WEDNESDAY SLIDE CONFERENCE 2013-2014

Conference 9

04 December 2013

CASE I: TVMDL 2012-02 (JPC 4018680).

**Signalment:** Six-month-old female Meerkat (*Suricata suricatta*).

History: The animal presented with one week history of visual impairment, intention tremor, head tilt, circling to right, absent menace reflex, and lack of proprioception of the pelvic limbs. The animal was treated with clindamycin, dexamethasone, and vitamin B with no clinical improvement. Euthanasia was opted due to poor prognosis.

Gross Pathology: None reported.

Histopathologic Description: The submitted slides contain sections of either the left or right cerebellar hemisphere, along with part of the vermis, and the underlying segment of the medulla. The main histologic lesions affected the folia of both hemispheres and the cerebellar vermis. These were characterized by areas of normal-appearing cerebellar folia that abruptly presented extensive and segmental Purkinje cell depletion. Affected areas were characterized by loss of neuronal bodies (empty baskets) that were surrounded by a proliferation of large numbers of astrocytes (Bergmann astrocytes). Cerebellar nuclei, the molecular layer, and the white matter

presented variable gliosis with occasional vacuolation of the latter. These were the main features in the submitted sections. Additional changes affecting Purkinje cells that are variably present in the slides include shrunken and hypereosinophilic cells showing an occasionally vacuolated perikaryon. A few cells had a swollen cytoplasm with dispersion of the Nissl bodies and displacement of the nucleus to the periphery (central chromatolysis). The sections of cerebellum of this meerkat were compared with an age-matched control that died of an unrelated cause. An apparent increase in the number of granular neurons was noticed in this animal, which also presented markedly increased numbers of astrocyte cell processes demonstrated with GFAP immunostaining. Astrocytic processes extended into both the molecular and granular layers. In the former, the processes presented a vague segmental distribution that corresponded to areas lacking Purkinje cells. GFAP also demonstrated marked astrocytosis and astrogliosis in the cerebellar nuclei.

Contributor's Morphologic Diagnosis: Cerebellum: Multifocal, marked, Purkinje cell degeneration and loss with vacuolation, chromatolysis, astrocytosis, and astrogliosis.
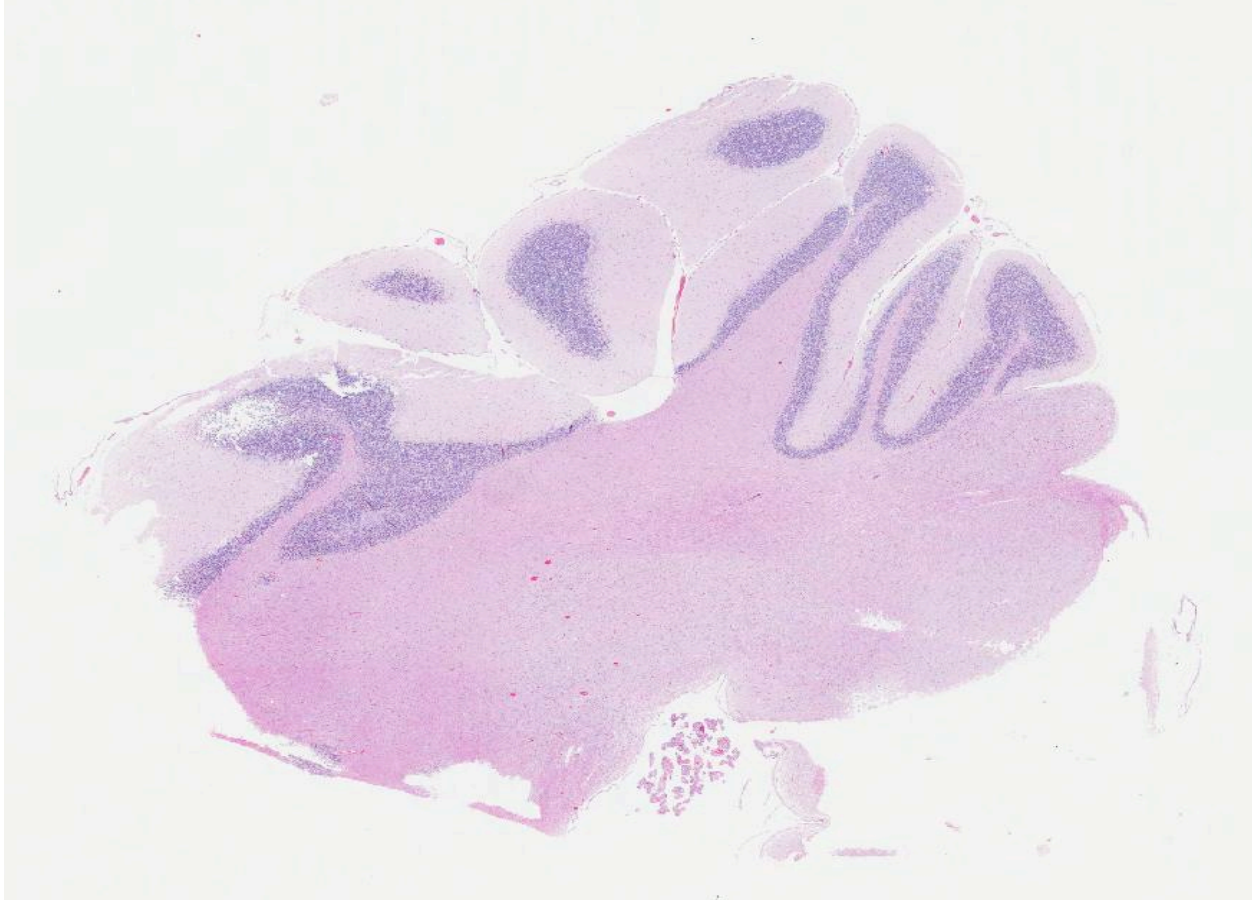
1-1. Cerebellum, meerkat: The cerebellar folia are diffusely flattened and the granular layer appears hypocellular. (HE 0.63X)

Contributor's Comment: The clinical history and histopathologic findings in the cerebellum of this meerkat are compatible with cerebellar abiotrophy. This term, which literally means lack of a life-sustaining nutritive factor, is used to denote diseases in veterinary neuropathology that share clinicopathological features with these seen in this case.[10] This condition has been described in several breeds of dogs, in horses, bovine, sheep, pigs, cats, in rabbits, and in an alpaca.[5,6,7,8,10] Reports of neurologic disease in meerkats are rare and confined to a case series of cholesterol granulomas and disseminated toxoplasmosis.[9,10] To our knowledge, cases of abiotrophy have not been reported in this species.

As the term abiotrophy implies, the microscopic lesions are not considered the result of an acquired insult (e.g. infectious disease or intoxication), but rather is the consequence of an intrinsic metabolic disorder with a suspected hereditary basis of transmission. Besides this animal, two other meerkats from the same zoo (three and six-months of age) presented with similar clinicopathologic findings suggesting an inherited disease. These animals belong to a small colony, in which inbreeding is very common. No histologic evidence of an infectious disease was detected in the examined sections of all three animals.

Abiotrophy is characterized by the spontaneous degeneration and loss of neurons prematurely, and it is viewed as affecting the organ after it has developed its full cellular component.[2,10] This differs from hypoplasia, in which the cerebellum fails to form completely during development as the result of infectious diseases (e.g. feline panleukopenia, bovine viral diarrhea, classical swine fever), toxicities (e.g. organophosphate trichlorfon in piglets), and malnutrition (e.g. hypocuprosis in goat kids and lambs).[5]

