Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2017-2018

C o n f e r e n c e 12

13 December 2017

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CASE I: 16L-2815 (JPC 4102118).

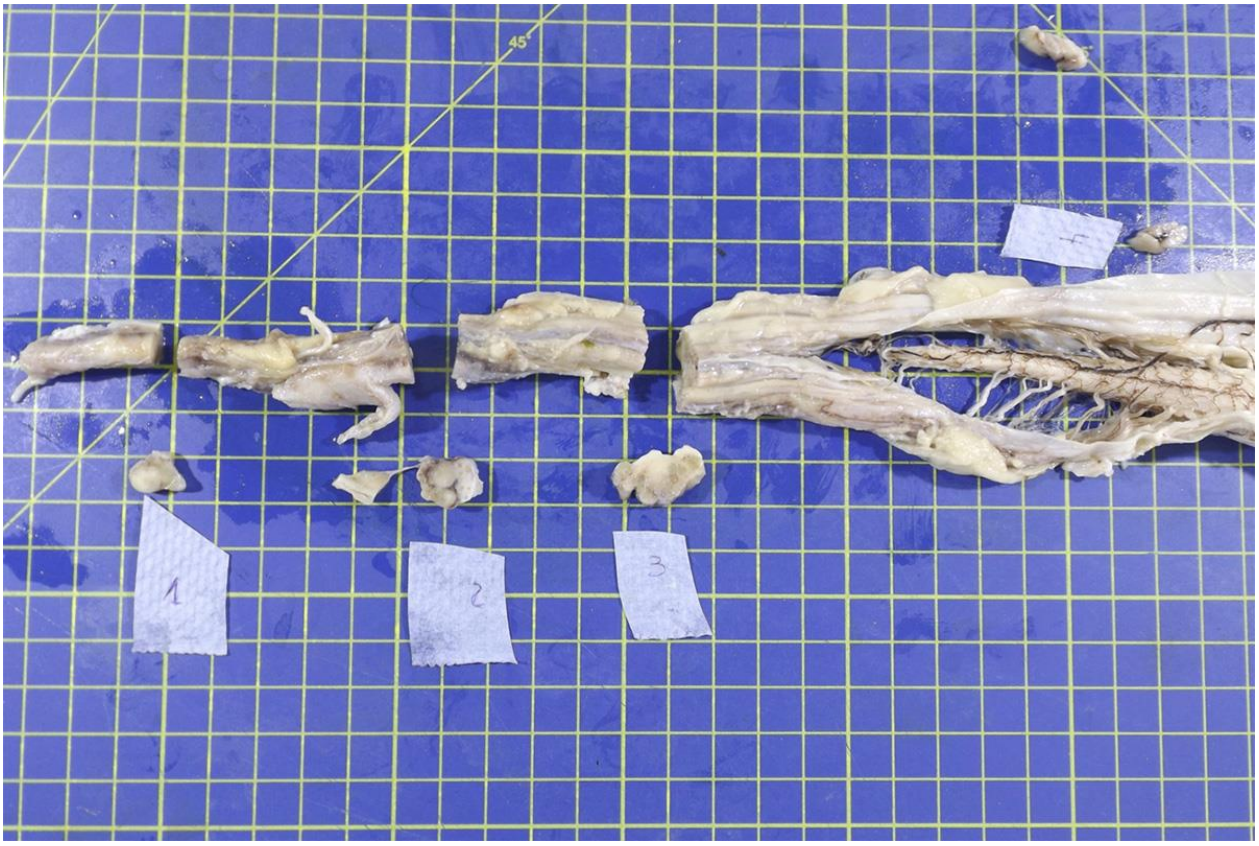
Signalment: 8 year-old, mare, Irish sport horse, *Equus caballus*, equine.

History: The animal presented with clinical signs consistent with cauda equina syndrome from 29th November 2015. Clinical neurological manifestations were an inability to pass feces and a progressive reduction in tail, anal and vaginal tone. Posteriorly there was a bilateral muscle atrophy of the muscles of the proximal hind limbs and the muscle of the tail head. Unilateral paresis of the right hind limb appeared and developed into a paraparesis. Steroids and non-steroidal anti-inflammatory drugs in combination with neuromodulator treatment were administered.

Gross Pathology: The lumbosacral spinal cord and extradural nerve roots (cauda equina) were markedly enlarged by an abundant grey to whitish irregular material with variable consistency and occasionally a nodular pattern. This material was consistently attached to the epineurium of the sacral nerve roots and was present from the *Conus medulari*, and between the nerve prolongations of the fillum terminale and cauda equina.

Laboratory results:
None provided.

Microscopic Description: Cauda equina: There are multiple cross sections of nerve roots, epidural adipose tissue and connective tissue. The majority of the fibrous tissue corresponds to the epineurium which is markedly expanded by an increased volume of dense irregular connective tissue (severe fibrosis). The fibrotic epineurium is infiltrated by a moderate to severe, multifocal inflammatory infiltrate composed of epithelioid macrophages, often surrounded by large numbers of lymphocytes, fewer plasma cells and occasional Langhans type multinucleated giant cells; there is multifocally mild hemorrhage. The majority of nerve roots are affected by a similar inflammatory process, the severity of these changes ranging from

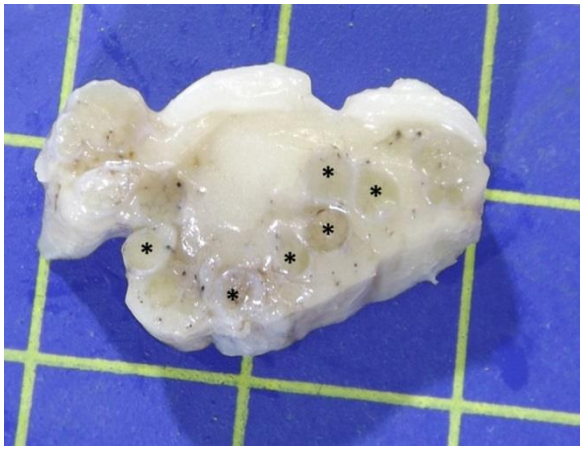


Cauda equina, horse. The lumbosacral spinal cord (right) and spinal roots are expanded and fused by abundant, variably mature fibrous connective tissue. (Photo courtesy of: Department of Veterinary Pathology, Infection and Public Health, Institute of Veterinary Science, University of Liverpool, Leahurst Campus, Chester High Road, Neston, CH64 7TE.

minimal to severe. The most severely affected nerve roots are entirely effaced by multifocal areas of necrosis characterized by large amounts of eosinophilic and basophilic amorphous material (myelin debris) and infiltrated by large numbers of inflammatory cells (macrophages, lymphocytes), and intact axons and myelin sheaths are rarely observed. In those nerves less severely affected, the epineurium and perineurium are markedly enlarged due to high number of infiltrating macrophages and lympho-cytes. There is a reduction of nerve fibers numbers, and nerves variably exhibit swollen axons (spheroid), moderately distended myelin sheets and occasionally phagocytic cells within areas of myelin degradation (digestion chambers). Other peripheral nerves are minimally affected or are unremarkable.

Contributor's Morphologic Diagnosis:
Horse, lumbosacral spinal nerve roots (cauda equina, fillum terminale): Multifocal, severe, chronic, granulomatous poly-radiculoneuritis with multifocal nerve necrosis, Wallerian axonal degeneration and epineurial fibrosis.

Contributor's Comment: Polyneuritis equi (cauda equina neuritis) is an uncommon sporadic disease of the horse of an unknown etiology but is considered likely to be an autoimmune or immune-mediated disorder; it is considered by some authors to be reactive condition from previous inflammatory or infectious episodes[2].



