

WEDNESDAY SLIDE CONFERENCE
2020-2021

Conference 8

14 October, 2020



Joint Pathology Center
Silver Spring, Maryland

CASE 1: 19764-13 (4048438-00)

Signalment:

3.75 year old neutered male Labrador retriever mix (*Canis lupus familiaris*)

History:

A gait change was first noted by the owner at 2.5 years of age. By January 2013, overt pelvic limb ataxia and nail wear was observed, and the patient was beginning to develop fecal and urinary incontinence. Second examination in May 2013 reveals worsening ataxia and reduced pelvic limb reflexes. By September 2013 the dog was non-ambulatory, paraparetic and was euthanized. At the time of euthanasia, the bladder had to be manually expressed and vocalizations were hoarse.

MRI revealed T2 intensification in the T7 vertebral body, but none in the spinal cord. CBC-chemistry values normal when tests were done in January. The dog was genotypically normal when tested for SOD1 mutation found in canine degenerative myelopathy in February 2013.

Gross Pathology:

No lesions were noticed during preparation of tissues from microscopic examination.

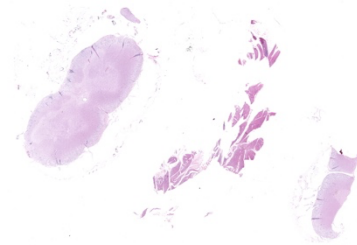
Laboratory results:

None.

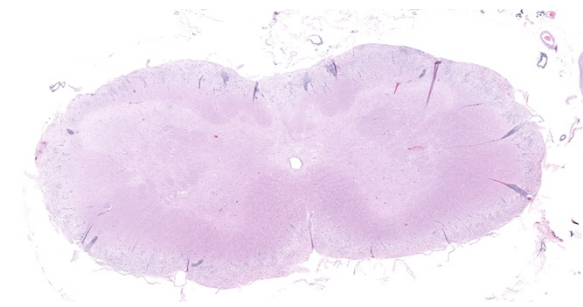
Microscopic description:

BICEPS FEMORIS MUSCLE: Scattered foci of lymphocytes and macrophages occur in the interstitium of the muscle. Degenerating and regenerating muscle fibers are associated with this inflammation. In addition, degeneration and necrosis of individual muscle fibers occur where inflammation is absent. These contain more frequent clusters of neutrophils. Protozoal trophozoites and cysts are present in the lesions. Some muscle bundles contain small angular fibers as well, and some of the nerves between muscle bundles appear to contain a reduced number of axons.

SPINAL CORD: Sections of spinal cord are characterized by vacuolation of the subpial white matter, with extensive astrogliosis. Penetrating coronal vessels have plump endothelium and are



Spinal cord and skeletal muscle, dog. A section of spinal cord and biceps femoris are presented for examination. (HE, 5X)



Spinal cord, dog. There is pallor and hypercellularity of the submeningeal white matter, with hypercellularity of Virchow-Robin's spaces. (HE, 13X)

surround by variably thick lymphohistiocytic cuffs. In the tissue, there are small clusters of plasma cells and rare nests of neutrophils. The leptomeningeal infiltrate consists largely of plasma cells. The affected tissue contains coccidian protozoal cysts with bradyzoites and less common individual tachyzoites. Enlarged astrocyte nuclei are evident in the affected areas. Similar lesions were present in the brainstem, cerebellum, pons and medulla with mild cerebral lesions. As well as occurring near the pial surface, lesions were common near the ependymal lining of the brain ventricles.

Contributor's morphologic diagnosis:

Widespread, surface-oriented lymphohistiocytic encephalomyelitis with plasmacytic meningitis and numerous coccidian protozoa

Widespread multifocal lymphohistiocytic myositis, with localized neurogenic atrophy and frequent coccidian protozoa

Contributor's comment:

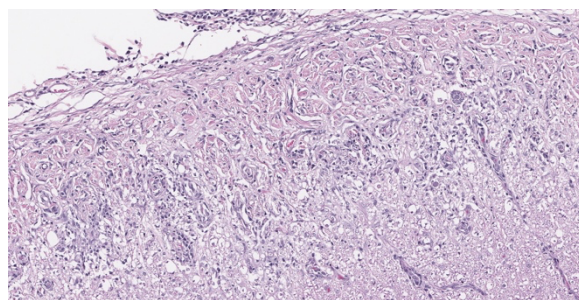
This dog developed slowly progressive ataxia, beginning as an adult. At the time of death, over a year later active proliferation of *Neospora* was apparent in muscle and throughout the neuraxis. This animal had no history of immunosuppressive therapy. It is not clear whether this case represents reactivation of a silent infection of primary progressive infection of an adult animal.

Neospora caninum is an apicomplexan protozoan parasite causing acute and recrudescent infections, primarily of dogs and cattle. Viable organisms can be isolated from cattle, water

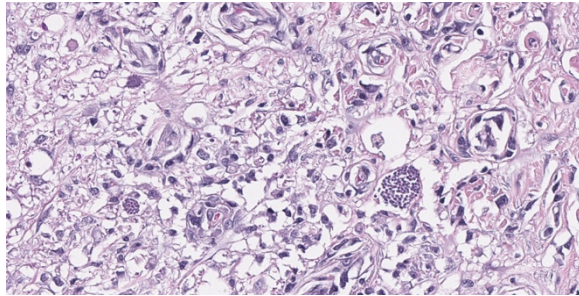
buffalo, sheep, dogs, horses, bison and miscellaneous wild ruminants and canids.⁶ Vertical transmission is considered variable in dogs and may not persist for repeat transmission during pregnancy.⁶ It is known that dogs can harbor encysted parasites in several organs, as detected by PCR.¹⁰ However, vertical transmission from the dam to puppies, sometimes with disease, has also been documented.^{8,12} But, experimentally, not all dogs given 10,000 oocysts shed organisms or seroconverted.² Horizontal fecal transmission has not been proved, but dogs can shed organisms after being fed infected tissue. Dogs can also be infected with *Toxoplasma gondii*, *Sarcocystis neurona*⁵ and *Sarcocystis* spp¹⁷ which can be confused with *Neospora* if immunohistochemical or PCR confirmation is not performed.

It is known that various isolates of *Neospora caninum* can vary in virulence.⁷ Using proteomics, Regidor-Cerrillo et al identified tachyzoite proteins associated with higher virulence¹⁵ that seemed to accentuate the gliding motility of the organism and increase oxidative stress. Some *Neospora* isolates killed about 10% of fetuses in a murine model of canine infection, while others were less pathogenic.⁴

Whether adult dogs can undergo reactivation of infection leading to clinical disease is a matter of speculation. *Neospora caninum* has been known to cause disease in adult dogs undergoing immunosuppressive therapy,^{9,14,16} and the contributor has observed an additional case that



Spinal cord, dog. The submeningeal white matter is edematous, contains numerous dilated myelin sheaths and contains numerous perivascular cuffs of lymphocytes and histiocytes that infiltrate the adjacent parenchyma. There are several intracellular cysts with numerous oval tachyzoites. (HE, 158X)



Spinal cord, dog. Higher magnification of three apicomplexan cysts within the affected white matter. (HE, 477X)

resulted in localized severe myositis with numerous organisms.

Detecting infection can be problematic: it has been estimated that up to 32% of Algerian pound dogs were infected as tested by PCR, and there was poor correlation between PCR results and seropositive test results among individuals.¹⁰

In cattle, abortions occur after oocysts are ingested during pregnancy or with reactivation of previous infection.⁶ There is an estimated 40-60% rate of vertical transmission, although not all fetal infections produce abortion. Most abortions occur at 5-6 gestational months, and less common CNS infection has been detected in calves less than 2 months of age. No predisposing immunosuppressive event is generally detected that might provoke reactivation.

Much has been done to try to distinguish reactivation from primary infection from chronic infection serologically. Cattle with antibody to both tachyzoite and bradyzoite antigen are suspected of reactivation. Anti-GRA7 (dense granule protein) antibody is found in acute infection or reactivation, while SAG4 (bradyzoite specific) antibody rise indicates chronic infection.² Cell-mediated immunity is potentially needed to control infection and is predominantly Th₁.¹³ Treatment with IFN- γ or TNF- α was shown to reduce tachyzoites.

Contributing Institution:

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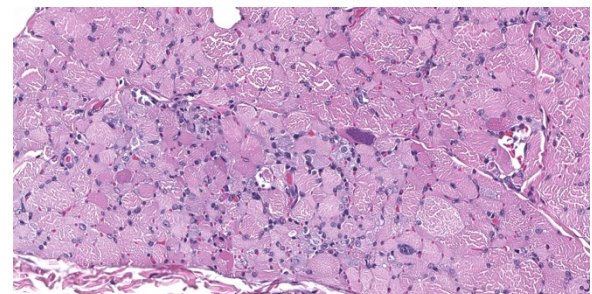
JPC diagnosis:

1. Spinal cord: Meningomyelitis, necrotizing and lymphohistiocytic, diffuse, moderate, with numerous apicomplexan cysts, Labrador retriever, canine.
2. Skeletal muscle: Myositis, lymphohistiocytic, multifocal, moderate, with intracellular apicomplexan cysts.
3. Skeletal muscle: Atrophy, segmental, marked.

