 2007;146:172–177.
13. Kipar A, Meli ML. Feline Infectious Peritonitis: Still an Enigma? *Vet Pathol.* 2014;51:505–526.
14. Licitra BN, Millet JK, Regan AD, et al. Mutation in Spike Protein Cleavage Site and Pathogenesis of Feline Coronavirus. *Em Infect Dis*. 2013;19(7):1066-1073.
15. Mora-Díaz JC, Piñeyro PE, Houston E, Zimmerman J, Giménez-Lirola LG. Porcine Hemagglutinating Encephalomyelitis Virus: A Review. *Front Vet Sci.* 2019;6:53.
16. Myrrha LW, Silva FMF, Peternelli EF de O, Junior AS, Resende M, Almeida MR de. The Paradox of Feline Coronavirus Pathogenesis: A Review. *Adv Virol*. 2011;2011:1–8.
17. Oma VS, Tråvén M, Alenius S, Myrmel M, Stokstad M. Bovine coronavirus in naturally and experimentally exposed calves; viral shedding and the potential for transmission. *Virol J*. 2016;13:100.
18. Omrani AS, Al-Tawfiq JA, Memish ZA. Middle East respiratory syndrome coronavirus (MERS-CoV): animal to human interaction. *Pathog Glob Health.* 2015;109:354–362.
19. Parker JC, Cross SS, Rowe WP. Rat coronavirus (RCV): A prevalent, naturally occurring pneumotropic virus of rats. *Archiv Gesamte Virusforschung*. 1970;31:293–302.
20. Pedersen NC, Allen CE, Lyons LA. Pathogenesis of feline enteric coronavirus infection. *J Feline Med Surg*. 2008;10:529–541.
21. Pedersen NC. A review of feline infectious peritonitis virus infection: 1963–2008. *J Feline Med Surg*. 2009;11:225–258.
22. Peiris JSM, Guan Y, Yuen KY. Severe acute respiratory syndrome. *Nat Med.* 2004;10:S88–S97.
23. Percy DH, Barthold DW. Chapter 2: Rat. In: *Pathology of Laboratory Rodents and Rabbits.* Wiley Blackwell; 2016:125-127.
24. Rissi DR. A retrospective study of the neuropathology and diagnosis of naturally occurring feline infectious peritonitis. *J Vet Diagn Invest*. 2018;30:392–399.
25. Uzal FA, Blattner BL, Hostetter JM. Alimentary System. In: Maxie MG, ed. *Jubb, Kennedy & Palmer's Pathology of Domestic Animals.* Vol. 2. 6th ed. Philadelphia, PA: Elsevier Saunders; 2016:253-255.
26. Wang YT, Su BL, Hseih LE, Chueh LL. An outbreak of feline infectious peritonitis in a Taiwanese shelter: epidemiologic and molecular evidence for horizontal transmission of a novel type II feline coronavirus. *Vet Res*. 2013; 44(57):1-9.
27. Wise AG, Kiupel M, Maes RK. Molecular characterization of a novel coronavirus associated with epizootic catarrhal enteritis (ECE) in ferrets. *Virology*. 2006;349:164–174.
28. Yoo D, Pei Y, Christie N, Cooper M. Primary Structure of the Sialodacryoadenitis Virus Genome: Sequence of

the Structural-Protein Region and Its Application for Differential Diagnosis. *Clin Diagn Lab Immunol.* 2000;7:568–573.
29. Zhang Y-Z, Holmes EC. A Genomic Perspective on the Origin and Emergence of SARS-CoV-2. *Cell*. 2020;181:223–227.
30. Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature.* 2020;579:270–273.

CASE IV:

Signalment:
4 year old, female, African pygmy hedgehog (*Atelerix albiventris)*

History:
A progressive mass was noted on the right side of the mouth, resulting in halitosis, tooth mobility, exophthalmus, scale loss, anorexia and weight loss. This was refractory to antibiotics (enrofloxacin) and anti-inflammatories (Meloxicam).