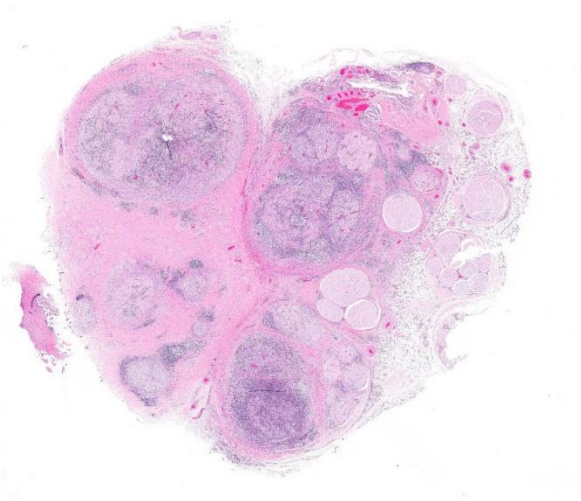
Cauda equina, horse. The entrapped spinal roots (asterisk) are fused within an irregular mass of proliferating fibrous connective tissue. (Photo courtesy of: Department of Veterinary Pathology, Infection and Public Health, Institute of Veterinary Science, University of Liverpool, Leahurst Campus, Chester High Road, Neston, CH64 7TE.)

Clinically, animals exhibit a slowly progressive peripheral neurological disorder, localizing to the sacrococcygeal nerves (cauda equina syndrome) and is most commonly observed in females[9]. Formerly known as cauda equina neuritis in horse, the name of the disease was updated to polyneuritis equi (PNE) based on descriptions in which the involvement of spinal nerves at various levels of the spine and cranial nerves were also reported[11].

Clinical examination allows the neuro-localization of lesions to the level of spinal nerve roots, frequently of the sacral spinal segments[4]. It represents an exclusion diagnosis with and is considered to be of idiopathic origin[1]. A case of verminous migration by *Halicephalobus gingivalis* has been described in association with the typical inflammatory reaction of cauda equina neuritis, however[5]. Equine neurotropic viruses such as equine herpesvirus 1 (EHV1) and West Nile virus have been ruled out as causes in cases reported in recent literature[1]. Some older studies have demonstrated an association between the lesion of PNE and infectious agents such EHV1, equine adenovirus type 1, equine arteritis virus, and *Streptococcus equi* ssp. *equi* with the suggestion that the lesion may have evolved through a bystander mechanism in which the presence of the inflammatory infiltrate damages the nerves, rather than the direct actions of these organisms as causal agents[9]. Additionally, exclusion of traumatic, developmental, neoplastic or toxic diseases should be ruled out before confirming the diagnosis[11].

The typical gross and microscopic lesions that characterize this disease are very much in keeping with the case we present. Typical gross lesions include thickening of the nerve roots of the sacral and coccygeal nerves which may be discolored by acute and / or chronic hemorrhage. Histologically a severe granulomatous neuritis with marked fibrosis and degenerative / necrotizing changes to nerve fibers is described[2].

Cauda equina syndrome also is described in dogs with a higher incidence in large breeds[9]. Nonetheless in this species it is defined as a compression of the spinal cord



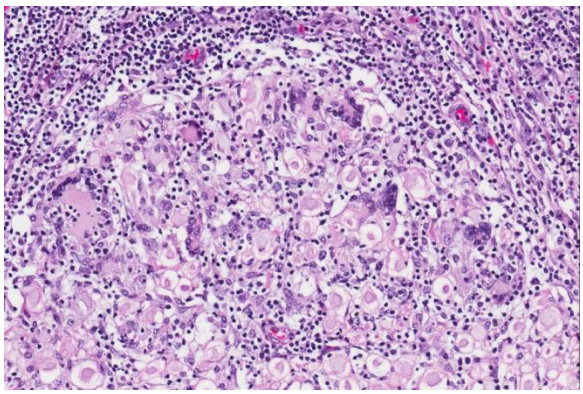
Cauda equina, horse. The epineurium of spinal nerve bundles is markedly expanded and fused with that of other nerve bundles, and the nerves are variably effaced by a cellular infiltrate. (HE, 6X)

due to stenosis of the lumbosacral central canal. Dogs and cats also develop acute polyradiculoneuritis with presumed autoimmune origin and low frequent affectation of the cauda equina[9].

In humans and laboratory animals, Guillain-Barre syndrome (GBS) and allergic neuritis (EAN) constitute infectious autoimmune diseases that have been compared with PNE in horses and acute polyneuritis in dogs. Descriptive studies including the characterization of the histopathological lesions in horses[3,10] are indicative of immune reactions against myelin in PNE. A 2008 study into the composition of the inflammatory infiltrates of PNE[9] demonstrated that both T and B lymphocytes were present within the lesions, along with macrophages. The authors consider the question as to whether there is a T-cell mediated immune response against myelin, or if the B-cells are producing an antibody against the P2 protein of myelin, or there may be a combination of both mechanisms. A 2015 study[3] of equine protozoal myeloencephalitis, caused by *Sarcocytis neurona*, demonstrated that some horses with antibodies against this organism also produce antibodies against myelin protein peptide. This suggests one possible aetiological relationship between an infectious organism and an immune-mediated peripheral neuritis.

JPC Diagnosis: Spinal cord, cauda equina: Polyradiculoneuritis, granulomatous, chronic, diffuse, severe with marked epineurial fibrosis and widespread axonal degeneration, Irish sport horse (*Equus caballus*), equine.

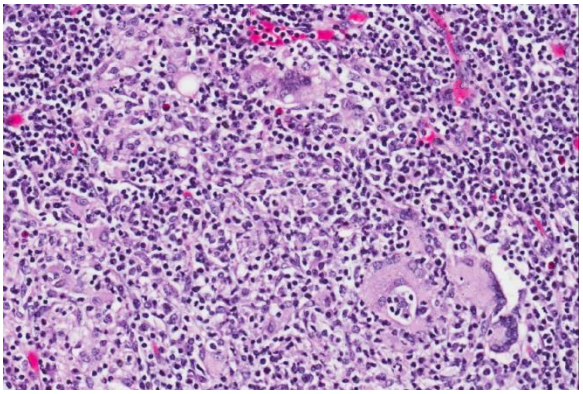
Conference Comment: The cauda equina is composed of roots of the sacral and coccygeal spinal nerves and is present in all mammalian species except for birds, whose



Cauda equina, horse. Cross section of an infiltrated nerve. Numerous lymphocytes and histiocytes infiltrate the nerve root endomysium occasionally replacing axons. Remaining axons sheaths are mildly dilated. There are moderate numbers of foreign body and Langhans type multinucleated macrophages. The encircling epineurium is expanded by numerous lymphocytes, fewer histiocytes and proliferating vessels, fibroblasts and collagen. (HE, 288X)

spinal cord extends throughout the entire spinal canal. These nerves exit the caudal end of the spinal cord and travel through the remainder of the spinal canal longitudinally to reach their intended intervertebral foramina. The cauda equina develops after birth as the vertebral column continues to grow, resulting in cranial displacement of spinal cord segments relative to their corresponding vertebrae (whereas during gestation the spinal cord segments are aligned with their corresponding vertebrae)[7].

First described in 1897 by Dexler as a combination of tail and anal sphincter paralysis due to chronic inflammation and fibrosis of the extradural portions of the nerve roots of the cauda equina, neuritis of the cauda equina has been identified throughout the years in numerous adult horses and ponies of various breeds. Many of the clinical signs (most of which are listed above) including urinary and fecal incontinence, perineal anesthesia, tail paralysis, muscle atrophy and weakness, and hind limb ataxia can be attributed to affected sacrocaudal nerve roots. However, animals often have additional clinical signs



Cauda equina, horse. Higher magnification of the infiltrate within an effaced nerve. No intact axons remain. (HE, 324X)

attributable to cranial nerve roots being affected such as facial paralysis, head tilt, and wasting of the masticatory muscles. The variety of affected nerves led to this syndrome being more aptly named "polyneuritis equi"[8]. In this case, attendees noted the variation of maturity of fibrous connective tissue throughout the lesion - ranging from hypertrophied fibroblasts and loosely packed immature fibrous connective tissue to dense mature collagen, denoting the diseases development over time.

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CASE II: N2015-0602 (JPC 4084210).

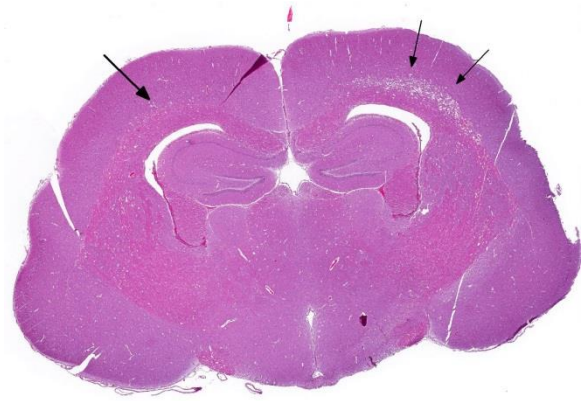
Signalment: 1.8 year-old, male, African pygmy hedgehog, *Atelerix albiventris*, hedgehog.