Most commonly, animals with abiotrophy are neurologically normal at birth but will progressively develop cerebellar deficits in the
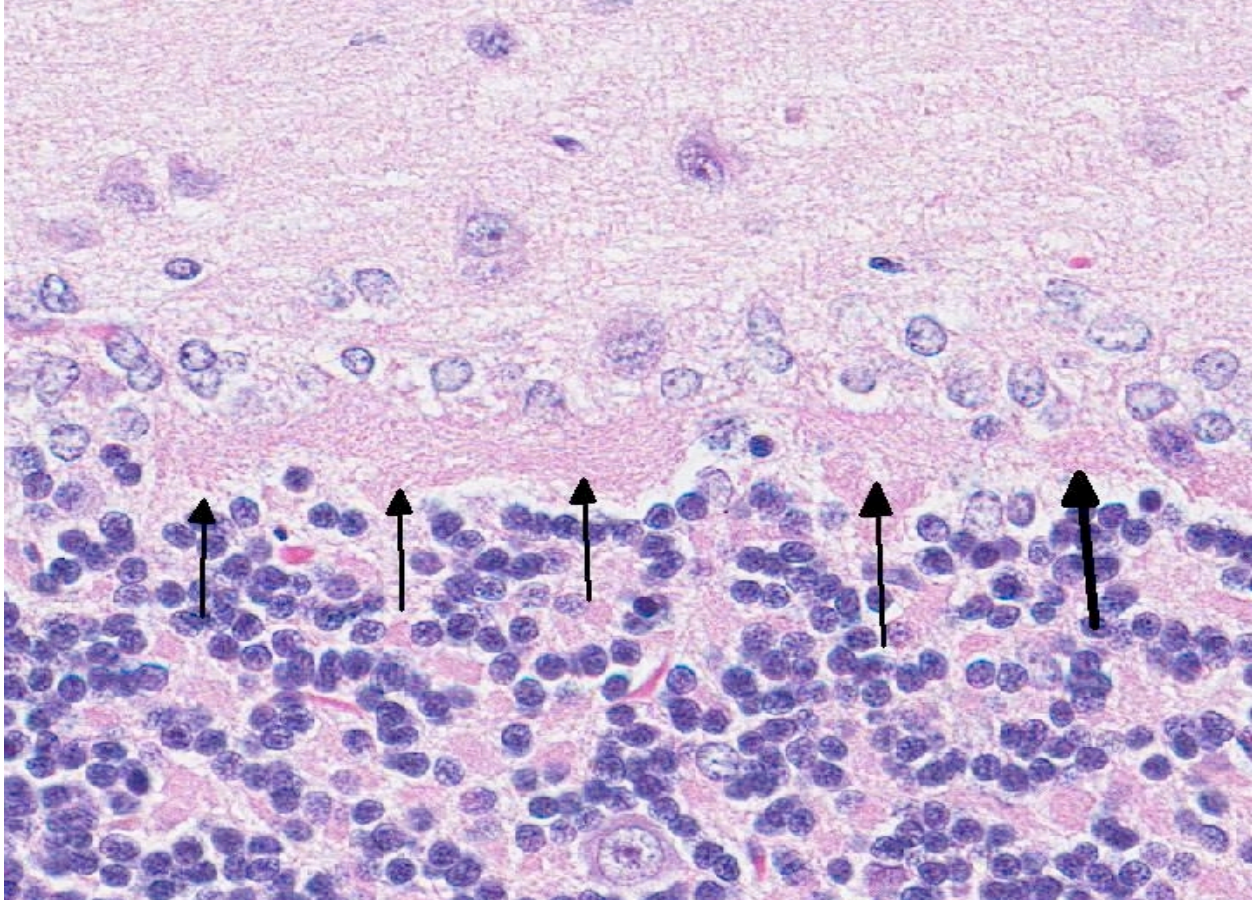
1-2. Cerebellum, meerkat: There is segmental and extensive loss of Purkinje cells, with "empty baskets" formed by the filling in of spaces previously occupied by Purkinje cells with a dense mat of astroglial fibers (arrows). Above the empty baskets are numerous large Bergmann astrocytes. (HE 192X)

postnatal period. However, some animal species may present a neonatal syndrome in which clinical signs are manifested in the immediate postnatal period (bovine and ovine) or can be delayed until time of ambulation (dog).[2,6] In postnatal syndromes, the onset and progression of clinical signs varies from a few days to months with a static course or slow progression.[3] Cerebellar ataxia, head tremor, truncal ataxia, symmetrical hypermetria, spacity, broad-based stance, and loss of balance are the most commonly described clinical manifestations in animals with cerebellar abiotrophy.[3] Besides visual and impaired proprioceptive positioning that were described by the field veterinarian, all the clinical signs evident in this meerkat are compatible with cerebellar disease.[3]

Grossly, the cerebellum can be normal or smaller, which is usually seen later in the course of the disease.[10] In animals that present gross changes of abiotrophy, cerebellar shrinkage can be noticeable with failure to fill the caudal part of the cranial vault, as well as with diminution of individual cerebellar folia, and broadening of sulci.[10] The involvement of the cerebellum is usually not uniform.[10] The cerebellum of the animal of this case was grossly unremarkable.

Microscopically, the distribution and characteristics of lesions vary depending on the species and breed of animals affected, but include: degeneration and loss of Purkinje cells, swelling of Purkinje cell axons, astrogliosis, gliosis of cerebellar nuclei, Wallerian degeneration of the white matter of the folia, and spheroids.[6,10] Proliferation of Bergmann astrocytes is seen in folia where significant Purkinje cell loss has occurred.[10] Because the integrity of the granule cell neuron is dependent on its synaptic relationship with the dendritic zone of the Purkinje neuron, loss of the latter is followed by reduction of the granule cell neurons.[2] The animal in this case presented an apparent increase in the number of granular neurons that was evident when compared with the cerebellum of

the age-matched control. The cause for this finding is undetermined. However, the other two meerkats that were diagnosed with cerebellar abiotrophy presented a decreased cellularity of the granular cell layer when compared with the control. Massive loss of Purkinje cells, which is accompanied by gliosis in the molecular layer, and atrophy of both molecular and granular layers are also features of poisoning in livestock that ingest several species of plants of the genus *Solanum*.[6] The consistency in the age of onset of clinical signs in these meerkats supported the diagnosis of abiotrophy. Extracerebellar lesions of abiotrophy have been described in the cerebellar cortex in the miniature Poodle, spinal Wallerian degeneration in rough-coated and Border Collies, and in Merino sheep.[6] The other sections of the CNS of all three meerkats were histologically normal.

JPC Diagnosis: Cerebellum: Purkinje cell loss, segmental, moderate, with Bergmann's astrocytosis.

Conference Comment: At the start of the conference, the moderator pointed out that most histological findings within the nervous system are, in reality, artifact. He cautioned participants that Purkinje cell degeneration, necrosis and chromatolysis are challenging to definitively identify, as Cytoplasmic darkening, unevenly dispersed Nissl substance and vacuolation are common artifacts in Purkinje cells. Participants briefly discussed the difficulty in differentiating normal gaps in Purkinje cells, which often exhibit irregular spacing, from the true loss observed in cerebellar abiotrophy. A key feature is the presence of increased numbers of Bergmann's astrocytes surrounding empty spaces where Purkinje cells are lost ("empty baskets"). Cerebellar astrocytes are classified broadly as bushy/velate protoplasmic (granular layer), smooth protoplasmic (granular and molecular layers) and Bergmann glial cells.[11] Bergmann glial cells are unipolar protoplasmic astrocytes located around Purkinje cells with long radial processes that enfold the synapses on Purkinje cell dendrites and traverse the molecular layer, terminating on the pial surface; their differentiation, migration and maturation is closely linked with that of the nearby Purkinje cells.[11] Immunohistochemical staining, specifically GFAP, is useful in demonstrating the "empty baskets" surrounded by Bergmann's gliosis that are often evident in cases of cerebellar abiotrophy.[5,11] Conference participants also debated the presence of decreased cellularity of the granular cell layer of the cerebellum, however they were subsequently informed that the contributor actually noted an apparent increase in the number of granular neurons when compared with the cerebellum of an age-matched meerkat control. This is an unexpected finding, as neurons of the granular cell layer are generally lost following the Purkinje cell degeneration and necrosis that characterizes cerebellar abiotrophy.

The contributor provides an excellent summary of cerebellar abiotrophy in various species of veterinary interest. Ruleouts for meerkat cerebellar abiotrophy include cerebellar hypoplasia due to in-utero/perinatal viral infection or toxin ingestion, neuroaxonal dystrophy and lysosomal storage diseases. Feline parvovirus, bovine pestivirus and ovine pestivirus have been shown to cause necrosis of the granular cell layer with resultant cerebellar hypoplasia in kittens, calves and lambs, respectively;[5] however, these viruses are not reported in meerkats. Additionally, when endogenous or exogenous factors such as infectious agents or toxins result in damage to fetal cerebellar components, the animal is typically affected at birth. On the other hand, with cerebellar abiotrophy the animal usually has normal cerebellar components at birth, but is subject to early-onset, hereditary, progressive cerebellar degeneration postnatally,[8] although as noted by the contributor there are exceptions to this generalization. Neuroaxonal dystrophy, reported in dogs, cats, horses and sheep, is a degenerative condition that occasionally affects the cerebellum and is characterized by nerve fiber degeneration and formation of large spheroids.[1,8] Lysosomal storage diseases occur when a lack of specific lysosomal enzymes causes various materials to accumulate in nerve cells and macrophages.[8] Neuroaxonal dystrophy and lysosomal storage diseases have not been reported in meerkats.

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CASE II: B10-1823-2L (JPC 4032319).

Signalment: 11-year-old male castrated Saint Bernard (*Canis lupus familiaris*).

History: The dog failed to recover normally following general anesthesia for a dental procedure.   Upon presentation to an overnight care facility, he was non-ambulatory, minimally responsive, and sedate.   Overnight, he had a generalized tonic/clonic seizure and developed anisocoria. By morning, he remained stuporous and unable to rise, but was transiently able to lift his head. Neurologic examination findings were consistent with lesions in the cerebrum and brainstem.   MRI and CSF analysis were not performed due to anesthetic concerns.   Previous bloodwork revealed severe hypothyroidism.

After weeks of supportive care and physical therapy, the dog was able to eat on his own. He continued to improve at home, though he lacked a menace response in the left eye and intermittently circled to the left. After five months, his neurologic status rapidly deteriorated, with incessant circling, hypersalivation, and multiple seizures.   The owners elected euthanasia and histopathologic evaluation of the brain.

