Joint Pathology Center

Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2016-2017

C o n f e r e n c e 12

14 December 2016

Mark T. Butt, DVM, DACVP
President, Tox Specialists (TPS), LLC
8420 Gas House Pike, Suite G
Frederick, MD

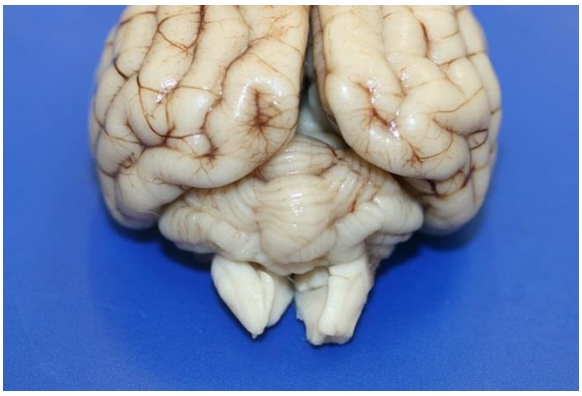
CASE I: 14-309/6 or 7 (JPC 4048850).

Signalment: Five-month-old, female, Coton de Tulear, dog (*Canis familiaris*).

History: The dog presented with ataxia evolving since the age of four months, with a rapid onset of clinical signs. Neurological examination oriented towards a cerebellar origin of the ataxia. Magnetic Resonance Imaging revealed a decreased size of the cerebellum, without signs of inflammation. The dog was euthanized after ataxia had worsened.

Gross Pathology: The only significant gross lesion at necropsy was a reduction in size of the cerebellum, with slight asymmetry between the two hemispheres. The gyri were diffusely sharply delineated and shrunken.

Laboratory results: N/A



Cerebellum, dog. The cerebellum was shrunken with slight symmetry between the two hemispheres. The folia appear diminished in size. (Photo courtesy of: Anatomie pathologique, Vetagro Sup, Campus vétérinaire, 1, avenue Bourgelat, 69280 Marcy l'etoile, FRANCE)

Histopathologic Description: Diffusely, the cerebellar folia are slightly flattened. Severe loss of cells within the granular layer is present, multifocally leading to complete absence of this layer. Rarely, granular cells are swollen and vacuolated (degeneration), multifocally associated with empty baskets. Diffusely replacing this layer are glial cells

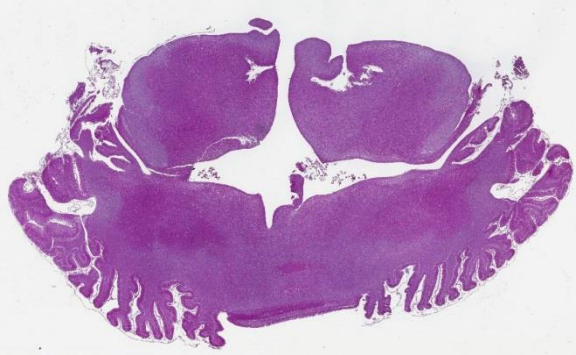
(mainly astrocytes). A discrete population of microglial cells is also present. The molecular layer is mostly normal in thickness, or more rarely thinner. Purkinje cells do not show remarkable degenerative changes.

Contributor's Morphologic Diagnosis: Cerebellum, Granular cell degeneration and loss, diffuse, severe, Coton de Tulear, canine.

Contributor's Comment: Cerebellar cortical abiotrophy is a spontaneous, premature and progressive degeneration and death of neurons without an intrinsically identifiable defect; it is well characterized in the dog and is described in several breeds. This condition is characterized by ongoing Purkinje neuronal cell degeneration and loss with reactive gliosis. Mostly, affected animals are healthy at the time of birth and develop clinical signs at several months of age, which worsen with time. In some breeds, a possible inherited genetic defect in the metabolism of the neurotransmitter glutamic acid has been proposed (or established).[2,11]

This case is an unusual form of a cerebellar degeneration in the Coton de Tuléar breed, characterized by a severe depletion in the granular cell layer, hence the name "cerebellar granuloprival degeneration" for this condition.[9] Rare cases of this condition in this breed have been published to date.[9] Similar to this case, all differ from the Purkinje cell atrophy reported in many canine breeds.

Some similarity between this Coton de Tuléar and cerebellar granuloprival hypo-plasia in cats caused by intrauterine parvovirus infection has been proposed. However, in Coton de Tuléars, there is no disorganization of the cerebellar cortex and



Cerebellum, dog. A section of cerebellum and brainstem is submitted. The paired caudal colliculi are at top, and cerebellar folia at left and right at the bottom. At subross magnification, the folia appear markedly thinned and hypocellular. (HE, 4X)

no lesions in the Purkinje cell layer. Parvovirus infection in dogs is not known to induce cerebellar changes.[9,12]

Contrary to what has been published, in this case there is no significant inflammatory change in the cerebellum.[9] The restriction of the disease specifically to Coton de Tuléar breed is favors a genetic basis for the lesions, but this hypothesis needs further analysis.

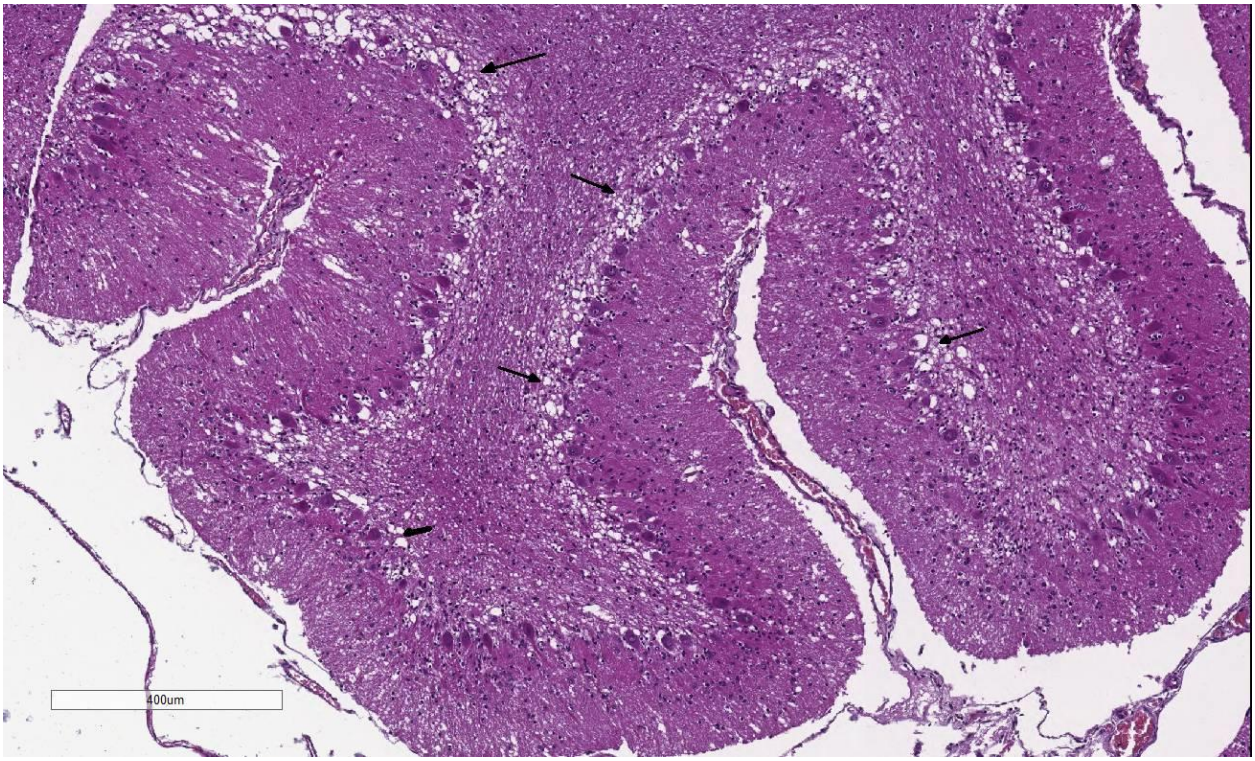
JPC Diagnosis: Cerebellum: Granular cell degeneration and loss, diffuse, severe, with spongiosis, and minimal multifocal Purkinje cell loss, Coton de Tulear, canine.