History: This hedgehog had an approximate 3-month history of progressive depression, weight loss, worsening limb tremors, paresis, and hunched posture. Clinical decline persisted despite supportive care and analgesic therapy (Meloxicam). The animal was euthanized.

Gross Pathology: The hedgehog was in good body and postmortem condition. Mild abrasions were present along the ventral trail. Absent gastric content and mild gall bladder distention suggested recent anorexia. There were no other significant gross findings.

Laboratory results:
None provided.

Microscopic Description: Multiple sections along the brain and spinal cord (cerebrum, thalamus, midbrain, cerebellum, brainstem, and spinal cord) were examined. Submitted slides include cerebrum and brainstem.



Cerebrum, pygmy hedgehog. There is extensive bilateral vacuolation of the white matter of the corona radiata. The very slight tangential section affects the amount of this structure at left. (HE, 6X)

Affecting white matter throughout the examined sections of central nervous system are regions of myelin degeneration. These areas are characterized predominantly by multifocal to clustered, variably sized (approximately 6-60 um-diameter), clear vacuoles (spongiosis), with occasional wispy residual eosinophilic myelin strands and subtle regional pale staining (rarefaction). There are mildly increased interspersed small basophilic glial cells (astrocytosis) with occasional satellitosis, few eosinophilic swollen axons (spheroids), rare vacuolated gitter cells and digestion chambers, and rare eosinophilic reactive astrocytes (gemistocytes). The myelin vacuolation is marked and variably symmetrical within the brainstem, is moderate and more unilaterally predominant within the cerebral cortex corona radiata, and is mild and scattered within the internal capsule, thalamus, midbrain, cerebellar white mater, spinal cord (thoracic, predominantly ventral funiculi), and optic nerve.

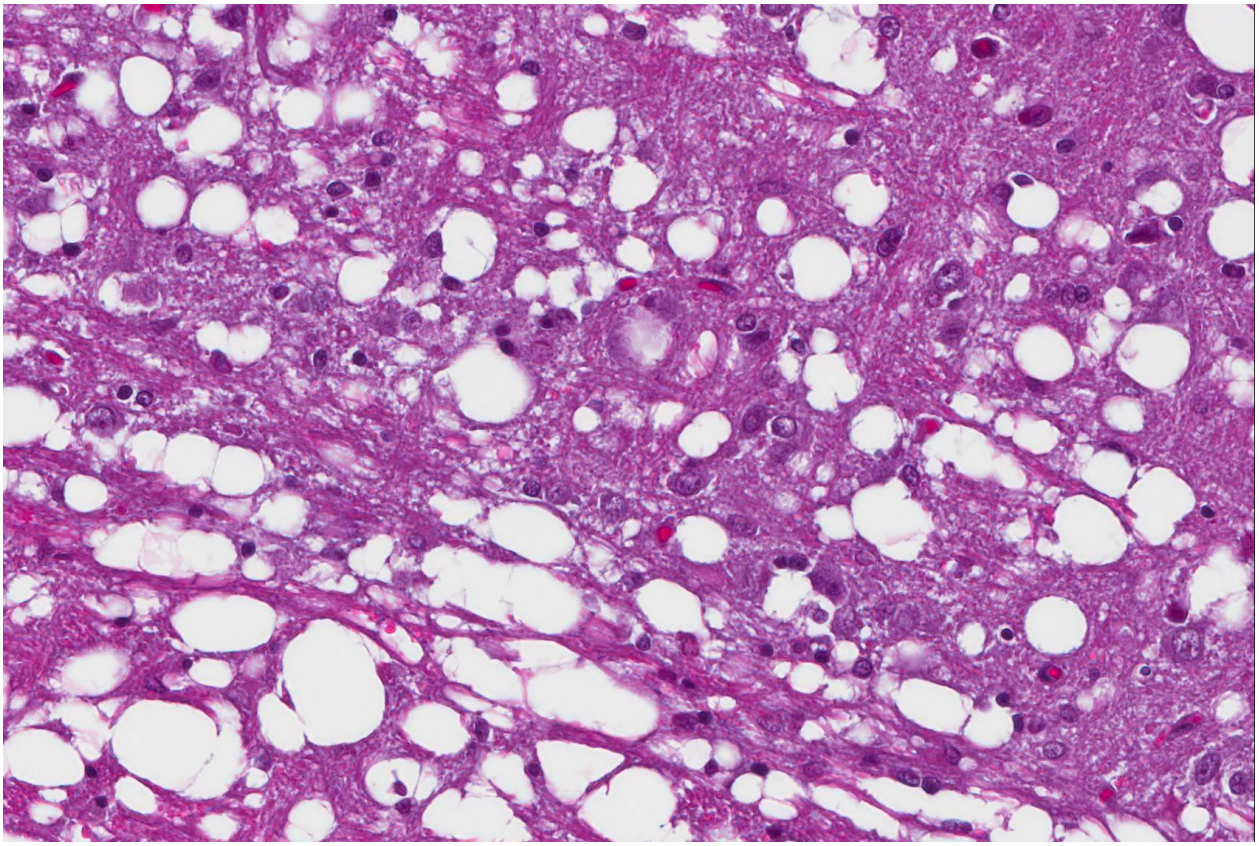
Contributor's Morphologic Diagnosis:
Brain and spinal cord, white matter: Myelin degeneration, multifocally extensive, mild (thalamus, optic nerve, spinal cord) to moderate (cerebral cortex, cerebellum) to marked (brainstem), with prominent spongiosis, mild gliosis, and rare axonal degeneration.

Contributor's Comment: This case demonstrates lesions of leukoencephalomyelopathy typical of "Wobbly Hedgehog Syndrome", characterized by demyelinating changes affecting the brain

and spinal cord. This syndrome is a progressive neuropathy of unknown etiology that affects African hedgehogs and is considered to have a familial tendency.

Wobbly Hedgehog Syndrome (WHS) has been recognized since the mid-1990s and is reported to affect approximately 10% of pet African hedgehogs in North America. The predominant clinical signs of WHS are progressive ataxia and paralysis, with onset often under two years of age. Disease progression is variable, but complete paralysis often occurs within 15 months. Other noted clinical abnormalities include tremors, exophthalmos, scoliosis, seizures, muscle atrophy, and self-mutilation.[4] In most cases, the progression of paralysis is ascending to tetraplegia. Weight loss is common and may relate to dysphagia in later stages of disease. Skin abrasions affecting feet and ventral body can relate to loss of motility. As in this case, affected animals are often euthanized due to quality of life concerns.

Histopathology is required for definitive diagnosis of WHS, with characteristic vacuolation of the white matter tracts of the brain (cerebrum, cerebellum, and brainstem) and spinal cord. Lesions are considered to begin with myelin loss, and progress variably to secondary axonal and even neuronal degeneration. In this case, myelin degeneration predominated, with minor axonal degeneration variably evident across sections. Degeneration of lower motor neurons of the ventral horns of the spinal cord, demyelination of ventral spinal rootlets, and neurogenic muscle atrophy are



Cerebrum, pygmy hedgehog. Vacuoles are distinct, range up to 40 um in diameter, and often have a single compressed hyperchromatic nucleus at the periphery, suggesting that the vacuoles represent markedly dilated myelin sheaths. (HE, 400X)

also reported.[4] The peripheral nervous system is unaffected. Hepatic lipidosis of varying severity has also been reported in some cases; in the animal of this report hepatocellular lipid vacuolation was mild.

The etiology of WHS is unknown. However, clustered cases and pedigree analysis suggest a hereditary basis. Relatives of this affected hedgehog had also succumbed to neurologic disease with pathologic findings supportive of WHS. Due to familial tendency, breeding hedgehogs with signs of WHS or their close relatives has been discouraged.[4] No treatments (antibiotics, vitamins and supplements, physical therapy regimes, etc.), have been confirmed to alter the course of disease.