Gross Pathology: There was marked atrophy of the entire left cerebral hemisphere, with the rostral portion most severely affected.   Blood vessels along the ventral surface of the left side of the brain were markedly thickened, white-tan, and gritty upon sectioning.
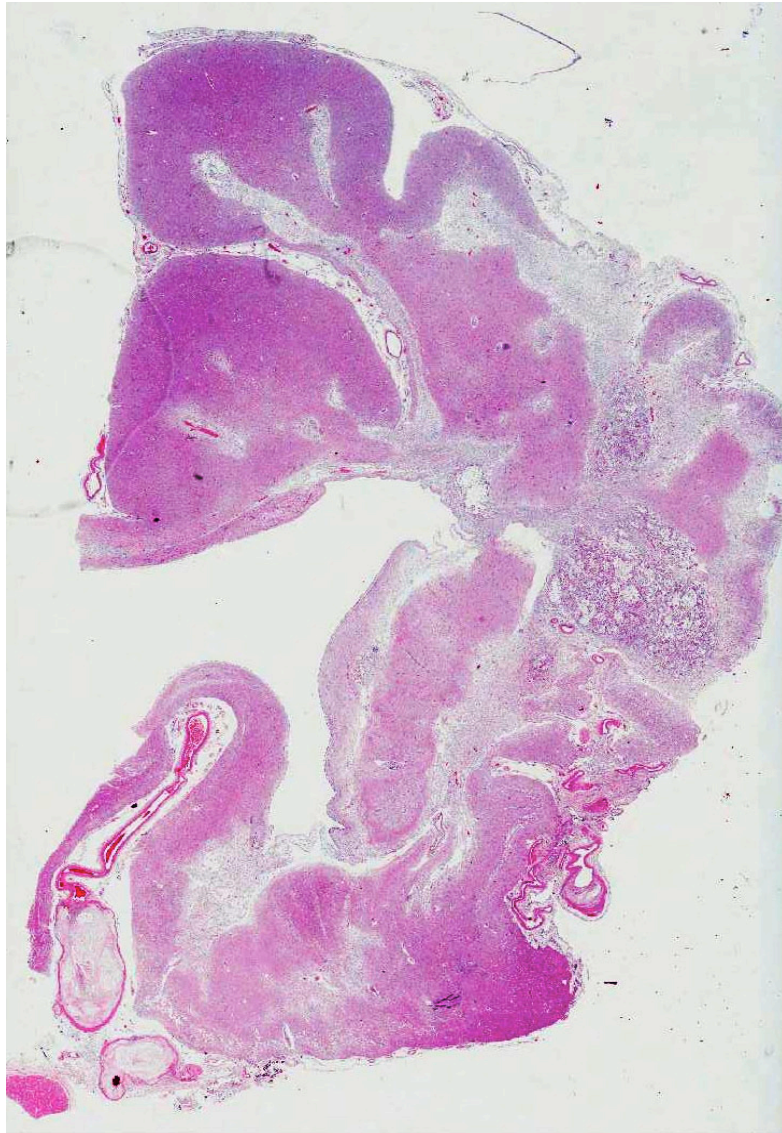
Histopathologic Description: Cerebral hemispheres: Affecting up to 40% of the grey and white matter of the left hemisphere are multiple coalescing foci of malacia, characterized by parenchymal loss with replacement by gitter cells, multinucleated giant cells, acicular (cholesterol) clefts, and scattered mineral (chronic infarcts).   The white matter tracts are most severely affected.   Adjacent to the malacic foci, the parenchyma is often vacuolated with increased numbers of glial cells (including reactive astrocytes), scattered necrotic neurons, and rare spheroids. The lateral ventricles are dilated (*hydrocephalus ex vacuo*), particularly the left. Within both the left and right hemispheres leptomeningeal blood vessels are often irregularly dilated. The tunica intima and media are expanded by cholesterol clefts and lipid-laden macrophages and leiomyocytes (foam cells) in a fibrous to amorphous eosinophilic fibrillar stroma (atherosclerotic plaques) that narrow the vascular lumina. Within these plaques are occasional foci of
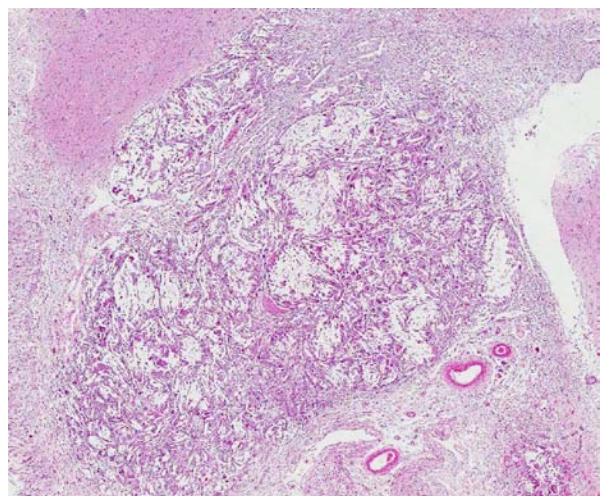

