CASE I – 4281 (AFIP 2886859)

Signalment: 6-year-old neutered-female Visla, canine, *Canis familiaris*.

History: The dog began having abnormal head movements 9 months previously, with noticeable neck stiffness, ataxia and circling noticed for 5 months before death. Over the 3 months prior to death, the animal developed whole body tremors, ataxia affecting all four limbs, and a reduced menace response. The animal was euthanized after treatment with corticosteroids did not result in improvement.

Gross Pathology: The entire fixed brain and fixed specimens from spleen, lung, liver, duodenum, pancreas and skeletal muscle were submitted for examination. Grossly, the brain was characterized by irregular, moderately symmetrical, demarcated regions in the periventricular white matter that were discolored gray-tan. (Figures 1 and 2) These extended slightly more forward in the left cerebral hemisphere, compared to the right side. Similar foci were present in the brainstem, pons and medulla. The specimen of spleen was thickened and contained numerous coalescing gray-tan foci.

Laboratory Results: Cisternal CSF contained 86 mg/dl protein and was Pandy positive, but the leukocyte count was not increased. Serologic tests for *Ehrlichia canis*, *Ehrlichia equi*, *Ehrlichia risticii*, *Rickettsia rickettsia*, *Toxoplasma*, *Neospora* and *Cryptococcus* antibodies were negative. Canine distemper IgM antibody was not found. MRI demonstrated contrast-enhancing multifocal periventricular foci,
but little alteration was evident on T1 or T2 non-contrast imaging. Fungal isolations were not done.

**Contributor’s Morphologic Diagnosis:** Multifocal to coalescing granulomatous encephalitis, predominantly periventricular, with fungal organisms.

**Contributor’s Comment:** Clusters of well-defined inflammation were found in the periventricular regions of the diencephalon and at other sites. Each consisted of a central region of debris in which fungal organisms were generally numerous. This core is surrounded by an inflammatory response that is dominated by macrophages and multinucleate giant cells. Perivascular cuffs away from the granulomas contain lymphocytes and plasma cells. Neutrophils and large areas of necrosis are not evident. Both slender, unbranching fungal pseudohyphae with parallel walls that are 3-5um in width and occasional yeast forms up to approximately 10um diameter are present, and one form dominated some of the lesions. Apparently viable multinucleate giant cells within granulomas frequently contained fungi. Organisms were better detected with PAS stain than H&E, and sections were stained with PAS. Similar organisms were found in numerous granulomas in the spleen and lung. The type of reaction and presence of pseudohyphae and yeasts is suggestive of Candida sp.

Although localized infection with Candida has been noted in dogs, only a few cases of generalized infection have been reported. Tissue from one of these cases contained many organisms in granulomas, while in the other, organisms were not observed in lesions. Brain involvement was mentioned in neither case, although it is thought to occur in dogs. Candida albicans readily causes meningitis in dogs when experimentally injected into the brain, and was lethal to neutrophil-depleted animals.

In contrast to the situation in dogs, CNS involvement is very common in human systemic candidiasis, itself a fairly common condition, and usually occurs in the context of systemic immunosuppression, hyperalimentation, or extreme prematurity. When encephalitis occurs, it is usually multifocal and bilateral, although neutrophils are more numerous in people than in this dog. Occasional cases of unilateral brain vasculitis have been found in the context of localized skin infection. This dog was unusual in that no underlying cause of immunosuppression was noted clinically, except for a short course of corticosteroids some months prior to death, after the onset of clinical signs. The disease affected multiple organs. In contrast to human cases, neutrophils are uncommon in lesions, and many of the surrounding macrophages appear viable. In vitro studies suggest that Candida is capable of intracellular growth or penetration of human cerebrovascular endothelial cells and can do so without killing the cell or perturbing the electrical resistance of the endothelial monolayer.
AFIP Diagnosis: Cerebrum: Encephalitis, granulomatous, multifocal to coalescing, marked, with fungi, Visla, canine.