Conference Comment: The contributor provides a compelling example of an atypical form of cerebellar abiotrophy in the canine. Cerebellar abiotrophy, also known as cerebellar cortical degeneration, has been described as a hereditary defect in several breeds of dogs,[1,3,9] Arabian horses,[8] rabbits,[7] an alpaca,[6] and recently in goats.[5] Histologically, the characteristic distribution of lesions includes the marked loss and degeneration of the Purkinje cell neurons, often with retrograde degeneration in granular cells due to failure of synaptogenesis between parallel nerve fibers

of the granular cell layer and Purkinje cells.[1,3,5] In this Coton de Tuléar dog, there is diffuse and severe degeneration and loss of the granular cell layer, with only scattered loss of Purkinje cells. This histomorphology has been rarely reported in the veterinary literature as cerebellar granuloprival degeneration in a number of different canine breeds, including the Coton de Tuléar, as discussed by the contributor.[3,4,9] Neonatal cerebellar ataxia in Coton de Tuléar dogs has also been reported as Bandera's syndrome, suggesting a breed-related hereditary disease.[3]

Abiotrophy is a spontaneous cerebellar degenerative disease process characterized by premature loss of neurons in the cerebellum.[2] Conference participants discussed how this differs from cerebellar hypoplasia, a condition in which the cerebellum does not completely form during embryogenesis due to in-utero viral infections from parvoviruses or pestiviruses. Examples include feline parvovirus (panleukopenia), bovine pestivirus (bovine viral diarrhea virus), classical swine fever (hog cholera/pestivirus), sheep and goat pestivirus (Border disease), and rat parvovirus (Kilham rat virus).[2] Additionally, certain toxicities, such as organophosphates, and malnutrition can also cause cerebellar hypoplasia.[3] In contrast to animals born with cerebellar hypoplasia, those affected with abiotrophy are neurologically normal at birth and develop early-onset progressive cerebellar proprioceptive deficits during the post-natal period, in the case of this dog at four to five months.[1,3,5] Typical neurologic deficits include ataxia, head tremor, intention tremors, symmetrical hypermetria, broad-based stance, and loss of balance.[2]

In addition to the diffuse and severe



Cerebellum, dog. There is an almost total loss of the granular cell layer, and spongiosis at its normal location (arrows) The Purkinje cell layer is considered within normal limits. (HE, 46X)

degeneration and loss of the cerebellar molecular cell layer, the conference moderator noted an increase in the number of hypertrophic astrocytes with large vesicular nuclei within the Purkinje cell layer, interpreted as Bergmann gliosis. This astrocytic reaction occurs predominantly in areas where Purkinje cells are lost, described by several conference participants as empty baskets.[4] Bergmann glial cells are astrocytes with cell bodies located in the Purkinje cell layer with long radial processes that surround the synapses on Purkinje cell dendrites and extend to the molecular layer, terminating on the pial surface of the cerebellum;[10] they are essential for the normal differentiation, migration and maturation of Purkinje cell and granular cell neurons. The immunohistochemical stain, glial fibrillary acidic protein (GFAP), is useful in demonstrating the empty baskets surrounded by Bergmann gliosis in cases of cerebellar abiotrophy.[10]

The confounding aspect of this case is the severe selective depletion of granular cells with only scattered loss of Purkinje cells. The pathogenesis of cerebellar granuloprival degeneration in this breed has not yet been elucidated; it is hypothesized to be the result of an inherited disorder of granular cell development, but most Purkinje cells survive since their main excitatory input is from the olivary nucleus.[4] However, some authors suggest that Purkinje cells can be lost as result of granular cell depletion in chronically affected dogs.[4]

Contributing Institution:
Anatomie pathologique,
Vetagro Sup, Campus vétérinaire
1, avenue Bourgelat,
69280 Marcy l'etoile, France
http://www.vetagro-sup.fr/en/

References:

1. Berry ML, Machado UB. Cerebellar abiotrophy in a miniature schnauzer. *Can Vet J*. 2003; 44:657-659.
2. Cantile C, Youssef S. Nervous system. Maxie MG ed. In: *Jubb Kennedy and Palmer's Pathology of Domestic Animals*. Vol 1. 6th ed. Philadelphia, PA: Elsevier Saunders; 2016:275-276.
3. Coates JR, O'Brien DP, Kline KL, et al. Neonatal cerebellar ataxia in Coton de Tulear dogs. *J Vet Intern Med*. 2002; 680-689.
4. Huska J, Gaitero L, Heindrich SN, et al. Cerebellar granuloprival degeneration in an Australian kelpie and a Labrador retriever dog. *Can Vet J*. 2013; 54:55-60.
5. Koehler JW, Newcomer BW, Holland M, Caldwell JM. A novel inherited cerebellar abiotrophy in a cohort of goats. *J Comp Path*. 153:135-139.
6. Mouser P, Levy M, Sojka JE, Ramos-Vara JA. Cerebellar abiotrophy in an alpaca (*Lama pacos*). *Vet Pathol*. 2009; 46:1133-1137.
7. Sato J, Yamada N, Kobayashi R, et al. Morphometric analysis of progressive changes in hereditary cerebellar cortical degenerative disease (abiotrophy) in rabbits caused by abnormal synap-togenesis. *J Toxicol Pathol*. 2015; 28:73-78.
8. Scott EY, Penedo MC, Murray JD, Finno CJ. Defining trends in global gene expression in Arabian horses with cerebellar abiotrophy. *Cerebellum*. 2016; Oct 5. [Epub ahead of print].
9. Tipold A, Fatzer R, Jaggy A, Moore P, Vanevelde M.

Presumed immune-mediated cerebellar granuloprival degeneration in the Coton de Tuléar breed. *J neuroimmunol*. 2010; 110:130-133.
10. Yamada K, Watanabe M. Cyto-differentiation of Bergmann glia and its relationship with Purkinje cells. *Anat Sci Int.* 2002; 77:94-108.
11. Zachary JF, McGavin DM. Nervous system. In: *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby; 2012:816, 856-857.
12. Zachary JF, McGavin DM: Mechanisms of microbial infections. In: *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby; 2012:229.

CASE II: N14-281 (JPC 4084300).

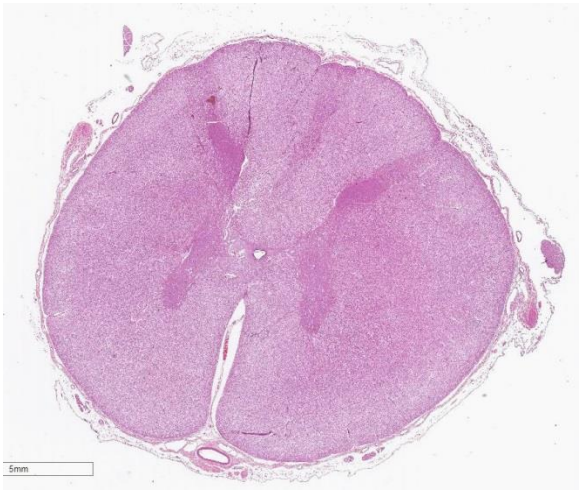
Signalment: 17-year-old, neutered male, quarterhorse, (*Equus caballus*).

History: Horse developed severe atrophy of facial muscles on the left side of the face 2 months prior to presentation to the teaching hospital. The weekend prior to submission the patient developed rear limb ataxia. Probing palpation of the cervical region revealed hyperesthesia and hyper-responsiveness. Probing around the mid cervical region did not elicit a response. Treatment with dexamethasone yielded some improvement in clinical signs. The horse was euthanized at the owner elected request**.**

Gross Pathology: The muscles on the left side of the face were diffusely atrophied and hemorrhage was present in the anterior compartment of the right eye. There was narrowing of the spinal canal between C2 and C3. The dura mater at the C2-C3 articulation was focally reddened. The third cervical vertebral body (C3) contained a 2.5 x 1.5 cm region of red and depressed tissue (bony sequestration) rimmed by thick white tissue (fibrosis) at its ventral border. The dorsal vertebral body of C2 also had a 1.5 cm linear band of firm white tissue (fibrosis) that traversed the bone in a dorsal-ventral direction.

