Joint Pathology Center

Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2016-2017
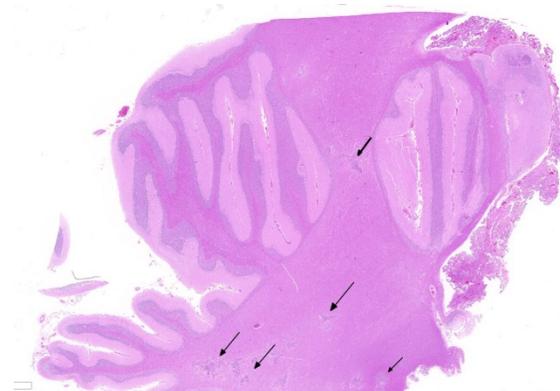
Conference 20

22 March 2017

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CASE I: W255-15 (JPC 4068726).

Signalment: Adult, thoroughbred gelding, (*Equus caballus*).

History: A mature, racehorse started to develop neurologic signs. When presented to the veterinarian, the horse showed severe ataxia and incoordination. The horse was also colliding with the box walls and displayed head pressing behavior. No significant changes were identified on hemogram or serum biochemistry. Blood collected for *Flavivirus* ELISA testing was negative. After unsuccessful treatment with dexamethasone and DMSO, the horse was humanely euthanized.

Gross Pathology: Gross examination of the brain on formalin-fixed cut sections revealed multifocal linear, beige discolorations within the thalamus and cerebellum (suspected areas of malacia). Within the brain stem, there was a focal loss of substance measuring. 0.8 x 0.6 x 0.6 cm.

Cerebellum with choroid plexus, horse. There are multiple areas of necrosis and inflammation within the cerebellar white matters (arrows). (HE, 5X)

Laboratory results: No laboratory analysis performed.

Histopathologic Description: Expanding the cerebellar white matter and infiltrating the granular and molecular layers of the cerebellar cortex, there is a focally extensive inflammatory reaction. This inflammatory focus is composed of coalescing accumulations of numerous epithelioid macrophages and multinucleated giant cells (of Langerhans and foreign body types), centered around sections of nematodes eggs

(ca. 10-15 x 30 μm), as well as transverse and longitudinal sections of nematode larvae and adults. The larvae measured 10 x 20 to 30 μm and the adults 15-20 μm x 250-300 μm in diameter with a smooth cuticle, platymyarian-meromyarian musculature, a rhabditiform esophagus, occasionally displaying a distinct corpus, isthmus and terminal bulb, and numerous deeply basophilic 2-3 μm internal structures within the pseudocoelom. Large numbers of lymphocytes and plasma cells are also seen throughout the granulomatous reaction, surrounding vessels and infiltrating the vessel wall. Within the adjacent molecular layer there is marked vacuolation and clear tissue separation (edema), along with numerous nematode sections.
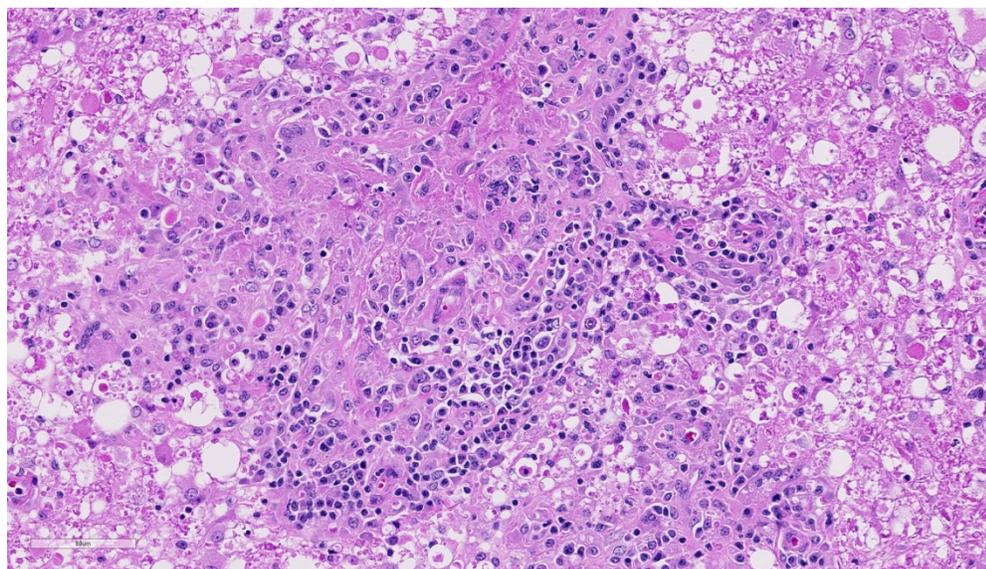
Within the cerebellar nuclei, there are multifocal, randomly distributed areas of necrosis, characterized by rarefaction of the neuropil, loss of architecture, and small amounts of eosinophilic cellular and karyorrhectic debris, admixed with several sections of nematodes and moderate numbers of gitter cells. Multifocally, perivascular infiltrates of macrophages, multinucleated giant cells, lymphocytes and plasma cells admixed with occasional nematodes expand the Virchow-Robin spaces and extend within the adjacent neuropil. Adjacent to the perivascular cuffs, there is marked vacuolation of the neuropil, dilated myelin sheaths and multiple round eosinophilic bodies (axonal spheroids). The meninges are multifocally infiltrated with low numbers of lymphocytes and macrophages.

Contributor's Morphologic Diagnosis: Brain: Granulomatous meningoencephalitis, marked, multifocal, with intralesional nematodes, horse (*Equus caballus*).
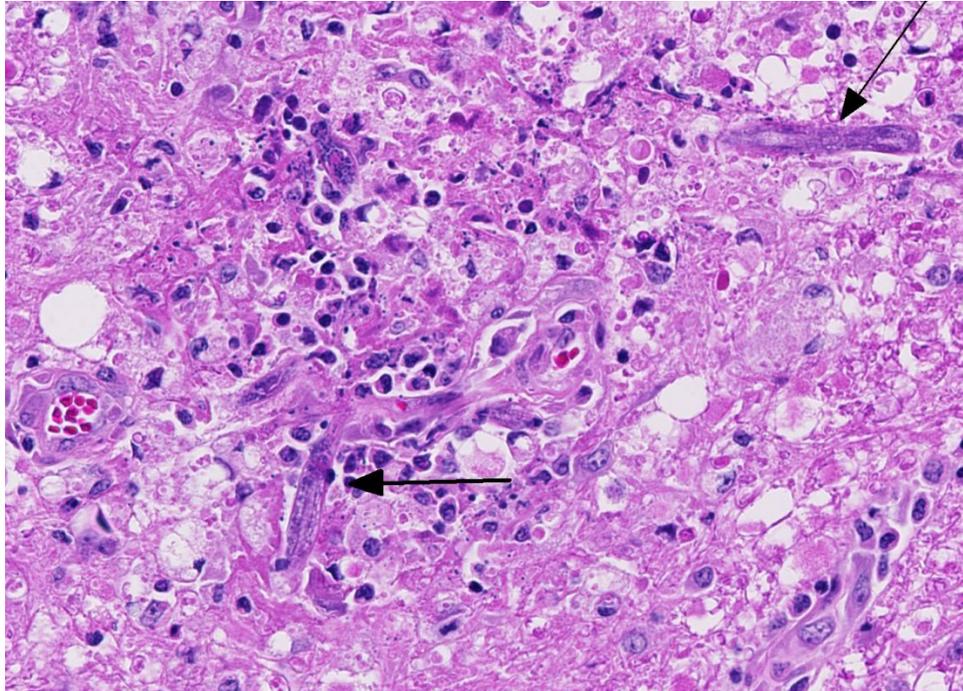
Contributor's Etiologic Diagnosis: Encephalitic nematodiasis.