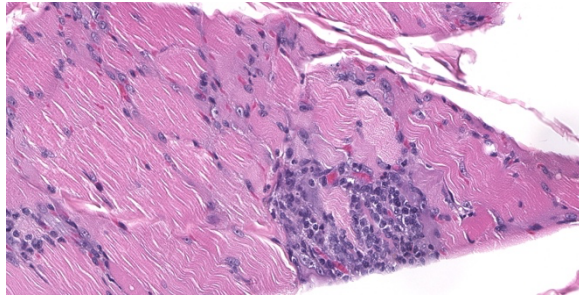
JPC comment:

There was discussion in conference that there may have been some slide variation, with some participants receiving sections of brainstem as well as spinal cord. The morphologic diagnosis will differ slightly, but the pathologic process remains the same.

The contributor provides a concise summary and explanation of this case. *Neospora* spp have three distinct infectious stages: tachyzoites, tissue cysts, and oocysts, with tachyzoites and tissue cysts found in both intermediate and definitive hosts. As stated by the contributor, *N. caninum* does not cause significant disease in adult cattle but causes abortion in beef and dairy cattle. Some common lesions in fetal calves include lymphocytic, plasmacytic, sometimes histiocytic hepatitis, pancarditis or myocarditis, myositis, and placentitis. The most common CNS lesion in bovine fetuses is multifocal discrete foci of necrosis, primarily in the brain, and the spinal cord to lesser extent. While capable of causing disease in dogs of all ages, the most common CNS lesions are necrotizing granulomatous, lymphoplasmacytic, and sometimes eosinophilic



Skeletal muscle dog. There is evidence of mild skeletal muscle degeneration with tinctorial change of fibers, hyperplasia of satellite nuclei, and infiltration of histiocytes within the endomysium. One fiber contains an intracellular apicomplexan cyst with numerous bradyzoites.



Skeletal muscle, dog. There are patchy areas of rhabdomyocyte necrosis with infiltration by numerous histiocytes and lymphocytes. (HE, 400X).

meningoencephalomyelitis, with tachyzoites often visible in lesions.³

The innate immune system is important in recognition of *Neospora caninum*. Both TLR2 and TLR3 play a role in initial recognition and induce production of IL-12 and IFN- γ . However, recent research has centered on nucleotide oligomerization domain (NOD)-like receptors (NLR). A specific group of NLRs sense multiplying pathogen associated molecular patterns (PAMP) or damage associated molecular patterns (DAMP) and initiate the formation of the inflammasome. The NLRP3 inflammasome sense extracellular ATP, nigericin, and uric acid crystals, as well as many bacteria, viruses, fungi, and parasites. Research using murine peritoneal macrophages showed that to activate the NLRP3 inflammasome, two signals are required. The first is usually provided by NF- κ B which results in increased transcription of NLRP3 and pro-IL-1 β and pro-IL-18. The second signal is downstream and initiated by the first step, and then causes inflammasome complex formation, resulting in caspase-1 proteolytically cleaving pro-IL-1 β and pro-IL-18 to their active forms. Concurrently, caspase-1 also triggers pyroptosis by cleaving gasdermin-D (GSDMD), resulting in GSDMD-formed pores in the plasma membrane. In murine macrophages exposed to *N. caninum*, gene expression was significantly increased for NLRP3 (and NLRC4 and NLRC5 to a lesser extent). In *Nlrp3*^{-/-} macrophages, significantly lower amounts of IL-1 β and IL-18 were produced, resulting in a less robust immune response to the pathogen.^{18,19}

Neospora caninum has been detected in various avians as well, such as the chicken, sparrow, magpie, and buzzard. A recent finding in Iran was that a number of hooded crow (*Corvus cornix*) brains were PCR positive for either *Neospora caninum*, *Toxoplasma gondii*, but no coinfections were detected. The crows most likely become infected after ingesting oocysts from infected carrion, but their role in the lifecycle remain to be elucidated more fully.¹

A potentially emerging disease in China, *Neospora caninum* has been detected in pigs and reported for the first time. Serum samples collected from January 2017 until December 2018 were tested and approximately 1.9% of all samples were positive for antibodies via a competitive-inhibition enzyme-linked immunosorbent assay (cELISA). Concurrently, PCR testing of brain samples was positive for *N. caninum* genes *Nc-5* and the ITS-1 region, finding five and eight in 600 samples harbored the parasite, respectively. The testing shows that pigs can serve as an intermediate host for *N. caninum*, but the epidemiological significance is not yet clear.¹¹

References:

1. Abdoli A, Arbabi M, Pirestani M, et al. Molecular assessment of *Neospora caninum* and *Toxoplasma gondii* in hooded crows (*Corvus cornix*) in Tehran, Iran. *Comparative Immunology, Microbiology and Infectious Diseases*. 2018; <https://doi.org/10.1016/j.cimid.2018.06.008>.
2. Aguado-Martinez A, Alvarez-Garcia G, Fernández-Garcia A, et al. Usefulness of fNcGRA7- and rNcSAG-based ELISA tests from distinguishing prim-infection, recrudescence, and chronic bovine neosporosis. *Vet Parasitol*. 2008;**157**:182-195.
3. Cantile C, Youssef S. Nervous System. In: *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, 6th Ed, Vol 1*. St. Louis, MO: Elsevier. 2016:387-388.
4. Dellarupe A, Regidor-Cerillo J, Jiménez-Ruiz E, et al. Clinical outcome and vertical transmission variability among canine neospora caninum isolates in a pregnant mouse mode of infections. *Parasitol*. 2014;**141**:356-366.

5. Dubey JP, Black SS, Verma SK, et al. *Sarcocystis neurona* schizonts-associated encephalitis, chorioretinitis and myositis in a two-month-old dog simulating toxoplasmosis, and presence of mature sarcocysts in muscle. *Vet Parasitol.* 2014; **202**:194-200.
6. Dubey JP, Schares G. Neosporosis in animals – the last five years. *Vet Parasitol.* 2011;**180**:90-108.
7. Dubey JP, Steekumar C, Knickman E, et al. Biologic, morphologic and molecular characterization of *Neospora caninum* isolates from littermate dogs. *Intern J Parasitol.* 2004;**334**:1157-1167.
8. Dubey JP, Vianna MC, Kwok OC, et al. Neosporosis in beagle dogs: clinical signs, diagnosis, treatment, isolation and genetic characterization of *Neospora caninum*. *Vet Parasitol.* 2007;**149**:158-166.
9. Fry DR, McSpooran JD, Ellis JT et al: Protozoal hepatitis associated with immunosuppressive therapy in a dog. *J Vet Intern Med.* 2009;**23**:366-368.
10. Ghalmi F, China B, Kaidi R, et al. Comparison of different serological methods to detect antibodies specific to *Neospora caninum* in bovine and canine sera. *J Vet Diagn Invest.* 2014;**26**:136-140.
11. Gui B, Lv Q, Ge M, Li R, Zhu X, Liu G. First report of *Neospora caninum* infection in pigs in China. *Transbound Emerg Dis.* 2019;00:1-4.
12. Heckerroth AR, Tenter AM. Immunoanalysis of three litters born to a Doberman bitch infected with *Neospora caninum*. *Parasitol Res.* 2007;**100**:837-846.
13. Jesus EE, Pinheiro AM, Santos AB, et al. Effects of IFN- γ , THF- α , IL-10 and TGF- β on *Neospora caninum* infection in rat glial cells. *Exp Parasitol.* 2013;**133**:269-274.
14. La Perle KM, del Piero F, Carr RF, et al. Cutaneous neosporosis in two adult dogs on chronic immunosuppressive therapy. *J Vet Diagn Invest.* 2001;**13**:252-255.
15. Regidor-Cerrito J, Álvarez-García G, Pastor-Fernández I, et al. Proteome expression changes among virulent and attenuated *Neospora caninum* isolates. *J Proteomics.* 2012;**75**:2306-22318.
16. Saev V, Martlé V, Van Ham L, et al. Neuritis of the cauda equina in a dog. *J Small Anim Pract.* 2010;**51**:549-552.
17. Sykes JE, Dubey JP, Lindsay LL, et al. Severe myositis associated with *Sarcocystis*

spp, infection in 2 dogs. *J Vet Intern Med.* 2011;**25**:1277-1283.

18. Wang X, Gong P, Zhang X, et al. NLRP3 Inflammasome Participates in Host Response to *Neospora caninum* infection. *Frontiers in Immunology.* 2018;9:1791.
19. Wang X, Gong P, Zhang X, et al. NLRP3 inflammasome activation in murine macrophages caused by *Neospora caninum* infection. *Parasites and Vectors.* 2017;10:266.

CASE 2: No label (4135756-00)

Signalment:

18-year-old, gelding, Friesian horse, Equus caballus

History:

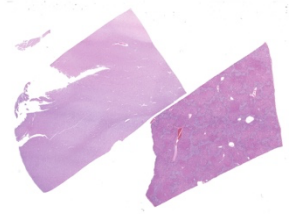
Depressed/dull mentation for the last 4 days; progressive neurologic signs, head pressing, weakness, falling down. Has had progressive weight loss over the winter.