2-1. Cerebrum, cross section at level of putamen, dog: There are multifocal to coalescing areas of pallor (necrosis) within both the gray and white matter, affecting up to 40% of the section, and the lateral ventricle is mildly dilated. (HE 0.63X)
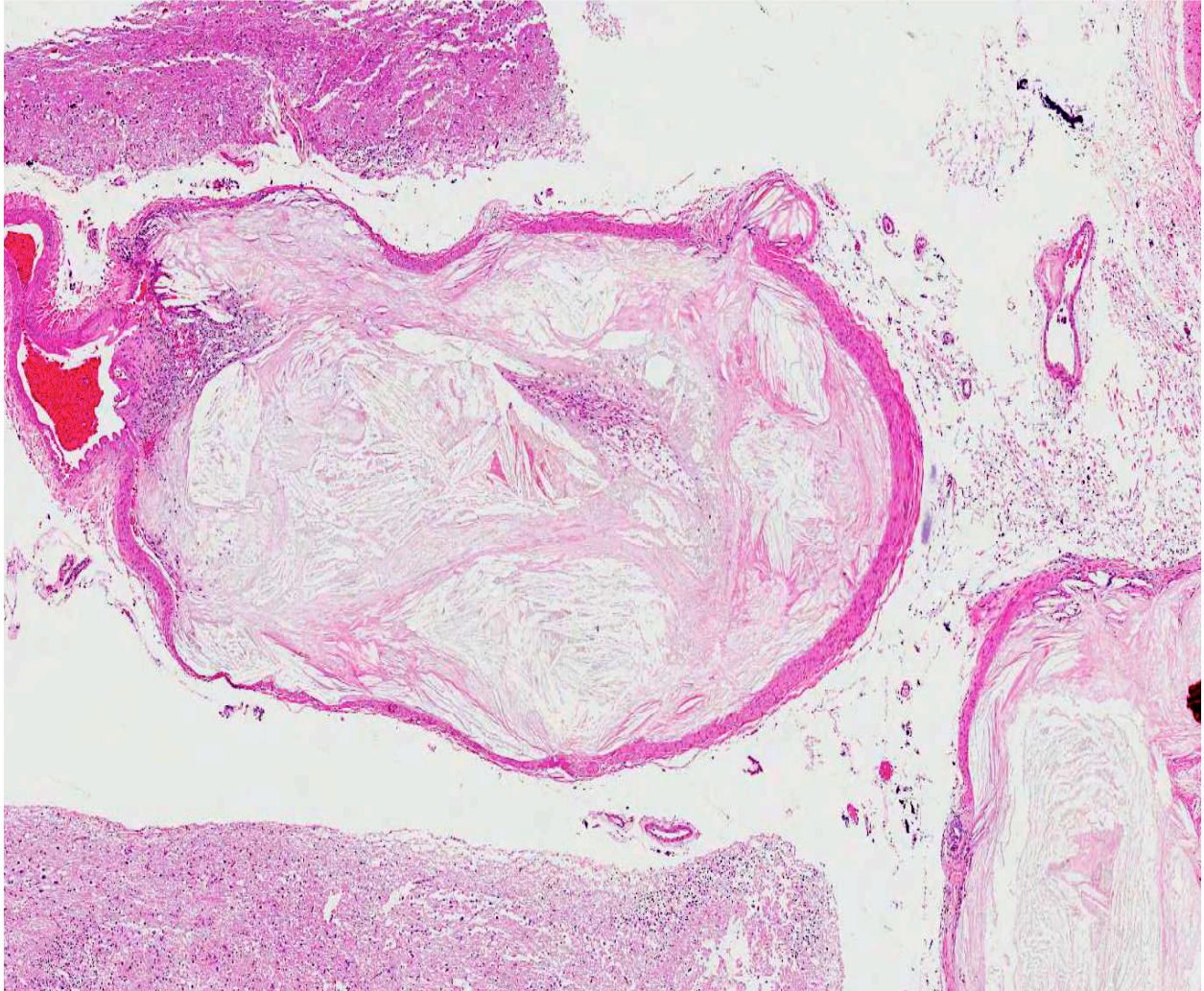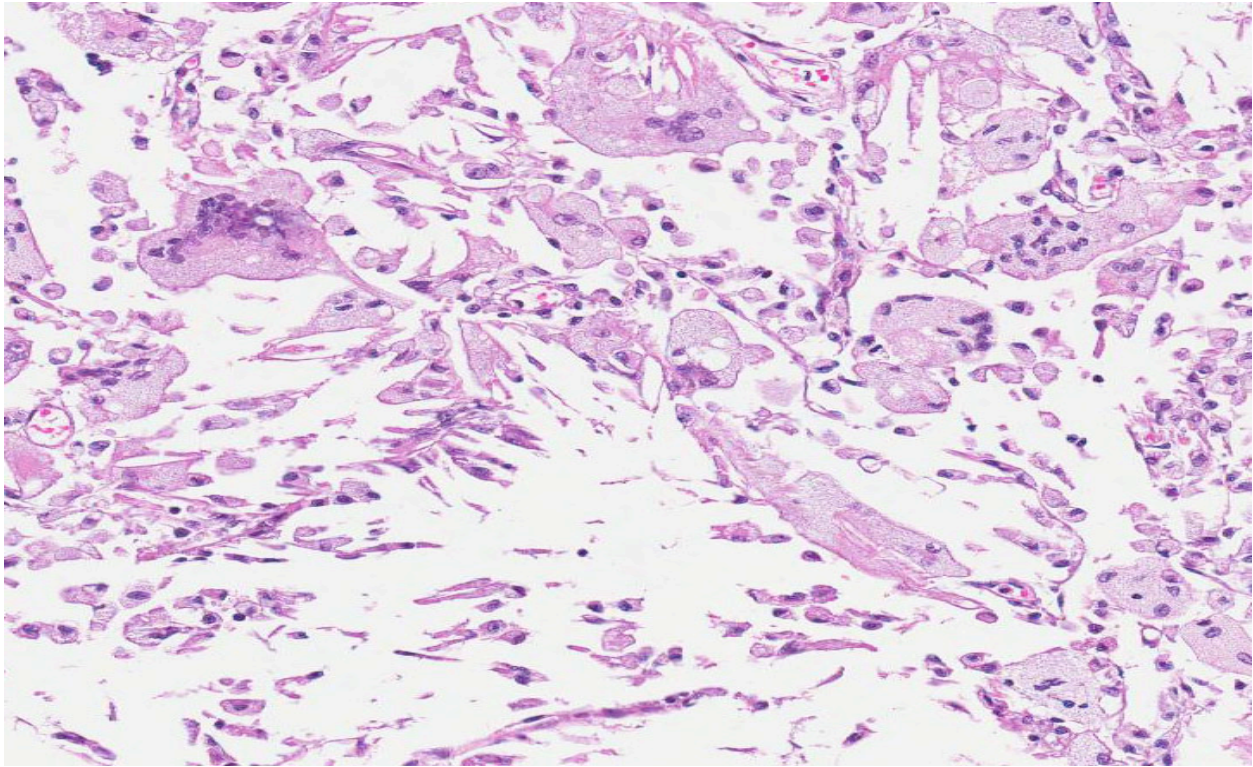
2-2. Cerebrum, cross section at level of putamen, dog: The walls of meningeal arteries at the base of the brain are markedly expanded, effacing the arterial lumens. (HE 23X)
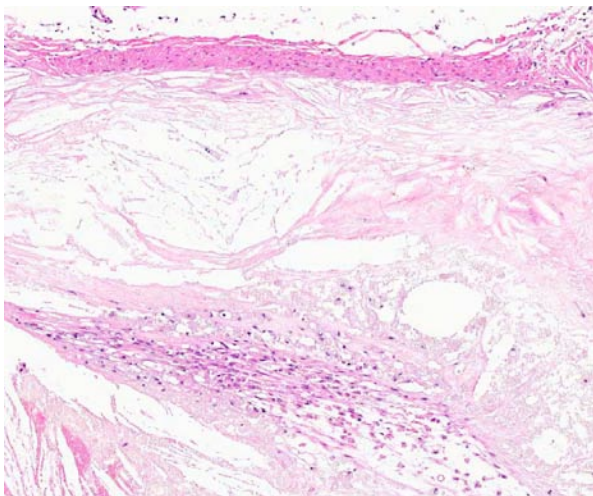
2-3. Cerebrum, cross section at level of putamen, dog: The arterial wall is effaced by abundant lipid, cholesterol clefts, and lipid laden histiocytes ("foam cells"). (HE 120X)

2-4. Cerebrum, cross section at level of putamen, dog: Occasionally, malacic areas are replaced by large cellular cholesterol granulomas. (HE 100X)

2-5. Cerebrum, cross section at level of putamen, dog: Higher magnification of one of the cholesterol granulomas, exhibit numerous multinucleated foamy macrophages admixed with numerous cholesterol clefts. (HE 300X)

hemosiderin- and hematoidin-laden macrophages and mineral. Low numbers of foamy macrophages, lymphocytes, and plasma cells are within the tunica adventitia and surrounding leptomeninges.

Contributor's Morphologic Diagnosis: Cerebral hemisphere (left): severe multifocal chronic encephalomalacia with cholesterol clefts, gitter cells, and multinucleated giant cells (chronic infarcts).

Leptomeningeal blood vessels: severe multifocal chronic atherosclerosis.

Contributor's Comment: The encephalomalacia in this case is consistent with an infarct secondary to severe atherosclerosis and luminal narrowing of the cerebral arteries, particularly the left rostral cerebral artery. Atherosclerosis is a vascular disease characterized by the formation of atheromas, or atherosclerotic plaques, in the vessel wall.[12] Atherosclerosis and its sequelae, including stroke, myocardial infarcts, and peripheral vascular disease, are leading causes of morbidity and mortality in humans.[10] However, atherosclerosis is infrequent in domestic animals.

The disease has been reproduced experimentally in pigs, chickens, and rabbits by feeding high cholesterol diets, but other species (including bovids, goats, cats, dogs, and rats) are considered resistant.[10,11] Naturally-occurring atherosclerosis has been described in aged pigs, birds, and dogs.[11]

Lesions in dogs have been described within the aorta and within small muscular arteries in a variety of organs, including the heart, lung, alimentary tract, spleen, eye, and urogenital and endocrine organs.[6,8] Arteries in the heart, brain, and kidneys are usually most severely affected. Clinical disease is rare and occurs when, as in humans, the atheromas occlude or rupture into the lumen, leading to ischemia, thrombosis, and/or lipid embolism.[10]

On gross examination, affected arteries are yellow-white with thick, irregularly nodular walls and narrowed lumina. Microscopically, early lesions consist of lipid-laden macrophages and leiomyocytes (foam cells) admixed with degenerating leiomyocytes within the tunica media.[8] With progression, the media becomes thicker with replacement of the normal architecture by intra- and extracellular lipid,
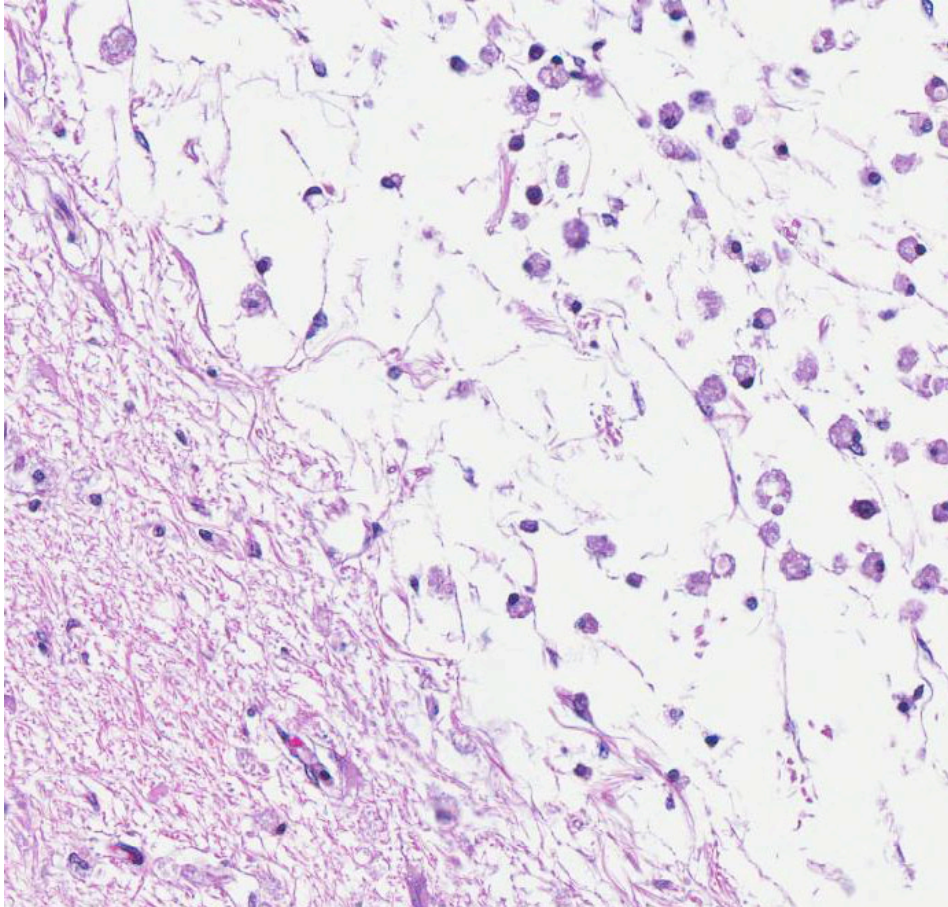
2-6. Cerebrum, cross section at level of putamen, dog: Within the cerebral white matter tracts there are large areas of liquefactive necrosis containing moderate numbers of Gitter cells. (HE 140X)

humans, a genetic predisposition to hyperlipidemia has been shown to increase the risk of atherosclerosis. While certain dog breeds, such as miniature schnauzers and Shetland sheepdogs, have a genetic predisposition to hyperlipidemia, additional studies are needed to determine the risk of atherosclerosis in these dogs.[5]