Cause: Presumed *Halicephalobus gingivalis*.

Cerebellum, horse. Foci of necrosis contain a range of inflammatory cells including numerous infiltrating macrophages, rare multinucleated giant cell macrophages, numerous lymphoycytes and plasma cells, but few if any neutrophils and eosinophilis. There is extensive edema at the periphera as well as dilated myelin sheaths and spheroids. (HE, 256X)

Contributor's Comment: Parasitic migratory encephalomyelitis is rare but represents an important cause of neurologic disease in horses and a number of parasites (protozoan and metazoan) have been described to be associated with central nervous system (CNS) disease.

Cerebellum, horse. Several, but not all foci of necrosis contain cross sections of adult nematodes as well as abundant cellular debris, hypertrophic astrocytes, numerous spheroids, and vessels are surrounded by low to moderate numbers of lymphocytes and plasma cells. (HE, 400X)

Granulomatous amebic encephalitis, caused by *Balamuthia mandrillairs* infection, occurs worldwide, albeit rarely, and has been described in horses.[10] Amebae resemble large macrophages with foamy cytoplasm, large eccentric nucleus but can be distinguished by positive staining with PAS.

Trypanosoma evansi infection was reported to cause necrotizing encephalitis in Brazilian horses, with the most severe lesions occurring within the cerebral white matter.[12]

A non-suppurative inflammation, similar to viral infections, is usually encountered in protozoal infections. Equine protozoal myeloencephalitis caused by *Sarcocystis neurona* (and much less commonly *S. hughesi*) occurs in North America in the geographical range of opposums (*Didelphis spp.*), as the latter represent the definitive host for *S. neurona*. The lesions with *Sarcocystis* infection more typically affect the cervical and thoracic spinal cord, but lesions in the brain stem can occur. *S neurona* schizonts are oval or irregularly round, have very thin walls and contain few basophilic ovoid merozoites.[4]

Horses are considered one of the less sensitive species to the pathogenic effect of *Toxoplasma gondii*, as a specific pathologic condition related to toxoplasmic infection has not been described under natural conditions or experimentally.[14]

The larvae of tapeworms of the family Taeniidae, such as *Coenurus cerebralis* (larval stage of *Taenia multiceps*), although rarely affecting horses, can migrate and reach various organs, in particular the liver and the CNS, where they form cysts of varying size, which can be sometimes very large.

In acute helminth infections, the inflammatory pattern is typically suppurative in nature with variable numbers of eosinophils, while chronic lesions develop a granulomatous component. Rare cases of cerebrospinal nematodiasis in horse have been attributed to *Strongylus vulgaris*.[3] *Angiostrongylus cantonensis* has been reported to induce neurological disease in foals.[15] *Parelaphostrongylus tenuis*,

commonly called meningeal worm or brain worm, is a common neurotropic parasitic nematode of white-tailed deer in eastern North America; however a recent report revealed that horses are also susceptible to infection, and developed lesions mostly within the cerebellar parenchyma and associated meninges.[13] The lesions within the CNS result from aberrant nematode larval migration, inducing areas of malacia (mostly in the white matter) with swollen axons and hemorrhages. The migration tracks induce reactive changes including vascular proliferation, invasion of macrophages, and perivascular cuffing containing varying numbers of eosinophils.

Similar lesions, caused by larval wandering of the microfilaria *Setaria sp.* in the brain and spinal cord are described in horses (aberrant hosts) in Asia. *Setaria digitata* is normally found as an adult in the peritoneal cavity of cattle and buffalo.[8]

Intracranial invasion by the nematodes *Halicephalobus gingivalis* is reported in horses in Europe, North and South America. Depending on the area of the CNS affected, lesions are granulomatous and eosinophilic meningoencephalitis, myelitis, poly-radiculitis or even cauda equina neuritis-like lesions. Parasitic granulomas in the kidney and gingiva may accompany the cerebral invasion.[1] *Halicephalobus gingivalis* has a characteristic rhabditiform esophagus, composed of a corpus, isthmus and bulb. Little is known about the life cycle and method of transmission of this nematode; however oral ingestion or wound contamination followed by hematogenous distribution is suggested.[7]

Based on the granulomatous nature and the lack of hemorrhages, the verminous encephalitis encountered in our case reveals a chronic stage. The presence of nematodes

with rhabditiform esophagus, in this case, suggests infection with the nematode *Halicephalobus gingivalis*. Attempts to definitively identify the organism with PCR were unsuccessful, and this could possibly be attributed to prolonged formalin fixation and processing procedures. Nevertheless, this case represents one of the rare equine verminous encephalitides reported in Australia.

JPC Diagnosis: Cerebellum: Encephalitis, necrotizing and granulomatous, multifocal, random, moderate, with numerous eggs, larvae, and adult rhabditid nematodes, thoroughbred, *Equus caballus*.

Conference Comment: The contributor provides a concise review of neurotropic parasite-induced disease in the equine central nervous system. *Halicephalobus gingivalis* (previously called *Micronema deletrix*, *Rhabditis gingivalis,* or *Tricephalobus gingivalis*) is a free-living and saprophytic rhabdditoid nematode that is a facultative (accidental) and opportunistic parasite primarily affecting horses.[3,16] There have been sporadic case reports of fatal disease in humans[11], rare reports in cattle[5], and a single case report in a zebra[9] with recurrent nematode-induced uveitis and disseminated granulomatous lesions.