Laboratory results: N/A

Histopathologic Description: Extending from just caudal of C1 to C5, there is a locally extensive area of rarefaction and multifocal to coalescing accumulations of glial cells and gitter cells, unilaterally involving the dorsal funiculus at the level of the gracile and cuneate fasciculi. Spinal cord inflammation is most concentrated at C3 and includes significant perivascular cuffing, few to moderate lymphocytes and plasma cells and scattered eosinophils. The associated spinal gray column is unilaterally affected with similar inflammation in these sections. Numerous swollen axons



Cervical spinal cord, horse. There is no visible lesion in the cord at subgross. This cross section is identifiable as cervical spinal cord due to the small ventral horns and the large dorsal funiculus (composed of ascending sensorimotor axons). (HE, 4X)

(spheroids) are present at C2 and C3 spinal cord sections. At the level of C2, there are occasional glial nodules in the contralateral dorsal funiculus as well. Small numbers of lymphocytes and plasma cells are diffusely present in the meninges and are more concentrated over the dorsolateral funiculus. The dorsal spinal nerve root ganglia are infiltrated with small numbers of lymphocytes and plasma cells at the level of C2 and C3.

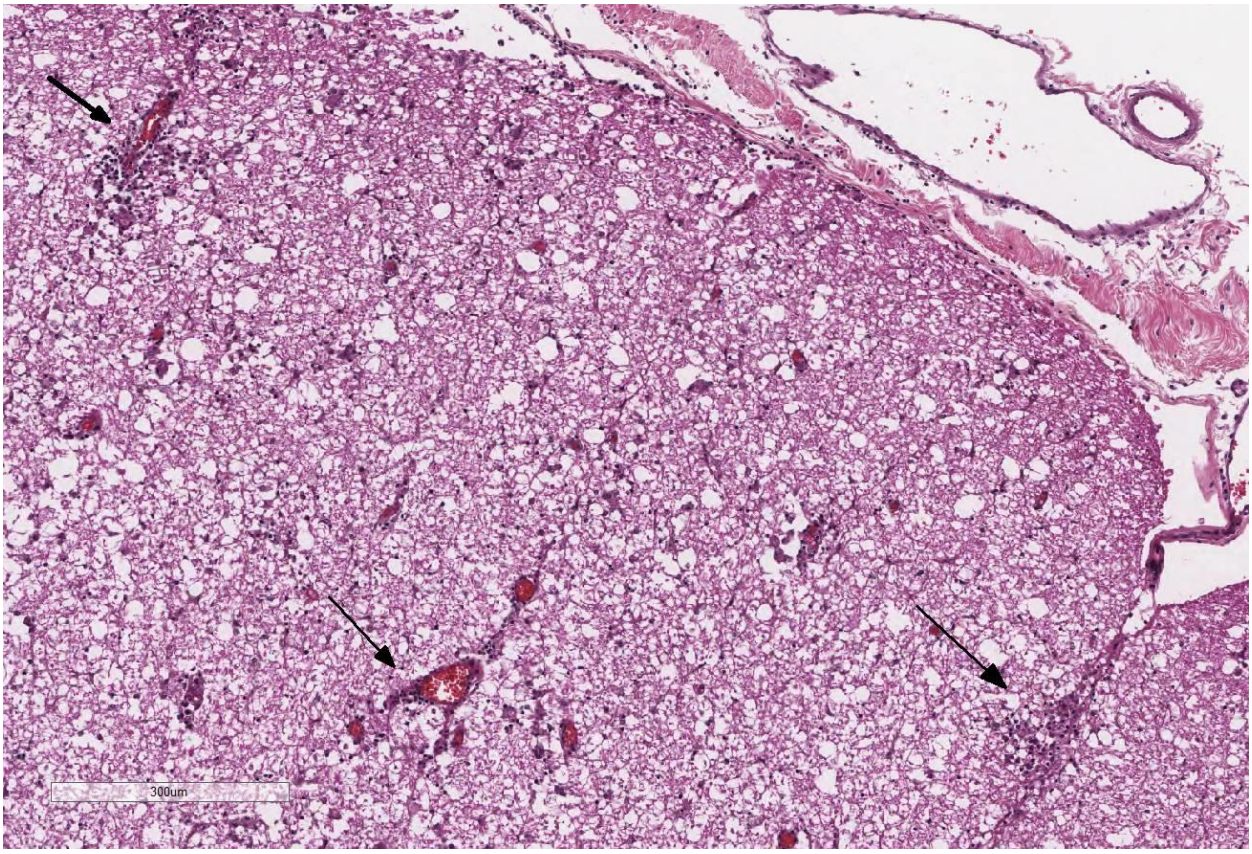
Contributor's Morphologic Diagnosis: Cervical spinal cord (C1-C5): Meningomyelitis, nonsuppurative and eosinophilic, unilateral, focally extensive, severe with spheroid formation, cervical spinal cord, dorsal funiculus

Contributor's Comment: The gross and histopathologic findings in this case are highly suggestive of cervical stenotic myelopathy. The unilateral distribution of the lesions coincides with the focus of stenosis observed in the spinal canal. Microscopic changes in the spinal cord include rarefaction, accumulations of glial and gitter cells, and lymphocytes, plasma cells, and scattered eosinophils. The horse, in this case, was 17 years old, considerably older than the typical case of cervical vertebral stenotic myelopathy (8-18 months and 1-4 years of age). However, several retrospective studies have documented this condition in horses up to 22 years of age.[3,4]

Cervical vertebral stenotic myelopathy, commonly referred to as "Wobbler's syndrome", is characterized by lesions in the spinal cord caused by narrowing of the spinal canal or compression by the vertebral articular processes.[6,7] There are two pathological syndromes: cervical vertebral instability (CVI) and cervical static stenosis (CSS). Clinical signs for both pathological syndromes include ataxia with the hindlimbs more commonly and more severely affected than the forelimbs.[7] Cervical vertebral instability is characterized by the narrowing of the spinal cord when the neck is ventroflexed. The cranial articular process of the vertebral bodies project in a ventromedial direction and impinge on the spinal cord. C-3 to C-5 of the spinal cord is the most affected area.[6] Young, rapidly growing horses between the ages of 8-18 months are predisposed to this condition. Breed dispositions include Thoroughbreds and Quarter horses, and males are affected more often than females. Contributing factors may be *ad libitum* feeding of high-energy and high-protein diet as well as copper deficiency.[6,7]

Cervical static stenosis is the less common syndrome. It is characterized by the compression of the spinal cord at the level of C-5 to C-7 due to the thickening of the ligamentum flavum and the dorsal laminae of the vertebral arches.[6] Predispositions are similar to those seen in CVI except horses aged one to four years are commonly affected.[3] The position of the neck does not determine whether or not the chord is compressed.

JPC Diagnosis: Spinal cord, dorsal medial fasciculi: Necrosis, focally extensive, asymmetric with lymphohistiocytic and eosinophilic perivasculitis and leptomeningitis, quarterhorse, *Equus caballus*.