Gross Pathology:

A complete gross necropsy was performed. Notable gross findings were limited to the liver, which was diffusely pale green to tan with a subtle reticular pattern that continued on cut surface. Diffusely the liver was markedly firm and contracted. No gross brain lesions were identified.

TRIGLYCERIDE	90	H	20 - 70 mg/dL
GLUCOSE	191	H	70 - 135 mg/dL
BUN	10		9 - 22 mg/dL
CREATININE	1.4		0.7 - 1.8 mg/dL
PHOSPHORUS	3.1		1.7 - 4.5 mg/dL
CALCIUM	8.9	L	11.5 - 14.0 mg/dL
MAGNESIUM	0.8	L	1.6 - 2.2 mg/dL
TOTAL PROTEIN	8.2	H	5.8 - 7.4 g/dl
ALBUMIN	2.1	L	2.9 - 3.7 g/dl
GLOBULIN	6.1	H	2.5 - 4.5 g/dl
A/G RATIO	0.3	L	0.7 - 1.5 RATIO
T-BILIRUBIN	3.0	H	0.4 - 1.8 mg/dL
AST	1131	H	185 - 375 IU/L
CK	6977	H	100 - 470 IU/L
GGT	708	H	10 - 25 IU/L
SDH	14	H	0 - 10 IU/L
SODIUM	127	L	132 - 142 mEq/L
POTASSIUM	3.28		2.7 - 4.8 mEq/L
CHLORIDE	84.3	L	97 - 104 mEq/L
BICARBONATE (HCO ₃ ⁻)	12.9	LL	26 - 33 mEq/L
ANION GAP	33	H	8 - 15 mmol/L
CALCULATED OSMOLALITY	257		mOsm/Kg
HEMOLYSIS	10		0 - 25 mg/dL
ICTERUS	5	H	1 - 3 mg/dL
LIPEMIA	4		0 - 14 mg/dL

Clinical laboratory data.



Cerebrum, liver horse. A section of cerebrum (left) and liver (right) are submitted for examination. At low magnification, a retiform pattern of pallor is seen within the section of liver. (HE, 5X)

Laboratory results:

See table.

Microscopic description:

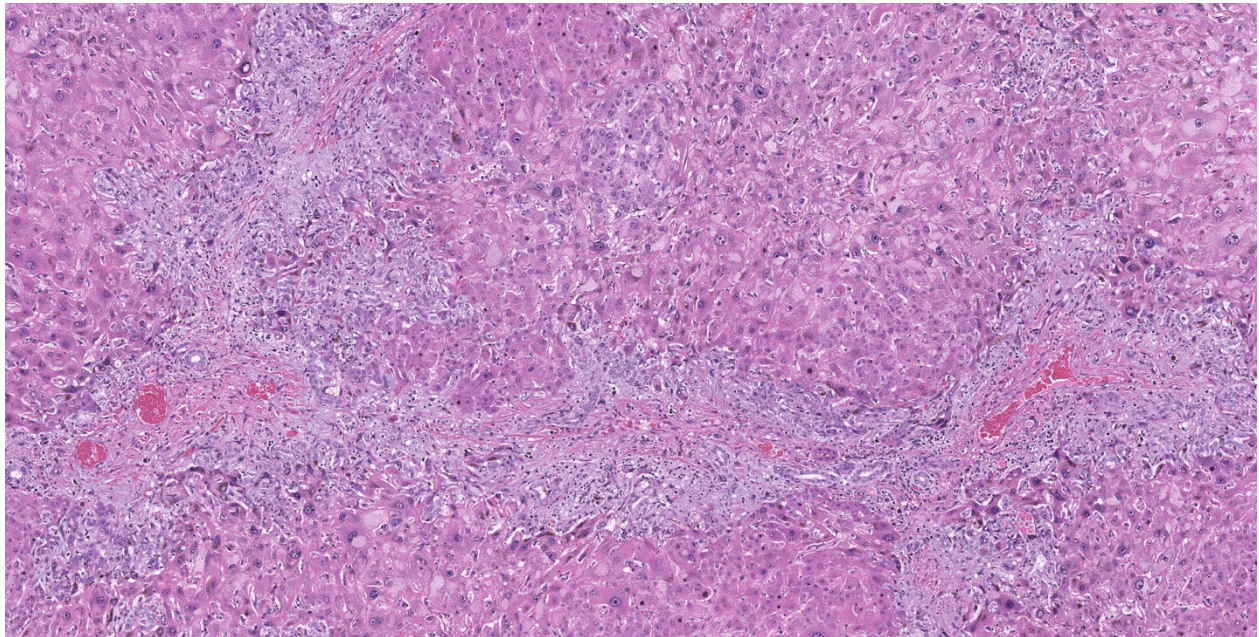
Liver: Diffusely hepatocytes are degenerative and dysplastic with marked variation in cellular morphology characterized by abundant, variable vacuolated, cytoplasm (cytomegaly) and marked nuclear variation with stippled chromatin and prominent nucleoli (karyomegaly). Hepatocytes occasionally have two to several nuclei. Rarely affected hepatocytes are necrotic with shrunken cellular profiles, hypereosinophilic cytoplasm, cellular rounding and pyknotic to karyorrhectic

nuclei. The interstitium is infiltrated by low numbers of individualized lymphocytes, plasma cells and pigment laden macrophages. Portal triads are diffusely expanded by an abundant amount of fibroplasia that dissects into the surrounding parenchyma and commonly connects adjacent portal triads (bridging fibrosis). Portal triads also have increased numbers of biliary profiles (biliary hyperplasia) and a moderate amount of oval cell hyperplasia. Multifocally hepatic lobules are hypercellular and expanded with loss of discrete architecture and portal triad drop out (nodular regeneration).

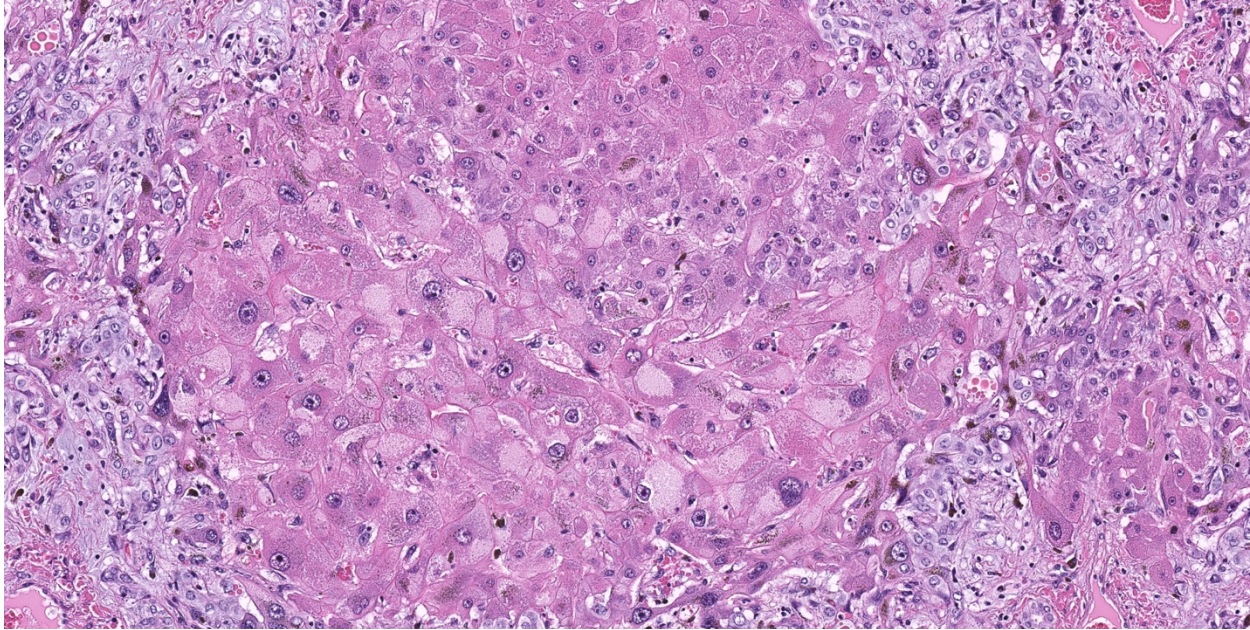
Cerebrum: Throughout the gray matter are an increased number of astrocytes with many pairs and clusters of astrocytes with enlarged vesicular nuclei that are multifocally surrounded by a non-staining rim (type 2 Alzheimer cells). Rarely, neurons in affected regions are hypereosinophilic and angular (necrotic). Small and medium caliber vessels have expanded Virchow-Robin spaces (vasogenic edema).