Conference participants identified numerous tangential cross sections of larval and adult rhabditid nematodes ranging in size from 10-25 um in diameter within areas of granulomatous and necrotizing inflammation randomly scattered throughout the cerebellum. *H. gingivalis* has a thin smooth cuticle, platymyarian-meromyarian musculature, a pseudocoelom, and a highly characteristic rhabditiform esophagus composed of a corpus, isthmus and bulb.[2,3,6,16,] Additionally, the intestinal tract

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is lined by uninucleate, low cuboidal cells and a single genital tract. Participants also recognized occasional 15-20 um ovoid embryonated eggs adjacent to adults and larvae.[3,6,16] Interestingly, only female adults, larvae, and eggs are identified within tissue sections. Free-living adult males have been found in the environment, but there are no reports of male *H. gingivalis* within host tissue. This likely indicates that *H. gingivalis* adults sexually reproduce in the environment and asexually within hosts.[2,3,6,16] The ability of the female parasite to reproduce parthenogenically within the host likely explains the massive numbers present within most affected tissue. Despite its small size, *H. gingivalis* can produce extensive tissue damage secondary to aggressive migratory behavior, typical of rhabditoid parasites.[2,6,16]

H. gingivalis usually causes severe perivascular granulomatous and eosinophilic inflammation. Organs other than the brain commonly infected by this parasite include the kidneys, lymph nodes, spinal cord, and adrenal glands. Additionally, tissue within the oral and nasal cavity also commonly contain granulomatous inflammatory lesions.[2,3,6,16] Lesions within the heart, liver, stomach, and bones are less common in horses. As mentioned by the contributor, little is known about the pathogenesis and life cycle of this parasite. Oral ingestion, inhalation or wound contaminations have all been proposed as portals of entry. There is also a single case report of probable transmission from a dam to a foal through the colostrum.[2,3,16] After entry, the nematode likely disseminates hematogenously throughout the body causing lesions in several organs. In this case, no additional lesions other than the brain are reported by the contributor. In horses, there are case reports with localized infection of the brain, without any other parasite associated lesions

elsewhere in the body. It is thought that in those cases, the parasite is inhaled into the nasal cavity and enters the brain via penetration through the cribriform plate.[2,3,16]

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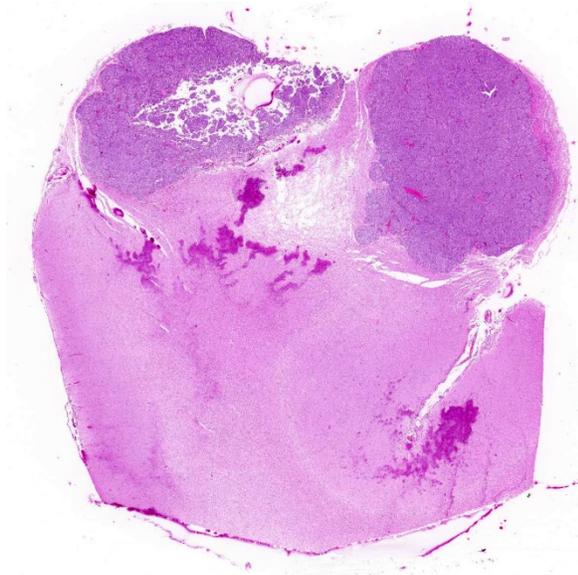
CASE II: 15842#185 (JPC 4086885).

Signalment: Nine-year-old female great Dane, (*Canis familiaris*).

History: The dog presented for clinical evaluation of progressive neurologic disease of two months duration and was diagnosed with canine cognitive dysfunction (CCD). Clinical signs include incoordination, weakness, lethargy, anorexia and locomotion difficulties. The animal was treated with antioxidants, tramadol, and propentophylin (vasodilator). Six months later, the dog's condition deteriorated with the onset of seizures and was euthanized.

Gross Pathology: The dog was in good body condition. There were no significant gross lesions of the thoracic, abdominal viscera, or eyes. In the left brain hemisphere, there was a 2x2.5 cm soft, grayish-tan, fixed mass with a velvet surface that protrudes from the fontal lobe. Following formalin fixation and sectioning, the mass extended 1cm into the gray matter of the brain parenchyma.

Laboratory results: No laboratory analysis performed.

Histopathologic Description: There is focally infiltration of the gray matter by a sharply demarcated but unencapsulated, multinodular, hypercellular mass compose by neoplastic cells arranged in concentric whorls separate into well-demarcated lobules by a delicate fibrous stroma. Neoplastic cells have variably distinct borders with varying amounts of eosinophilic microvacuolar cytoplasm, oval to spindle-shaped vesicular nuclei with finely stippled chromatin and predominantly one large open-faced nucleoli. Mitoses are rare with less than one mitotic figure per high power field. Occasionally necrotic foci

Cerebrum, dog: The meninges are expanded by a large, multilobular neoplasm which compresses the underlying cortex. (HE, 7X)

are present. There is no evidence of vascular invasion.

Brain tissue immediately adjacent to the mass is markedly compressed, and there are few lymphocytes and some pigment (hemosiderin) laden macrophages.

Contributor's Morphologic Diagnosis: Brain: Meningioma, transitional.

Contributor's Comment: The incidence of brain tumors in canine may approach 3.0%. It has been reported that meningiomas are the most common intracranial tumors in dogs, followed by glial tumors (astrocytoma and oligodendroglioma).[9] Meningioma is also the most common type of intracranial tumor in the cat. Meningiomas are rare in cattle and not recorded in horses.[10] Neoplastic cells arise from the cap cells covering the arachnoid, particularly at the point where they project into the venous sinuses[4] and pia mater of the nervous system.[3]

Older dogs may have a predisposition for primary brain tumors. Meningiomas occur in dolichocephalic breeds, especially German shepherds, golden retrievers and Labrador retrievers, with no consistent sex predisposition.[7,9]

Meningiomas may be present especially in the rostral cerebrum for a prolonged period before clinical signs of intracranial disease become obvious to the owner. Further, owners may attribute more subtle signs of forebrain disease (such as pacing or mentation change) to the normal aging process in a geriatric pet.[5] The majority of animals with brain tumors present with a variety of mild or ill-defined neurological signs.

Canine meningiomas clinically display variable behavior that currently makes outcome difficult to predict. The behavior of canine meningiomas varies widely from case to case. Individual cases receiving basic therapies (corticosteroids and supportive therapies, such as anticonvulsant drugs) can have a much better outcome than other individuals that undergo more aggressive therapeutic regimens, such as surgery and post-operative radiation.[1]