Gross Pathology:
Around the maxillary dental arcade was a poorly demarcated, firm, multilobulated, off-white to tan mass; within the oral cavity, this

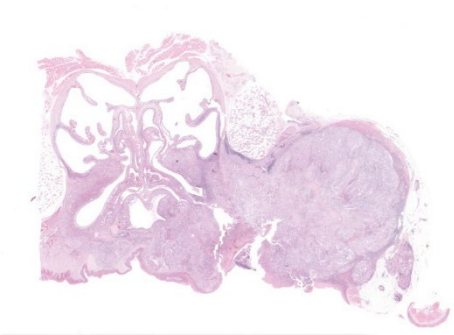

Figure 4-1. Cross section of head, hedgehog. An infiltrative neoplasm arises from the oral cavity and effaces the bones and soft tissues of the face and infiltrates the sinuses dorsally. (HE, 5X)

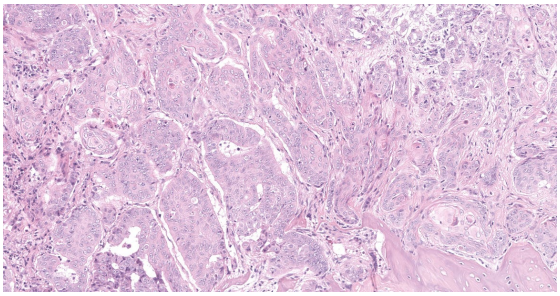

Figure 4-2. Head, hedgehog. Neoplastic squamous epithelium is arranged in nests, cords and trabeculae, and keratinizes poorly within the center of nests. (HE, 170)

was approximately up to 25 mm x 14 mm x 12 mm. The surface was ulcerated and only one molar tooth remained, which was very loose. There was a focal area of ulceration of the hard palate (up to 5 mm x 4 mm). The left maxillary dental arcade was missing several cheek teeth and the remaining teeth were loose. On transverse sectioning of the skull, the mass extended dorsally, replacing much of the right side musculature and the maxillary bone, and extending medially, invading the right nasal cavity (the maximum size of the mass was ~25 mm x 14 mm x 20 mm).

Laboratory Results:
No reported laboratory results.

Microscopic Description:
The tissue is markedly effaced and replaced by a poorly demarcated proliferation of atypical squamous epithelial cells arranged in variably sized cords, trabeculae and nests. Individual cells are oval to polygonal, with variably distinct cell borders and a moderate amount of eosinophilic cytoplasm. They have oval to polygonal, hypochromatic, vesicular nuclei, with one or multiple prominent nucleoli. There is moderate anisocytosis and marked anisokaryosis, and frequent binucleate and multinucleate cells. Atypical cells often palisade around the margin of lobules, and central cells often undergo abrupt keratinisation (hypereosinophilic cells with poorly discernible nuclei) or are markedly swollen. There are occasional individualised necrotic

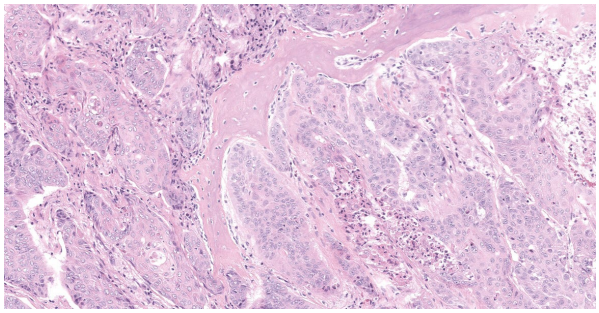

Figure 4-3. Head, hedgehog. Neoplastic cells effaced facial bones. (HE, 170X)

cells (hypereosinophilic cells with karyorrhectic or pyknotic nuclei), clusters of necrotic cells with infiltrates of neutrophils, or apoptotic cells (shrunken cells with pyknotic nuclei). The mucosal surface is thinned (eroded), lost (ulcerated) or is degenerate, with marked vacuolated keratinocytes (ballooning degeneration). The subepithelial tissue contains moderate number of lymphocytes, plasma cells and fewer granulocytes.