Although there are no reports of WHS transmission between unrelated hedgehogs, an infectious cause is not entirely excluded. A similar paralytic and demyelinating syndrome has also been reported in European hedgehogs, but with slightly different histologic and epidemiologic features, for which a viral etiology was suspected.[7] More recently, nonsuppurative encephalitis with vacuolation of the white matter was reported in conjunction with positive detection of pneumonia virus of mice (family paramyxoviridae) in an African hedgehog suspected of WHS, but the relationship between the disease findings and links to causality require further investigation.[6]

Demyelinating conditions of animals and humans may be acquired or inherited/genetic. These diseases result from abnormalities affecting myelin sheaths or myelin forming cells. Axonal or neuronal degeneration is a secondary occurrence. Causes of acquired demyelination include: infectious agents (e.g., canine distemper virus and small ruminant lentiviruses), immune-mediated inflammation (e.g., multiple sclerosis in humans), metabolic derangements (e.g., central pontine myelinosis associated with rapid correction of hyponatremia in various species, and hepatic encephalopathy in various species), and toxic exposures (e.g., hexachlorophene, stypandrol, and others in various species).[2,4-6] In some cases, hypoxia/ischemia and compressive lesions may also predominantly affect oligodendrocytes and myelin sheaths.[5] Hereditary demyelinating conditions include identified genetic defects in humans (e.g., Canavan's disease leukodystrophy due to mutation causing deficiency of aspartoacylase enzyme) and animals (e.g., canine spongiform leukoencephalomyelopathy in Shetland sheepdogs and Australian cattle dogs associated with cytochrome b mitochondrial DNA mutation; and maple syrup urine disease in Hereford, polled Hereford, and polled Shorthorn calves, linked to autosomal recessive mutation causing deficiency in branched-chain alpha-ketoacid decarboxylase complex). Additionally, multiple other presumed heredofamilial conditions causing myelin vacuolation in animals are reported for which specific genetic links are yet to be confirmed (e.g., in horned and polled Hereford calves, Samoyed and Border Terrier puppies, Egyptian Mau and ragdoll cats, and Silver Foxes).[2,4]

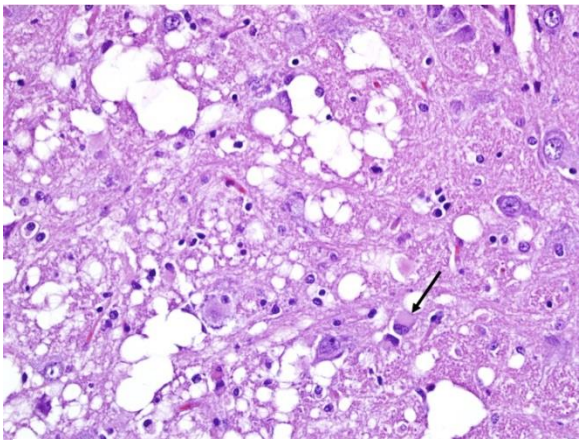
Other reported causes of progressive neurologic signs in hedgehogs include brain tumors (e.g., astrocytoma, microglioma, mixed glioma), intervertebral disk disease, and systemic diseases such as hepatic encephalopathy.[1,4,8] None of these conditions was apparent in this case.

JPC Diagnosis: There were two different sections submitted:

1. Brainstem, white matter: Myelin degeneration, bilaterally symmetrical, severe with neuronal degeneration and gliosis, African pygmy hedgehog (*Atelerix albiventris*), hedgehog.
2. Cerebrum, diencephalon, corona radiata: Myelin degeneration, bilaterally symmetrical, moderate.

Conference Comment: Hedgehogs and gymnures belong to the order *Insectivora* and make up the *Erinaceidae* family. There is very little reported infectious diseases in the African (*Atelerix albiventris*) or European (*Erinaceius europaeus*) hedgehog. Of note, enteritis caused by *Salmonella* sp., pneumonia caused by *Corynebacterium* sp., and upper respiratory disease caused by *Pasteurella* sp. and *Bordetella bronchiseptica* are the most frequent offenders. Additionally, insectivores are potential reservoirs for bloodborne pathogens that are transmitted by parasite vectors such as: *Rickettsia* spp., *Borrelia burgdorferi*, *Babesia microti*, and *Anaplasma phagocytophilum*. Fungal diseases reported include: adiaspiromycosis, cryptococcosis, paecilomycosis, histoplasmosis, and dermatophytosis. Viral diseases are extremely rare, but there have been reported cases of infection with herpes simplex virus 1 in African and European hedgehogs.[3]

Neoplasia is the most common of the noninfectious diseases in hedgehogs with malignant mammary adenocarcinoma, lymphoma, and oral squamous cell carcinoma being most frequent. In African hedgehogs specifically males over one-year-old there is a high incidence of cardiomyopathy which is usually diagnosed late in the disease process at which time it is often fatal.[3]



Brainstem, pygmy hedgehog. Within vacuolated areas, there are rare gemistocytes. (Photo courtesy of: Wildlife Conservation Society , www.wcs.org) (HE, 400X)

Clinical neurologic signs in hedgehogs (incoordination, inability to roll into a ball, seizures, paralysis) are often diagnosed (even in clinical settings) as "wobbly hedgehog syndrome" which is a progressive and potentially hereditary disease that results in myelin degeneration in the central nervous system. An important differential diagnosis is intervertebral disk disease which is much less common but has been reported in hedgehogs.[3]

Within examined sections of cerebral cortex, there are multifocal areas within the superficial cortex and pyriform lob which contain numerous bright red, shrunken neurons which were interpreted by the JPC staff and moderator as acutely necrotic; a minimal glial reaction was present. This change is most consistent with ischemic neuronal necrosis; the necrotic neurons do not appear to be associated with areas of white matter degeneration and may represent a second concurrent disease process.

Contributing Institution:
Wildlife Conservation Society
www.wcs.org

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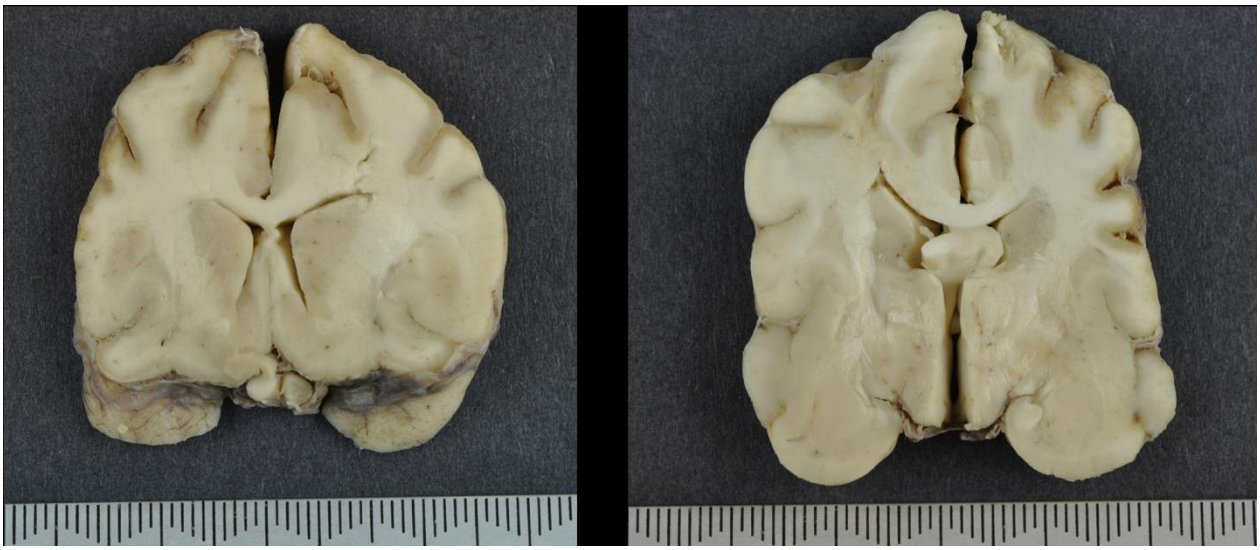
CASE III: C973 (JPC 4020995).

Signalment: 9 year-old male neutered greyhound, *Canis familiaris*, canine.