Meningiomas usually grow as well-demarcated, often lobulated, firm, granular masses that usually have a broad-based or pedunculated attachment to the overlying meninges. Less commonly they can form plaque-like masses over the meninges.[3,7] In some cases, canine meningioma shows a large cystic cavity or aggregation of severely vacuolated neoplastic cells as a consequence of ischemic events.[7] Meningiomas are gray, sometimes yellow on cut surface, firm, and may be gritty. These tumors grow either by compression or, less commonly, by infiltration of the adjacent brain. In dogs, meningiomas are found commonly in the
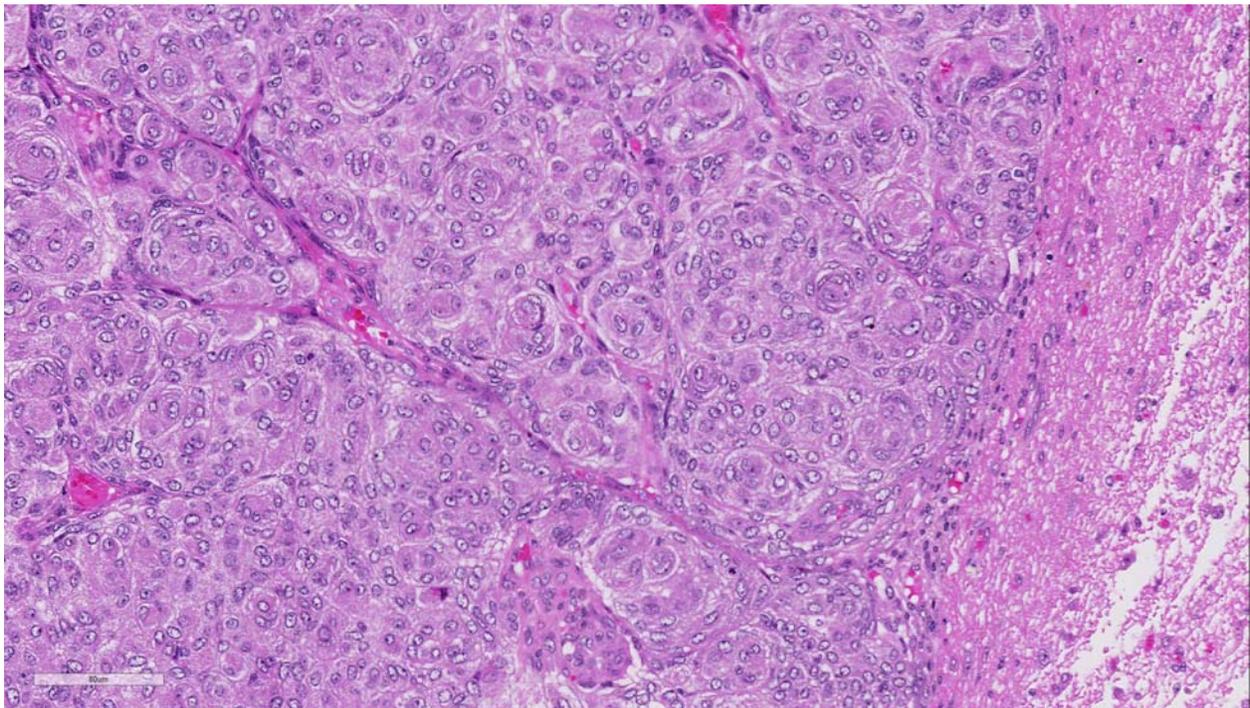
region of the olfactory bulb and frontal lobes, but can occur anywhere over the surface of the cerebral hemispheres. Supratentorial meningiomas are more common over the meninges of the convexities than in basilar sites.[3]

Due to the embryonic origin of the meninges, meningiomas exhibit highly variable morphological and immuno-phenotypic patterns.[7] A classification based mainly on the WHO classification of human histological subtype describes the following histological patterns: meningothelial, fibroblastic, transitional (mixed), psammo-matous, angiomatous, papillary, granular cell, myxoid, and anaplastic. All of these histological variants except anaplastic (malignant) meningiomas have similar biologic behavior. They are slow growing and cause clinical signs by compression of underlying nervous tissue.[5] Areas of chondroid, osseous, myxoid, and xanthomatous-like tissue can be found in the meningothelial and transitional forms.[3]

Most intracranial meningiomas are benign as indicated by low frequency of metastases and failure to invade the brain. By these criteria, ~ 2.5% are malignant and may metastasize. In contrast, extracranial meningiomas, which occur mainly in the paranasal region and orbit, are anaplastic and locally aggressive.[7,9,10]

In some cases, it can be difficult to distinguish meningiomas from other neoplasms, such as astrocytomas, oligo-dendrogliomas, metastatic carcinomas, germ cell tumors, and peripheral nerve sheath tumors. In these cases, a basic immuno-histochemical panel consisting of vimentin, CD34, and E-cadherin has been proposed for the characterisation of canine and feline meningiomas.[7] Meningiomas also stain sparse to moderate positively for cyto-keratin, NSE, and S-100 and negative for synaptophysin and GFAP.[10]

JPC Diagnosis: Brain, cerebrum: Meningioma, transitional, Great Dane, *Canis familiaris*.

Cerebrum, dog: Neoplastic cells are arranged in lobules separated by fibrovascular septa. Neoplastic cells are arranged in nests and whorls. Compressed neuroparenchyma is at right. (HE, 288X)

Conference Comment: The contributor provides an excellent review of the biologic behavior, gross findings and, histopathologic patterns associated with the many different classifications of meningiomas in dogs and cats. Meningiomas arise from the meningo-thelial cells that line the arachnoid villi and cover the central nervous system.[2,3] As mentioned by the contributor, this is the most common type of intracranial tumor in dogs and constitutes between 30-50% of all primary intracranial tumors in this species.[2,3] Additionally, meningiomas account for approximately 60% of all primary intracranial neoplasms in cats. The vast majority of meningiomas occur either within the cranium (82%) or spinal cord (15%).[3] Most intracranial meningiomas occur within the olfactory bulb, but they have also been reported in the cerebral and cerebellar convexity, parasagittal and parasellar area, cerebellopontine angle, and foramen magnum.[2,3] Conversely, only about 2-3% occur in the retrobulbar or periorbital space.[2] Readers are encouraged to review 2015 Wednesday Slide Conference #20 Case 4 for an outstanding example of a periorbital meningioma in a dog.

With the exception of the less common invasive and atypical grade II and malignant and anaplastic grade III types, meningiomas rarely invade into the neuroparenchyma.[2,3] Instead, they will grow expansively within the cranium, as in this case, causing compression necrosis of the neuropil.[2,3,8] Canine meningiomas bear a striking similarity in both histomorphology and biologic behavior to human meningiomas. As a result, there has been widespread application of the World Health Organization (WHO) classification scheme for humans in scientific reports of canine meningiomas.[2-6,8] Conference participants discussed the nine histologic types of meningiomas, including: meningothelial, fibrous (fibroblastic), transitional (as in this case), psammomatous, angiomatous, papillary, granular cell, myxoid, and anaplastic. Other than the anaplastic type, all are slow growing and have a relatively benign biologic behavior.[4,5,8] However, regardless of the histomorphology, in meningiomas in domestic animals, malignancy is still based on mitotic rate, cellularity, growth pattern, and tumor invasion into the underlying nervous tissue.[2-5,8]

Atypical, choroid, and clear cell types are subcategories of grade II meningiomas. Grade II meningiomas require 4 mitoses/ 10 high powered fields and at least three of the following features: loss of architectural pattern replaced by sheets of cells, small cell formation with a high nuclear: cytoplasmic ratio, nuclear atypia or macronuclei, hypercellularity, and spontaneous necrosis.[2,3] The clear cell category is characterized by sheets of epithelial cells with abundant PAS positive cytoplasm, indicating glycogen accumulation. Grade III malignant meningiomas are comprised of papillary, anaplastic, and and rhomboid types and are characterized by extreme cellular anaplasia, >20 mitoses/10 high powered fields, necrosis, and neuroinvasion.[3]

Conference participants discussed doublecortin (DCX), Ki-67, E-cadherin, N-cadherin, and beta-catenin as potential immunohistochemical (IHC) stains that would help differentiate malignant from benign meningiomas. A relatively recent study in *Veterinary Pathology* used the previously mentioned IHC stains to predict the biologic behavior of meningiomas.[4] The authors found that doublecortin (DCX), which is important in neuroblast migration during development, is highly expressed in

invasive brain tumors, including malignant meningiomas. Additionally, Ki-67 staining, a standard marker of cell proliferation, is significantly higher in anaplastic meningiomas compared to benign varieties and is widely used to evaluate tumor grade and malignancy in humans.[4] The authors also found that there is positive correlation between DCX and N-cadherin expression and conversely a negative correlation between E-cadherin and N-cadherin expression. Additionally decreased E-cadherin expression is associated with increased nuclear beta-catenin expression suggesting that decreased E-cadherin, increased N-cadherin, DCX expression, and nuclear beta-catenin staining are all associated with malignant meningiomas.[4,8]

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CASE III: 16N74 (JPC 4085376).