Cervical spinal cord, horse. The dorsal funiculus contains moderate numbers of dilated myelin sheaths and a lymphoplasmacytic and eosinophilic perivascular infiltrate. (HE, 84X)

Conference Comment: This interesting case generated spirited discussion amongst conference participants. While attendees essentially agreed with the contributor's histopathologic description and morphologic diagnosis, there was no consensus for the histogenesis of the necrotizing lesion in the spinal cord of this horse. The conference moderator offered an alternative interpretation of an infectious cause, with *Sarcocystis neurona* causing acute onset weakness, ataxia, and a focally extensive area of necrosis in the dorsal medial spinal cord with corresponding lymphohistiocytic and eosinophilic leptomeningitis. The conspicuous eosinophilic component of the perivasculitis and leptomeningitis in this case, may suggest a parasitic etiology. *S. neurona* is an apicomplexan protozoan parasite which causes equine protozoal myeloencephalitis (EPM), a relatively common and severe neurologic disease in horses. Opossums are the definitive host for the parasite and spread the disease by fecal shedding of sporocysts into the environment.[1] Unfortunately, no apicomplexan schizonts or merozoites were observed in any examined tissue sections. Other potential etiologies offered by conference participants included acute intervertebral disc rupture and fibrocartilaginous embolism, although disk material was not visualized within the section.

As noted by the contributor, the age of the horse (17-years-old) is a highly atypical presentation for both cervical stenotic myelopathy and cervical static stenosis.[2,3,5] While most cases of cervical stenotic

myelopathy involve ventral compression of the spinal cord and spinal nerves with Wallerian-type degeneration of the white matter of the dorsal and ventrolateral spinal cord affecting the descending spino-cerebellar tracts of both the pelvic and thoracic limbs,[2,5] in this section, the ventral and lateral spinal cord is relatively unaffected. The lesions seen in the submitted section of spinal cord are primarily located in the dorsomedial spinal cord at the level of the fasciculus gracilus and fasciculus cuneatus.

Some conference participants noted occasional scattered 2x5 um filamentous bacilli multifocally throughout the neuro-parenchyma. The brain and spinal cord are exquisitely susceptible to post-mortem autolysis and putrefaction. As a result, the conference moderator cautioned attendees against overinterpreting artifactual bacterial overgrowth within post-mortem tissue samples of the central nervous system, especially when they are not associated with inflammation, as in this case.

Contributing Institution:
College of Veterinary Medicine
1200 West Montgomery Road
Tuskegee University
Tuskegee Institute, Alabama 36088
http://www.tuskegee.edu/academics/colleges/cvmnah/school of veterinary medicine.aspx

References:

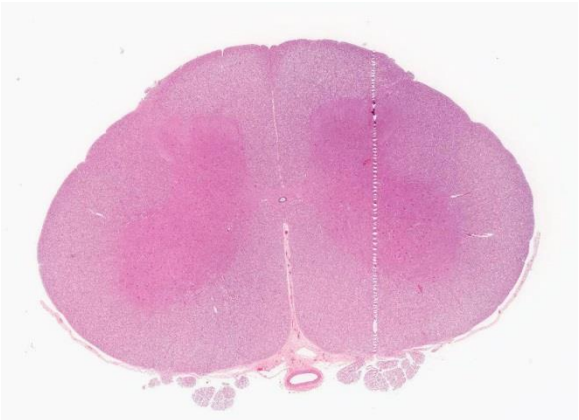
1. Bowman DD. Protozoans. In: *Georgis Parasitology for Veterinarians*. 9th ed. St. Louis, MO: Saunders Elsevier; 2009:104-105.
2. Janes JG, Garrett KS, McQuerry KJ, et al. Cervical vertebral lesions in equine stenotic myelopathy. *Vet Pathol*. 2015; 52:919-927.
3. Levine JM, Adam E, MacKay RJ, et al. Confirmed and presumptive cervical compression myelopathy in older horses: A retrospective study (1992-2004). *J Vet Intern Med*. 2007; 21:812-819.
4. Levine JM, Scrivani PV, Divers TJ, et al. Multicenter case-control study of signalment, diagnostic features, and outcome associated with cervical vertebral malformation-malarticulation in horses. *J Am Vet Med Assoc*. 2010; 237(7):812-822.
5. Reed SM. Cervical vertebral stenotic myelopathy: Pathogenesis. *Proceedings of the International Equine Neurology conference*. College of Veterinary Medicine, Cornell University, New York. 1997:45-49.
6. Thompson K. Bones and joints. In: *Pathology of Domestic Animals*, 5th Edition. Edinburgh : Saunders; 2007: 44-46.
7. Zachary JF, McGavin DM. Nervous system. In: *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby; 2012:816, 833-835.

CASE III: W1111-03 or W1112-03 (JPC 4006284).

Signalment: Six-month-old, male castrated, cross breed ox, (*Bos taurus*).

History: An outbreak of neurological disease occurred in a herd of 99, 6 to 12-month-old, mixed breed, beef cattle. The herd was grazing pasture with a daily supplementary ration of 2 kg/head of sprouted barley. Fifty-seven animals were affected and eighteen animals were euthanized after becoming recumbent and

unable to rise. The cattle had been fed hydroponically sprouted barley for seven months before the outbreak. Over the last few weeks of feeding, a green-blue downy mold had grown on the barley, its development had coincided with some unseasonably warm and humid spring weather. The mold did not have any effect on the appetite of the cattle which were being fed approximately 2 kg/head daily when adverse signs were first noticed. The first indication of toxicity was noticed by the owners when a 12-month-old cross-bred heifer showed signs of what was thought to be colic, based on her hunched posture and reluctance to walk. The next day another two cattle were noticed sick and three more the following day. At this stage the feeding of the sprouted barley was discontinued and the cattle were moved to another paddock. New cases continued to develop over the next 18 days. By six weeks, only a few of the affected cattle had not recovered, though all had lost considerable weight. A range of clinical signs was observed. Mildly sick cattle were instantly recognizable by the arching of their backs. Unless disturbed, most appeared to graze normally. Ataxia, knuckling of the hind fetlocks and hypermetria of hind limbs were also obvious in affected cattle. Some cattle developed progressively worsening ataxia with generalized muscle tremors. The cattle that were euthanized had progressed to posterior paralysis, recumbency and were often polypneic. One sternally recumbent animal appeared to be blind. Another was found in lateral recumbency displaying opisthotonos.



Spinal cord, ox. There is no visible lesion at subgross magnification. The prominent ventral horns and ventral emergence of the spinal nerves suggests that this is lumbar cord. (HE, 4X)

The mold on the barley sprouts was identified as *Aspergillus clavatus* (Agrifood Technology, Princes Hwy Werribee, Victoria 3030). *A. clavatus* is identifiable by its long, smooth conidiophore and huge club-shaped vesicle to which are attached uniseriate phialides.[3] The mould was confirmed as *A. clavatus* and a specimen is held at the DPI, Plant Diseases Herbarium, Knoxfield Victoria.

Gross Pathology: There were no remarkable gross changes seen in the internal organs of the necropsied animals.

Laboratory results: Biochemical analysis of sera from the two recently recumbent cattle found mild to moderate elevations in GLDH (428 and 30 U/L; reference range < 20 U/L) and LDH (2318 and 2395 U/L; reference range 50-400 U/L). CK was mildly elevated in one animal, as was AST in the other.