History: The animal presented with a 2-month history of progressive generalized ataxia and hypermetria in all 4 limbs. Multifocal left forebrain, cerebellar and brainstem signs were observed. Magnetic resonance imaging revealed multifocal white and grey matter lesions, notably worse on the left parietal lobe. Cerebrospinal fluid analysis revealed no significant abnormalities. Serology was positive for *Toxoplasma gondii* at 1:200 and *Neospora caninum* at 1:800. Treatment with steroids and clindamycin was initiated; however the dog deteriorated and was subsequently euthanized. Brain tissue was polymerase chain reaction (PCR) positive for *Neospora caninum*.

Gross Pathology: The brain was submitted for post mortem examination. The cerebral hemispheres appeared slightly asymmetrical. Prosection revealed expansion of the left frontal dorsal white matter. In multiple sections, the dorsal and lateral cerebral cortices were focally thinned and discoloured with a tan to brown appearance.

Laboratory results:
Serology was positive for *Toxoplasma gondii* at 1:200 and *Neospora caninum* at 1:800. Treatment with steroids and clindamycin was initiated; however the dog deteriorated and was subsequently euthanized. Brain tissue was polymerase chain reaction (PCR) positive for *Neospora caninum*.



Cerebrum, dog. Serial sections of the fixed cerebrum demonstrate irregularly but markedly thinned and darkened superficial gray matter in the dorsal and lateral cortex. (Photo courtesy of: The Royal Veterinary College, Hatfield, England)

Microscopic Description: Multiple brain sections revealed a widespread, bilateral necrotizing inflammatory lesion affecting mainly the dorsal and dorsolateral cerebral cortices, characterised by multifocal extensive cerebrocortical necrosis. The necrotic grey matter was replaced by fibrillary gliosis or cavitated and contained many gitter cells, with some slides exhibiting more destructive lesions than others. Multifocal moderate to marked lymphocytic and lesser plasmacytic aggregates were seen within the lepto-meninges and the necrotic cortices, forming variably sized perivascular cuffs. Multiple reactive (proliferating) vessels were observed. The corona radiata in areas appeared degenerate or necrotic with many areas of myelin vacuolation.

Found within and adjacent to the areas of cerebrocortical necrosis and more widely in the brain were abundant round to oval protozoal cysts measuring up to 90 um in diameter with a 2-4 um thick eosinophilic cyst wall enclosing numerous 2-3 um basophilic bradyzoites.

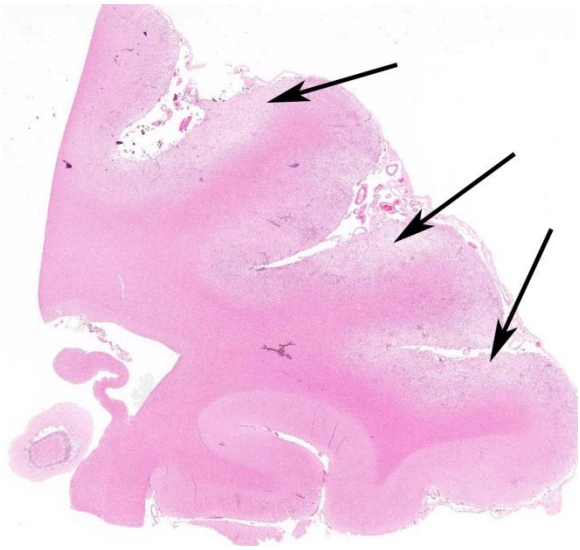
<u>Additional sections</u>

Minimal to milder inflammation and gliosis was observed surrounding the lateral, third and fourth ventricles and deeper tissue and within the mesencephalon. Severe bilateral degeneration characterised by myelin vacuolation, few intramyelinic macro-phages, and gliosis was noted in the crus cerebri and pyramids. A small area of the cerebellar vermis exhibited vacuolation and some disruption of the cortex with mild loss of Purkinje neurons.

Immunohistochemistry: cysts within sections of the cerebrum were positive in stains for *N. caninum*.

Contributor's Morphologic Diagnosis:
Brain, cerebral cortex: Meningoencephalitis, necrotizing, chronic-active, multifocally extensive, severe, with protozoal cysts.

Contributor's Comment: *Neospora caninum* is a cyst-forming coccidian parasite in the phylum Apicomplexa, family Sarcocystiidae and like other coccidians, is an obligate intracellular parasite[12,13].



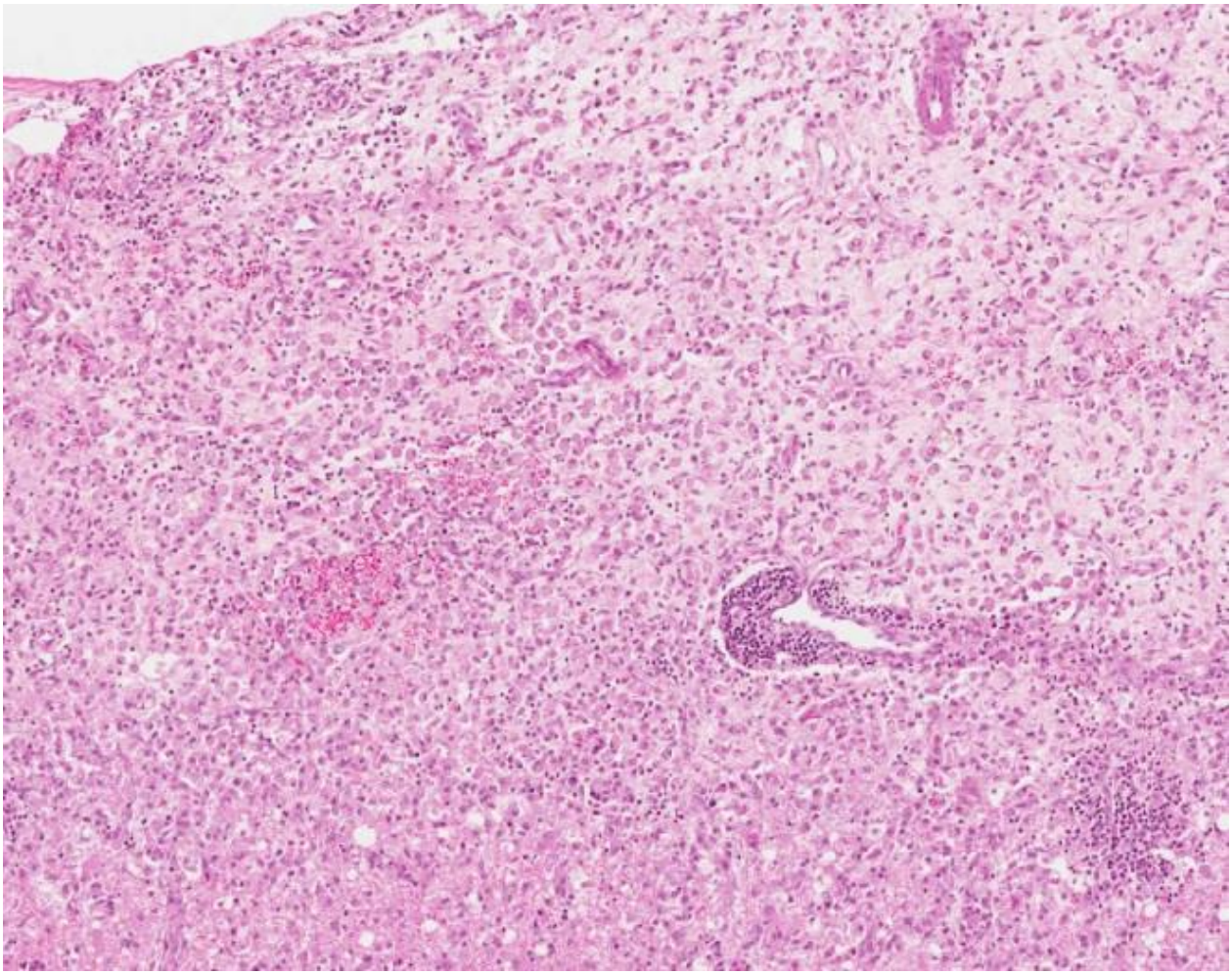
Cerebrum, dog. The superficial grey matter is thin and demonstrates segmental pallor (arrows). 3-3.