Signalment: 15-month-old castrated male Nelore calf, (*Bos taurus indicus*).

History: This calf was part of a herd of 70 calves of about the same age that was shipped from another farm six months ago. The owner reported that the onset of neurological cases occurred one month after the calves' arrival on the farm. During a five-month period, three more calves (including the one of this report) died. The calf of this report presented for neurological disease with a 5-day fatal clinical course. Clinical signs observed were fever (41°C), apathy, blindness, drooling, grinding of the teeth, locked jaw, and flaccidity of the tongue.

Gross Pathology: Significant gross findings were restricted to the brain. In the cortex of the parietal and frontal lobes of the telencephalic hemispheres, there were bilaterally symmetrical depressed, red and soft areas and extensive areas of severe leptomeningeal hemorrhages (Fig.1). On cut section, the frontal cerebral cortex contained extensive, dark brown and granular areas of hemorrhage and malacia (Fig. 2).

Laboratory results: None available.

Histopathologic Description: Main lesions affected the frontal, temporal, and parietal cortex, the last of which is represented in the submitted slides. In these sites, there are intense leptomeningeal mononuclear infiltrates and multifocal areas with loss of neuropil and cavitation, with accumulation of large numbers of foamy macrophages (gitter cells). Segments of marked submeningeal hemorrhages are observed above these necrotic areas. In adjacent

areas, the more acutely affected neuropil contained extensive areas of edema with astrogliosis and segmental areas of laminar neuronal necrosis where neurons exhibit hypereosinophilic and shrunken cytoplasm and pyknotic nuclei. Multifocally through-out the adjacent neuropil and neuro-parenchyma, blood vessels have prominent endothelial cells, and perivascular spaces had cuffs (up to 5-cell thick) of lymphocytes and plasma cells that extend to the leptomeningeal vessels. The neuropil underlying the leptomeninges is partially collapsed and contain areas of fragmentation with accumulation of large numbers gitter cells. Large numbers of gitter cells are also seen in the perivascular space (interpreted that these cells are probably migrating from the necrotic areas into perivascular spaces to be reabsorbed then into the general circulation). Multifocal areas of edema eventually coalesce to dissect the cortical grey matter (spongiosis), sometimes in a laminar pattern. Occasional astrocytes contain large intranuclear amphophilic viral inclusions that pushed the chromatin peripherally. The neuropil within the necrotic areas is sprinkled with basophilic, granular chromatin-like material. Multifocal narrow ring hemorrhages are seen perivascularly

Cerebrum, calf. The parietal and frontal lobes are bilaterally depressed with extensive leptomeningeal hemorrhage. (Photo courtesy of: Laboratory of Anatomic Pathology, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil.
https://www.ufms.br/)

and extend into the neuropil. The subcortical white matter is loose due to edema and also diffusely infiltrated by gitter cells. Similar perivascular cuffing is present in both gray and white matter. Perivascular cuffing is also present in the occipital cortex, mesencephalon, pons, medulla and cerebellum.
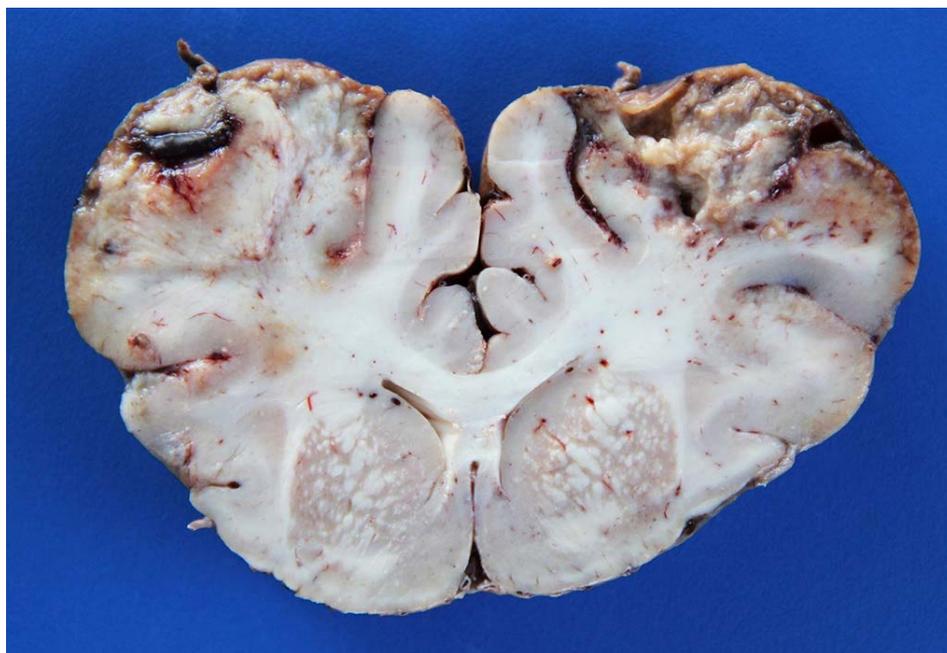
Contributor's Morphologic Diagnosis:
Brain, necrotizing meningoencephalitis, focally extensive, acute to subacute, severe, with intranuclear inclusion bodies in astrocytes.

Contributor's Comment: The gross and microscopic findings reported in this calf are typical of those observed in cases of meningoencephalitis due to either bovine herpesvirus (BoHV) either by type 1 virus
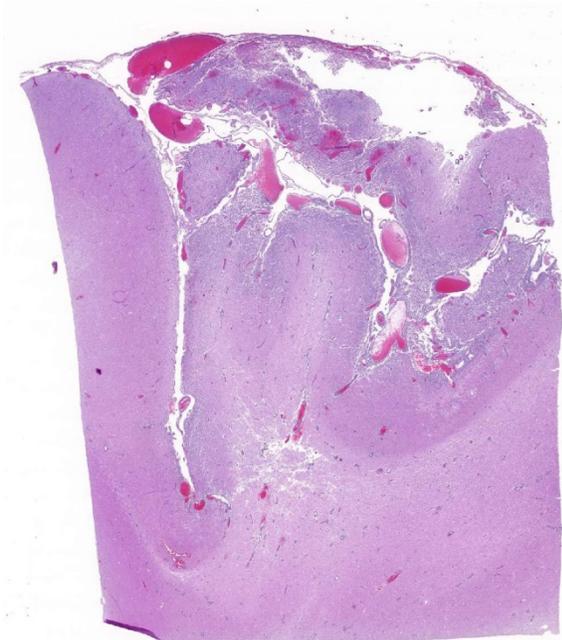