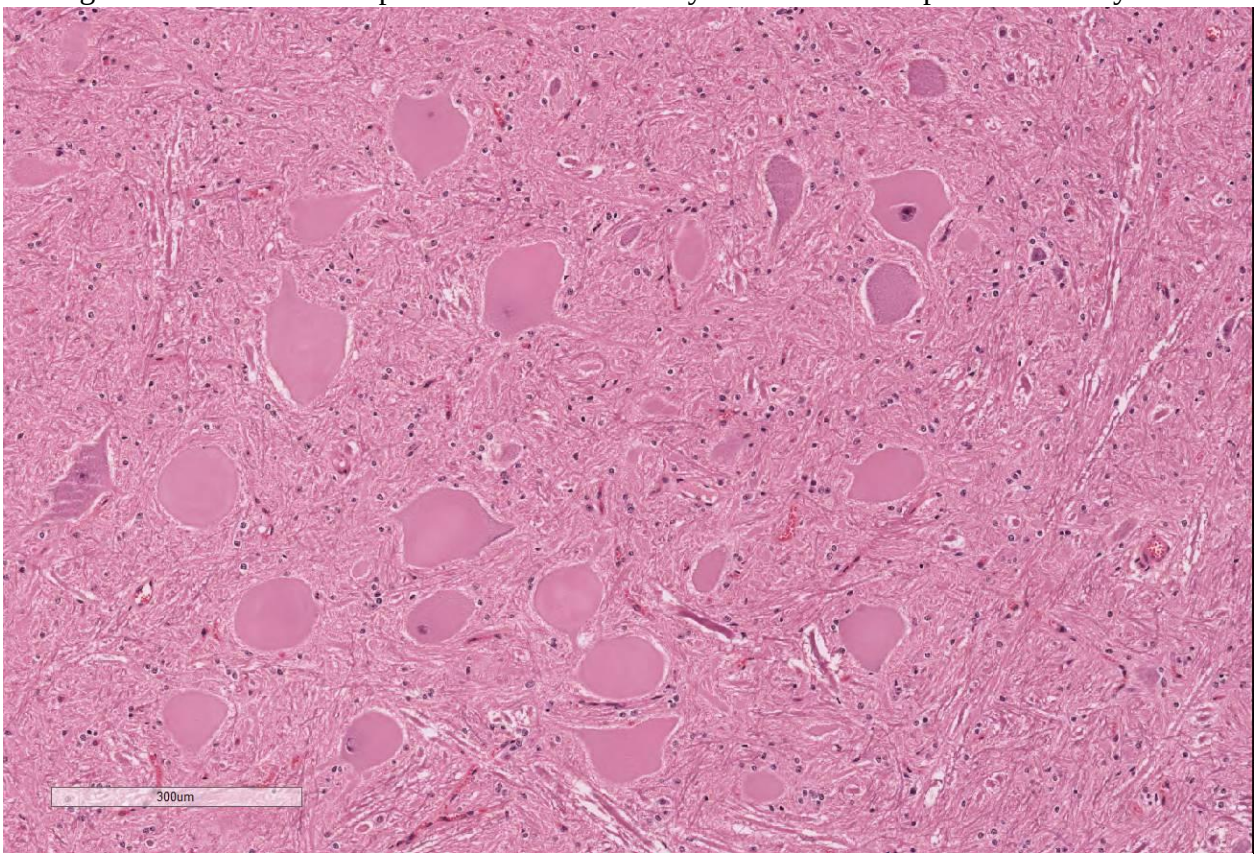
Histopathologic Description: Significant histological abnormalities are limited to the central nervous system. Neuronal changes are apparent in the brain and spinal cord (spinal cord sections submitted for WSC). Affected neurons are swollen and rounded, completely chromatolytic with pale or acidophilic cytoplasm, and the few nuclei captured in section are peripheral, shrunken and often pressed against the cell membrane. Many nuclei are affected, especially the red nuclei, dorsal motor nucleus of the vagus, reticular nucleus of the medulla and alpha motor neurons among the lateral and medial

motor neurons of the cervical and lumbar intumescences. Small gamma efferent neurons are not involved. There is no apparent cell loss or glial reaction. Within the brain and spinal cord, there is minor axonal swelling and myelin vacuolation with astrocytic swelling. Otherwise, the integrity of myelin and axons is preserved. The peripheral nervous system, including autonomic ganglia, peripheral nerves, and craniospinal nerve roots, shows no abnormality.

Contributor's Morphologic Diagnosis: Brain and spinal cord: chromatolytic neuronal degeneration, severe multifocal acute, with mild edema of myelin.

Contributor's Comment: The epidemiology, clinical signs, histological findings in the brain and spinal cord and mycological examination in this case[3] are consistent with those previously described and caused by *Aspergillus clavatus* tremorgenic neurotoxicosis.[5,6]

A clavatus is present in soil and commonly isolated from cereal grains and their germinated seeds and other feedstuffs. It also occurs commonly in pigeon droppings.[6] Tremorgenic syndromes have been experimentally reproduced in ruminants by feeding *A clavatus* pure cultures and *A clavatus* contaminated grains. A number of toxins have been isolated from A clavatus including cytochalasin E, tryptoquivaline, tryptoquivalone, patulin, but none have been tested in ruminants to determine responsibility for the tremorgenic syndrome. Toxic extracts from sorghum beer residue capable of reproducing the tremogenic syndrome in sheep dosed orally did not



Spinal cord, ox. Neuronal cell bodies of the ventral horn are diffusely and markedly swollen with dispersal of Nissl substance (central chromatolysis). The cytoplasm is illed with numerous and distinct vacuoles , and nuclei are often peripheralized and occasionally hyperchromatic. (HE, 88X)

contain patulin, trypotquivalone or nortryptoquivalone so the identity of the toxin(s) remains undetermined.[6]

The case is submitted to the WSC with the kind permission of the surviving author, Dr C El-Hage, University of Melbourne.

JPC Diagnosis: Spinal cord, grey matter: Neuronal degeneration, multifocal, severe, with chromatolysis and vacuolar degeneration, cross breed ox, *Bos taurus*.

Conference Comment: The contributor provides a striking example of severe toxin-induced swelling and central chromatolysis of neuronal cell bodies (soma). In this case, the pathogenic process is most severe in the grey matter of the ventral horns. The ventral horn of the spinal cord contains nuclei for lower motor neurons that supply motor input to somatic muscle via axons in the white matter of the ventral funiculus. Typically, lesions in lower motor neurons produce flaccid paralysis, rather than the muscular tremors and hyperesthesia present in this case; however, as mentioned by the contributor, the pathogenic mechanisms of neuromycotoxicosis in *Aspergillus clavatus* have not yet been determined.

Some authors consider the mycotoxin patulin, produced by *Aspergillus* sp. and *Penicillium* sp, to be the major contributor to the neurotoxicity induced by *A. clavatus*.[1,4] Patulin has been implicated in previously reported cases of neurotoxicity in animals. In the brain and spinal cord, patulin inhibits acetylcholinesterase and the Na+/K+-ATPase resulting in a buildup of the stimulatory neurotransmitter acetylcholine.[1,4] Decreased breakdown of acetylcholine at the neuromuscular junction results in convulsions, tremors, stiffness, impaired locomotion, and hyperesthesia, seen clinically in this case.[4] Additionally, inhibition of the Na+/K+-ATPase may have devastating effects on the cell, including ionic depolarization of the cell membrane resulting in neuronal signal transduction deficiencies and disruption of the cell's concentration gradient causing increased osmolarity and cellular swelling.[4]

Conference participants discussed the degenerative changes associated with chromatolysis in the central nervous system. Chromatolysis represents a change in the histomorphologic appearance of the soma due to the central or peripheral dispersal pattern of the Nissl substance.[2] The chromatolyic pattern in this case is consistent with central chromatolysis. Central chromatolysis occurs in the large neurons of the brainstem, spinal motor neurons, and peripheral ganglia and is characterized by central clearance of Nissl granules and marked cellular swelling with pale, eosinophilic, homogenous, ground-glass appearance to the cytoplasm.[2] Soma nuclei are often peripheralized and possess prominent nucleoli. Typically, central chromatolysis occurs secondary to axonal injury and represents a reparative response in the soma, incorporating increased free ribosomes for protein synthesis, lysosomes, and mitochondria. This anabolic response required for axon regeneration is referred to as the axon reaction.[2] Central chromatolysis occurs in a number of neurodegenerative diseases, including copper deficiency in sheep and goats, grass sickness in horses, avian encephalomyelitis virus in chickens, and feline dysautonomia, known as Key-Gaskell syndrome, in cats. Peripheral chromatolysis is generally associated with cell body shrinkage rather than swelling. It is characterized by Nissl granules surrounding the nucleus and is a relatively nonspecific degenerative lesion.[2]

Contributing Institution:

University of Melbourne
Faculty of Veterinary Science
250 Princes Hwy
Victoria Australia, 3030
http://www.vet.unimelb.edu.au/

References:

1. Brotha CJ, Legg MJ, Truter M, Sulyok M. Multitoxin analysis of *Aspergillus clavatus*-infected feed samples implicated in two outbreaks of neuromycotoxicosis in South Africa. *Ondersepoort J Vet Res*. 2014; 12: doi:10.4102/ojvr.v81i1.848.
2. Cantile C, Youssef S. Nervous system. In: In: Maxie MG, ed. *Jubb Kennedy and Palmer's Pathology of Domestic Animals*. Vol 1. 6th ed. Philadelphia, PA: Elsevier Saunders; 2016:252-255,327.
3. Hage C M; Lancaster M J: Mycotoxic nervous disease in cattle fed sprouted barley contaminated with *Aspergillus clavatus*. *Aus Vet J*. 2004; 82:639-641.
4. Loretti AP, Colodel EM, Driemeier D, et al. Neurological disorder in dairy cattle associated with consumption of beer residues contaminated with *Aspergillus clavatus*. *J Vet Diagn Invest*. 2003; 15:123-132.
5. Maxie MG, Youssef S. Nervous system. In: *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Maxie MG, ed. 5th ed, Edinburgh UK: Elsevier Saunders; 2007:369.
6. McKenzie RA, Kelly MA, Shivas RG, et al. *Aspergillus clavatus* tremorgenic neurotoxicosis in cattle fed sprouted grains. *Aus Vet J*. 2004; 82:635-638.

