The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
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CONFERENCE 22
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CASE I – 01-5598 (AFIP 2788841)

Signalment: Three-year-old, male, Siamese cat, Feline

History: This cat was normal when acquired in December of 1999, with the exception of an old wound on the dorsum. In August of 2000 the cat started chewing at its tail head and seemed painful. A skin biopsy revealed superficial dermatitis. Seizure-like activity began in October 2000. Clinical pathology revealed leukopenia and a low BUN. Treatment with phenobarbitol was initiated, but by the end of October 2000, the cat had altered mentation, lost balance, seemed blind (ran into walls) and had a loss of appetite. In November 2000, serology was negative for toxoplasma (titer < 1:8), and negative for FIP in February 2000. The cat was euthanatized in March 2001 because of excessive seizures.

Gross Pathology: No remarkable findings apart from subjective widening of the sulci and relative narrowing of the gyri.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Brain, cerebrum and hippocampus, neurons and glia: Eosinophilic intracytoplasmic inclusions, with neuronal degeneration, diffuse cerebrocortical atrophy, and astrocytic hypertrophy/hyperplasia.

Contributor's Comment: Neuronal feline ceroid-lipofuscinosis is a rare entity that had been documented in Siamese cats, a cat from Japan, and a Domestic shorthair cat from Canada. The exact etiopathogenesis of feline ceroid-lipofuscinosis remains undetermined. Ceroid-lipofuscinosis in other species is inherited, as are most other lysosomal storage diseases, which usually are transmitted as autosomal recessive traits. Although the genetic lineage of this cat is unknown, inbreeding of

barn cats would be likely to result in increase of recessive traits. Clinically, ceroid-lipofuscinosis results in severe progressive neurologic disease. Clinical signs include blindness, seizures, and decreased mentation. Both eyes of this cat showed severe diffuse retinal atrophy and degeneration (slides not submitted). The pigment autofluoresced intensely when unstained paraffin sections were examined under the fluorescent microscope. Staining characteristics were consistent with those published by Bildfell et al., 1995. The pigment granules stained pink with Periodic acid-Schiff (PAS), dark blue with Luxol (LFB), and black with Sudan black B (SB), but were negative with Acid fast (AF) stains.

Microscopically, there is diffuse, mild degree of cerebrocortical atrophy characterized by thinning of the gray matter and a relative decrease in the number of cortical neurons when compared with cerebrum from another cat approximately the same age. Neurons throughout the brain, especially in the cerebrum, contained various amounts of granular to globular cytoplasmic granules that ranged from 2-8 um in diameter. The pigment granules varied from light brown to bright red with H & E stain. Comparable pigment granules were found within glial cells and astrocytes or free in the neuropil (hippocampus). A reactive astrocyosis is markedly present in the cerebral cortex. Microgliosis was multifocally prominent within the cerebral cortex and hippocampus.

AFIP Diagnosis: Cerebrum and hippocampus at the level of the occipital lobe: Neocortical atrophy, diffuse, moderate, with neuronal loss, astrocytosis, and abundant intraneuronal pale yellow to eosinophilic granular material, Siamese, feline.

Conference Comment: Neuronal ceroid lipofuscinoses are a group of inherited conditions characterized by the accumulation of autofluorescent lipopigments that histologically resemble, and stain similarly to, ceroid and lipofuscin. The condition is described in dogs, cats, cattle, sheep, goats, horses, mice, nonhuman primates, and humans. In many, but not all of these conditions, the primary material stored is subunit c of mitochondrial ATP synthase. While the exact neurodegenerative process is unclear, it is suggested that the primary defect lies within the mitochondria, rather than a defect in lysosomal proteolysis. Subunit c protein, which is highly hydrophobic, has a propensity to bind lipids and form para-crystalline complexes, rendering it refractory to catabolism. Its accumulation is well described in English Setter dogs, South Hampshire sheep, and characterizes late infantile, juvenile, and adult onset forms in humans.

A second group of ceroid lipofuscinoses is described in which the primary materials accumulated are sphingolipid activating proteins A and D, rather than subunit c. The likely defect is a deficiency in the lysosomal enzyme palmitoyl protein thioesterase. This form occurs congenitally in Swedish Landrace lambs and in Miniature Schnauzer dogs as an adult onset.

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CASE II – WN01/1351 (AFIP 2789368)

Signalment: 7-month-gestation calf, female, Angus, *Bos taurus*, bovine.