Contributor's morphologic diagnosis:

Liver: Marked, chronic, toxic hepatocellular degeneration with portal and bridging fibrosis, biliary hyperplasia, megalocytosis and



Liver, horse. Portal areas are markedly expanded by fibrous connective tissue which often bridges between adjacent portal areas as well as extends into the peripheral hepatic lobule. Even at this low magnification, enlarged hepatocellular nuclei of megalocytes can be seen. (HE, 71X)



Liver, horse. Portal areas (left and right) efface the periphery of the lobule with a combination of disordered fibrosis, plump fibroblasts, and proliferation of bile ducts lined by hypertrophic biliary epithelium. Remaining centrilobular and midzonal hepatocytes exhibit marked hydropic degeneration and cytoplasmic vacuolation which effaces sinusoids. Several megalocytes exhibit markedly enlarged nuclei. (HE, 300X)

megalokaryosis, rare individual hepatocyte necrosis and nodular regeneration.

Brain: Moderate, chronic, diffuse Alzheimer type 2 astrocytosis with multifocal neuronal necrosis and degeneration.

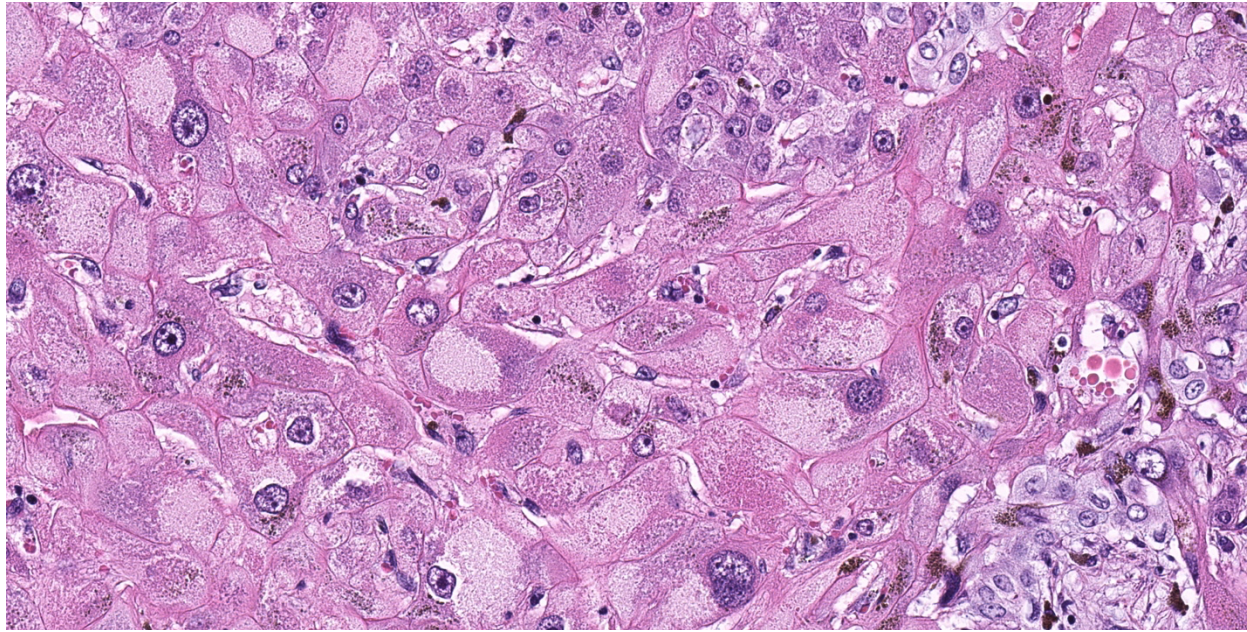
Contributor's comment:

The triad of changes appreciated in the liver, namely the fibrosis, biliary hyperplasia, and megalocytosis with karyomegaly, are nearly pathognomonic for pyrrolizidine alkaloid (PA) toxicosis in horses. PAs are found within many different plants, particularly in the Boraginaceae, Compositae and Leguminosae families.¹ PA is thought to be produced by plants to dissuade foraging by grazing animals. Many animals will not choose to eat PA containing plants unless better forage is not available. Several plant species are particularly problematic for domestic species, which includes *Senecio*, *Crotalaria*, *Heliotropium*, *Amsinckia*, *Echium*, and *Cynoglossum* species.^{3,5} In this particular case the exact plant species was not identified.

PA is an indirect hepatotoxin requiring phase 1 biotransformation from zone 3 hepatocytes

utilizing P450 cytochrome (mainly CYP3A and CYP2B6).⁷ Three common pathways of PA metabolism have been identified including hydrolysis of PAs to release necines and necic acid, N-oxidation to form PA N-oxides, and oxidation of PAs to produce dehydropyrrolizidine (pyrrolic ester) derivatives.^{2,7} The two former pathways are considered pathways of detoxification that lead to excretion of the PA via the urinary system. This is thought to partially determine differences in species susceptibility with rats lacking the required esterase activity and thus being very susceptible, while guinea pigs are highly resistant to toxicity.² Biotransformation via the third pathway of PA forms the dehydropyrrolizidine alkaloid (dPA). DPA is extremely reactive which can covalently bind to DNA or protein forming DNA adducts, protein adducts and DNA and protein cross links.⁷

The cumulative effects of the toxic products lead to DNA replication but inhibition of successful mitosis. This excessive DNA replication without cell division leads to the microscopically appreciated cytomegaly and karyomegaly.^{1,3,5} However, hepatocytes are not uniformly affected



Liver, horse. High magnification of affected hepatocytes with hydropic swelling, abundant intracytoplasmic lipofuscin, occasional intracytoplasmic protein droplets and megalocytic nuclei. (HE, 377X)

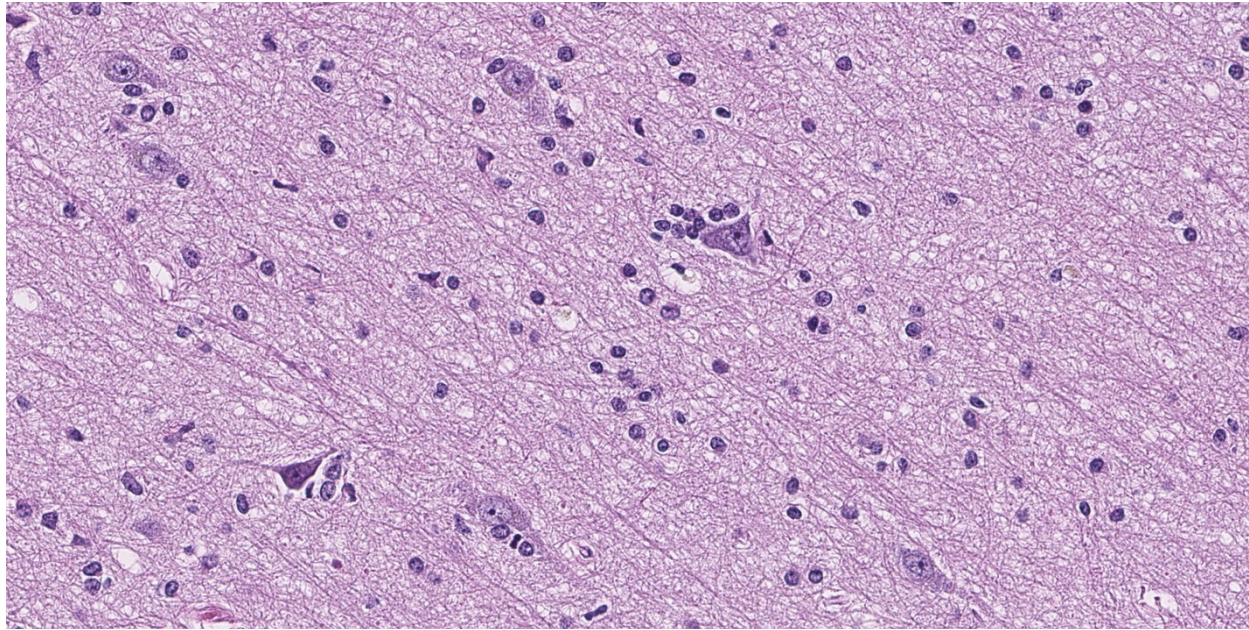
with some cells retaining the ability to successfully replicate. This leads to the gross and histologic manifestation of small nodules of more typical regenerative hepatocytes. The corresponding biliary hyperplasia, typically prominent in PA toxicity, is thought to be a response to the local growth factors and stimuli released with chronic-active hepatocyte injury. Fibroplasia in these cases can be extremely varied with some species-specific findings with sheep having minimal fibroplasia and cattle displaying marked fibrosis.