Molecular analysis shows that *N. caninum* is closely related to *Toxoplasma gondii*[12]. Both coccidian parasites have proliferative (tachyzoite) and tissue cyst (bradyzoite) phases with tachyzoites proliferating by endodyogeny. Some differences in morphology and life cycle exist; *N. caninum* has a thicker cyst wall and does not develop within a host cell parasitophorous vacuole as does *Toxoplasma gondii*. Differentiation by light microscopy alone is unreliable. Electron microscopy, immunohisto-chemistry and molecular techniques are required for definitive diagnosis[15]. Co-infection with *T. gondii* can theoretically occur and it should be considered as a differential diagnosis[8].

Naturally occurring neosporosis has been reported in a variety of animals including dogs, cats, cattle, sheep, goats, deer, water buffalo, antelope, a rhinoceros and horses (*Neospora hughesi*). In general, neosporosis is primarily a disease of cattle and dogs where the parasite causes abortion and CNS/PNS/muscle disease respectively[5,6,7,15]. The domestic dog is the definitive host for the parasite; however, experimental studies have shown that the Australian dingo and the coyote are also definitive hosts[8,15]. The mechanism of natural infection in dogs is incompletely understood, however ingestion of neural and muscle tissue containing cysts is considered the most likely source of infection[8]. In cattle, *N. caninum* is very efficiently transmitted transplacentally (vertically) but consumption of bovine foetal membranes can also act as a source of infection in dogs[7]. Transplacental transmission in the terminal stages of gestation and post-natal transmission via milk occurs in dogs. Infected dogs produce environmentally resistant oocysts which play an important role in the epidemiology of neosporosis[14]. To date, viable fecal oocysts have been demonstrated only in naturally infected dogs and the gray wolf[8]. The ingestion of sporulated *N.caninum* oocysts from the environment is the natural method of infection in juvenile and adult cattle which are the main intermediate hosts[14].

The *Neospora* lifecycle involves three infectious stages: (1) Oocysts produced in the feces of dogs following ingestion of bradyzoites; (2) The tachyzoite, a rapid multiplying stage which initiates lesion development by multiplying in and rupturing cells; (3) with onset of host immune defense, tachyzoites differentiate into bradyzoites to form tissue cysts mainly in the central nervous system (CNS) and muscle of dogs and in the intermediate host[2].

N. caninum tachyzoites can invade a variety of cell types in many organs including those of the monocyte-macrophage system. The most likely method of spread to the CNS occurs through infected leukocytes crossing the blood-brain barrier. Findings typical of pathogens with endothelial tropism such as vascular swelling and injury, tissue ischemia



Cerebrum, dog. The superficial gray matter is markedly hypercellular, with infiltration of numerous Gitter cells, multifocal hemorrhage, and numerous lymphocytes and plasma cells within Virchow-Robins space. (HE, 69X)

and multifocal infarction are often observed in CNS lesions caused by *N. caninum*[14,15].

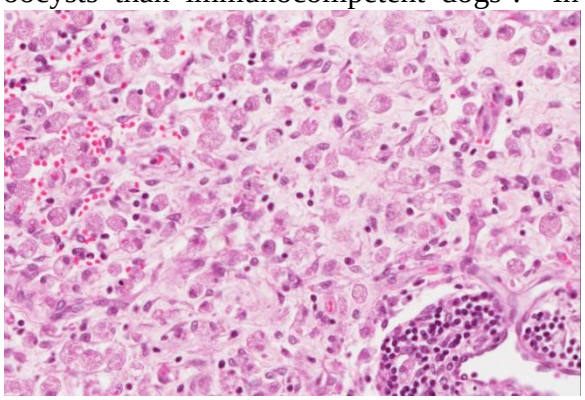
Clinical disease can affect dogs of all ages, however the most severe cases of *N. caninum* typically occur in congenitally infected puppies over 3 weeks of age[8], involve several animals in a litter and present as a progressive ascending neuromuscular paralysis caused by encephalomyelitis, polymyositis and especially polyradiculitis[1]. Neurological signs are dependent on the site that is parasitized and may include such features as; rigidity following muscle denervation and contracture leading to rigid hyperextension of the pelvic limbs, cervical weakness, and dysphagia[1,5]. Pelvic limb contracture is one of the most consistently reported signs in pups[8]. Widespread involvement of the CNS and other organs occurs in adult dogs with signs of disseminated disease including pneumonia, polymyositis, myocarditis, dermatitis, and hepatitis[1,5,15].

Gross CNS lesions can be present throughout the white and/or gray matter with the periventricular white matter being affected in some cases. Peracute lesions

consist of foci of hemorrhage and necrosis adjacent to blood vessels. Chronic lesions have a granular yellow-brown to grey appearance. Early microscopic lesions include tachyzoite infection of and proliferation within endothelial cells, leading to ischemia and necrosis of surrounding neuropil[15]. Later lesions are characterized by nonsuppurative encephalomyelitis and the presence of tachyzoites and tissue cysts in neurons and neuropil with the amount of necrosis, gliosis, neovascularization and white matter injury dependent on the duration of the lesion[1]. More chronic lesions contain prominent lymphocytic and histiocytic perivascular and leptomeningeal aggregates. Over time, due to host immune defense mechanisms tachyzoites change to bradyzoites that replicate more slowly and form tissue cysts[15].

Diagnosis of neosporosis involves histological detection of lesions, immuno-histochemistry illustrating tachyzoite and bradyzoite phases, PCR detection of parasite DNA and serology[8].

Treatment with corticosteroids is con-traindicated in cases of neosporosis[11] as immunosuppressed dogs may shed more oocysts than immunocompetent dogs[7]. In this greyhound, the numerous cysts within the CNS were presumably a consequence of corticosteroid treatment. In studies, dogs that had been given corticosteroids shed more than 100,000 oocysts after being fed with infected murine brains. Various pathological manifestations of neosporosis have been reported in dogs on immuno-suppressive therapy, including cerebellar inflammation and atrophy (which can occur in the absence of steroid treatment), the presence of tachyzoites in cerebrospinal fluid and protozoal hepatitis[9,10,11]. Treatment with currently available drugs including clindamycin is considered only partially effective. None of the currently available drugs are considered capable of killing tissue cysts[8].

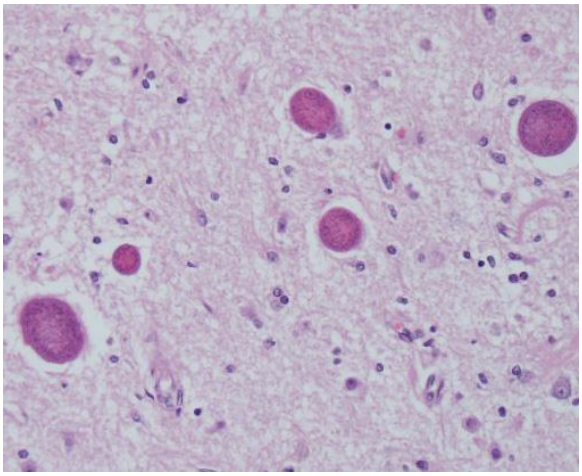


Cerebrum, dog. Higher magnification of the gray matter, showing the large number of Gitter cells within an edematous neuropil with numerous proliferating capillaries lined by hypertrophic endothelium. (HE, 69X)

The predominant cerebrocortical distribution is an unusual neuroanatomic pattern for this infection. We believe that the white matter degeneration within the crus cerebri is secondary following the cerebral cortical necrosis while focal Purkinje cell loss in the midvermis is probably due to brain herniation. While the neocortical inflammation and necrosis in this case is due in large part to the parasites, prolonged seizures may also have contributed to this pattern of necrosis.

JPC Diagnosis: Cerebrum: Meningo-encephalitis, necrotizing, segmental, severe with numerous intracellular and extracellular apicomplexan cysts, Greyhound (*Canis familiaris*), canine.