Conference Comment: Candida sp. is a dimorphic fungus, of which the yeast phase is a normal inhabitant of alimentary, upper respiratory, and genital mucosal surfaces of animals. They reproduce by budding and proliferate as blastoconidia (budding yeast-like cells), or are present as pseudohyphae (segmentally constricted at points of attachment of individual yeasts) and branched, septate hyphae. Candida sp. may cause infection in young or debilitated animals, or as a complication of antibiotic therapy. Candida albicans is the most commonly isolated species.9,10,11

In general, candidiasis of the oral cavity (thrush) is the most frequent manifestation of infection in mammals, and is characterized by a gray-green pseudomembrane over an intact mucosal surface.10

In piglets, candida most often invades the squamous mucosa of the stomach, usually along with the oral cavity and esophagus. In calves, lesions are seen most commonly in the rumen, but may also involve the omasum, reticulum, and abomasum. In foals, candidiasis most often involves the esophagus and squamous epithelium of the stomach, with ulceration adjacent to the margo plicatus.13

In birds, candida affects the mucosal surfaces of the mouth, esophagus, crop, and proventriculus. The characteristic lesions are raised white mucosal plaques with a catarrhal or mucoid exudate.12

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References:
CASE II – 03-256 (AFIP 2888698)

Signalment: 5-month-old Angus heifer.

History: The calf was found in lateral recumbency and a neurologic examination revealed tetraparesis, no response to deep pain in the left hind limb, and absence of the panniculus muscle reflex caudal to T3/T4. The calf was euthanatized.

Gross Pathology: A 6 cm section of spinal cord between T2 and T3 had focally extensive softening and gray to brown, streaking discoloration of the right lateral and ventral portions of the cord (Fig. 1).

Laboratory Results: None reported.
Contributor's Morphologic Diagnosis: Myelomalacia with intravascular fibrocartilaginous emboli, spinal cord.

Contributor's Comment: This is a case of fibrocartilaginous emboli causing infarction of the spinal cord and has not been reported in the bovine. The spinal cord has extensive necrosis of gray and white matter with hemorrhage. Areas of white matter degeneration with axonal swelling and loss and macrophage accumulation surround the necrotic area (Fig. 2). Occasional blood vessels in the meninges and parenchyma are occluded by homogeneous lavender to basophilic material (Fig. 3). This material stains positive with alcian blue (Fig. 4) and a von Kossa stain (Fig. 5) and is consistent with intervertebral disk material. The affected vessels have fibrinoid necrosis and neutrophil inflammation within the wall.

The pathogenesis of fibrocartilaginous emboli within the vasculature of the spinal cord is uncertain and five mechanisms have been proposed. These are 1) dissection of the nucleus pulposus through a degenerate dorsal annulus fibrosis into areas of neovascularization associated with the degenerate annulus; 2) direct penetration of the spinal arteries or veins following rupture of the nucleus pulposus through the dorsal annulus fibrosis; 3) herniation of disk material through the vertebral end plate into the marrow cavity and venous sinuses; 4) herniation of disk material into persistent embryonal vasculature of the annulus fibrosis; and 5) herniation into anomalous vasculature or arteriovenous fistulae. Trauma is probably a factor in several of these mechanisms.

Fibrocartilaginous emboli occur most commonly in large breed dogs that are predisposed to type II intervertebral disk disease. Extrusion of the nucleus pulposus through a degenerating annulus fibrosis is the most likely mechanism of embolism in these cases. The disease has not been reported in the bovine and the mechanism in this case is more likely to be extrusion of nucleus into anomalous vasculature.

AFIP Diagnosis: Spinal cord, ventral gray and white matter: Infarct, focally extensive, with fibrocartilaginous emboli, Angus, bovine.

Conference Comment: Fibrocartilaginous embolism has been described in dogs, pigs, horses, cats, sheep, and humans. As the contributor mentions, the origin of the embolus is most likely the nucleus pulposus, but the annulus fibrosis and vertebral growth plate cartilage have also been suggested as sources for emboli. The contributor gives a concise review of the proposed pathogenesis of this disease.
This disease is described in large breed dogs but not in chondrodystrophic breeds, which are most prone to develop intervertebral disk prolapse. No age or sex predisposition has been identified in dogs. In one report in pigs, genetic, performance, and behavioral characteristics were believed to be predisposing influences. These pigs had heavier market weights than usual and were excitable and prone to sudden movements with vigorous muscle contraction, all which are believed to contribute to increased pressure on intervertebral disks.

