The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
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CONFERENCE 18
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CASE I – A (AFIP 2861856)

Signalment: 4-year-old, male-castrated, *Canis familiaris*, Belgian terrier

History: Rapid onset (weeks) of weight loss, lethargy, listlessness, inappetence, and exercise intolerance. These clinical signs worsened in spite of supportive care, followed by vomiting, oliguria, and tachycardia.

Gross Pathology: There was bilateral enlargement and diffuse pallor of the kidneys.

Laboratory Results:
Serum biochemistry: elevated BUN, creatinine, and phosphorus.
Urinalysis: isosthenuria with hematuria (+4) and proteinuria (+3).
Kinetics-based ELISA (KELA): positive for Lyme disease with a titer of 510
(interpretation of Lyme ELISA units: >499, very high positive).
Serology: negative for *Ehrlichia* and heartworm.

Contributor's Morphologic Diagnoses: Kidney: 1. Chronic glomerulonephritis.
2. Chronic tubulointerstitial nephritis.

Contributor's Comment: Lyme Disease (borreliosis) is a systemic infection caused by the spirochete *Borrelia burgdorferi*, which is transmitted via ticks of the genus *Ixodes*. *Borrelia burgdorferi* infects a variety of mammals, including humans and dogs, and causes diseases of multiple organ systems, including the skin, central nervous system, heart, and musculoskeletal systems[1, 4]. Renal disease associated with *Borrelia burgdorferi* infection has been described in the dog and is characterized by glomerulonephritis and tubulointerstitial changes[2, 3]. *Borrelia burgdorferi* are rarely detected in histologic sections of effected kidneys. Etiologic diagnoses are dependent on serologic techniques such as indirect fluorescent antibody[5], ELISA or KELA, or Western immunoblotting. Following early diagnosis, infection with *Borrelia burgdorferi* has been successfully treated with antibacterial compounds such as amoxicillin or

tetracycline. However, dogs with renal borreliosis usually present clinically with renal failure and protein-losing nephropathy, warranting a guarded to grave prognosis[4]. Prevention of Lyme disease in the dog has been attempted through vaccination of susceptible dogs with a bacterin, commercially available since being conditionally licensed in 1990 and completely licensed in 1992[4].

AFIP Diagnoses: 1. Kidney: Glomerulonephritis, membranoproliferative, global, diffuse, moderate, with tubular dilation, proteinosis, necrosis and regeneration, and multifocal mild chronic lymphoplasmacytic interstitial nephritis, Belgian Terrier, canine. 2. Kidney, tubules and Bowman's capsules: Mineralization, multifocal, mild.

Conference Comment: The important features to microscopically distinguish canine Lyme nephritis from other causes of renal disease are the simultaneous presence of diffuse glomerulonephritis, tubular dilation with tubular necrosis and regeneration, and interstitial lymphoplasmacytic nephritis. The exact pathogenesis of the tubular changes in canine Lyme nephritis is unknown, but immune-mediated glomerular disease is likely the primary inciting event. The subsequent tubular changes may arise from a combination of decreased perfusion and hypoxia, and the toxic effect of profound proteinuria.

Ixodes ticks have a three-host, two-year life cycle. Larvae and nymphs feed on the white-footed mouse (*Peromyscus leucopus*) and other small rodents/mammals, whereas the adult ticks feed on white-tailed deer (*Odocoileus virginianus*) and other large mammals. The two canine breeds that are over-represented in studies of Lyme nephritis are Labrador and Golden Retrievers.

Recently, in contrast to the indirect fluorescent antibody assay or enzyme linked immunosorbent assay, Western immunoblotting was proven capable of differentiating naturally exposed dogs from those with vaccinal antibody titers, or dual-status dogs by detecting differences in antibody response to *B. burgdorferi* antigens, including outer surface proteins (OSP) A and B.

Conference participants discussed possible causes for soft tissue mineralization; the differential diagnosis includes renal secondary hyperparathyroidism, hypervitaminosis-D including ingestion of cholecalciferol-containing rodenticide, and dystrophic calcification.

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CASE II - N01-406 (AFIP 2834686)

Signalment: Adult feral pig.

History: Tissue collected from one of several feral pigs slaughtered at a local meat processing plant. The pigs were heavily infested with various metazoan parasites.

Gross Pathology: Examination of the serosal surface of the small intestine revealed multifocal, 0.5 cm, firm, white, slightly elevated nodules in the wall of the intestine. Within the lumen of the intestine, contiguous with these nodules, were milky-white, pseudosegmented, flat worms measuring 4-12 cm long and 0.5 cm wide. The anterior end of each worm was embedded deep into the wall of the intestine. Other parasites observed grossly in these animals included lungworms, roundworms, spargana and kidney worms of swine.

Laboratory Results: Not available.

Contributor's Morphologic Diagnosis: Small intestine: Granuloma, eosinophilic, focal with intralesional adult acanthocephalan, etiology consistent with *Macracanthorhynchus hirudinaceus*.