History: Owner found a premature calf in a paddock of first calf heifers. The calf died shortly before necropsy examination.

Gross Pathology: No abnormalities detected.

Laboratory Results:
Stomach/aerobic culture: Mixed flora (not significant).
Stomach/*Campylobacter* sp. culture: Negative.
Pericardial fluid: 345 ug IgG/ml (> 121 ug IgG/ml is elevated. This finding is consistent with antigenic stimulation in utero).
Simbu group ELISA: Positive. The Simbu ELISA is a test for the Simbu group of viruses which includes Akabane, Aino, Peaton, Douglas, Tinaroo, Thirmi, and Facey's Paddock viruses.

Contributor's Morphologic Diagnosis: Encephalitis, nonsuppurative, moderate, with focal malacia: caudal brainstem.

Akabane disease.

Contributor's Comment: In the caudal midbrain,and medulla extending to the obex, there is extensive mild cuffing of medium-sized vessels by lymphocytes cells, occasionally associated with localised areas of neuropil disruption and malacia.

Akabane virus is a member of the Simbu group within the Bunyaviridae, and is a teratogenic arbovirus, that causes outbreaks of congenital arthrogryposis and hydranencephaly in cattle in Australia. There is serological evidence of Akabane infection in cattle in Africa, the Middle East and Southeast Asia, but clinical disease is confined to the fetus. When a susceptible pregnant female is infected the outcome of the resulting fetal infection depends on the stage of gestation: encephalomyelitis (173 days-full term), arthrogryposis (104-173 days) and hydranencephaly (76-104 days). The virus is neurotropic in the fetus (arthrogryposis is secondary to neurological disease limiting limb movement in utero).

Culicoides brevitarsis, the highly efficient vector of Akabane virus, is distributed in the northern half of Australia, and outbreaks of Akabane disease occur when non-immune pregnant cattle are introduced from southern areas to the northern *C. brevitarsis*-endemic areas. Also, in drought times the distribution of *C. brevitarsis* contracts to the north-east, and female cattle born in the outer parts of the conventional endemic *C. brevitarsis* area may not experience their usual protective natural infection before reproductive age. With the return of favourable climatic conditions and the vector to the endemic area, the population of non-immune, locally bred, pregnant cattle are susceptible to Akabane infection and an outbreak of congenital Akabane disease.

AFIP Diagnosis: Cerebellum and pons: Encephalitis, nonsuppurative, multifocal, mild to moderate, with myelin sheath swelling, axonal degeneration, intramyelinic macrophages, and gliosis, Angus (*Bos taurus*), bovine.

Conference Comment: Fetal malformations due to Akabane virus follow infection of naive pregnant animals early in the gestation period. Virus spreads hematogenously to placental trophoblastic cells where it persists and replicates. Displaying a tropism for rapidly dividing cells, the virus invades the fetus infecting cells of the central nervous system. Destruction of germinal cells within the brain results in the characteristic porencephaly and hydranencephaly. Cerebellar hypoplasia can be a subtle finding while no degenerative changes are typically found in the midbrain, brainstem, or spinal cord.

Denervation atrophy of muscles secondary to lower motor neuron degeneration results in dysplastic muscular changes that were at one time tentatively termed runt-muscle disease. Grossly skeletal muscles are poorly developed and undergo contraction fixing the joints in flexion. Histologically muscle fibers appear as single cells and lack cross striations. Adipose tissue is increased and in some cases appears to replace muscle bundles. Associated deformities include torticollis, scoliosis, and kyphosis.

Conference participants commented on the presence of what appeared to be rare protozoal organisms within the neural parenchyma and discussed the possibility of a coinfection. Additional diagnostics would be of interest in this case.

Contributor: Regional Veterinary Laboratory, NSW Agriculture, Wollongbar NSW Australia 2477

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CASE III – D01-110 (AFIP 2790774)

Signalment: 1-year-old, female, Quarter Horse, Equine

History: This filly had been purchased with a history of "trauma to the back" prior to purchase. It was loaded on to a trailer and taken to its new owner apparently normal, but once unloaded and in the barn it became spastic in all four limbs and went down. Spastic paresis was worse in the hindlimbs and remained severe over a period of 12 days during which the filly was frequently down. Also during this time it was treated with banamine and antibiotics for a seroma on the right hind limb. On the 11th day it developed a rectal prolapse. On the 12th day, the rectum

was reportedly torn during attempts to reduce the prolapse and the owner opted for euthanasia.