JPC Diagnosis: Cerebrum at the level of the putamen, gray and white matter: Necrosis, multifocal to coalescing, severe with cholesterol granulomas.

Brain, cerebral arteries: Atherosclerosis, diffuse, severe.

cholesterol clefts, mineral, cellular debris, and fibrous connective tissue that may become hyalinized.[6,8,10] Severe lesions lead to extension into the adventitia, as well as disruption of the internal elastic lamina and extension into the intima. This is in contrast to human atherosclerosis, where lipid deposition occurs primarily in the intima.[10]

In almost all canine cases, atherosclerosis occurs in association with hypothyroidism and/or diabetes mellitus.[10] Hypercholesterolemia is a common finding in both of these endocrinopathies and is thought to lead to the development of atherosclerosis in affected dogs.[5,10] However, in some dogs, serum cholesterol is normal and no endocrinopathy can be identified.[5] Furthermore, some thyroidectomized dogs on a high-cholesterol diet do not develop atherosclerosis.[10] Thus, other factors, such as genetics, may play a role in the development of canine atherosclerosis. In

Conference Comment: Spontaneous, diet-induced atherosclerosis is a common condition in pet birds, especially psittacines, White Carneau pigeons and Japanese quails.[2] It is also occasionally reported in captive reptiles.[13] Additionally, the Watanabe heritable hyperlipidaemic (WHHL) rabbit strain, which has a heritable gene mutation that results in hypercholesterolemia and atherosclerosis, has been developed as a model for human atherosclerosis.[1] Atherosclerosis is rare in dogs and it is typically associated with hypercholesterolemia or hyperlipidemia; its development is thought to result from abnormal lipid metabolism, although the exact pathogenesis has not been determined.[8]

There are five major plasma lipids in domestic animals: cholesterol, cholesterol esters, triglycerides and phospholipids are transported as

lipoproteins, while nonesterified fatty acids are bound to albumin for transportation.[4]   In mammals, dietary lipid is digested by pancreatic lipase and emulsified by bile acids into monoglycerides and free fatty acids. Micelles, which are formed from monglycerides, fatty acids, cholesterol, bile salts and fat soluble vitamins, are absorbed by jejunal enterocytes, where they are degraded.[4]   Chylomicra are synthesized within enterocytes from triglycerides (formed from glycerol and fatty acids), cholesterol esters, cholesterol, phospholipid and apolipoprotein.   These chylomicra are then secreted into intestinal lacteals, and they eventually reach the plasma via lymphatics and the thoracic duct. Chylomicra are hydrolyzed by lipoprotein lipase to fatty acids and glycerol, which are absorbed by adipocytes/hepatocytes and stored as triglycerides.[4] Lipid is transported in plasma bound to apolipoprotein, which is synthesized and secreted by the liver. Lipoproteins are classified (with increasing density) as chylomicra, very low density (VLDL), intermediate density (IDL), low density (LDL) or high density (HDL) lipoprotein.[4]

Very low density lipoprotein is synthesized in the liver; in addition to triglyceride, cholesterol and phospholipid (in a ratio of 4:1:1), it has three apolipoproteins: B-100, C and E.   ApoC and ApoE are acquired from HDL.   The primary function of VLDL is to deliver triglycerides to adipose tissue and striated muscle.[4,13] Within the capillaries of these tissues, VLDL is cleaved by lipoprotein lipase, producing IDL, which retains ApoE and ApoB-100; IDL can then follow two possible pathways: 1) the hepatocyte LDL receptor recognizes ApoB-100 or ApoE, allowing hepatic uptake and recycling to form VLDL, or 2) conversion to LDL by hepatic lipase (in hepatic sinusoidal capillaries) with removal of ApoE and most of the triglyceride.   The LDL can then be cleared by the liver via LDL receptor recognition of ApoB-100, or it can be cleared by other methods, especially the monocyte/macrophage system, which contributes to the pathogenesis of atherosclerosis.[4,7,13] Defects in the LDL receptor, such as the inherited LDL receptor defect in Watanabe rabbits or in people with familial hypercholesterolemia,[1] result in increased monocyte/macrophage-mediated clearance of excess LDLs, promoting the formation of xanthogranulomas and atherosclerosis.

Hepatic LDL receptor binding occurs within a clathrin-coated pit where LDL is internalized within a vesicle that fuses with the lysosome, resulting in degradation of ApoB-100 to its constituent amino acids, and the catabolism of cholesterol esters into free cholesterol.[7] The LDL receptor is subsequently recycled to the surface, while free cholesterol exits the lysosome via a process mediated by NPC1 and NPC2. This free cholesterol plays several roles in lipid metabolism.   It can be utilized to form cell membrane components, steroid hormones and/or bile salts; it also activates acyl-coenzyme A, which leads to esterification and storage of excess cholesterol.[7]   Free cholesterol within hepatocyte cytoplasm also inhibits LDL receptor synthesis, and inhibits HMG CoA reductase, which is the rate-limiting enzyme in cholesterol synthesis.[7]

In humans most cholesterol is transported as LDL, the plasma concentration of which is the most important risk factor for development of atherosclerosis.[14] The lipoprotein profile of swine bears the closest resemblance to that of humans, while dogs and cats have a higher proportion of HDL; as a result dogs and cats are generally considered to be resistant to atherosclerosis.[4] The exact pathogenesis of atherosclerosis in association with canine hypothyroidism has not yet been characterized.[14]

New data suggest an important role for chemokines and chemokine receptors in atherosclerosis,[13] and a recent study showed that advanced glycation end products (AGEs) may play a role in the pathogenesis of atherosclerosis in humans and dogs.   Advanced glycation end products belong to group of compounds resulting from glycation and oxidation of proteins, lipids and nucleic acids; specifically, hyperglycemia and oxidant stress contribute to increased formation of AGEs.[3] In humans and dogs, deposition of AGEs has been demonstrated immunohistochemically in atherosclerotic lesions and it has been suggested that AGEs may contribute to the development of atherosclerosis via effects on lipoproteins, extracellular matrix proteins, inflammatory mediators, smooth muscle and vascular endothelial cells.[3]   Advanced glycation end products may also suppress cellular antioxidants and induce expression of two important oxidized LDL (OxLDL) receptors: macrophage scavenger receptors class A and CD36. Increased expression of these receptors leads to enhanced uptake of

OxLDLs, resulting in the transformation of macrophages to foam cells.[3]

Rule-outs for canine atherosclerosis include arteriosclerosis (i.e., age related, often incidental intimal fibrosis of large elastic arteries), arterial calcification (e.g., due to renal disease, vitamin D toxicosis, ingestion of calcinogenic plants or infectious diseases) or lysosomal storage diseases such as Mucopolysaccharidosis-I (MPS-I). Mucopolysaccharidosis-I is an inherited, autosomal recessive deficiency of α-L-iduronidase that causes lysosomal accumulation of glycosaminoglycans (GAGs) in a variety of cell types, including those within the vascular system (see WSC 2013-14, conference 5, case 2).[9] A similar disease (i.e., Hurler/Scheie syndrome) affects humans.[7] Some dogs with MPS-I develop occlusive plaques affecting the tunica intima of medium to large arteries near branch points, or areas of low shear stress, such as the distal aorta or coronary and mesenteric arteries.[9] Histologically, intimal plaques consist of abundant extracellular matrix admixed with foamy (i.e., GAG-laden) macrophages, fibroblasts and smooth muscle cells.[9]

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CASE III: NIAH 2013 1 (JPC 4035522).

Signalment: 110-days of gestation male mixed-breed stillbirth fetus, pig (*Sus scrofa domesticus*).

History: From September to October 2005, a total of 6 sows in a herd of 13 aborted.   On the morning of October 12, 2005, a litter of 19 piglets from one sow was found as stillbirth feti, including two cases of mummification.   Two of those stillborn piglets were in fair postmortem condition and were submitted for necropsy.   Sows had been vaccinated for Aujeszky's disease virus (ADV) but not for Japanese encephalitis virus (JEV).