Cerebrum, calf. On cut surface, the frontal cerebral cortex contained extensive, dark brown and granular areas of hemorrhage and malacia. (Photo courtesy of: Laboratory of Anatomic Pathology, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil. *https://www.ufms.br/)*

(BoHV-1) or type 5 (BoHV-5).[5,6,7] The history, clinical signs, and characteristic lesions in the brain of this calf warrant a presumptive diagnosis of necrotizing

meningoencephalitis by BoHV-1 or BVH-5. This diagnosis was further supported by the finding of characteristic intranuclear inclusion bodies in astrocytes.[7] The occurrence of intranuclear astrocytic or neuronal viral inclusions may vary among cases, but when present they are important in the presumptive diagnosis of bovine herpes viral infection.[7]

Meningoencephalitis due to BoHV-1 or BoHV-5 shares many similarities regarding epidemiological and clinicopathological findings, and the diagnostic confirmation should be performed using FAT, viral isolation.[8,9] However, the differentiation between neurological infection caused specifically by BoHV-1 or BoHV-5 can only be achieved through the use of molecular ancillary tests such as the glycoprotein C–based PCR.[8,9] Results obtained from studies using this latter method of differentiation in south Brazil showed that BoHV-1 and BoHV-5 may be interchangeably involved in very similar clinicopathological conditions that were previously attributed to a single strain (either BoHV-1 or BoHV-5).[8,9] These tests are used only for research purposes in our diagnostic routine, and the con- firmation of bovine herpesviral infection by either FAT or viral isolation is usually sufficient for confirmation of herpesviral

Cerebrum, calf. There is extensive cavitation and hypercellularity of the submeningeal cortex. (HE, 5X)

infection (but not viral typification) in the diagnostic routine service. In the present case, none of these ancillary tests were available.
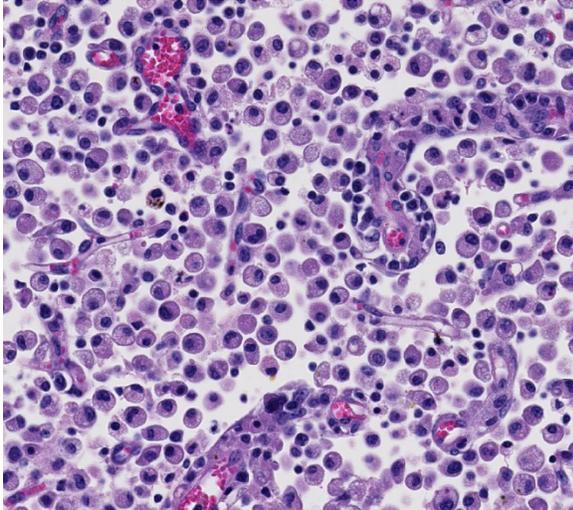
The clinical course in cases of meningoencephalitis due to BoHV varies from 1 to 15 days, and affected cattle may develop a wide range of clinical signs such as severe depression, serous ocular and nasal discharge, grinding teeth, circling, blindness, incoordination, head pressing, nystagmus, recumbence, paddling, opistho-tonus, and seizures.[5-8]

Based on the geographical location and gross findings in this calf, the main differential diagnoses at the time of necropsy included infection by bovine herpesvirus (BoHV-1 or BoHV-5)

meningoencephalitis, polioencephalomalacia (PEM), and rabies.[7] Severe cases of PEM may rarely present with gross lesions that resemble those described for this calf. Gross findings in cases of bovine rabies are usually absent.[5-7]

Necrotizing meningoencephalitis due to BoHV affects mainly calves (as is the case on this report) and young adults submitted to environmental or management-related stressors, including weaning, high concentration of cattle, transportation, and introduction of new animals into the herd.[6-7] The stressors conditions precipitating the disease in the present case were not identified. However, the calf has been transported some months before the onset of clinical disease, which most likely provided close contact with other animals and might have facilitated infection or reactivation of a latent herpesviral infection.[3,4]

BoHV-1 and BoHV-5 are genetically and antigenically related viruses that belong to the family Herpesviridae, subfamily Alpha-herpesvirinae, genus *Varicellovirus*.[2] BoHV-1 has been historically associated with abortion, respiratory, and genital disease, namely infectious bovine rhino-tracheitis and infectious pustular vulvovaginitis or balanoposthitis. Although BoHV-5 has been classically associated with neurological disease in cattle, it is currently believed that both BoHV-1 and BoHV-5 cause identical neurological disease in endemic areas and cases of meningoencephalitis caused by BoHV-1 are not as uncommon as previously suspected.[7,9]

Cerebrum, calf. Areas of cavitation are populated by innumerable Gitter cells (transformed microglial which clean up myelin debris) (HE, 288X)
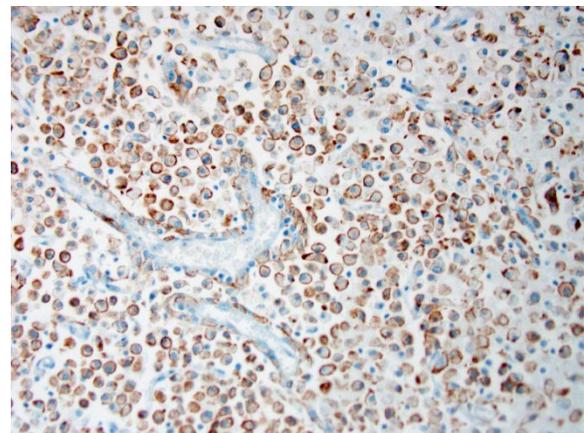
Necrotizing meningoencephalitis due to BoHV has been described worldwide, particularly in South America.[4,5,9] The reason for the lower incidence of meningoencephalitis due to BoHV in other parts of the world is unknown, but it has been proposed that widespread vaccination against BoHV in North America and Europe protects susceptible animals and prevents clinical disease in these areas.[10]

Viral transmission occurs via direct or indirect contact among susceptible individuals, with primary viral replication occurring in the ocular and oropharyngeal mucosal epithelium. Following primary replication, viral particles reach the rostral portions of the brain and sensory ganglia via axonal retrograde transportation and direct invasion through the olfactory bulb and trigeminal nerves.[2]

Viral invasion into the brain may result in secondary replication and neurological disease or subclinical infection and viral latency in the trigeminal ganglia and central nervous system.[3] Latently infected individuals will become an important

source of virus to other susceptible cattle in the case of virus reactivation.[3,4] A hematogenous route of infection has been proposed, but it seems less likely due to the characteristic distribution of the lesions in the frontal areas of the brain, which support direct viral invasion through the olfactory bulb.[3,7]