In affected animals, the involved spinal cord segments are often brown-red and soft. Both gray and white matter may be affected, and the lesions are commonly asymmetrical. Microscopically, the diagnostic finding is occlusion of blood vessel(s) by an embolus of fibrocartilage. This appears grayish on H&E stain, magenta with PAS, tan with PTAH, and blue with Alcian blue stain. Since there is abundant collateral circulation to the spinal cord, numerous vessels must be occluded to produce an infarct. If some blood flow continues to the affected area, the infarct will become hemorrhagic.

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References:

CASE III – 02-7876 (AFIP 2889973)

Signalment: 11-26 days of age mixed gender lambs (ovine).

History: Nursing lambs were being found dead with no clinical signs observed.

Gross Pathology: No lesions were found in 3 of 4 lambs submitted for necropsy. A fourth lamb had approximately 10 ml of clear pericardial fluid.
Laboratory Results: None reported.

Contributor’s Morphologic Diagnosis: Necrosis and edema, brainstem and cerebellar peduncles, multifocally extensive (encephalomalacia).

Contributor’s Comment: Microscopic lesions in these brain sections consist of multifocal areas of neuronal necrosis and rarefaction (edema) in the neuropil. Most of the slides submitted with this case are from only one half of the brainstem, however the lesions were bilaterally symmetrical. The areas affected were primarily in the anterior brainstem and cerebellar peduncles.

These microscopic brain lesions are diagnostic for focal symmetrical encephalomalacia, which in the literature is described as a chronic neurological manifestation of enterotoxemia. Focal symmetrical encephalomalacia has been produced in experimental enterotoxemia by infusion of epsilon toxin of *Clostridium perfringens* type D. While this is referred to as a chronic manifestation of enterotoxemia, the clinical syndrome in these lambs was peracute, with lambs found dead with no clinical signs observed.

The course of enterotoxemia is usually very short, often less than 2 hours and never more than 12 hours. Lambs are often found dead, although some individuals may show clonic convulsions prior to death. Lambs that survive for a few hours may have green, pasty diarrhea, staggering, recumbency, opisthotonos, and convulsions.

The disease can be reproduced experimentally by injection into the duodenum of whole culture of *C. perfringens* type D, or by intravenous infusion of epsilon toxin. *Clostridium perfringens* type D normally inhabits the alimentary tract of sheep, but only in small numbers. If there is passage of large quantities of starch granules into the duodenum when sheep overeat, or consume large quantities of milk, organisms can multiply and toxin production proceeds to the point where toxemia occurs. Lambs on well-fed, heavy-milking ewes are particularly susceptible.

The epsilon toxin increases the permeability of the intestinal mucosa to this and other toxins, thereby facilitating its own absorption. A receptor for epsilon toxin has been identified on vascular endothelial cells, with a result of vascular damage and increased vascular permeability. Vascular damage results in perivascular and intercellular edema in the basal ganglia, thalamus, internal capsule, substantia nigra, subcortical white matter, and cerebellum. Protein-rich fluid accumulations also occur in the pericardial sac and lung.
Hemoconcentration and hyperglycemia occur in enterotoxemia. The increase in blood glucose is proposed to be caused by mobilization of hepatic glycogen by hepatocyte-bound epsilon toxin, or release of catecholamines due to stimulation of the sympathetic division of the autonomic nervous system because of brain edema. There may be profound hyperglycemia and glucosuria in affected lambs.

Other histologic changes of enterotoxemia include degeneration and necrosis of the epithelium of the proximal convoluted tubules in the kidney, secondary to endothelial damage by the epsilon toxin.

Diagnosis of enterotoxemia can be challenging, as gross and microscopic lesions often are minimal and obscured by postmortem autolysis. Detection of epsilon toxin in intestinal contents by mouse neutralization testing and ELISA assays has been evaluated, with inconsistency in the various techniques for detection of the toxin.

Vaccination of pregnant ewes 3 weeks prior to lambing is a highly effective tool in prevention of enterotoxemia in newborn lambs to at least 12 weeks of age, whereas vaccination of neonatal lambs may not provide added protection. Ewes and lambs in this flock of sheep were not vaccinated, as the owner was attempting to raise the sheep under natural conditions without the use of “artificial” measures of husbandry.