Conference Comment: The protozoal organisms in the phylum Apicomplexa that result in encephalomyelitis are *Toxoplasma*, *Hammonidia*, *Sarcocystis*, and *Neospora*. Of the four, *Neospora* is the most recent addition. First identified in 1988 by Drs. Dubey, Carpenter, and Speer[4,6] canine

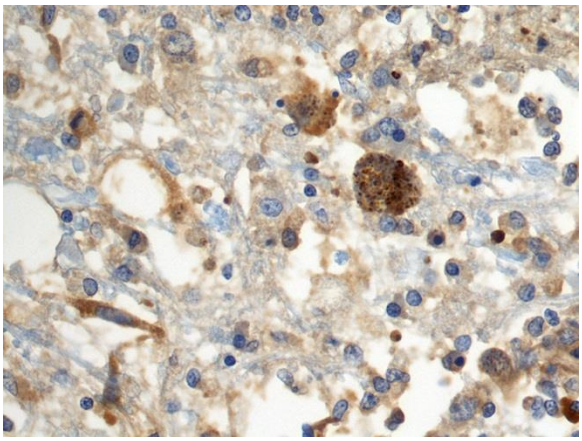


Cerebrum, dog. There are numerous apicomplexan cysts scattered throughout the gray and white matter consistent with Neosporum caninum. (HE, 400X)

protozoan encephalomyelitis was originally thought to be caused by *Toxoplasma.*

Toxoplasma gondii which is still an important differential diagnosis (as mentioned above) uses domestic cats and other Felidae as its definitive host, whereas all other warm-blooded animals can act as intermediate host. Interestingly, cats can also act as intermediate hosts where the parasite continues in an extraintestinal cycle. Infection can occur in one of three ways: (1) ingestion of meat containing tissue cysts, (2) ingestion of food contaminated with cat feces which contain sporulated oocysts, and (3) infection in utero. Transplacental infection is most common in sheep, among domestic animals, where infection begins as a primary placentitis affecting predominately the cotyledons with progressive seeding of the organism to the fetus. Late abortions are common with tissue cysts present in the fetal brain and myocardium. Areas of mineralization may accompany cysts in the brain most likely due to hypoxia from placental insufficiency during pregnancy. Following ingestion of oocysts or tissue cysts by intermediate hosts, the organism forms tissue cysts within the CNS, skeletal and heart muscles most commonly. These cysts may survive for the life of the host and can either: (1) become latent and cause no clinical signs or (2) result in acute, necrotizing disseminated infections affecting CNS, lung, myocardium, liver, pancreas, skeletal muscle, and lymph nodes. The organ affected depends mostly on the host. For instance, in cats severe interstitial pneumonia is common, and in adult dogs, concurrent polymyositis and encephalomyelitis is the most common scenario. In puppies and young dogs, however, polyradiculoneuritis is the most common pattern. In the case of latent infections, tissue cysts may show up as incidental findings on necropsy or, if the animal is immune suppressed, may convert to an active infection. There is an association in dogs with *Morbillivirus* infection and toxoplasmosis, presumably because of the immunosuppressive action of the virus.[13]

Neospora caninum was identified by Dubey and his colleagues in a retrospective study of 23 cases of canine "toxoplasmosis" in which this new organism was distinguishable from *Toxoplasma* only by electron microscopy and immunohistochemistry[4,6]. Ultrastructurally, *Neospora* tachyzoites lack



Cerebrum, dog. Cysts are immunopositive for N. caninum . (anti-N. caninum, 400X)Photo courtesy of: The Royal Veterinary College, Hatfield, England) (HE, 400X)

micropores, but have numerous micronemes and more rhoptries than *Toxoplasma gondii*. Additionally, *Toxoplasma* replicates exclusively in parasitophorous vacuoles, whereas, *Neospora* can use a parasitophorous vacuole or replicate free within the cell cytoplasm[3].

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CASE IV: O291/16 (JPC 4101762).

Signalment: 8 month-old, female, intact, Pug, *Canis familiaris*, canine.

History: The dog had two generalized seizures within a week. On clinical

Cerebrum, dog. There is segmental primarily perivascular hypercellularity of the deep superficial cortex (arrows) at the junction of gray and white matter. (HE, 6X).

examination, the dog presented with abnormal posture and right-sided circling. The dog also had generalized muscle twitching, most notably in the face. Menace response was absent. The dog was subsequently admitted to the animal hospital for intensive care treatment with phenobarbital, glucocorticosteroids and clindamycin, after which it became semi-comatose. As symptoms did not subside, the dog was humanely euthanized.

Gross Pathology: The dog was brought directly to necropsy. No gross findings were evident.

Laboratory results:
Hematology, biochemistry, electrolytes and C-reactive protein (CRP) were within normal reference ranges. Toxoplasma serology was negative.

Microscopic Description: Brain, parietal cortex: In the grey-white matter interface, with predominance in the cortical grey matter and sparing of deep cortical white matter, there are multifocal to diffuse inflammatory lesions, distributed within the neuropil and centered around perivascular spaces (perivascular cuffs). The neuronal parenchyma exhibits moderate to severe vacuolation (rarefaction) with scattered glial cells and vessels outlined by hypertrophic endothelium. Perivascular inflammatory infiltrates are composed primarily of lymphocytes, plasma cells, macrophages and occasional binucleated cells. These cells can also be seen in the leptomeninges and around meningeal vessels.

Multifocally, phagocytic cells engulfing neuronal debris (neuronophagia) are evident (not in all slides), as well as neurons exhibiting pyknotic nuclei, with shrunken cellular outline and hypereosinophilic cytoplasm (neuronal necrosis). Glial nodules and diffuse gliosis can be seen throughout the aforementioned areas, as well as neuronal loss and satellitosis. Clefting of perivascular spaces from surrounding parenchyma is also observed (perivascular edema).

Contributor's Morphologic Diagnosis:
Brain: Polioencephalitis and meningitis, non-purulent and necrotizing, multifocal to coalescing, severe.

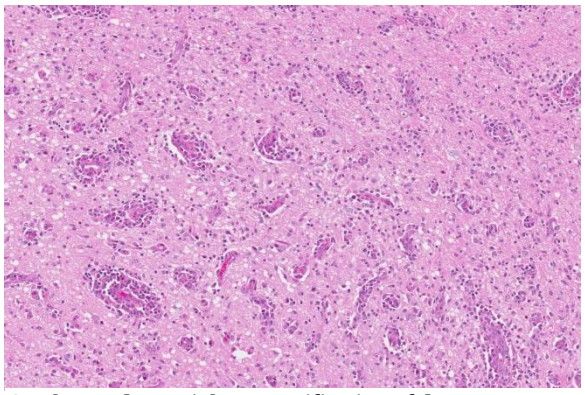
Name the Condition: Necrotizing meningoencephalitis

Contributor's Comment: Necrotizing meningoencephalitis (NME), formerly known as "pug dog encephalitis", is an idiopathic disorder primarily affecting small breed dogs such as Pugs, and less commonly Pekingese,[3] Chihuahua,[8] Maltese,[3] Shih Tzu and other small breed species[4]. Dogs present

with neurological signs at ages ranging between 6 months to 7 years, with a mean age of onset at 29 months.[18] Affected Pug dogs often present at a median age of 18 months, and the disease is most often seen in young females.[9] Occasional reports of NME in large breed dogs exist, but is a rare occurrence.[5] Affected dogs usually present with sudden onset of prosencephalic clinical signs including seizures and depression, often with fatal outcome.[18]

A commonly discussed differential diagnosis of NME is granulomatous meningoencephalomyelitis (GME). GME also affects small breed dogs, such as terriers and toy breeds, but may occur in larger breeds as well, with an age range of 6 months to 12 years.[3]

The anatomical distribution of lesions in NME and GME varies. NME usually affects the cortical grey matter, with relative sparing of deeper periventricular tissues. The lesions are often confluent over large areas, and may be evident grossly (but not always). Multifocal swelling and yellow foci of malacia can be observed bilaterally, but asymmetrically, in the cerebral hemispheres. GME usually produces milder gross changes, but granulomatous foci are occasionally seen.[17] Another variant of idiopathic necrotizing meningoencephalitis is necrotizing leukoencephalitis (NLE). NLE is a condition primarily affecting Yorkshire terriers, Boston terriers and Chihuahuas.[3] As for NLE, malacic foci are observed but, in contrast to NME, are centered in the white matter of the cerebral hemispheres.