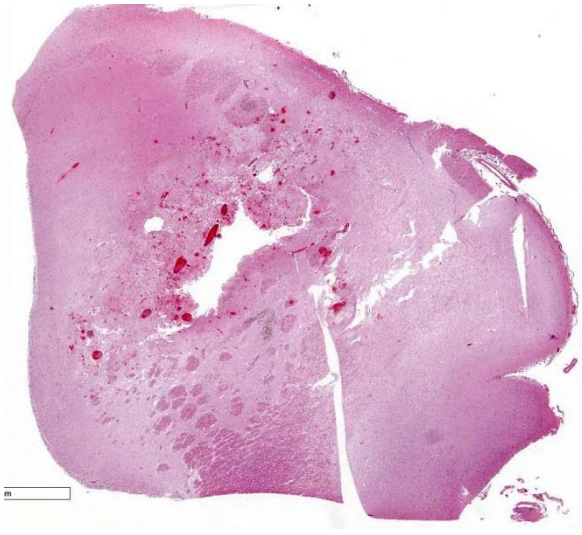
CASE IV: A16-6925-12 (JPC 4083741).

Signalment: 7-month-old male Angus ox (*Bos taurus*)

History: A previously healthy bull calf was presented to the Veterinary Teaching Hospital the day it was found down and unresponsive.

Gross Pathology: The brain is wet and soft with mildly dilated ventricles and excessive transparent watery cerebrospinal fluid. A 1 cm x 1.5 cm dark pink, softened and cavitated focus is in the left cerebral hemisphere, adjacent to the caudate nucleus and rostral to the optic chiasm. Less distinct foci of reddening and softening are found elsewhere in the meninges and parenchyma of the brain and spinal cord.

Mandibular, parotid, and cervical lymph nodes are enlarged to 2 cm in diameter. Petechiae and ecchymoses are scattered



Cerebrum, ox. Approximately 20% of the section is composed of a large area of hemorrhage and infarction. (HE, 5X)

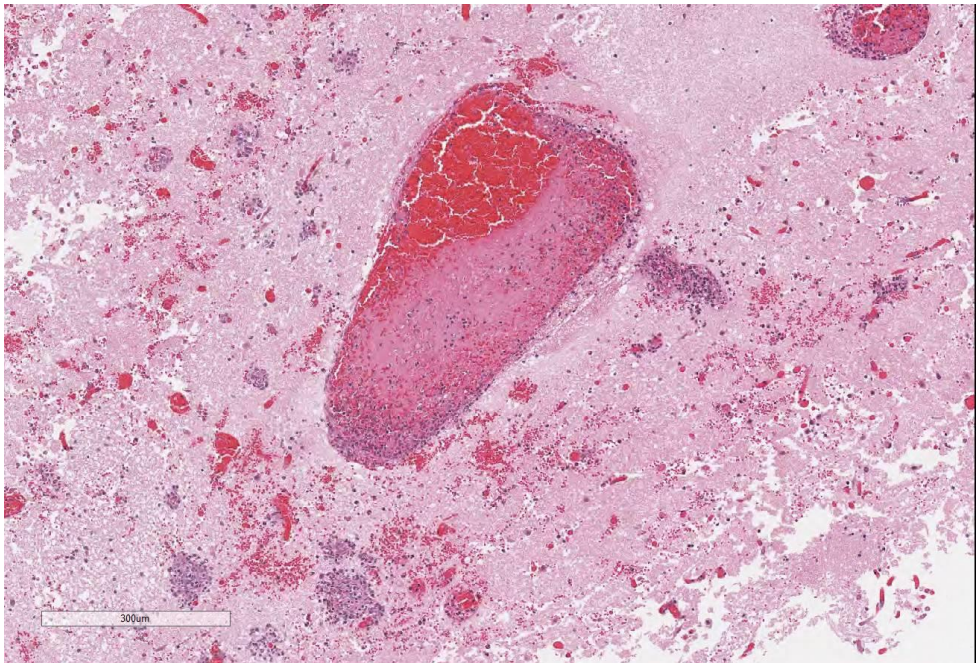
through many skeletal muscles, esophageal adventitia, pulmonic trunk adventitia, visceral and parietal pleura, small intestinal serosa, and urinary bladder. The lungs are reddened. One liter of transparent yellow watery fluid is in the abdominal cavity. The small intestine, spiral colon, and descending colon have multifocal mucosal red foci. Several cestodes, up to 70 cm long, are in the jejunal lumen. Blood-tinged mucus is in the lumen of the descending colon.

Gross lesions are not observed in the pituitary gland, trigeminal nerves and ganglia, oral cavity, larynx, trachea, heart, thyroid gland, aorta, stomach, spleen, liver, gallbladder, pancreas, common bile duct, forestomachs, abomasum, adrenal glands, kidneys, testes, joints, or bone marrow.

Laboratory results: Aerobic culture of brain: *Histophilus somni*

Bovine herpesvirus fluorescent antibody test: Negative
Negative virus isolation
Fluorescent antibody test for rabies (Indiana State Department of Health): Negative
Fecal flotation: Numerous trichostrongyle-type eggs as well as eggs/ova of *Moniezia benedeni*, *Nematodirus* spp., *Eimeria* spp.

Histopathologic Description: In sections of cerebrum (submitted slide), brain stem, and spinal cord, many vessels (mainly veins and venules) have poorly organized thrombi rich in neutrophils. A few venules contain bacteria. The endothelium in affected vessels is disrupted with transmural extension of neutrophils and fibrinoid material or frank hemorrhage into Virchow-Robin space and beyond. Thrombi and hemorrhage are also in the leptomeninges. Surrounding neuroparenchyma is rarefied with hemorrhage, necrosis, and infiltration by neutrophils with fewer lymphocytes or macrophages. Thrombi are also in inflamed vessels of the ventricular myocardium and skeletal muscles. Neutrophils with fewer lymphocytes and macrophages infiltrate adjacent musculature. Myocytes are necrotic with hypereosinophilic sarcoplasm and pyknosis or karyorrhexis. Multifocal hemorrhage, necrosis, and aggregates of



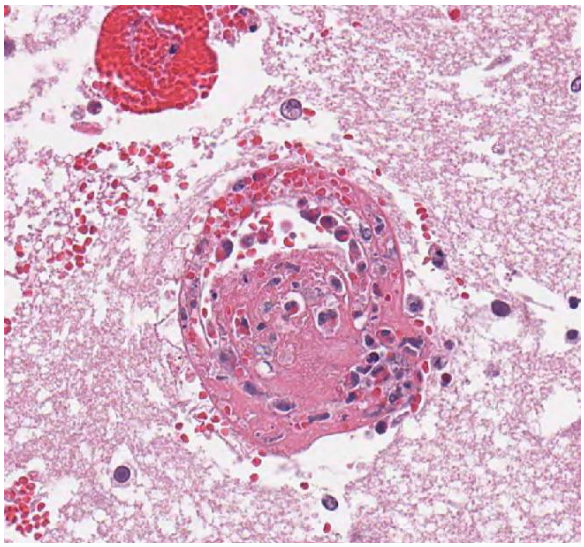
Cerebrum, ox. Vessels within the area of hemorrhage are partially to totally occluded by fibrincellular thrombi, and there is infiltration of inflammatory cells and hemorrhage within the walls and into the surrounding perivascular space (vasculitis). (HE, 84X)

neutrophils are in the tunica media of the pulmonic trunk.