Contributor's Morphologic Diagnoses:
Oral squamous cell carcinoma.

Contributor's Comment:
Oral squamous cell carcinomas (SCCs) are commonly diagnosed in the African pygmy hedgehog, and are the third most common neoplasm in this species, presenting with clinical signs including tooth mobility, edentulism and gingivitis.[11] Oral SCCs in African pygmy hedgehogs have been previously described as commonly locally infiltrative with rare metastases, though metastases to distant sites have been reported.[5] In addition to oral SCCs, cutaneous SCCs have also been described in African pygmy hedgehogs.[2] There are few studies characterizing oral SCCs in African pygmy hedgehogs.

Oral squamous cell carcinomas have been characterized in greater detail in other domestic animals, and is the most common oral tumor in cats and the second most prevalent in dogs.[14] These present frequently on the tongue and gingiva, with local invasion, bone invasion and a variable survival, with the greatest mean survival time (MST) following removal of invaded bone.[7] Canine SCCs have been classified further into Conventional (82.1% of cases), Papillary and Basaloid (5.95%), Adenosquamous (3.6%) and spindle cell SCCs (2.4%).[8] However, there is little data to determine variance in prognosis for these different histological subtypes.[7] In any case, spread of oral SCCs to local lymph nodes often occurs late in the disease process[4] and local reoccurrence is the most common form of treatment failure.[12]

Currently, the pathogenesis of oral SCCs is thought to be following long term epithelial hyperplasia from chronic gingivitis irritation.[14] In dogs though there may be a tentative link with canine oral papillomavirus (CPV-1) producing papillomas which undergo malignant transformation.[13] In humans, multiple factors have been implicated in the development of oral SCCs including environmental factors such as tobacco consumption, alcohol consumption and chronic inflammation which leads to dysplasia of the local area and multiple alterations to tumor suppressor genes (p16, $p14^{ARF}$, FHIT, RSSFIA and p53) leading to "field cancerization" and development of SCCs.[1] "Field cancerization" refers to a theory based on the frequent observation of epithelial dysplasia at the periphery of human invasive oral cancers in situations of chronic exposure to carcinogenic insult, which may leads to increased risk of malignant transformation in the entire area affected by the insult.

Contributing Institution:
Easter Bush Pathology – Royal (Dick) School of Veterinary Studies. https://www.ed.ac.uk/vet/services/easter-bush-pathology

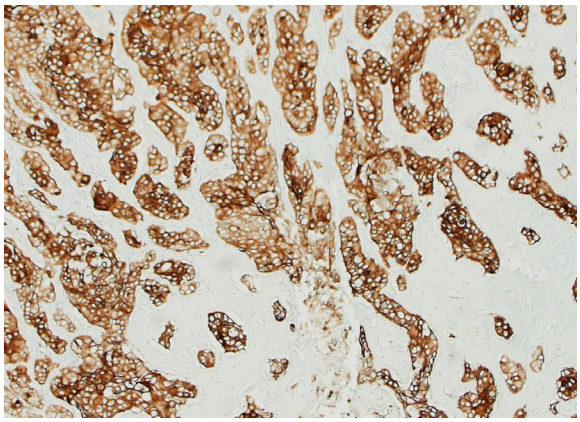

Figure 4-4. Head, hedgehog. Neoplastic cells demonstrate strong immunopositivity for cytokeratin. (anti-AE1/AE3, 400X)

JPC Diagnosis:
Cross section of head: Squamous cell carcinoma.