Three clinical forms of PA toxicity have been well established and include acute, phasic and prolonged exposure. The acute form is rare, as animals generally avoid ingesting large quantities of plants that contain high levels of PA due to poor palatability. This form usually occurs with cases of starvation (when other forage is not available) or with experimental induction. It manifests with marked acute centrilobular to submassive necrosis with hemorrhage. The second, and most common form, is phasic or seasonal exposure. This form occurs with seasonal low-level grazing of plants with PA and manifests, as demonstrated in this case, with megalocytosis, biliary hyperplasia, and fibroplasia. The third form is prolonged exposure

and is almost exclusively induced by *Heliotropium*, usually in sheep. This form leads to a small markedly fibrotic liver, which lacks the characteristic regenerative hepatocytes or megalocytosis.^{3,5}

As mentioned previously, there is a noticeable variation in presentation amongst species, which is multifactorial. Ruminants are considered more resistant than monogastrics due to the degradation of the PA within the ruminal fermentation processes. Amongst ruminants, sheep are considered to be the most resistant, requiring 20 times the concentration by oral exposure when compared to cattle.³ When affected, sheep classically present with the prolonged form of toxicosis due to *Heliotropium*. Pigs are considered to be the most susceptible species, while horses are moderately susceptible and present with either the acute or more phasic forms.^{1,3,5}

Although the liver is commonly considered the primary tissue affected by PA toxicity other tissues can be injured, some of which have certain plant specificities. It has been demonstrated that there is injury to endothelial cells within the liver, leading to the large degree of hemorrhage appreciated within the acute form. The injury



Cerebrum horse. Neurons are often bordered by multiple glial nuclei (satellitosis). (HE, 400X)

here has been speculated to be from leakages of reactive PA from adjacent hepatocytes.^{2,3} Some PA containing plants, such as *Crotalaria*, have a wider tissue effect, commonly affecting renal convoluted tubules and Clara cells within the lungs. These tissue toxicities are thought to be due to the biotransformative properties of these cells.

As mentioned, horses have intermediate susceptibility to PA toxicity, but have a high chance of developing hepatic encephalopathy in the face of a toxic insult, as demonstrated in this case. Hepatic encephalopathy is due to the increased concentration of ammonia and other liver-related metabolites. Ammonia is considered directly neurotoxic, with astrocytes serving as an important cell for ammonia detoxification. Astrocytes detoxify ammonia via the synthesis of glutamine via glutamine synthase. Increased levels of glutamine in astrocytes cause astrocyte swelling, forming the classic type 2 astrocytes.⁸ Interestingly, horses with hepatic encephalopathy don't present with spongiform change at the white and gray matter junction. The exact pathogenesis of spongiform change, and why horses don't have this manifestation, have not been clarified.

Contributing Institution:

Colorado State University

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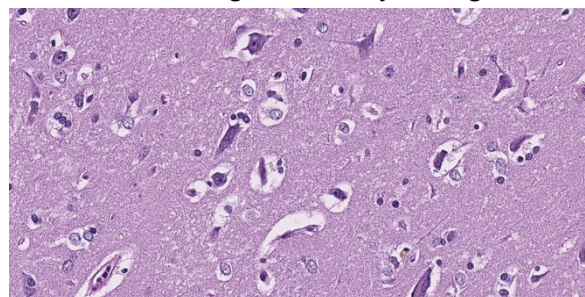
JPC diagnosis:

Liver: Fibrosis, portal and bridging, diffuse, moderate, with hepatocellular loss, megalokaryosis, megalocytosis, cholestasis, and ductular reaction (biliary hyperplasia), Friesian, equine.

Cerebrum: Alzheimer's type 2 astrocytosis, diffuse, moderate, with gliosis, neuronal necrosis and satellitosis.

JPC comment:

During conference discussion, there was lively debate surrounding the severity of degenerative



Cerebrum, horse: Enlarged astrocyte nuclei are suggestive of Type II Alzheimer's cells. (HE, 400X)

neuronal changes, which was largely a result of slide variation among participants.

While pyrrolizidine alkaloid (PA) compounds cause harm to veterinary species, they also have detrimental effects in humans. While animals often graze at the margins of pasture, or have PA containing plants co-harvested with feed plants, human PA toxicity is often more direct. In some areas of the world, dehydropyrrolizidine alkaloid (DHPA) producing plants have been eaten or used as part of herbal medicine for generations. There are a number of recent herbal products and remedies that contain DHPA compounds and are not regulated in any way (similar to nutrition supplements in the United States). Thousands of people were poisoned in Afghanistan and Tajikistan when grain, and subsequently grain, was contaminated. However, the scope of the problem is enormous, with more than 3000 plant species used in more than 50,000 herbal products, as of 2016. A number of government health organizations and agencies have developed regulations and recommendations for products containing DHPA compounds, including the United States, United Kingdom, Germany, European Food Safety Authority, Dutch National Institute for Public Health, World Health Organization, and Food Standards Australia New Zealand.⁶

Based on the reactions required to metabolize DHPA compounds, it is reasonable to ponder whether they have carcinogenic or genotoxic effects. Testing individual DHPA compounds is difficult, because plants often contain only 2-3% of a compound and may be part of numerous toxic compounds. However, research has been conducted on riddelliine in rats and mice, because *Senecio riddellii* contained approximately 18% riddelliine and nearly no other DHPA compounds. Multiple murine studies have found dose dependent increases in the rates of neoplasia in subjects, with the most common finding hepatic hemangiosarcoma. A study using p53 knockout mice replicated those findings and found that even exposure at the lowest dose (5 mg/kg/day) for only 14 days resulted in an increased incidence of neoplasia. While evidence is incomplete, it is reasonable to assume other

untested DHPA compounds may produce similar effects.⁶

Donkeys have recently been used to research two species of *Crotalaria* and determine if they affect the same tissues in a similar manner. However, in the tested donkeys, *Crotalaria retusa* exposure was only associated with liver lesions at either the low or high dose, while *Crotalaria juncea* exposure primarily affected the lungs at high doses. Because the primary DHPA compounds in the two plants are different, this result suggests either that a toxic DHPA metabolite produced in the liver is released to systemic circulation and causes damage in the lung, or that the whole DHPA compound avoids metabolism in the liver and is oxidized by cytochrome P450 systems of club cells in the lung. In the study donkeys, because there were few liver lesions, it may be more likely that the majority of the DHPA compounds bypass the liver and are metabolized in the lung.⁴

References:

1. Brown DL, Wettene AJV, Cullen JM, Hepatobiliary system and exocrine pancreas. in Zachary JF. Pathologic Basis of Veterinary Disease. St. Louis, MO: Mosby; 2017: 449-450
2. Chen, T., Mei, N., & Fu, P. P. (2010). Genotoxicity of pyrrolizidine alkaloids. Journal of Applied Toxicology. doi:10.1002/jat.1504
3. Cullen JM, Stalker MJ, Liver and Biliary system. in: Jubb KV, Kennedy PC, & Palmer N. Pathology of domestic animals. San Diego, CA: Academic Press; 2015: 337-338
4. Pessoa CRM, Pessoa AFA, Maia LA, et al. Pulmonary and hepatic lesions caused by the dehydropyrrolizidine alkaloid-producing plants *Crotalaria juncea* and *Crotalaria retusa* in donkeys. *Toxicol.* 2013;71:113-120.
5. Stalker MJ, Hayes MA. Liver and biliary system. In: Maxie MG, ed. Jubb, Kennedy, and Palmers Pathology of Domestic Animals. Vol 2. 5th ed. Philadelphia, PA: Elsevier; 2007:373-374
6. Stegelmeier BL, Colegate SM, Brown AW. Dehydropyrrolizidine Alkaloid Toxicity, Cytotoxicity, and Carcinogenicity. *Toxins (Basel)*. 2016;8(12):356. Published 2016 Nov 29. doi:10.3390/toxins8120356

7. Wiedenfeld, H., & Edgar, J. (2010). Toxicity of pyrrolizidine alkaloids to humans and ruminants. *Phytochemistry Reviews*, 10(1), 137-151. doi:10.1007/s11101-010-9174-0
8. Zachary JF: Nervous system. In: Zachary JF, ed. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby; 2007:814-815

CASE 3: PV200 (4119010-00)

Signalment:

A 4.5 years old, male castrated, Yorkshire terrier (*Canis familiaris*)

History:

Vomiting. Mass observed in the ileocecal junction on ultrasound. Resection and anastomosis of a distal portion of ileum and cecum with a mass on exploratory abdominal surgery.

Gross Pathology:

On exploratory surgery, a mass in the intestinal wall, at the ileocecal junction.

Laboratory results:

Normal serum protein.

Microscopic description:

Ileocecolic junction. There are multifocal areas of saponification and cholesterol clefts present along the serosal margin, in the tunica muscularis and occasionally in the submucosa. These areas are surrounded by macrophages and multinucleated giant cells, with peripheral reactive fibrous tissue (granulomas). There is mild dilation of lymphatics in the submucosa and there is rare lymphangiectasia in the lamina propria.