Transmural hemorrhages in the small intestine are associated with submucosal fibrin thrombi, pleocellular leukocytic infiltration, and segmental necrosis of intestinal mucosa. The lung is congested with edematous interlobular septa. Alveolar spaces contain increased number of macrophages. A few poorly organized thrombi are in small pulmonary vessels.
Evaluated sections of lymph node, spleen, liver, kidney, adrenal gland, rumen (several ciliated protozoa), colon, trigeminal nerve and pituitary gland are within normal limits.

Contributor's Morphologic Diagnosis: Thrombotic meningoencephalitis with neutrophilic vasculitis.

Contributor's Comment: Rabies had been included in the clinical differential diagnosis, but the differential diagnosis at autopsy, based on the presence of multiple malacic hemorrhages in the brain and spinal cord, included thrombotic meningo-encephalitis and herpesviral



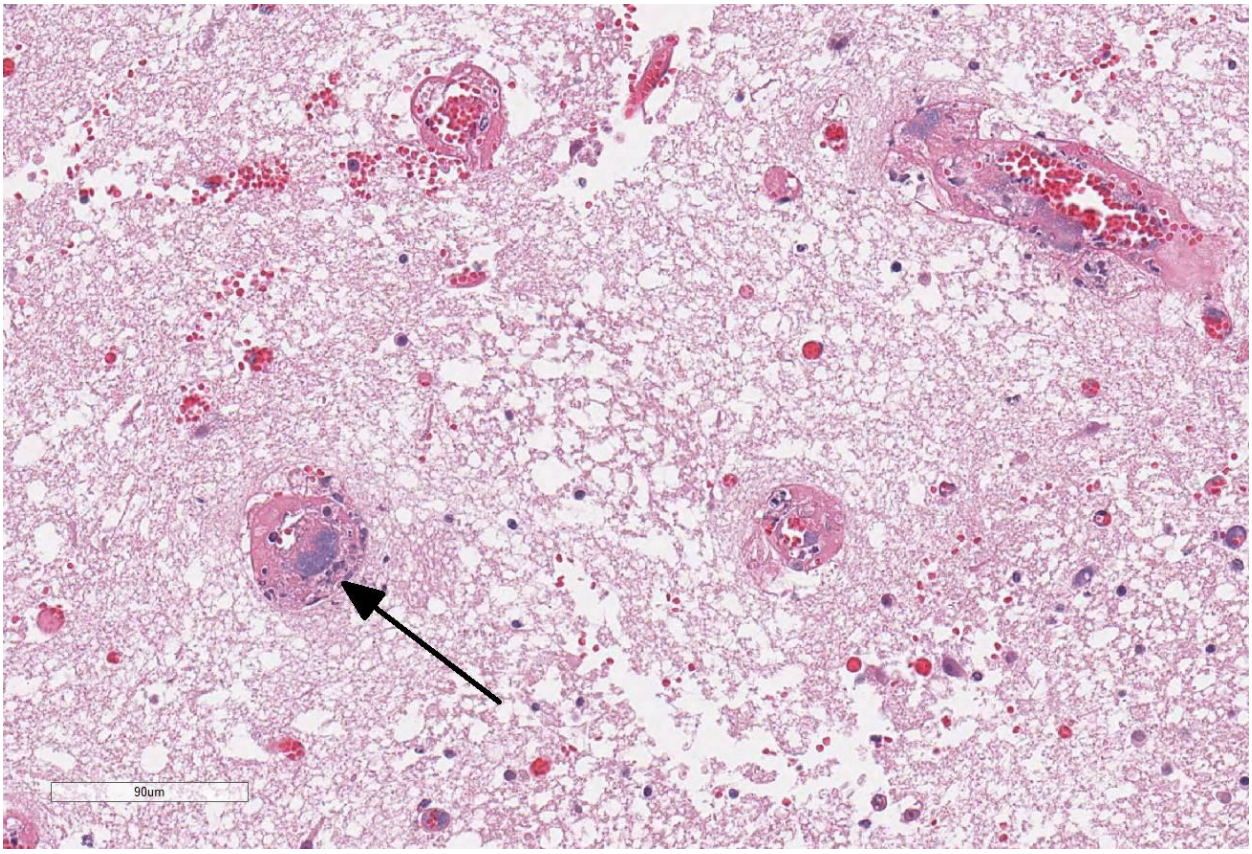
Cerebrum, ox. Multifocally, vessels are occluded and the wall is expanded by polymerized fibrin, hemorrhage, degenerate neutrophils, and cellular debris (fibrinoid necrosis). (HE, 360X)

encephalomyelitis. Histologically, the prominent and neutrophilic phlebitis with bacteria in the lumen of venules, plus the absence of trigeminal ganglionitis (and a negative FA test for rabies virus), prompted submission of brain for culture for *Histophilus somni*, which was isolated. Lesions of vasculitis, thrombosis, and inflammation were also detected histologically in the myocardium, pulmonic trunk, and skeletal muscles, but lung lesions were minimal in this case.

Histophilus somni is the cause of bovine thrombotic meningoencephalitis (TME), formerly known as thromboembolic meningoencephalitis (TEME). Currently, vasculitis (mainly phlebitis) is considered a primary lesion with thrombosis secondary to the local vasculitis, rather than the result of embolization from a distant site.[2] In fact, the tendency to induce thrombosis is a key feature of *H. somni*, and entails interactions of the bacterium with endothelial cells, leukocytes, and platelets.[1] The disease is reportedly more common in older calves and yearlings, and in late fall and early winter;[6] this 7-month-old calf died on the 23rd of November.

Lipooligosaccharide (LOS) is considered the major virulence factor of *H. somni*.[1,2] Its diverse activities contribute to the pathogenicity of *H. somn*i. Caspase-mediated apoptosis of endothelial cells (and of other host cells)[1] triggered by LOS (probably by its lipid A component), is thought to initiate the vasculitis of TME.[4] LOS is also thought to play a role in antigenic mimicry, inflammation (via Toll-like Receptor-4), resistance to phagocytosis and killing by leukocytes, and evasion of the immune response.[3]

Although most vaccine studies have been focused on the bovine respiratory disease

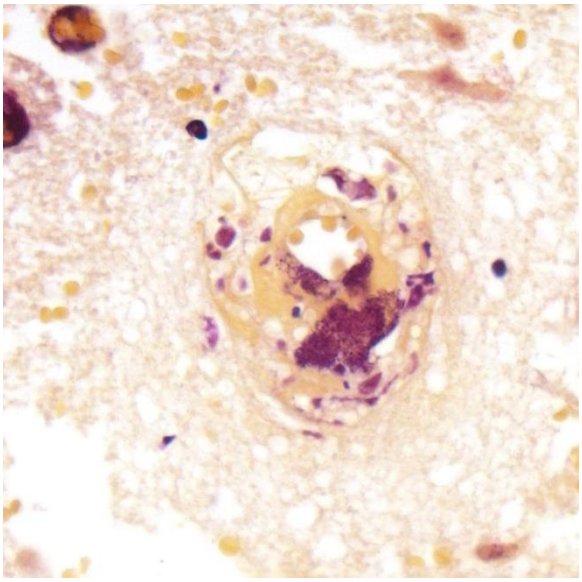


Cerebrum, ox. Thrombosed vessels often contain large colonies of coccobacilli (arrow). (HE, 348X)

complex, results suggest a role for humoral immunity. Macrophages that ingest *H. somni* are soon killed by the bacteria, so are unlikely to play a long-term role in dissemination of infection. This may also suggest that Th1 immunity is less important in disease control than humoral immunity.[3]

JPC Diagnosis: Brain: Vasculitis, fibrinous and necrotizing, multifocal, severe, with thrombosis, infarction, and numerous colonies of coccobacilli, Angus, *Bos taurus*.