The gross and microscopic findings reported in this calf are typical of those observed in cases of meningoencephalitis due to either BoHV-1 or BoHV-5. In addition, cases of neurological disease by BoHV with neither gross nor microscopic lesions despite the development of severe neurological disease have been observed.[7,8] These variations in the pathological

Cerebrum, calf. Gitter cells exhibit strong cytoplasmic reactivity for IBA-1 (a macrophage marker). The cytoplasm is diminished by the presence of numerous phagosomes containing myelin debris. (anti-IBA-1, 200X)

presentation of neurological disease due to BoHV are attributed to potential differences in the neurovirulence or to individual susceptibility of animals to viral infection.[1,8]
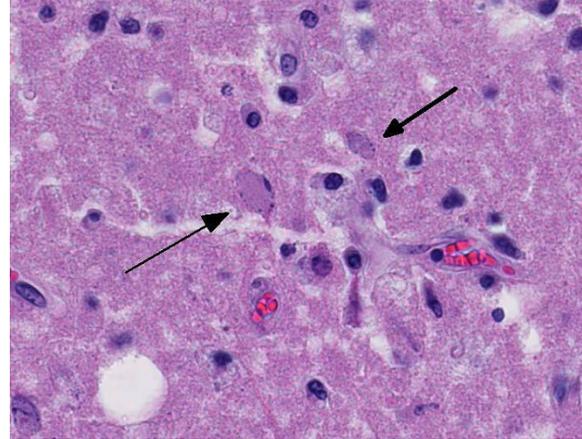
JPC Diagnosis: Cerebrum, telencephalon: Meningoencephalitis, necrotizing, multifocal to coalescing, severe, with marked gliosis, multifocal vasculitis and rare intraglial

intranuclear viral inclusion bodies, Nelore calf, *Bos taurus indicus*.

Conference Comment: We thank the contributor for the thorough review of the epidemiology, clinical presentation, gross and histologic lesions, and pathogenesis associated with neurotropic bovine herpesvirus (BoHV) infection in a calf. In addition, the excellent quality gross photographs provided by the contributor demonstrate the typical lesions of bilaterally symmetrical severe multifocal to coalescing encephalomalacia and hemorrhage affecting the rostral cerebrum. As mentioned above, both BoHV-1 and 5 are antigenically related members of the *Alphaherpesvirinae* subfamily and are part of genus *Varicellovirus*. Additionally, they both infect epithelial cells at the portal of entry and establish latent infection in the sensory trigeminal ganglia.[2,3,6,12,15] This is a relatively uncommon disease that primarily affects young and immunosuppressed cattle. Severe clinical illness is most frequently reported in South American, with a high prevalence in Brazil and Argentina.[2] Although conference participants were not given the country of origin of this animal before the conference (Brazil), most identified rare intranuclear inclusion bodies within degenerate neurons and astrocytes, highly characteristic of BoHV infection.[2]

Attendees also described numerous microglial phagocytic cells with an abundant amount of foamy eosinophilic cytoplasm (gitter cells) infiltrating into the necrotic areas. Gitter cells are microglial cells that transformed into phagocytic macrophages within the central nervous system and function to engulf cellular debris and degenerate myelin.[2] Microglial phagocytes also surround degenerate neurons (satellitosis) and phagocytose degenerate neuronal cell bodies (neuronophagia) during neurotropic viral infection. In severe lesions, as in this case, gitter cells can arise from peripheral blood monocytes as well as microglial cells.[2] These cells are nicely highlighted by intense membranous and

Cerebrum, calf. Astrocyte nuclei within the adjacent gray matter are expanded by amphophilic intranuclear viral inclusions.

cytoplasmic immunoreactivity for ionized calcium binding adapter molecule 1 (IBA-1), an immunohistochemical stain commonly used to identify microglial cells and macrophages run by the Joint Pathology Center prior to the conference.

Conference participants discussed differential diagnoses for laminar cortical necrosis and polioencephalomalacia in cattle. These include sulfur intoxication, sodium chloride intoxication/water depri-vation, lead intoxication, and thiamine deficiency. Participants also discussed other infectious diseases that can cause neurologic disease in cattle, such as rabies virus, malignant catarrhal fever (MCF), listeriosis, and the neurotropic amoeba, *Naegleria fowleri*.[2,8,9,15]

The conference moderator noted that this case has excellent examples of ferruginated neurons. These are deeply basophilic dead neurons that become encrusted and replaced with finely beaded material interpreted as

mineral, rather than being removed by phagocytosis. The mineral is composed of both calcium and iron and affected neurons are highlighted by both von Kossa and *Perls' Prussian blue* staining techniques. These neurons are reported to occur in areas of ischemic and hypoxic damage in the central nervous system, particularly in juveniles and neonates.[5]

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CASE IV: A00629 (JPC 4066658).

Signalment: Four-month-old male Labrador retriever, (*Canis familiaris*).

History: A 3-month-old Labrador retriever pup developed progressively worsening tetraparesis with a spastic swimming-puppy-like position of the thoracic limbs and a flattened chest. One month later mild vestibular signs and myoclonic jerks in the head and cervical region became obvious. General clinical examination was within normal limits. Neurological examination revealed absent patellar reflexes, weakness on the 4 limbs with an abnormal spasticity of the thoracic limbs and mild generalized muscle atrophy. During the second visit a vestibular strabismus in the right eye, a mild right-sided head tilt and regular myoclonic jerks at the head and thoracic limbs were noticed.

Electrophysiological examination was normal. RX of the thorax only confirmed the dorsoventral flattening of the thorax. Due to the worsening neurological signs further examinations were declined by the owner and the pup was euthanized at the age of 4.5 months.

Gross Pathology: Except for the dorso-ventral flattening of the thorax, no gross abnormalities were noted at necropsy.

Laboratory results: Blood examination and cerebrospinal fluid analysis were within normal limits.

Histopathologic Description: Cerebrum: Within the white and gray matter of the brain, some blood vessels are surrounded by numerous short, perpendicularly oriented, hypereosinophilic, amorphous intraastrocytic accumulations, varying in diameter from 4 to 20 μm (Rosenthal fibers). These Rosenthal fibers are also found in the astrocytic endfeet in the subpial tissue and to a lesser extend throughout the parenchyma. Mainly in the white matter, there is proliferation of abnormal astrocytes with large nuclei, prominent nucleoli and glassy eosinophilic to pale cytoplasm. Occasionally, there are binucleated astrocytes.

GFAP-staining: All Rosenthal fibers are strongly immunopositive for GFAP.