AFIP Diagnosis: Thalamus, dorsal and lateral: Necrosis, multifocal, breed not specified, ovine.

Conference Comment: The contributor gives a thorough review of the disease caused by Clostridium perfringens type D, often also called “overeating disease” or “pulpy kidney disease.”

There are five strains of Clostridium perfringens (A, B, C, D, and E), each of which causes enterotoxemia, among other diseases. There are four principle lethal exotoxins elaborated by C. perfringens that are important in its pathogenicity – alpha, beta, epsilon, and iota. Alpha toxin acts on cell membranes and produces hemolysis or cell necrosis. Beta, epsilon, and iota toxins cause necrosis and increased vascular permeability. In addition to the epsilon toxin, C. perfringens type D also produces alpha toxin.

Clostridium perfringens type A, which produces alpha toxin, is the most common cause of gas gangrene in both humans and animals. It also rarely causes acute intravascular hemolysis and icterus in calves and lambs and is known as “yellow lamb disease”. Colitis X is a rapidly fatal diarrheal disease in horses with
an uncertain etiology, but it has been associated with *C. perfringens* type A. Additionally, food-borne illnesses in humans are commonly linked to type A.\(^2,5,6\)

*Clostridium perfringens* type B elaborates alpha, beta, and epsilon toxins and is a cause of enterotoxemia in lambs, calves, and foals. The syndrome in lambs is called lamb dysentery, affects lambs less than 2 weeks old, and causes acute hemorrhagic enteritis. Occasionally it also causes ulceration with subsequent intestinal perforation and peritonitis. In older lambs, this disease is known as “pine” (in England) and causes depression, unthriftiness, and reluctance to suckle. In calves, this organism causes acute hemorrhagic enteritis with mucosal necrosis in animals younger than 10 days of age. It affects foals within the first two days of life, and also causes hemorrhagic and ulcerative enteritis.\(^2,5\)

*Clostridium perfringens* type C produces alpha and beta toxins and causes disease in adult sheep, goats, and feedlot cattle, and neonatal lambs, calves, foals, and piglets. In adult sheep, the disease is called “struck”. The lesions include hemorrhagic enteritis with ulceration, and peritonitis with a large volume of clear yellow fluid in the peritoneal cavity. The disease in goats and cattle is similar to sheep. Calves, lambs, and foals are affected by hemorrhagic and necrotizing enteritis during the first few days of life, and piglets during the first 8 hours of life. Antemortem clinical signs in neonatal animals may not be observed.\(^2,5,6\)

*Clostridium perfringens* type E produces alpha and iota toxins. It causes intestinal disease in calves, lambs, and rabbits.\(^2,5\)

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**References:**
CASE IV – X26871 (AFIP 2888447)

Signalment: Adult female raccoon (Procyon lotor).

History: This wild raccoon was found recumbent in a driveway and was euthanized by an intramuscular injection of Ketamine® and an intrathoracic injection of Euthanyl®.

Gross Pathology: The animal was obese with normal muscle mass and very abundant subcutaneous and internal adipose tissue stores. There were several, multifocal to coalescing, firm, raised skin masses in the right shoulder region. They ranged in diameter from 2 – 5 cm, and the epidermis overlying the largest one was ulcerated. On cut surface, they were uniformly white and extended from the superficial dermis into the underlying subcutaneous tissues. The right prescapular lymph node was moderately enlarged. The stomach was empty and the descending colon contained normal formed feces.

Laboratory Results:
1. T-lymphocyte CD3 marker (Avidin biotin complex – Peroxidase NCL-CD3-12 Novocastra®) – Positive
2. B cell CD79a marker (Avidin biotin complex – Peroxidase HM47-A9 Neomarker®) – Negative
3. Class 2 MHC Marker (Avidin biotin complex – Peroxidase TAL.1B5 Dako®) – Negative

Immunohistochemistry was performed on sections of skin and brain. There was diffuse infiltration of class 2 MHC marker positive cells in the skin mass with more marked staining of the meningeal cellular infiltrate. However, it was not the neoplastic cells that were staining. The positive cells were interpreted to be within the inflammatory cell population admixed with the tumor cells, and, as a result, this test was considered negative. Using extensive cell conditioning and dropping the primary antibody dilution, much of the effects of prolonged fixation of the tissues in formalin on the CD3 stain were overcome. The entire sheet of tumor cells in the skin and the clusters of neoplastic cells within the meninges were CD3 positive.
The cellular morphology was not ideal but adequate to make a diagnosis of a T cell tumor using this protocol. CD79a B cells were not detected in either tissue.