Gross Pathology: At necropsy a mucosal rectal prolapse was present but no tear was found, and there was no obvious underlying cause for the prolapse. No gross lesion to account for tetraparesis was evident in the cranium, brain, vertebral column, spinal canal or spinal cord.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Spinal cord – Chronic diffuse symmetrical myelopathy with axonal degeneration and extensive astrogliosis, and mild to moderate axonal dystropy.

Consistent with equine degenerative myeloencephalopathy

Contributor's Comment: Histologic changes were extensive in the spinal cord and caudal brainstem. In the spinal cord the most striking change is axonal and myelin loss, most intense around the periphery of the cord, and with marked reactive astrogliosis. There are also scattered axons in the process of degeneration and fragmentation with associated reactive macrophages (Wallerian-like degeneration), and occasional slate gray spherical swollen axonal segments. These changes are particularly severe in the dorso-lateral (spino-cerebellar) tracts and somewhat less so in lateral and ventral sulco-marginal tracts. In the spinal grey matter there are variably numerous eosinophilic axonal spheroids ranging in size from approximately 30um to greater than 100um in diameter, which when large are often vacuolated or have variably dispersed skein-like contents. Unfortunately these are not present in every section. These spheroids appear to occur most frequently in the zones immediately lateral to the central canal. In addition, occasional chromatolytic neurons can be found. The spinal cord changes were equally severe throughout thoracic and lumbar segments, but somewhat less severe in the cervical segment.

In the caudal brainstem axonal spheroids were numerous bilaterally in several locations, including the gracilis, cuneate, accessory cuneate nuclei (few), the medial vestibular nuclei (many), and in the most rostral portions of the fasciculus gracilis and cuneatus (many). In addition scattered degenerating axons were evident throughout the reticular formation.

A major differential diagnosis in this animal was some form of focal compressive cervical myelopathy, but the pattern and nature of the lesions is consistent with a diagnosis of equine degenerative myeloencephalopathy (EDM), and represents ongoing diffuse degenerative changes over a long period. In this case, advanced and irreversible changes are well in evidence. Not conspicuous in

this case was lipofuscinosis, which is reported to be a frequent feature of the pathologic changes.

Although clinical signs are usually expressed in the first two years of life, and as early as 6 months of age, they may not appear until later in rare cases. The syndrome is seen in many pure and mixed breeds, and is also recorded in the zebra and Mongolian wild horse. The pathogenesis of EDM has yet to be entirely clarified since it was first reported in the 1970s, and it remains a morphologic diagnosis with a number of contributory etiologic factors.

A role for Vitamin E is well established by several criteria, along with evidence for some genetic predisposition possibly linked to requirement for Vitamin E. Supplementation of pregnant mares and new born foals with Vitamin E can prevent the disease in predisposed families, and can partially or totally reverse clinical disease when administered early.

At Cornell several cases have been seen in which there was an overlap pathologically between EDM and Motor Neuron Disease (EMND), which typically affects horses greater than two years of age. The overlap occurred in horses 2 years old or less, and the clinical signs were consistent with EDM in three cases, and with EMND in one case (T.J. Divers – personal communication). However in general there has been no clear connection between the two diseases, despite the demonstrated role of Vitamin E in both.

A possible association with environmental toxicants or drugs has also been suggested. In the US the incidence of EDM may have (anecdotally) declined over the past decade.

AFIP Diagnosis: Spinal cord, thoracic, lateral and ventral funiculi: Axonal loss and astrocytosis, subpial, bilaterally symmetrical, moderate to severe, with dorsal spinal cerebellar nucleus spheroids, Quarter Horse, equine.

Conference Comment: The differential diagnosis discussed in conference included conditions with clinical presentations similar to that of equine degenerative myeloencephalopathy (EDM). These include cervical stenotic myelopathy (CSM), equine protozoal myeloencephalitis (EPM), and equine herpesvirus 1 infection (EHV1).