Gross Pathology: The brain was slightly swollen and edematous at necropsy.   No obvious gross abnormalities were seen in other examined organs.

Laboratory Results:

- No significant bacteria or viruses were isolated from the fetus.
- JEV Hemagglutination inhibition (HI) titers from the body fluid of stillborn piglets: ~10 to~640.
- JEV HI titers from sows that aborted: ~640 to ~5120.
- Serological tests for porcine reproductive respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), ADV and toxoplasmosis were all negative.
- PCR for PRRSV and PPV were negative.

Histopathologic Description: Cerebrum: There was nonsuppurative encephalitis characterized by perivascular cuffing with mononuclear cells, multifocal gliosis and severe neuronal necrosis. Mild meningitis was also observed in the cerebral hemisphere.   In the brainstem, there was severe multifocal necrosis with hemorrhage in addition to the nonsuppurative inflammation.   Basophilic granular material was noted multifocally within necrotic lesions.   These basophilic granules stained black with Von Kossa and were identified as calcification.   JEV antigen was immunohistochemically detected in the cytoplasm of nerve cells within the lesions.

Spinal cord (not included): There was nonsuppurative poliomyelitis characterized by perivascular cuffing with mononuclear cells, diffuse and multifocal gliosis and severe neuronal necrosis.   JEV antigen was immunohistochemically detected in the cytoplasm of nerve cells within the lesions.

Histological lesions and viral antigen were restricted to the central nervous system.   There were no lesions in the other examined organs including the liver, spleen, kidney, heart, lung and lymph node.

Contributor's Morphologic Diagnosis: Cerebrum: Meningioencephalitis, nonsuppurative, necrotizing, diffuse, moderate to severe, Japanese encephalitis, stillbirth fetus, pig.
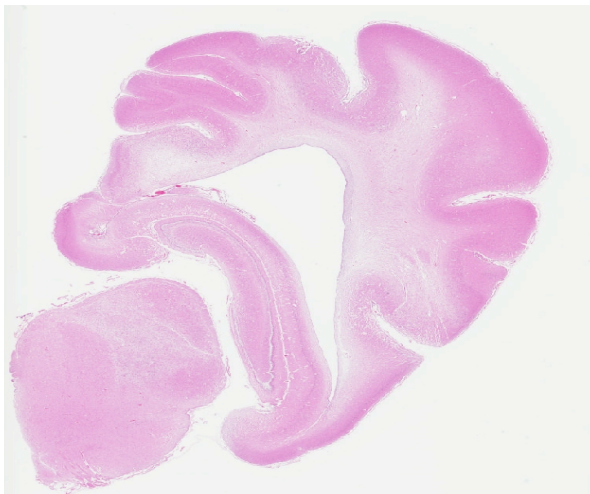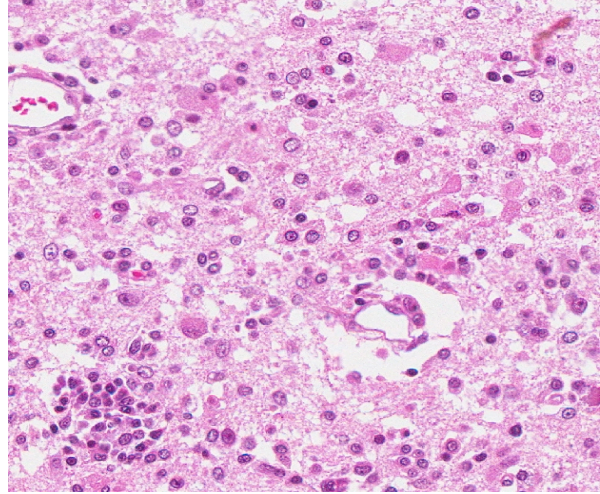

3-1. Cerebrum at level of hippocampus, one-day old piglet: At subgross inspection there is marked pallor of the cerebral white matter. This is considered an appropriate level of myelination at this age. (HE 0.63X)
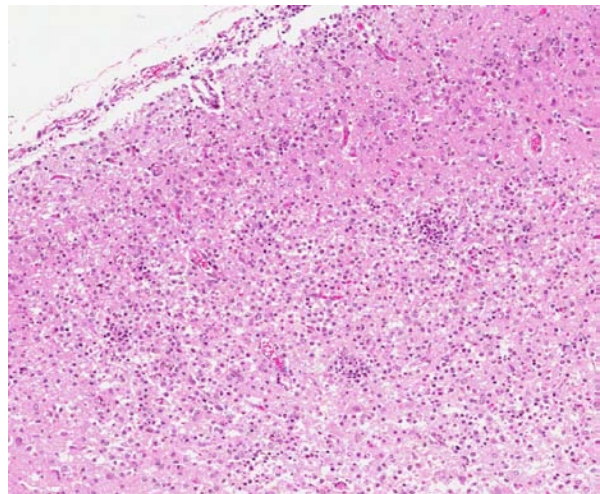
3-2. Cerebrum at level of hippocampus, one-day old piglet: There is marked inflammation of the superficial cortex as well as the overlying meninges. (HE 140X)
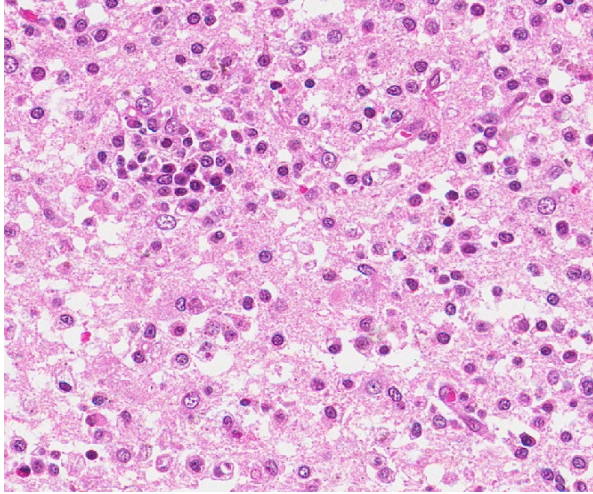
3-3. Cerebrum at level of hippocampus, one-day old piglet: Within areas of inflammation, neutopil is spongiotic and infiltrated with low to moderate numbers of histiocytes and lymphocytes, which are concentrated in perivascular areas. There are also increased numbers of reactive astrocytes within these areas. (HE 325X)

3-4. Cerebrum at level of hippocampus, one-day old piglet: Within areas of inflammation there are numerous reactive astrocytes with abundant eosinophilic cytoplasm (gemistocytes). (HE 400X)

Contributor's Comment: Japanese encephalitis virus (JEV) is one of four major encephalitic flaviviruses of public health importance; the other three are West Nile virus (WNV), St. Louis encephalitis virus (SLEV) in North America, and Murray Valley encephalitis virus (MVEV) in Australasia.[6] JEV is transmitted by mosquitoes of the genus *Culex*.[3,10] A wide range of domestic and wild avian and mammalian species including humans, horses, and swine are infected in nature.[10] Pigs are considered to be amplifier hosts with viremia that makes the virus available to mosquitoes.[3] Adult, non-pregnant swine typically do not exhibit overt signs of infection.[10] Reproductive failure may occur in non-immune females that become infected prior to 60-70 days gestation.[10] Pregnant sows may abort, produce mummified fetuses or give birth to stillborn or weak piglets.[10] Nonsuppurative fetal encephalitis has been described in cases of JEV abortion.[1]

In the present case, JEV antigen was immunohistochemically detected in the cytoplasm of intralesional nerve cells. An immunohistochemical method for diagnosing JEV in formalin-fixed tissues from infected pigs would be useful as a simple and rapid diagnostic test. JEV antigen was immunohistochemically detected in the nerve cells within areas of nonsuppurative encephalitis in 3-week-old pigs experimentally infected with JEV.[12,13] However, postmortem changes prevent immunohistochemical detection of viral antigen in many cases of aborted fetuses. Therefore, there are limited reports of immunohistochemical detection of JEV antigen in aborted fetuses. The present case shows that immunohistochemical detection of JEV antigen is also of diagnostic importance in those aborted fetuses with a relative lack of postmortem change.

In the present case, the virus was not isolated from stillbirth fetus. It appears that successful isolation of JEV from pigs of abnormal litters is dependent on the time that pigs were exposed to the virus in utero. It was reported that JEV was successfully isolated from feti of 3 litters that were collected at 7 to 22 days after experimental inoculation of sows with JEV, but not from affected pigs of 2 litters that were collected 62 and 84 days after infection of sows.[11] Detection of JEV specific antibody in body fluids of aborted fetuses by HI is a useful method for diagnosis.[10] The present case was diagnosed as Japanese encephalitis based on clinical history, histopathological findings, detection of JEV specific antibody by HI and immunohistochemical detection of JEV antigen in the brain lesions.

JPC Diagnosis: Cerebrum at the level of the hippocampus: Meningoencephalitis, necrotizing, nonsuppurative, focally extensive, marked, with gliosis.