Conference Comment: The contributor provides an outstanding example of the hallmark lesions of *Histophilus somni* in the brain of a feedlot calf. Conference participants localized the examined tissue section to the corpus striatum of the cerebrum due to the heterogeneous mix of white and grey matter tracts. *H. somni* is a facultative anaerobic gram-negative coccobacillus that is a normal commensal bacterium of the bovine genital tract and nasal cavity.[2] In 6 to 12-month-old calves, infection usually occurs following a stressor, such as transportation, inclement weather, crowding, or changes in diet.[2,3,6] Virulent strains of *H. somni* often cause septicemia resulting in a wide variety of lesions secondary to vasculitis and thrombosis caused by virulence factor, lipooligo-saccharide (LOS), discussed by the contributor above. Typical lesions associated with *H. somni* include thrombotic meningoencephalitis, myocarditis, mastitis, metritis, orchitis, conjunctivitis, necrotizing laryngotracheitis, and polyarthritis. *H. somni* also causes fibrinopurulent broncho-pneumonia as part of the bovine respiratory disease complex.[2,3,6] Readers are encouraged to read 2016 Wednesday Slide Conference



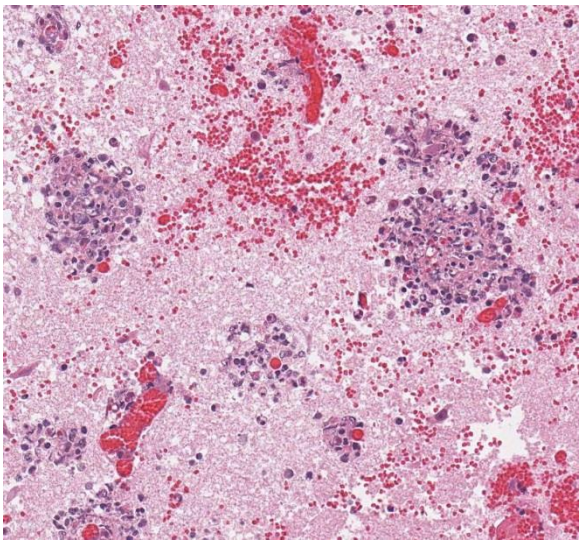
Cerebrum, ox. Bacterial colonies are better visualized with a Brown-Hopps tissue Gram stain. (BH, 400X)

#2 Case 4 for a review of the bovine respiratory disease complex. Small ruminants, bighorn sheep (*Ovis canadensis*), and North American bison (*Bison bison*) can also be affected; although the clinical manifestations are often not as severe likely due to less intensive management practices in these species.[6]

While the histologic lesions of *H.somi* are widespread, the bacteria has a tropism for the small venules of the cerebral vascular tissue and the most severe changes often occur within the brain, as in this case.[2] Affected calves often acutely die without treatment. In this case, there is severe fibrino-necrotic vasculitis and thrombi containing numerous coccobacilli resulting in a focally extensive infarct of the neuroparenchyma. Numerous colonies of bacteria are also present in the neuropil. Most conference participants agreed that the hemorrhage and infarction of the brain is a result of fibrinonecrotic vasculitis rather than a primary necrosuppurative meningoencephalitis.

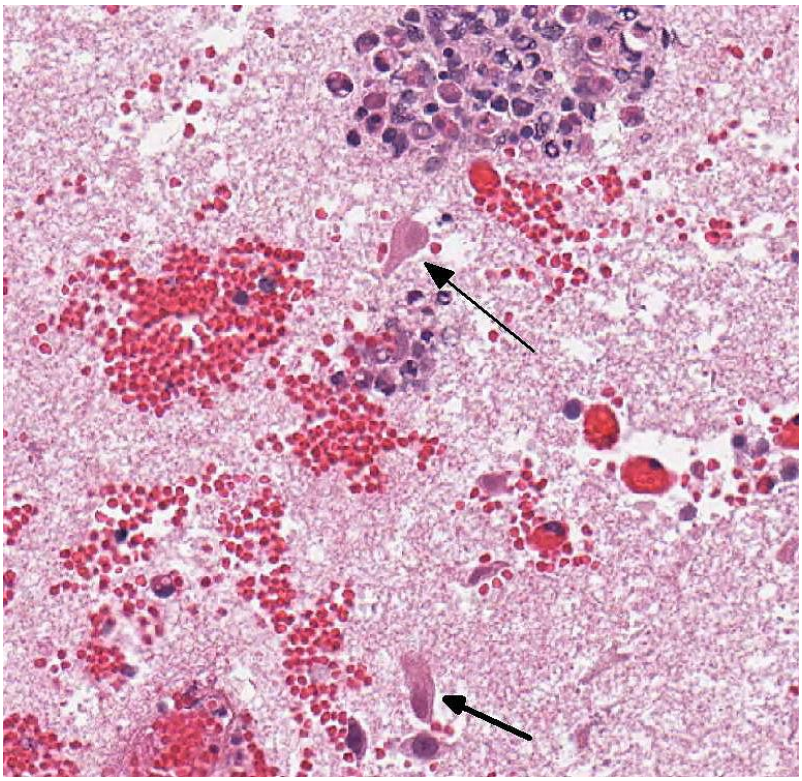
As mentioned by the contributor, *H. somi* secretes an endotoxin (LOS) causing caspase 3-mediated apoptosis of endothelial cells leading to vasculitis and thrombus formation.[2] Recent studies indicate that *H. somni* can also stimulate endothelial cell tissue-factor (factor 3) activity and disrupt intercellular junctions enhancing pro-coagulant activity on the endothelial surface.[1] *H. somni* and LOS also activate bovine platelets, which further enhances tissue factor activity on the endothelial surface, upregulates leukocyte adhesion molecules (P-selectin, E-selectin, and ICAM-1), and initiates endothelial cell apoptosis via the FasL (caspases 8 and 9).[2,5] They also induce endothelial cell cytokine and reactive oxygen species production.[3,4,5] The mechanisms of *H. somni* induced vasculitis and thrombus formation are complex and research is ongoing to fully elucidate the pathogenesis.

Contributing Institution:
Purdue University
Indiana Animal Disease Diagnostic Lab
Purdue University Dept. Comparative



Cerebrum, ox. Numerous vessels are effaced by degenerate neutrophils and macrophages. (HE, 280X)

Pathobiology
406 S. University St.
West Lafayette, IN 47907
Animal Disease Diagnostic Laboratory: http://www.addl.purdue.edu/
Department of Comparative Pathobiology: http://www.vet.purdue.edu/cpb/



Cerebrum, ox. There are numerous necrotic neurons within the areas of infarction. (HE, 320X).

References:

1. Behling-Kelly E, Rivera-Rivas J, Czuprynski CJ. Interactions of *Histophilus somni* with host cells. *Curr Top Microbiol Immunol*. 2016; 396:71-87.
2. Cantile C, Youssef S. Nervous system. Maxie MG ed. In: *Jubb Kennedy and Palmer's Pathology of Domestic Animals*. Vol 1. 6th ed. Philadelphia, PA: Elsevier Saunders; 2016:364-365.
3. Corbeil LB. Host immune response to *Histophilus somni*. *Curr Top Microbiol Immunol*. 2016; 396:109-129.
4. Inzana TJ. The many facets of lipooligosaccharide as a virulence factor for *Histophilus somni*. *Curr Top Microbiol Immunol*. 2016; 396:131-148.
5. Kuckleburg CJ, McClenahan DJ, Czuprynski CJ. Platelet activation by *Histophilus somni* and its LOS induces endothelial cell pro-inflammatory responses and platelet internalization. *Shock*. 2008; 29:189-196.
6. O'Toole D, Sondgeroth KS. Histophilosis as a natural disease. *Curr Top Microbiol Immunol*. 2016;396:15-48.