Cervical stenotic myelopathy is the result of stenosis of the cervical spinal canal with compression of the spinal cord. The condition is further subdivided into two syndromes based on the pathogenesis. Cervical static stenosis typically affects the C5-C7 region of young adult horses and is the result of acquired narrowing of the spinal canal due to progressive osteosclerosis of the dorsal laminae and the deposition of fibrovascular tissue in the ligamenta flava. Cervical

vertebral instability typically affects the C3-C5 region of young horses and is the result of a functional narrowing of the spinal canal upon flexion of the neck. Clinical signs are generally abrupt in onset with a progressive course. Lesions depend on the suddenness and degree of compression with severe compression resulting in necrosis and loss of parenchymal architecture affecting gray matter, white matter, or both. Rostral to th

History: In the 4 to 5-month-old white-faced lambs involved in this case, the clinical history involved approximately 50 lambs that were "showing signs of depression, severe weight loss, and will only eat the weeds that are involved. Sheep seem to have an addiction...and it seems to be worse in the fall when the weeds green back up." Gross necropsy lesions in two lambs examined were limited to evidence of emaciation, such as an absence of depot fat in one lamb and serous atrophy of fat in a second lamb.

Gross Pathology: No gross lesions other than severe emaciation.

Laboratory Results: Not available.

Contributor's Morphologic Diagnosis: Brain: mild to moderate multifocal neuronal cytoplasmic vacuolation (conference participants may have sections of cerebrum, cerebellum, or medulla oblongata).

Etiology: Swainsonine toxicity.

Disease: Locoism.

Contributor's Comment: Microscopically in each of the two lambs examined, there was foamy cytoplasmic vacuolation in multiple neurons and renal cortical tubule epithelial cells, resulting in a diagnosis of locoism. (In some sections of brain tissue, autolysis has resulted in "rupture" of the swollen neurons, resulting in a more foamy histologic appearance.)

Locoweed poisoning costs the animal industry millions of dollars worldwide and occurs when livestock graze certain species of *Astragalus* or *Oxytropis* containing swainsonine. Swainsonine (an indolizidine alkaloid) inhibits lysosomal alpha-mannosidase, resulting in a lysosomal storage disease. Swainsonine also inhibits Golgi mannosidase II, which is involved in N-linked glycoprotein processing. Inhibition of the Golgi mannosidase II results in abnormal glycosylation and, therefore, processing, of many oligosaccharides. Changes occur in cellular adhesion and receptor-ligand interactions such as altered excretion of intestinal and pancreatic enzymes.

Because swainsonine has been shown to cause changes in antigen processing and a variety of lymphocyte functions, toxicity may result in increased susceptibility to other diseases. While short-term exposure has been reported to have minimal ill effects, exposure long-term results in permanent neurologic damage.

Clinical signs of poisoning include: nervousness, proprioceptive deficits, intention tremors, seizures, muscle weakness, infertility/abortion, emaciation, depression, decreased weight gain concurrent with decreased dietary intake and anorexia, cardiovascular disease, and death. In experiments involving the feeding of locoweed, rams developed transient degeneration of the seminiferous tubules, the epithelium of the epididymus, and the vas deferens. Reduced production of significantly more abnormal sperm were produced concurrent with locoweed ingestion. In another experimental setting, there was an increased incidence of brisket disease/high mountain disease, resulting in right-sided heart failure in calves fed *Oxytropis sericea* or swainsonine.

Swainsonine is rapidly absorbed, leading to a high serum concentration of swainsonine and inhibition of serum alpha-mannosidase activity. Diagnosis of swainsonine intoxication has, until recently, been limited to detection of altered urinary oligosaccharides or by assaying alpha-mannosidase activity in leukocytes. A recent study has found that the t1/2 of serum/blood swainsonine clearance is short (approximately 20 hours) thus preventing measurement of serum/blood swainsonine from becoming a useful diagnostic testing method. Within a few days of being removed from the source, animals have low or undetectable concentration levels of swainsonine in their serum, even if permanent neurologic damage has occurred. Tissue levels in liver and kidney remain elevated for a longer period of time in sheep, with a tissue concentration half-life (t1/2) of 60 hours and 51 hours, respectively.

Diagnosis is confirmed by the characteristic histopathologic changes which include vacuolar degeneration of neurons and parenchymal cells such as kidney tubular epithelial cells, interstitial cells and pericytes of the myocardium (in rats), exocrine pancreas, thyroid follicular epithelium, Purkinje cells of the cerebellum, Kupffer cells, macrophages in the spleen and lymph nodes, and urinary bladder transitional cell epithelium. It is hypothesized that GABAnergic neurons have an increased susceptibility to swainsonine. In rats it has been shown that the swainsonine-induced vacuoles contain mannose-rich oligosaccharides.

AFIP Diagnosis: Brain: Neuronal degeneration and swelling, diffuse, moderate to severe, with abundant confluent clear cytoplasmic vacuoles, breed not specified, ovine.

Conference Comment: The contributor has provided a concise review of this entity.

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