Conference Comment: The family *Flaviviridae* is composed of three genera: Flavivirus, Pestivirus and Hepacivirus.[7] The genus

Flavivirus contains multiple viruses of veterinary importance, many of which are mosquito or tick-borne, including Japanese encephalitis virus, West Nile virus, Wesselsbron virus, and several less well-described flaviviruses in central Europe and South America.[7]   Other flaviviruses of public health significance include Yellow-fever virus, dengue virus, St. Louis encephalitis virus, and Murray Valley encephalitis virus.[7] The flaviviral genome is composed of positive-sense ssRNA with a 5' terminal cap structure.[7]

As its name implies, Japanese encephalitis virus primarily occurs in Asia and Southeast Asia, especially in areas with extensive freshwater marshes or irrigated rice fields.   The primary vector is the *Culex* mosquito.[7]   Swine do not typically develop clinical disease when infected with this flavivirus (with exceptions as noted by the contributor); they function as disease amplifiers and they are often used as sentinels for monitoring Japanese encephalitis virus in endemic areas. In addition to swine, wild birds have been implicated as amplifying hosts in virus transmission.[3] Viral titers in horses and humans are likely insufficient for re-infection of mosquito vectors, so these species are considered dead-end hosts.[3] Japanese encephalitis virus is one of the most frequent causes of human encephalitis in Asia[3] and it can also cause fatal encephalitis in horses.[12]   Routes of flaviviral neuroinvasion remain somewhat controversial.   Historically, hematogenous spread to the CNS was the presumed route of infection, however recent studies also support retrograde axonal transport via olfactory nerves.[2,13]

West Nile virus is also transmitted by *Culex* mosquitoes, with wild birds functioning as the primary amplifying hosts.[4] American crows and other corvids (blue jays, magpies), passerines, raptors, shorebirds and flamingos are highly susceptible, while domestic poultry and psittacines are generally resistant to fatal infection with WNV.[8] Common histopathological findings in WNV infected wild birds include meningoencephalitis, myocarditis, splenic and bone marrow necrosis; viral antigen is most commonly detected in the kidney, brain and heart.[4] In contrast to the high-titer viremia and widespread necrosis and inflammation found in wild birds, horses tend to have a transient, low-titer viremia with primarily central nervous system involvement.[4]   Histological lesions in equids typically include lymphocytic polioencephalomyelitis, predominantly within the lower brain stem and ventral horns of the thoracolumbar spinal cord, with perivascular cuffing and scattered hemorrhage.[2] In addition to wild birds, horses, mules, donkeys and pigs,[5] clinical disease due to WNV has also been reported in reindeer,[9] and Eastern fox squirrels and humans.[5]

Wesselsbron virus, a flavivirus transmitted by *Aedes* mosquitoes, is important in sub-Saharan Africa, where it causes acute disease resembling Rift Valley fever in sheep.[7] Wesselsbron disease causes high mortality in newborn lambs and kids, with subclinical infection or sporadic abortions in adults.[7]

The mosquito-born flaviviruses described above are typically neurotropic, however yellow fever virus and Dengue virus, which affect humans and monkeys, are viscerotropic, causing hemorrhagic fever rather than encephalitis.[4] Yellow fever virus was originally transported (via its *Aedes* mosquito vector) to the "New World" on slave ships, where it decimated the populations of many coastal cities in the 18th and 19th centuries.[7]   Disease is biphasic, beginning with fever, headache and nausea, followed by eventual hepatic and renal failure.   The widespread icterus often observed with this disease led to the name "yellow fever."[7] Old world monkeys tend to have subclinical infections, while New World monkeys develop clinical disease with high mortality.[7] Infection is characterized by widespread midzonal hepatic necrosis, acute renal tubular necrosis and multifocal lymphoid necrosis.   Dengue fever is one of the most important viral arthropod-borne hemorrhagic diseases in the world today. African monkeys have been implicated as the original reservoir of the dengue virus, and may still play a role in transmission, however the urban mosquito *Aedes aegypti/albopictus* is currently the most widely recognized vector in most outbreaks.[7]

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CASE IV: F1021947 (JPC 4002855).

Signalment: 11-year-old spayed female Pembroke Welsh Corgi dog (*Canis lupus familiaris*).

History: One year duration of immune-mediated hemolytic anemia (IMHA) with recent resolution of pneumonia. The dog presented to Colorado State University Veterinary Teaching Hospital for acute onset of panting and reluctance to move.

Gross Pathology: The abdomen is pendulous with approximately 20 milliliters of brown, thick fluid which is confined to the left cranial dorsal quadrant. This fluid corresponds to a full thickness, elliptical perforation of the gastric fundus measuring 1 ½ cm in length with regional serosal fibrinous adhesions to the omentum. There are multifocal hemorrhages within the greater leaf of the omentum and greater curvature of the stomach. The liver is mild to moderately enlarged diffusely with rounded edges and contains multiple tan, soft, round nodules that exude thick viscous material and measuring 1mm-2cm in diameter. Within the left frontal lobe there is a moderately well demarcated, soft, gelatinous, gray to tan foci measuring no more than 2cm in diameter. There are multifocal renal, capsular pits which correspond with collapse and loss of cortical parenchyma.

Laboratory Results:

Complete blood count:

Parameter	Result	Reference Range
Nucleated cells	$2.9 \times 10^3/\mu l$	$4.5\text{-}15.0 \times 10^3/\mu l$
Segmented neutrophils	$1.9 \times 10^3/\mu l$	$2.6\text{-}11.0 \times 10^3/\mu l$
Lymphocytes	$0.2 \times 10^3/\mu l$	$1.0\text{-}4.8 \times 10^3/\mu l$
Eosinophils	$0.1 \times 10^3/\mu l$	$0.1\text{-}1.2 \times 10^3/\mu l$
nRBCs	$0.1 \times 10^3/\mu l$	$0.0 \times 10^3/\mu l$
RBC	$4.1 \times 10^3/\mu l$	$5.5\text{-}8.5 \times 10^3/\mu l$

HGB	9.7 g/dl	13.0-20.0 g/dl
PCV	30.0%	40.0-55.0%
MCV	75.0 fl	62.0-73.0 fl
MCHC	32.0 g/dl	33.0-36.0 g/dl
Reticulocytes	85,050/µl	0.0-60,000/µl

Serum chemistry:

Parameter	Result	Reference Interval
Total protein	7.3 gm/dl	5.3-7.2 gm/dl
Globulin	3.9 gm/dl	2.0-3.8 gm/dl
ALP	437 IU/l	20-142 IU/l
ALT	179 IU/l	10-110 IU/l
GGT	68 IU/l	0-9 IU/l
Glucose	534 mg/dl	75.0-130.0 mg/dl
Anion gap	26	13-22

Blood gas (venous):

| Parameter	Result	Reference Interval
pH	7.119	7.3-7.5
pCO2	40.3 mmHG	24.0-39.0 mmHG
pO2	166.0 mmHG	67.0-92.0 mmHG
HCO3	12.5 mm/L	15.0-24.0 mm/L
Anion gap	24 mEq/l	13.0-22.0 mEq/l

Histopathologic Description: Brain: Focally expanding approximately 25% of the section there is rarefaction and effacement of the neuropil by marked numbers of inflammatory cells, karyorrhectic debris and abundant numbers of brown pigmented fungal hyphae. Hyphae have parallel walls and are occasionally septate and

branching. Inflammatory infiltrates consist primarily of epithelioid macrophages, occasionally multinucleated cells and intact and degenerative neutrophils. Hyphae are typically extracellular but a few are intimately associated with multinucleated cells. Vessels within this region are lined by prominent, hypertrophied endothelium, surrounded and disrupted by macrophages, lymphocytes, plasma cells and lesser neutrophils. Multifocally there are patches of perivascular fibrin exudate. At the periphery of this region there are scattered neurons with pale nuclei and dispersion of Nissl substance (chromatolysis). The leptomeninges contain moderate perivascular lymphocytes and plasma cells.

Contributor's Morphologic Diagnosis: Brain: Pyogranulomatous encephalitis, focally extensive, marked with intralesional dematiaceous fungal hyphae and vasculitis.