JPC Comment:
With the growing popularity of hedgehogs as pets, numerous retrospective studies have described the prevalence of neoplasia within captive populations, each with slightly different results. In a study of pet hedgehogs in the US, the most common neoplasms were mammary neoplasia, lymphoma, and oral squamous cell carcinoma.[11] In a study in Japan, the most common neoplasms were endometrial stromal tumor, fibrosarcoma, and mammary neoplasms.[9] In a study of 63 hedgehogs from the Tai Pei Zoo, the three most common neoplasms were oral squamous cell carcinoma, lymphoma, and pulmonary adenocarcinoma.[10] The authors speculated that geographic variability may be due to in-breeding of closed populations due to movement restrictions imposed due to the species' susceptibility to foot and mouth disease.[10]

A study of captive hedgehogs in Chile focused specifically on oral masses found that 17 of 27 oral masses were squamous cell carcinoma. These neoplasms were generally ulcerated (13 cases) and occurred most commonly (12 cases) in the caudal aspect of the right maxilla.[3] Gingival hyperplasia was the second most common diagnosis (8 cases). Gingival hyperplasia was found in varied location, manifested as pedunculated or sessile masses, and was non-ulcerated.[3]

Hedgehogs are one of a few non-cloven hooved species which can be naturally infected by foot and mouth disease virus. During a series of in England in the 1940s, several infected wild hedgehogs were found with vesicles on the feet, and researchers subsequently demonstrated that cattle could experimentally infect hedgehogs.[6] The authors concluded that hedgehogs had some role in spreading infection between cattle. [6]

References:

1. Choi, S. & Myers, J. N. Molecular pathogenesis of oral squamous cell carcinoma: Implications for therapy. *J. Dent. Res.* 87, 14–32 (2008).
2. Couture, É. L., Langlois, I., Santamaria-Bouvier, A. & Benoit-Biancamano, M. O. Cutaneous squamous cell carcinoma in an African pygmy hedgehog (Atelerix albiventris). *Can. Vet. J.* 56, 1275–1278 (2015).
3. Del Aguila G, Torres C, Carvallo FR, Gonzolaz CM, Cifuentes FF. Oral masses in African pygmy hedgehogs. *Jour Vet Diag Invest*. 2019; 31(6): 864-867.
4. Grimes, J. A. et al. Histologic evaluation of mandibular and medial retropharyngeal lymph nodes during staging of oral malignant melanoma and squamous cell carcinoma in dogs. *J. Am. Vet. Med. Assoc.* 254, 938–943 (2019).
5. Heatley, J., Mualdin, G. & Cho, D. A Review of Neoplasia in the Captive African Hedgehog (). *Semin. Avian Exot. Pet Med.* 14, 182–192 (2005).

6. McLaughlan JD, Henderson WM. The occurrence of foot-and-mouth disease in the hedgehog under natural conditions.*J Hyg (Lond).* 1947; 45(4): 474-479.
7. Meuten D.J. *Tumors in domestic animals.* 5th ed. Ames, IO: John Wiley & Sons, Inc. 2002.
8. Nemec, A., Murphy, B., Kass, P. H. & Verstraete, F. J. M. Histological Subtypes of Oral Non-tonsillar Squamous Cell Carcinoma in Dogs. *J. Comp. Pathol.* 147, 111–120 (2012).
9. Okada K, Kondo H, Sumi A, Kagawa Y. A retrospective study of disease incidence in African pygmy hedgehogs (*Atelerix albiventris*).
10. Pei-Chi H, Jane-Fang Y, Lih-CHiann W. A Retrospective Study of the Medical status on 63 African Hedgehogs (*Atelerix albiventris*) at the Taipei Zoo from 2003 to 2011. *J Exot Pet Med*. 2015; 24:105-111.
11. Raymond, J. T. & Garner, M. M. Spontaneous tumours in captive African hedgehogs (Atelerix albiventris): A retrospective study. *J. Comp. Pathol.* 124, 128–133 (2001).
12. Riggs, J. et al. Outcomes following surgical excision or surgical excision combined with adjunctive, hypofractionated radiotherapy in dogs with oral squamous cell carcinoma or fibrosarcoma. *J. Am. Vet. Med. Assoc.* 253, 73–83 (2018).
13. Thaiwong, T. et al. Malignant transformation of canine oral papillomavirus (CPV1)-associated papillomas in dogs: An emerging concern? *Papillomavirus Res.* 6, 83–89 (2018).
14. Uzal F, Plattmer B. Alimentary System. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol 2. 6th ed. Philadelphia, PA: Elsevier Saunders. 2016: 25–26.