Contributor's morphologic diagnosis:

Focal mural intestinal lipogranulomatous lymphangitis

Contributor's comment:

Lipogranulomatous lymphangitis is inflammation of the intestine and lymphatic vessels and surrounding tissues caused by chronic leakage of lipid-laden chyle.⁶ The most common cause of this type of finding is lymphangiectasia

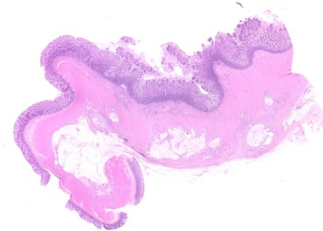
with protein losing enteropathy. The finding is usually diffuse along the serosa and mesentery of the small intestine.^{4,6}

Lymphangiectasia has been described in the dog as one of the most common cause of malabsorption/protein losing enteropathy.⁴ Dilated intestinal lacteals result in lymph leakage into the small bowel lumen with subsequent protein-losing enteropathy with chronic diarrhea, wasting, hypoproteinemia, lymphopenia, hypercalcemia and hypocholesterolemia. Lymphangiectasia is associated with a syndrome that includes peripheral edema, ascites and hydrothorax, the result of hypoalbuminemia.⁴

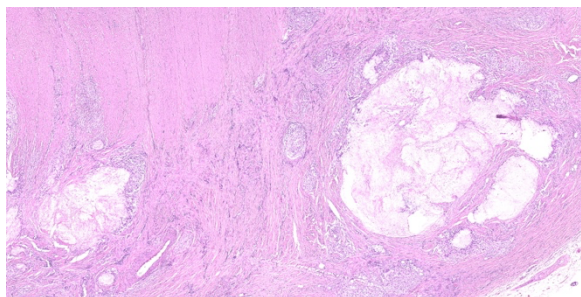
Breed predisposition includes Yorkshire terriers and Norwegian Ludenhunds, in which lymphangiectasia is part of the syndrome of protein losing enteropathy with inflammatory bowel disease and in which gastritis in gastric neoplasia may be concomitant.^{4,6}

In this case however, the dog had normal serum protein and the lesion was restricted to the ileocecal junction. There are two relatively recent reports^{2,5}, of dogs with distal small intestinal thickening or masses, with evidence of lipogranulomatous lymphangitis. These dogs had normal total protein, albumin, globulin and cholesterol concentrations. The dogs presented with diarrhea, vomiting, abdominal pain and weight loss.

Abdominal ultrasound indicated thickening of the distal small intestine or a mural mass in the ileocecal junction. There was no evidence of diffuse lymphangiectasia.



Ileocecal junction, dog. Within the cecal serosa and external muscularis, there are lakes of degenerate lipid and the submucosa is expanded and hypercellular. (HE, 5X)



Ileocecal junction, dog. Higher magnification of effaced lymphatics within the muscularis and serosa, with leakage of lipid-laden chyle and resultant granulomatous inflammation. (HE, 31X)

The histologic lesions were similar in all dogs. The lesions were in the distal small intestine. The wall of the intestine was expanded by inflammation, dilated lymphatics and fibrosis. There was formation of granulomas with central saponified fat or cholesterol clefts surrounded by neutrophils, foamy macrophages and multinucleated giant cells.

Postoperative outcomes ranged from remission of clinical signs without treatment for 10 to 12 months in two dogs, postoperative management with medical and nutritional management in three dogs and no outcome for one case.

Contributing Institution:

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JPC diagnosis:

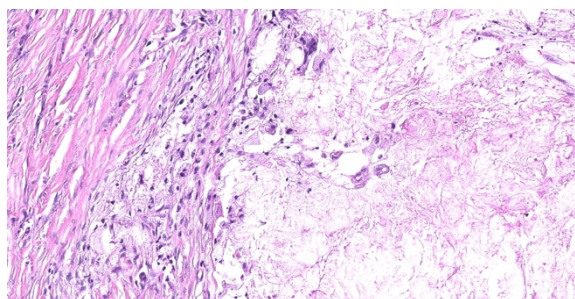
Ileum, cecum: Lymphangitis, lipogranulomatous, diffuse, chronic, severe, with mild multifocal lymphangiectasia, Yorkshire terrier, canine.

JPC comment:

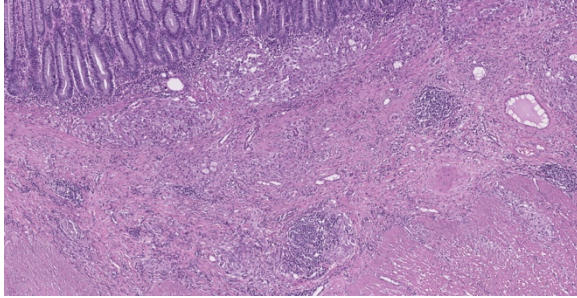
The contributor provides a concise summary of lipogranulomatous lymphangitis in the dog. As they often are found concurrently, a short overview of lymphangiectasia is now provided. There are reported cases of both primary and focal canine lymphangiectasia, the latter being discussed by the contributor.

While there are tools and algorithms for standardized scoring of endoscopic biopsies of stomach, duodenum, and the colon from dogs and cats, they capture only one dimension of lymphatic characterization. The WSAVA guidelines classifies villous lacteal width into normal (up to 25% width of villous lamina propria), mildly dilated (up to 50% width), moderate dilation (up to 75% width), and marked lacteal dilation (up to 100% of villous lamina propria).¹ Chronic inflammatory enteropathy (CIE) is applied to gastrointestinal tract conditions that persist for at least 3 weeks' duration, under which intestinal lymphangiectasia may be classified. In addition to villous lacteal dilation, non-villous mucosal lacteal dilation data may help increase classification of disease or inform prognosis.⁷ Recent research has shown there may be a statistically significant correlation between both villous and non-villous lacteal widths in the ileum and dogs that have CIE with protein losing enteropathy. Unfortunately, while WSAVA recognizes important and relevant information that can be gained from ileal biopsies, there is not yet a standardized template for evaluation.⁸

Lymphatic endothelium is derived from venous progenitor cells and express certain antigens that distinguish them from blood vessel endothelium. Depending on availability, useful immunohistochemical stains include the nuclear transcription factor human prospero homeobox (Prox-1), lymphatic vascular endothelial hyaluronic acid receptor (LYVE-1), and podoplanin (D2-40).⁷



Cecum, dog: Higher magnification of effaced lymphatics with abundant degenerate lipid surrounded by foamy macrophages and concentric lamellae of collagen. (HE, 259X)



Cecum, dog: The submucosa is expanded by granulomatous inflammation centered on lymphatics as well as nodular submucosal aggregates of lymphocytes. (HE, 69X)

Dogs (and other animals) with intestinal lymphangiectasia often have derangements of nutritional absorption that is clinically measurable, including hypoproteinemia, hypogammaglobulinemia, and lymphopenia. It has been found that dogs with intestinal lymphangiectasia have fewer total plasma cells in the orad portions of the intestine, and higher plasma cell concentrations in the aborad sections. While changes in distribution of plasma cells, as well as the distribution of IgA, IgM, and IgG secreted are apparent, the significance remained undetermined.³

References:

1. Day MJ, Bilzer T, Mansell J, et al. Histopathological standards for the diagnosis of gastrointestinal inflammation in endoscopic biopsy samples from the dog and cat: A report from the World Small Animal Veterinary Association Gastrointestinal Standardization Group. *J Comp Path.* 2008;138:S1-S43.
2. Lecointre A et al. Focal intestinal Lipogranulomatous lymphangitis in 10 dogs. *J Small Anim Practice*, 2016, 57(9):465-71.
3. Suter MM, Palmer DG, Schenk H. Primary intestinal lymphangiectasia in three dogs: A morphological and immunopathological investigation. *Vet Pathol.* 1985;22:123-130.
4. Uzal FA et al. Alimentary system. In: Jubb, Kennedy and Palmer's *Pathology of Domestic Animals*. Ed: M Grant Maxie. 6th ed. vol. 2, Elsevier, 2016:90.
5. Van Kruinigen HJ, et al. Lipogranulomatous Lymphangitis in Canine Intestinal Lymphangiectasia. *Vet. Pathol*, 1984, 21: 377-383.
6. Watson VE, et al. Focal Intestinal Lipogranulomatous Lymphangitis in 6 Dogs (2008–2011). *J Vet Intern Med*, 2014; 28:48–51.
7. Wennogle SA, Priestnall SL, Suarez-Bonnet A, Soontarak S, Webb CB. Lymphatic endothelial cell immunohistochemical markers for evaluation of the intestinal lymphatic vasculature in dogs with chronic inflammatory enteropathy. *Journal of Veterinary Internal Medicine*. 2019;33:1669-1676.
8. WSAVA International Gastrointestinal Standardization Group. Endoscopic, Biopsy, and Histopathologic Guidelines for the Evaluation of Gastrointestinal Inflammation in Companion Animals. *J Vet Intern Med*. 2010;24:10-26.

CASE 4: N-864-18 (4153559-00)

Signalment:

Four-day-old Aberdeen Angus male calf, bovine (*Bos taurus taurus*)

History:

A male Angus calf was delivered by a heifer, without any abnormalities in the calving process. Initially the animal was clinically normal, and ingested colostrum and milk adequately; however, on the third day of life the calf started presenting clinical signs including anorexia,



Eye, calf. The calf presented with unilateral hypopyon. (Photo courtesy of: Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Setor de Patologia Veterinária, <http://www.ufrgs.br/patologia>)



Liver, calf. Numerous raised pyogranulomas are present throughout the liver. (Photo courtesy of: Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Setor de Patologia Veterinária, <http://www.ufrgs.br/patologia>)

diarrhea, and apathy. The clinical picture rapidly progressed to persistent lateral recumbence and neurological manifestations characterized by nystagmus, teeth grinding, opisthotonus, and paddling movements. In addition, unilateral left hypopyon was observed. In the next day (fourth day of life, second day of clinical progression), euthanasia was elected due to the poor prognosis. The calf was referred from an extensive cow-calf operation with around two hundred dams located in the state of Rio Grande do Sul, southern Brazil. Two other newborn calves were reported to become ill in the comprehended period, one of which died; however, necropsy was not performed in these cases.