Contributor's Morphologic Diagnosis: Cerebrum, gray and white matter, encephalopathy, multifocal, chronic,
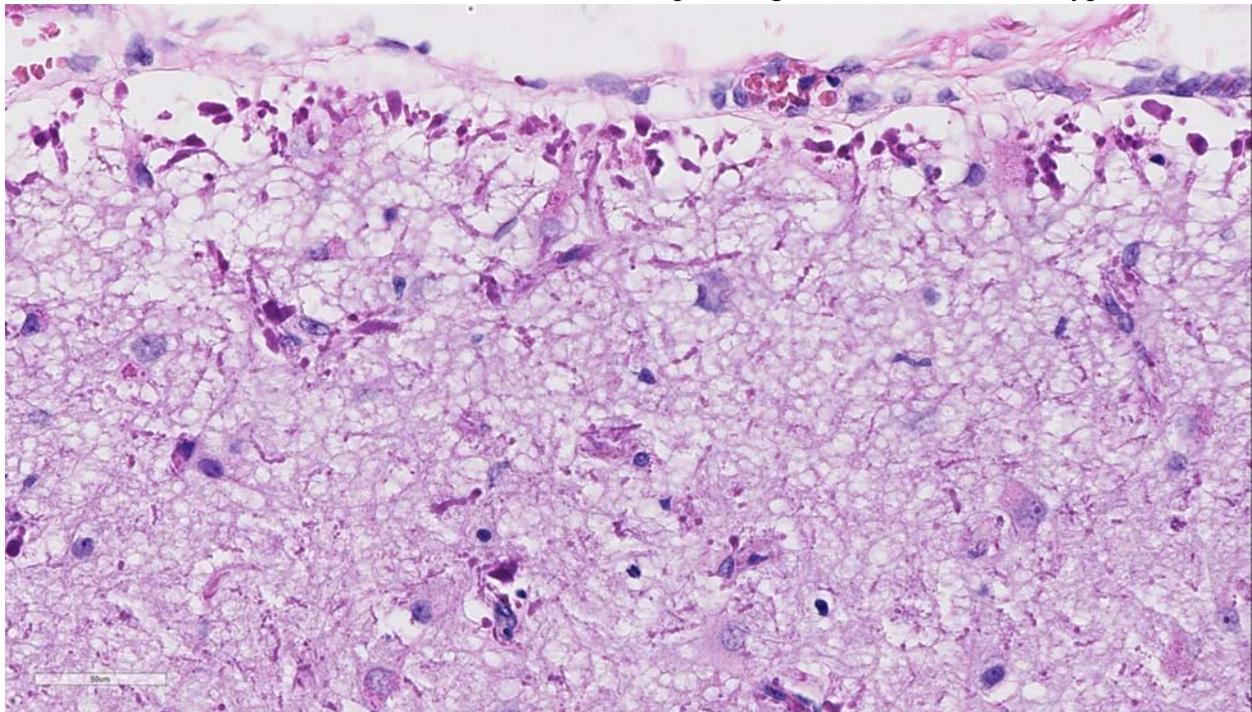

Cerebrum, puppy. A section of cerebrum and ventricle is presented for examination. (HE, 5X)

moderate, perivascular and subpial accumulation of Rosenthal fibers, astrocytosis and astrocytic hypertrophy.

Contributor's Comment: In humans, Rosenthal fibers are found in Alexander disease (AxD), and, albeit in greatly reduced numbers in chronic reactive astrocytosis and low-grade astrocytomas.[4,5] They are seldom encountered in animal neuropathology. Alexander disease, or fibrinoid leukodystrophy, is a rare neurodegenerative disorder of astrocyte dysfunction in human. In veterinary medicine, Alexander disease is very rare and has been reported in a few dogs (two Labrador Retrievers, one Scottish Terrier dog, one Miniature Poodle, three Bernese Mountain dogs, one Bernese Mountain cross breed, one French bulldog, and one Chihuahua) and four sheep (one white Alpine sheep and three Merino sheep).[1-7]

There are no specific gross lesions of AxD. The classic histological lesions are Rosenthal fibers. These fibers are deeply eosinophilic, irregularly shaped, elongated, round to oval intra-astrocytic aggregates. Rosenthal fibers have been shown to be ubiquinated aggregates of GFAP, αβ-crystalin and HSP27.[3,4]

In humans AxD is classified based on the age of onset as infantile, juvenile and adult. Recently, Prust *et al.* proposed a reclassification in two age-dependent clinical subtypes: type I, characterized by an early age of onset, seizures, macrocephaly, encephalopathy, developmental delay, paroxysmal deterioration, failure to thrive and typical MRI features, and type II, characterized by a later age of onset, autonomic dysfunction, bulbar symptoms, ocular movement abnormalities and atypical MRI features.[5] The characteristic pathological feature of both types of AxD

Cerebrum, puppy. Astrocytic endfeet within the subpial cortex and projecting onto superficial cortical vessels are swollen and brightly eosinophilic (Rosenthal fibers). Additionally, astrocytes are hypertrophic and often contain brightly eosinophilic granules within their cytoplasm. (HE, 400X)

are widespread and abundant Rosenthal fibers.

All known genetic causes of AxD are attributed to GFAP mutations (explaining more than 95% of the cases), mostly *de novo* dominant missense mutations with hotspots at R79 and R239, the latter one inducing the most aggressive form.[1]

JPC Diagnosis: Cerebrum: Astroglial dystrophy, diffuse, severe, with marked subpial, subependymal, and perivascular Rosenthal fiber formation, Labrador retriever, *Canis familiaris*.

Conference Comment: The contributor provides a concise review of Alexander disease (AxD), a rare neurodegenerative disorder previously reported in dogs, sheep, and humans.[1-7] Conference participants identified the large brightly eosinophilic and irregularly shaped Rosenthal fibers (RF) with astrocytes scattered throughout the white matter and aggregated in the subpial, subependymal, and perivascular spaces. This is the classic histologic lesion distribution associated with previously reported cases of AxD in all reported species.[1,7] As mentioned by the contributor, these accumulated fibers consist of large aggregates of glial fibrillary acidic protein (GFAP), αB-crystallin, heat shock protein (hsp-27) and ubiquitin, within markedly expanded astrocytic processes distributed throughout the central nervous system.[1,4,7] Prior to the conference, the Joint Pathology Center ran a GFAP immunohistochemical stain which demonstrated intense immuno-staining of the RF surrounding vessels and in the subpial and subependymal areas. RF have also been reported to be immuno-positive for ubiquitin. The presence of RF is not pathognomonic for AxD and has been reported in glial scars and multiple sclerosis in humans; however, the distribution of the RF in the subpial, subependymal, and perivascular areas in this and other reported cases is unique to AxD. In addition to Rs, other common histologic lesions of AxD include white matter demyelination and astrogliosis.[1,7]

As mentioned by the contributor, it is thought that a mutation in GFAP leads to glial intermediate filament disorganization, decreased solubility, and defective degradation of the protein.[1,3,7] This results in accumulation of the aberrant and misfolded protein, leading to cellular stress and the unfolded protein response (UPR) in the endoplasmic reticulum. Cellular stress and the resulting UPR are postulated to be the initiating factor for the production of ubiquitin and heat shock proteins (αB-crystallin, hsp-27) accumulating with GFAP and forming the RFs seen histologically.[1,4,7] Accumulation of these insoluble fibers is likely progressively toxic to astrocytes and degrades oligodendrocyte function, affecting myelin formation in the white matter. As a result, animals affected with AxD typically present as juveniles with rapidly progressive depression, ataxia, paresis, generalized tremors, decreased spinal reflexes, and seizures.[1]

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