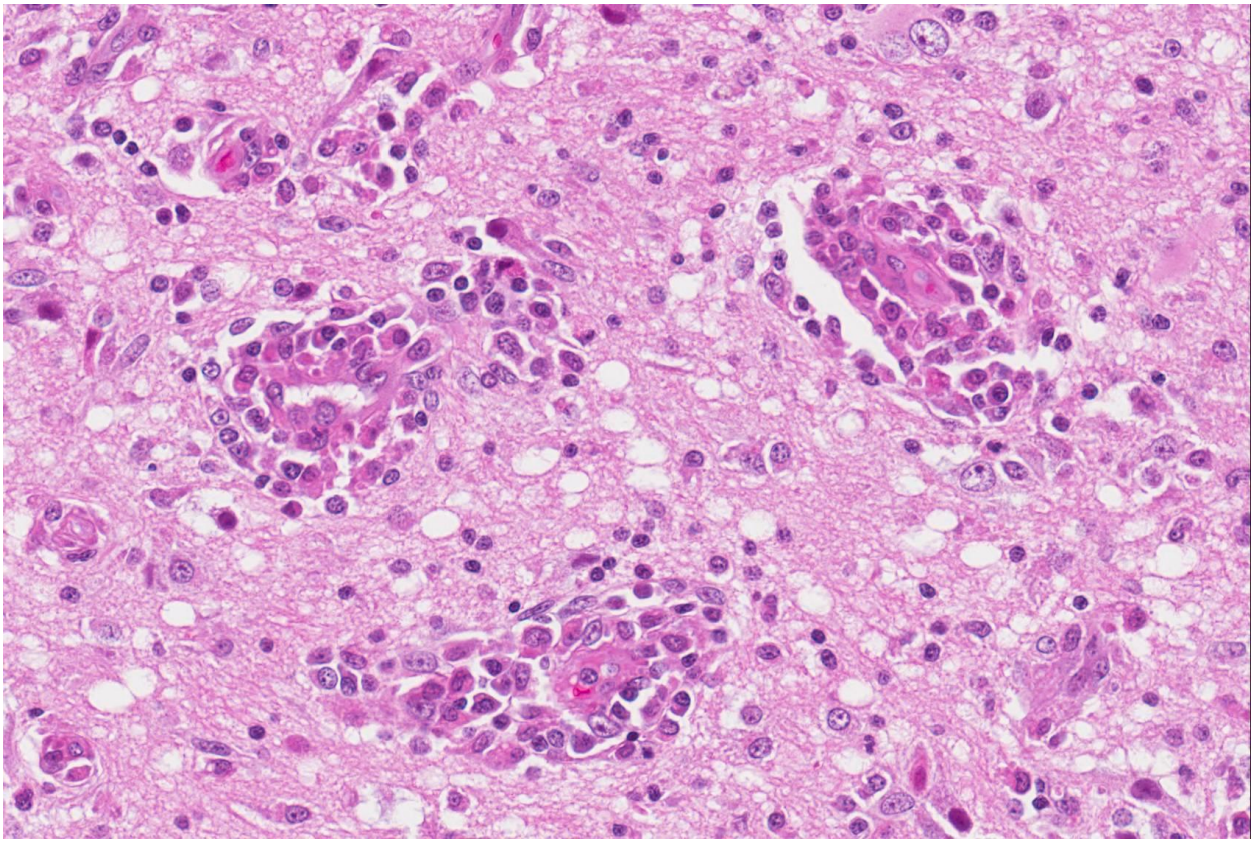


Cerebrum, dog. Higher magnification of deep gray matter with cuffing of small vessels by 2-4 layers of histiocytes, lymphocytes, and fewer neutrophils. Inflammatory cells migrate in low numbers into the adjacent vacuolated neuropil. (HE, 144X).

Histologically, NME primarily affects the cortical grey matter. Areas of rarefaction, vacuolation and neuronal necrosis are significant features of the disease, accompanied by non-purulent inflammatory infiltrates of lymphocytes, plasma cells and macrophages, arranged diffusely and as perivascular cuffs.[3] In contrast, histologic changes in GME are multifocal and patchy, with lesions centered in the basal parts of the brain, particularly the white matter of the brain stem and spinal cord.[18] GME often produces a more cellular infiltrate consisting of numerous macrophages and giant cells, with or without mitotic activity.[3] These infiltrates may be arranged in a granulomatous fashion with formation of epithelioid cells.[17] However, large malacic foci are rarely seen in GME, and for this reason, GME may be hard to distinguish from brain malignant histiocytosis.[3]

Histopathological evaluation is required to distinguish between all subsets of idiopathic meningoencephalitis.[18]

A definitive etiology has not been identified for either NME or GME. For NME and GME, immune-mediated reactions against brain tissue have been suggested, including anti-astrocyte antibodies in NME and GME,[10] and anti-GFAP antibodies specifically in NME patients.[11] GFAP has been found to be one of the more commonly targeted auto-antigens in Pug dogs.[16] According to immunohistochemical studies, a predominance of CD3- positive T-cells has been detected in GME.[12] CD163-positive



Cerebrum, dog. Still higher magnification of deep gray matter. Inflammatory cells around vessels are of normal morphology. There is vacuolation of the intervening neuropil, likely the result of edema and a mild gliosis with occasional hypertrophic astrocytes (arrow) (HE, 400X).

cells (macrophages) can be detected in both NME and GME,[12] but lysozyme immuno-reactive cells are more abundant in NME than GME.[17] However, the distribution of the macrophage cell population varies from diffuse in NME to granulomatous and perivascular in GME.[12] On protein level, a marked increase of IFN-γ and IL-17 has been observed in NME and GME, respectively.[13]

The inheritance pattern of NME has been investigated. Two loci have been identified in NME-affected dogs, one of these being associated with dog leukocyte antigen class II.[2, 7] Common genetic backgrounds have been proposed in dogs with NME regardless of breed. However, the implication of these traits may differ between dog breeds.[15] In Pug dogs specifically, a strong familial inheritance pattern has been demonstrated.[6]

Infectious etiologies for these encephalitides have also been discussed. According to one study, *Mycoplasma canis* has been identified in some cases of NME and GME (4/25 and 1/25 respectively).[1] However, the role of *Mycoplasma* in the pathogenesis of these diseases remains uncertain. Regarding viral causes, alpha-herpesviruses are proposed as possible agents.[3] However, no association between NME and viral pathogens such as herpes-, adeno- or parvoviruses has been found in many studies, including one pertaining to approximately 5000 studied individuals.[1,6,14]

JPC Diagnosis: Cerebrum: Meningo-encephalitis, lymphohistiocytic, multifocal

to coalescing, moderate with edema, neuronal loss, and astrocyte hypertrophy, Pug (*Canis familiaris*), canine.

Conference Comment: Necrotizing meningoencephalitis (NME, fully described above) results in malacia of the cerebral cortex and mononuclear cell infiltration in the meninges and perivascular spaces that is bilateral but asymmetrical and mostly affecting the gray matter. The main differential, granulomatous meningoencephalitis (GME), is characterized by histiocytic inflammation predominately within the cerebral white matter that can form granulomas with chronicity.[3]

Both NME and GME have currently unidentified causes and are active areas of research. Many recent works are detailed above, and a recent study in pug dogs reviewed individuals that had undergone extrahepatic portosystemic shunt attenuation surgery to identify if there were increased postoperative neurologic complications, including NME. Only four dogs were necropsied, none of which had evidence of NME.[20]

In this case, attendees were not certain that this represented a case of NME as no definitive areas of necrosis were present in the submitted slide. Given the lack of demonstrable parenchymal necrosis and the extent of concurrent white matter lesions, conference participants discussed the possibility that this may actually case represent a case of GME. Another topic of discussion not mentioned by the contributor is the presence of numerous clustered hypertrophic astrocytes clustered which demonstrated large vesicular nuclei and abundant pink cytoplasm. Participants discussed whether the term "Alzheimer type 2 astrocytes" was appropriate in this case, a term has been traditionally reserved for reactive astrocytes in hepatic encephalopathies which are induced by accumulation of ammonia and other endogenous toxins, or cases involving cerebral amyloid.[3] Preferring to be conservative in such matters which are known to enflame true neuropathologists, the "hypertrophic astrocytes" was settled upon in this case.

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