Gross Pathology:

The animal presented good body condition and a small amount of orange feces was observed adhered to the perianal area. Externally, the main visible abnormality consisted of accumulation of white material in the anterior chamber of the left eye (unilateral hypopyon). At the opening of the thoracic and abdominal cavities, multiple soft, white to yellow nodules (0.2-1.0 cm in diameter) were observed in the liver, spleen, kidneys, mesenteric lymph nodes, heart, and lungs. Cross sectioning of the cerebellum revealed a reddish, locally extensive, soft to friable, irregular area, effacing great part of the organ. Multifocal reddish circular areas ranging from 0.5 to 1.0 cm in diameter, similar to those described in the cerebellum, were also observed in the basal

nuclei, thalamus, mesencephalon, and telencephalon. Cross sectioning of the left eye revealed anterior peripheral synechia, posterior synechia, diffuse white discoloration and thickening of the choroid, and accumulation of white, friable material in the anterior chamber (hypopyon).

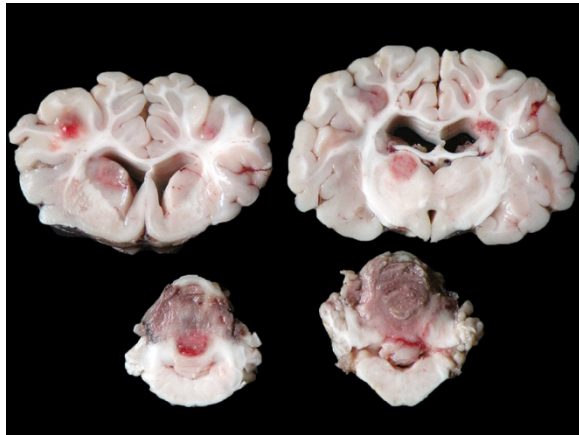
Laboratory results:

Fresh liver and brain fragments were collected and inoculated in Sabouraud dextrose agar (Kasvi®), MacConkey agar (Kasvi®) and Blood agar base (Kasvi®) with 5% sheep blood. The plates were incubated aerobically at 37 °C. MacConkey and Sabouraud plates did not have any growth after 72 hours. In both blood agars (brain and liver), within 24 hours of incubation, the growth of white to gray, downy, and fluffy fungal colonies was observed. To identify the fungus, total DNA was extracted from these colonies, as previously described.² The partial 18S rDNA was amplified by PCR reaction as described elsewhere,⁹ using the following primers: EukA-F: 5'-AACCTGGTTGATCCTGCCAGT-3' EukA-R: 5'-GATCCWTCTGCAGGTTACCTAC-3'. PCR amplicons were analyzed by Sanger sequencing.

The generated sequences were compared using BLAST (NCBI database), and matched to *Mortierella wolfii*, showing 99% of identity in the 18S rDNA partial sequence. For confirmation of



Kidney, calf. Numerous raised pyogranulomas are present throughout the kidney. (Photo courtesy of: Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Setor de Patologia Veterinária, <http://www.ufrgs.br/patologia>)



Brain, calf. Multiple areas of hemorrhage and malacia are scattered throughout the brain, and the cerebellar vermis is particularly affected. (Photo courtesy of: Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Setor de Patologia Veterinária, <http://www.ufrgs.br/patologia>)

the identity of the fungus, a phylogenetic analysis was performed within the phylum Zygomycota. *M. wolfii* isolate was positioned in the Mortierellomycotina subphylum clade together with other *M. wolfii* sequences.² Samples of blood serum, spleen, and lymph nodes were also submitted to the reverse transcription-PCR assay to identify the bovine viral diarrhea virus (BVDV);¹ however, the results were negative for all tested samples.

Microscopic description:

Each submitted slide presents a section of cerebellum. The normal trilaminar cerebellar aspect has been nearly completely effaced by multifocal to coalescing areas of marked necrosis and neuropil rarefaction, which are seen both in the white and grey matter. These areas are associated with intense accumulation of necrotic cellular debris, fibrin deposition, hemorrhage, and marked inflammatory infiltrate of viable and degenerate neutrophils, foamy macrophages (gitter cells), lymphocytes, plasma cells and rare multinucleated giant cells. These inflammatory cells are present within necrotic areas, frequently markedly expanding Virchow-Robin spaces, as well as extending and severely expanding the adjacent leptomeninges.

Amidst the necrotic areas, marked multifocal fibrinoid necrosis and hyalinization of blood

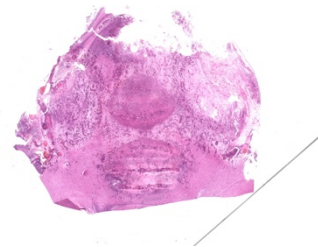
vessels are observed. Affected vessels present the wall markedly expanded by accumulation of amorphous proteinaceous material and inflammatory infiltrate of neutrophils. In addition, marked multifocal thrombosis is commonly seen associated with these blood vessels.

Numerous hyaline hyphae are observed intermixed with inflammation and necrotic areas. These fungal structures are large (3-8 μ m wide), present scarce septations, non-parallel walls, non-dichotomous branching, and occasionally show bulbous dilatations. Multifocally, numerous hyphae are also frequently observed surrounding as well as invading and expanding the wall of blood vessels, associated with the mentioned vascular changes. Fungal characteristics are highlighted with Grocott methenamine silver stain.

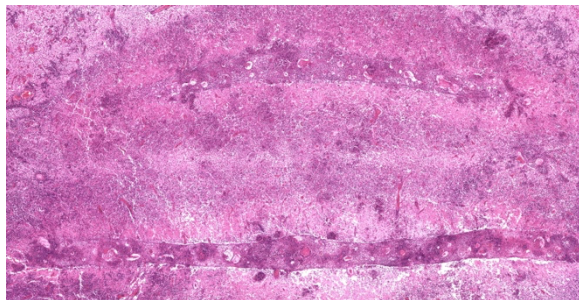
Some slides present sections of choroid plexus, in which multifocal, mild inflammatory infiltrate of lymphocytes, macrophages, and neutrophils is seen. In addition, similar multifocal areas of marked necrosis and inflammation associated with fungal hyphae were seen in several organs, including the liver, left eye, spleen, kidneys, heart, thyroids, adrenal glands, mesenteric lymph nodes, as well as other regions of the encephalon.

Contributor's morphologic diagnosis:

Necrotizing and pyogranulomatous meningoencephalitis, marked, multifocal to coalescing, subacute, associated with severe multifocal vasculitis, thrombosis and numerous fungal hyphae.



Cerebellum, calf. A section of cerebellum is presented for examination. At subgross examination, large areas of necrosis and hemorrhage obscure the normal architecture and markedly expand the cerebellar meninges. (HE, 5X)



Cerebellum, calf: Higher magnification of affected cerebellum demonstrating the extent of necrosis and largely perivascular suppurative inflammation effacing the cerebellar parenchyma and expanding the folial leptomeninges (HE, 44X)

Lymphohistiocytic and neutrophilic choroid plexitis, subacute, mild, multifocal.

Contributor's comment:

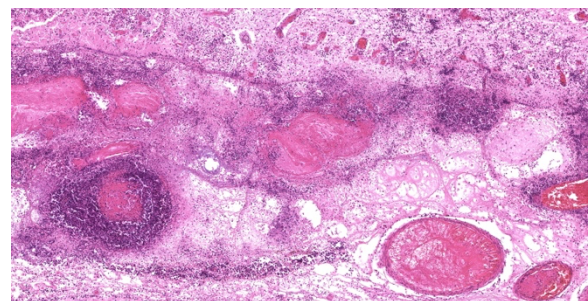
Mortierella wolfii is a saprophytic fungus, which belongs to the zygomycetes class.^{6,11} This agent is found in the environment, including in spoiled feeds such as moldy silage and hay,^{7,11} however, it can also be found in the soil and pastures.⁷ Zygomycetes are often implicated as a cause of abortion in cattle,⁷ and *M. wolfii* is the most common agent in New Zealand, where it has been considered the causative agent of a condition named “distinctive mycotic abortion-pneumonia syndrome”³, though it rarely induces systemic disease in adult cattle^{6,11} or in neonatal calves.¹² To the best of the author’s knowledge, this is the first description of this condition affecting cattle of this age group outside Oceania.²

In this case, the alterations observed in the brain, as well as in several other organs, are associated with vascular lesions, and indicate a probable hematogenous dissemination of the fungus. Tropism of zygomycetes for blood vessels occurs through a process called angioinvasion. Hyphae have the ability to invade endothelial cells, gain access to the circulatory system, and spread to other parts of the body. The presence of infarcts, necrosis, and vasculitis are considered typical lesions that occur secondarily to the fungal invasion of the blood vessels,^{11,17} as observed in this case.