Contributor's Comment: Phaeohyphomycosis in domestic animals is an uncommon, opportunistic infection caused by a variety of fungal species.[4] Characteristic of phaeohyphomycotic fungi is the presence of variable melanin pigment in the cell wall giving it a distinct brown color.[4] Production of melanin pigment is thought to contribute to the organism's virulence.[4,9] DHN-melanin (1, 8-dihydroxynaphthalene) and DOPA-melanin (L-3, 4-dihydroxyphenylalanine) are two specific melanin pigments thought to play a role in the pathogenicity of dematiaceous fungi.[9] The role of such pigment has been implicated in contributing to organism's resistance to ultraviolet radiation, extreme temperature variation, oxidation, enzymatic degradation, as well as structural functions and guarding against desiccation.[2,9,14]

The various fungal species are not morphologically distinct on routine H&E histologic examination.[4] Culture, fungal
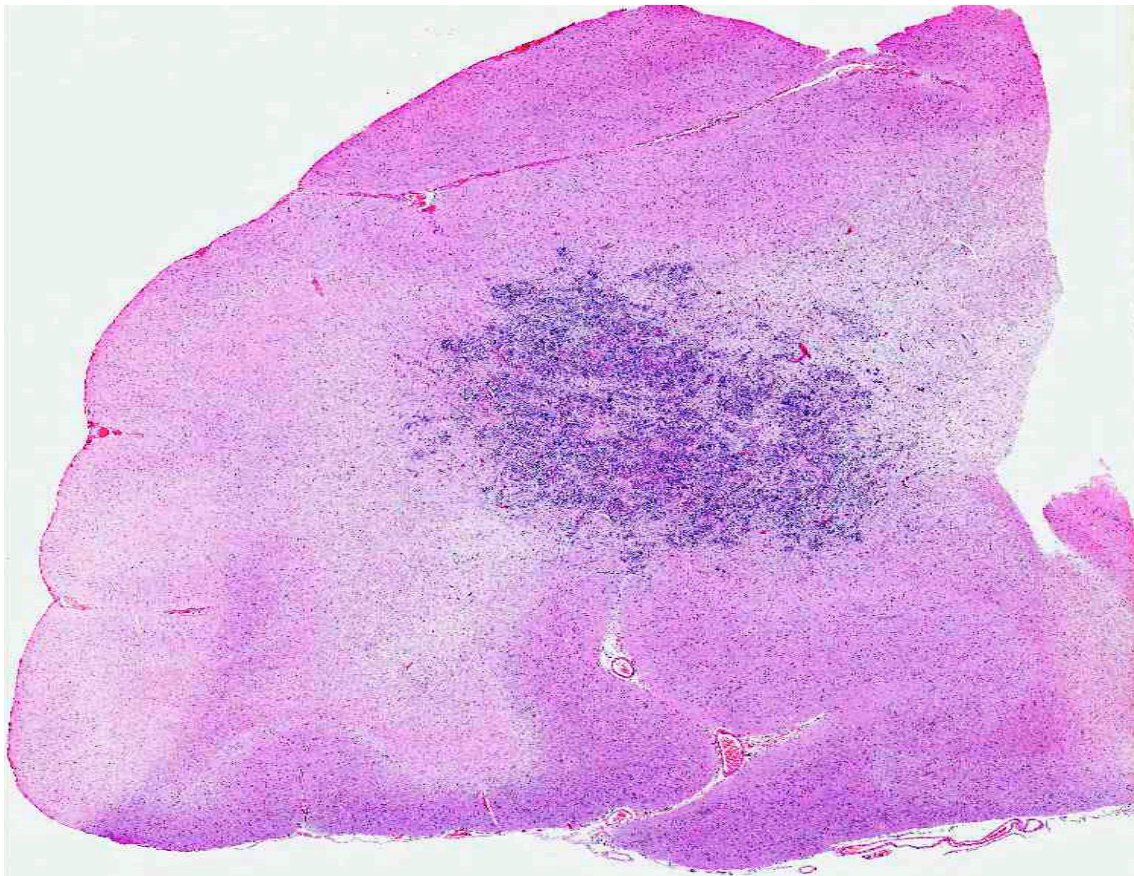
4-1. Cerebrum, dog: Within the frontal lobe there is a focally extensive area of inflammation and necrosis. (HE 0.63X)
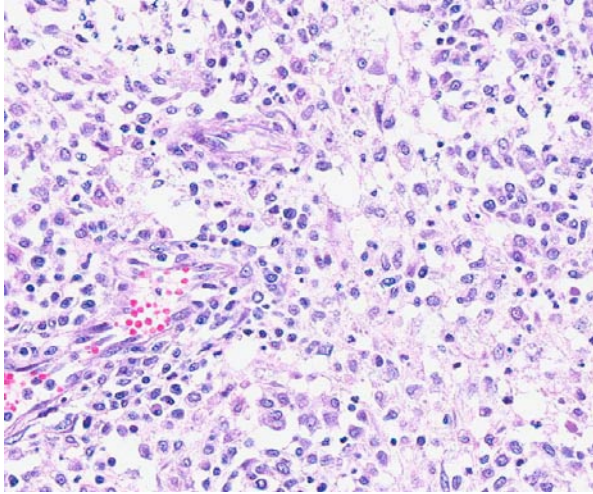
4-2. Cerebrum, dog: Necrotic foci are infiltrated by large numbers of histiocytes, moderate numbers of neutrophils, and rare multinucleated macrophages. Inflammation is concentrated in perivascular areas; vascular endothelium is markedly hypertrophic. (HE 200X)
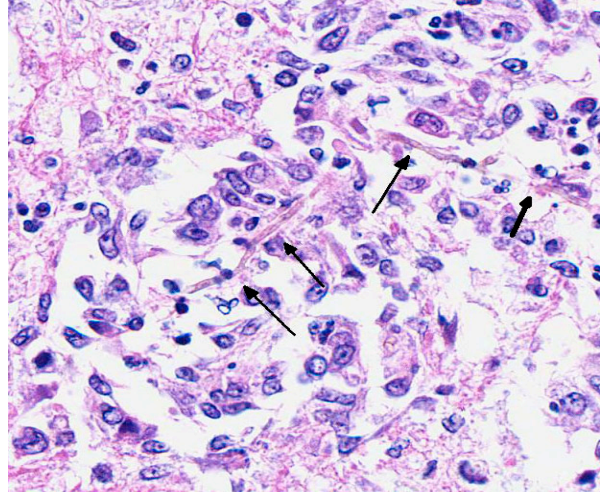
4-3. Cerebrum, dog: Scattered throughout areas of necrosis and inflammation are rare 3-5 diameter, pauciseptate, lightly pigmented fungal hyphae with parallel walls, consistent with dematiaceous fungi. (HE 400X)

morphology or molecular techniques are generally required for speciation.[6] Organisms in general have a dark brown to light yellow pigment, although degree of pigmentation can vary, and have 2-6 μm in width septate hyphae that can be branched or unbranched.[4,7] If melanin pigment is not identifiable, Fontana-Masson histochemical stain may assist in identification of subtle pigment.[4,7]

Phaeohyphomycotic fungi are composed of more than 100 different dematiaceous species presenting clinically as cutaneous, central nervous system and disseminated infections.[6,14,16] Reported most frequently in cats, phaeohyphomycosis has been occasionally documented in horses, dogs, cattle and goats.[5,7,12,15] Typically feline infections are limited to subcutaneous tissues although disseminated and cerebral forms do occur and are most often associated with immunologic compromise.[14,16] Cutaneous forms are thought to occur due to introduction of the organism via a penetrating wound.[14,16] Specifically, *Cladophialophora bantiana*, (also known as *Cladosporium bantianum*, *Cladosporium trichoides*, and *Xylohypha bantiana)* has a tropism for nervous tissue and has been reported as a cause for cerebral phaeohyphomycosis in both dogs and cats.[11,12]

Immunosuppression likely contributed to the pathogenesis of cerebral phaeohyphomycosis in this dog with a history of cyclosporine and prednisone administration for treatment of IMHA. Route of infection was unclear at the time of necropsy although ultimately hematogenous dissemination was suspected due to the presence of fungal organisms within the liver. Cutaneous infection may have been the route of entry although lesions were absent at necropsy examination. Ingestion of the fungus and entry via the compromised stomach was considered but unlikely due the absence of histologic identification of the organism within sections of the stomach at the location and distant to the perforation.

JPC Diagnosis: Cerebrum: Encephalitis, necrotizing and granulomatous, multifocal to coalescing, severe, with vasculitis, mild lymphohistiocytic meningitis and rare dematiaceous fungal hyphae.

Conference Comment: Several species of dematiaceous fungi demonstrate a predilection for the central nervous system and phaeohyphomycosis (cerebral and/or disseminated) has been reported in an alpaca, a snow leopard, reptiles, including a tortoise and an iguana and leafy and weedy seadragons,[1,3,8,13] in addition to the domestic species previously listed.

As noted in the summary, immunosuppression secondary to repeated administration of cyclosporine and prednisone for treatment of immune mediated hemolytic anemia likely predisposed this dog to fungal infection. With

that in mind, conference participants briefly discussed the clinical pathologic findings in this case, which were consistent with the history of treatment for IMHA and the diagnosis of disseminated phaeohyphomycosis. Macrocytic, hypochromic, regenerative anemia is an expected finding in dogs with IMHA.[10] Corticosteroid treatment typically induces lymphopenia, and while neutrophilia is a common finding in cases of hemolysis and corticosteroid administration, the neutropenia observed in this case probably occurred due to the disseminated fungal infection.[10] Globulin concentration also increases in infection and inflammation.[10] Anemia induced hypoxic injury to centrilobular hepatocytes may result in elevation of ALT, while increased ALP and GGT may occur following corticosteroid-induced hepatocellular glycogen accumulation.[10] Corticosteroids also induce hyperglycemia.[10]

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