**Contributor’s Morphologic Diagnosis:** T-cell lymphosarcoma with skin, lymph node and brain involvement.

**Contributor’s Comment:** In this section of brain, the subarachnoid space and spaces of Virchow-Robin are moderately to markedly distended by a pleocellular cell infiltrate with a population of relatively uniform round cells predominating. The round cells have fairly well defined cell borders, moderate amounts of finely granular basophilic cytoplasm and round nuclei with a condensed to euchromatic chromatin pattern; up to three-fold anisokaryosis; and a prominent nucleolus. Single cell necrosis within the population is common. Mitotic figures are common (four mitoses observed in 10 random HPF). A variable number of lymphocytes, plasma cells, eosinophils and macrophages are admixed with the round cell population. The normal architecture of the right prescapular lymph node and skin of the right shoulder region was effaced by similar neoplastic and inflammatory cell populations. In the skin, the neoplastic lymphocytes had a perifollicular distribution in the superficial dermis, but there was no clear evidence of tumor cells infiltrating the overlying epidermis or follicular-adnexal epithelium.

Lymphoid tumors are among the most common neoplasms in domestic animals, but cutaneous lymphoma is rare in all species\(^1\). In the literature, there are two reports of lymphosarcoma in wild raccoons\(^2,3\), neither of which describes cutaneous involvement. However, both of these cases also involved females, and the tumor in one case also involved the brain. The raccoons described in these two reports were in poor body condition, as opposed to the present raccoon which was obese. This would suggest that the neoplastic disease had not been a long term problem for this animal.

Cutaneous lymphoma is traditionally divided into epitheliotropic and nonepitheliotropic forms\(^4\). In the dog, either form can be of T-cell origin. However, in epitheliotropic tumors, the neoplastic cells have an affinity for the epidermis and adnexal epithelium; are typically CD8\(^+\) T cells; and originate in the skin, metastasizing only late in the disease. Whereas in the nonepitheliotropic tumors, the neoplastic cells grow deeply in the dermis and subcutis; are predominately CD3\(^+\) T cells; and may be multicentric in origin. Based on this information, the cutaneous lymphoma in this raccoon was considered to be of the nonepitheliotropic form.

T-cells secrete cytokines that activate and attract other inflammatory cells. This would account for the pleocellular population of inflammatory cells admixed with the neoplastic cells in this case.
The cause of lymphosarcoma in raccoons has not been identified. Viral, environmental and genetic etiologies are described for lymphosarcoma in domestic animals.

**AFIP Diagnosis:** Cerebrum: Lymphoma, raccoon (*Procyon lotor*), procyonid.

**Conference Comment:** Based on the pattern of a dense infiltrate within the meninges and Virchow-Robbins space, conference attendees favored a neoplastic process over an inflammatory process. A parenchymal distribution would be more characteristic of an inflammatory process.

One inflammatory condition with a predominantly perivascular distribution was, however, discussed. Granulomatous meningoencephalomyelitis (GME) is characterized by a perivascular accumulation of well-differentiated lymphocytes, monocytes, plasma cells and, often, epithelioid cells within the white matter. These inflammatory cells are sometimes arranged in whorls around vessels and, as the disease progresses, these cells expand to form coalescing perivascular cuffs which compress, but usually do not infiltrate, the intervening parenchyma.

Lymphoma infiltrating the central nervous system is described in cattle as part of enzootic bovine lymphoma. In adult cattle, lymphoma is associated with bovine leukemia virus (type C retrovirus) and has a predilection for several sites, one of which is the epidural fat. This B-cell lymphoma may infiltrate the lumbar spinal cord and spinal nerve roots, causing posterior paralysis. Other sites commonly associated with bovine leukemia virus-associated lymphoma include retrobulbar fat, abomasum, myocardium (most frequently the right atrium), and uterus.

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