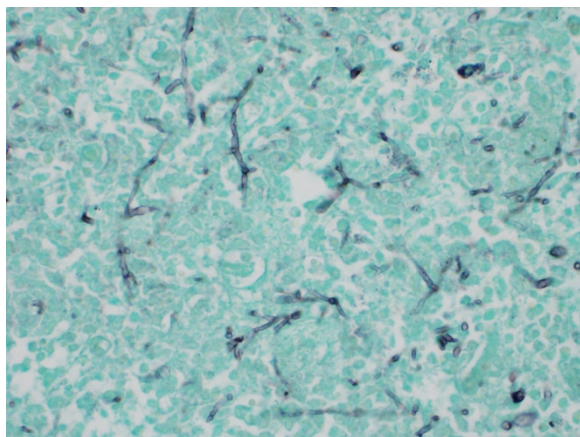
It is suggested that *Mortierella* infection may occur *in utero*.^{12,14} The fungus establishes a primary respiratory infection in the dam, enters the bloodstream and reaches the uterus, where it can induce acute metritis and placentitis, leading to abortion due to fetal hypoxia.^{11,14} Even though subacute or chronic fungal metritis and placentitis may allow fetal infection, calves are usually born alive and clinically normal, and may develop lesions and clinical disease a few days after calving,¹⁴ similar to what is described in this case. In older calves and adult cattle, disseminated mycoses may occur due to a primary gastrointestinal or respiratory infection.⁵

We highlight that the culture using blood agar base showed pure fungal growth, while no growth occurred in the Sabouraud agar, which is likely related to the tropism of *M. wolfii*. Moreover, the 18S rDNA partial sequencing and analysis was able to confirm the identity of the isolated fungus as *M. wolfii*, with 99% of identity in the matching BLAST search. Because of the presence of neurological clinical signs and hypopyon; bacterial septicemia is the main differential diagnosis of this case. This has been mostly observed in neonatal calves that did not ingest colostrum adequately, and this condition is predominantly associated with *Escherichia coli* infection.⁸ Infectious thrombotic meningo-encephalitis caused by *Histophilus somni* is characterized by similar gross lesions in the brain, however histological lesions are distinct.^{6,10}

Furthermore, other fungi, especially *Aspergillus* spp. and zygomycetes, may cause similar lesions as those observed in this case, and the differential



Cerebellum, calf: There is thrombosis of arteries and veins, profound vasculitis, and marked expansion of the meninges. (HE, 75X)



Cerebellum, calf. Scattered throughout areas of necrosis as well as within thrombosed vessels, there are numerous 4-6µm diameter pauciseptate non-dichotomously branching fungal hyphae. (Grocott's methenamine silver, 400X)

diagnosis should be done mainly through culture and molecular tests.^{6,11,14}

Contributing Institution:

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Setor de Patologia Veterinária
<http://www.ufrgs.br/patologia>

JPC diagnosis:

Cerebellum: Meningoencephalitis, necrotizing and fibrinosuppurative, diffuse, acute, severe, with necrotizing vasculitis, thrombosis, hemorrhage, and numerous fungal hyphae, Aberdeen Angus, bovine.

JPC comment:

The contributor provides a succinct synopsis of *Mortierella wolfii*. As stated, these fungi exhibit angiotropism, leading to clotting of blood in vessels, and subsequent infarction and extensive necrosis of tissues, a trait shared among most zygomycetes.¹⁵ *Mortierella* spp, along with zygomycetes of the *Rhizopus*, *Rhizomucor*, *Absidia*, and *Mucor* genera, are most often implicated in mycotic bovine abortion, gastrointestinal infections, ulcerative lesions and mesenteric lymphadenitis. It is also notable for causing respiratory, CNS, and visceral infection as a result of hematogenous dissemination. While they often cause no problems to carriers, stress from dietary changes, malnutrition, antibiotic use

resulting to alteration of gastrointestinal flora, concurrent infections, recent parturition, or other physical trauma may increase the likelihood of infection.⁴

A recent, less common, presentation of mycotic ulcerative keratitis in a thoroughbred horse in Japan was shown to be due to *Mortierella wolfii*. In this case, the ventral two thirds of the right cornea were opaque with intercellular epithelial edema, with multifocal, punctate, gray opacities in the epithelium of the axial cornea and a superficial corneal ulcer. Direct and indirect pupillary light reflexes were normal, there was no aqueous flare in the anterior chamber, and no abnormalities were detected in the posterior segment. Cytologic evaluation of corneal scrapings is often useful in the diagnosis of keratomycosis, with nonseptate hyphae easily visualized.¹⁶

There are several characteristics that increase pathogenicity of some of the zygomycetes, and specifically those of Mucorales. They possess the ability to suppress IFN- λ and RANTES (CCL5) in natural killer cells. They acquire iron through siderophores (iron chelators) and high-affinity iron permeases, and likely also extract iron from hemoglobin. Increased iron concentrations allow for rapid fungal growth, as well as impairing phagocyte function and decreasing IFN- λ secretion (in a mouse model). In order to facilitate vascular invasion, Mucorales adhere to endothelial cells by binding host receptor GRP78 with ligand spore coat homolog (CotH) proteins, leading to endocytosis of the fungus.¹³

One interesting aspect of the contributor's laboratory analysis is that no growth in Sabouraud's agar was noted. Typically, *Rhizopus* spp, *Rhizomucor* spp, *Absidia* spp, *Mucor* spp, and *Mortierella* spp grow rapidly on Sabouraud's agar at room temperature.⁴ Additional elucidation of the pathogenesis of *Mortierella wolfii* may provide insights into this finding.

References:

1. Bianchi MV, Konradt G, de Souza, SO, et al. Natural outbreak of BVDV-1d-induced mucosal disease lacking intestinal lesions. *Vet Pathol.* 2016;54(2):242–248.

2. Bianchi RM, Vielmo A, Schwertz CI, et al. Fatal systemic *Mortierella wolfii* infection in a neonatal calf in southern Brazil. *Cienc Rural*. 2020;50(1):1–5.
3. Carter ME, Cordes DO, di Menna ME, Hunter R. Fungi isolated from bovine mycotic abortion and pneumonia with special reference to *Mortierella wolfii*. *Res Vet Sci*. 1973;14:201–206.
4. Chengappa MM, Pohlman LM. Agents of Systemic Mycoses. In: eds. McVey DS, Kennedy M, Chengappa MM, *Veterinary Microbiology*, 3rd Ed. Ames, IA:John Wiley and Sons, Inc. 2013:342.
5. Chihaya Y, Matsukawa K, K Ohshima, et al. A pathological study of bovine alimentary mycosis. *J Comp Pathol*. 1992;107:195–206.
6. Curtis B, Hollinger C, Lim A, Kiupel M. Embolic mycotic encephalitis in a cow following *Mortierella wolfii* infection of a surgery site. *J Vet Diagn Invest*. 2017;29(5):725–728
7. Knudtson WU, Kirkbride CA. Fungi associated with bovine abortion in the Northern plains states (USA). *J Vet Diagn Invest*. 1992;4:181–185.
8. Konradt G, Bassuino DM, Prates KS, et al. Suppurative infectious diseases of the central nervous system in domestic ruminants. *Pesq Vet Bras*. 2017;7(8):820–828
9. Medlin L, Elwood HJ, Stickel S, Sogin, ML. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*. 1988;71:491–499.
10. Momotani E, Yabuki Y, Miho H, Ishikawa Y, Yoshino T. Histopathological evaluation of disseminated intravascular coagulation in *Haemophilus somnus* infection in cattle. *J Comp Pathol*. 1985;95:15–23.
11. Munday JS, Laven RA, Orbell GMB, Pandey, SK. Meningoencephalitis in an adult cow due to *Mortierella wolfii*. *J Vet Diagn Invest*. 2006;18:619–622.
12. Munday JS, Wolfe AG, KE Lawrence, Pandey, SK. Disseminated *Mortierella wolfii* infection in a neonatal calf. *N Z Vet J*. 2010;58:62–63.
13. Petrikos G, Tsioutis C. Recent Advances in the Pathogenesis of Mucormycoses. *Clinical Therapeutics*. 2018;40(6):894–902.
14. Vasconcelos DY, Grahn BH. Disseminated *Rhizopus* infection with ocular involvement in a calf. *Vet Pathol*. 1995;32:78–81.
15. Voigt K, Vaas L, Stielow B, de Hoog GS. The zygomycetes in a phylogenetic perspective. *Persoonia*. 2013;30:i-iv. doi:10.3767/003158513X666277
16. Wada S, Ode H, Hobo S, et al. *Mortierella wolfii* keratomycosis in a horse. *Veterinary Ophthalmology*. 2011;14(4):267–270.
17. Zachary JF. Mechanisms of microbial infections. In: Zachary JF. Pathologic basis of veterinary disease. 6th. St. Louis, Missouri, USA: Elsevier; 2017:132–241.