Contributor's Comment: The submitted slides contain cross and/or tangential sections of an adult female acanthocephalan embedded in the wall of the small intestine. Some sections demonstrate the parasite's anterior end (proboscis) in the center of the lesion. Acanthocephalans can be distinguished from other helminths by the lack of a digestive tract, a thick hypodermis, lacunar channels and specialized structures such as the proboscis and lemnisci. Their common name, thorny-headed worm, refers to the retractable proboscis armed with rows of hooks that are used to attach to the gut of the host. The hypodermis serves in the uptake and digestion of

nutrients. They also have 1-3 pairs of lemnisci, which are covered with muscles and may function in proboscis extension. The eggs of *M. hirudinaceus* are oval and have three envelopes, a thin outer membrane and a thick, 2-layered mottled shell and an inner fertilization membrane.

Intestinal acanthocephaliasis occurs when a vertebrate (definitive) host ingests an invertebrate (intermediate) host containing the infective acanthor (cystacanth stage). Disease ranging from mild to fatal is produced by the mechanical presence of the adult worms embedded in the intestinal wall where they induce pyogranulomatous inflammation and may result in perforation of the intestine. Adult worms may vary from a few centimeters to 1 meter in length. Grossly the worms produce firm white nodules several millimeters across which are visible from the serosal surface of the intestine. The parasites are flat when alive and may be confused with tapeworms, but round up forming a cylindrical appearance once fixed. Microscopically the head of the parasite can be found embedded deep in the submucosa and tunica muscularis surrounded by necrotic cellular debris and a heavy infiltrate of neutrophils and eosinophils. The necropurulent center of the lesion is rimmed by fibroblasts and a mixture of macrophages, lymphocytes and plasma cells.

Macracanthorhynchus hirudinaceus is a common parasite of wild swine. Pigs typically become infected by ingesting beetles of the Family Scarabaeidae (e.g. dung beetles, June beetles) when rooting for grubs. *M. hirudinaceus* is also the most common cause of human acanthocephaliasis, especially in rural areas of Asia where various kinds of beetles are consumed as food or medication. Another acanthocephalan of veterinary importance is *Prosthenorchis elegans. P. elegans* is a highly pathogenic parasite of Central and South American New World monkeys in the wild and in captivity. Overcrowding and unsanitary conditions may result in heavy infestations. Rodents are the common definitive host for *Moniliformis moniliformis,* but infections have also been reported in chimps, great apes and man. Most other acanthocephalans are found in the gastrointestinal tract of marine vertebrates.

AFIP Diagnosis: Small intestine: Enteritis, ulcerative, necrotizing and pyogranulomatous, focally extensive and transmural, severe, with adult acanthocephalan, etiology consistent with *Macracanthorhynchus hirudinaceus*, feral pig, porcine.

Conference Comment: The contributor provided an excellent summary of acanthocephaliasis. The unique feature that distinguishes the phylum from all other parasites is the presence of lemnisci, which presumably assist in eversion and retraction of the spined proboscis. Acanthocephalans of marine mammals include *Bulbosoma* sp. Wild carnivores are the usual hosts of *Macracanthorhynchus ingens* and *Oncicola canis*.

Conference participants listed other well-known porcine metazoan parasites. These include nematodes: stomach worm (*Hyostrongylus rubidus*), roundworm (*Ascaris suum*), strongyle (*Oesophagostomum dentatus*), whipworm (*Trichuris suis*), kidney worm (*Stephanurus dentatus*); lungworm (*Metastrongylus apri, M. salmi, M. pudendotectus*); cestodes: (*Taenia hydatigena, T. solium/Cysticercus cellulosae,*

Echinococcus granulosus); and trematodes: liver flukes (*Fasciola hepatica, F. gigantica*), and the pulmonary trematode (*Paragonimus kellicotti*).

Contributor: Comparative Pathology, AFRL/HEDV, 2509 Kennedy Cir, Brooks AFB, TX 78235-5118

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CASE III – WN 01/3959 (AFIP 2840887)

Signalment: 16-month-old, female, Merino sheep (*Ovis aries*).

History: Over a 10-day period this animal showed progressive neurological signs. It stood apart from the rest of the flock and appeared to have impaired vision. It had a decreased menace response, and when restrained, tended to collapse to one side. It did not respond to parenteral treatment with thiamine and was euthanized.

Gross Pathology: No gross lesions were observed at necropsy. In transverse sections of formalin-fixed cervical cord at C1-2, the grey matter was swollen, and irregularly dark and soft.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Meningoencephalomyelitis (cranial cervical cord), necrotising pyogranulomatous, severe, subacute, with Gram-positive bacilli consistent with *Listeria* sp.

Contributor's Comment: The cranial cervical cord has extensive malacia of grey matter, including large areas infiltrated by neutrophils and hypertrophic microglia

extending into the adjacent white matter. Vessels in all areas including the leptomeninges have hypertrophic endothelium and mild to marked perivascular cuffing by lymphocytes, macrophages, plasma cells and variable numbers of neutrophils. Within the cytoplasm of individual cells scattered through the necrotic areas are small numbers of short plump Gram-positive bacilli consistent with *Listeria* sp. At all levels of the brain there was minimal to mild lymphocytic cuffing of meningeal vessels. In the midbrain and medulla there was marked (3-6 cells deep) lymphoplasmacytic cuffing of parenchymal vessels, and in the medulla granulomatous inflammation extended into the adjacent neuropil, which contained scattered swollen degenerate axons. There was no history of silage consumption. This appears to be a sporadic individual case of ovine listeriosis.

L. monocytogenes can gain access to the central nervous system from damaged oral, nasal, or ocular mucosa via the trigeminal nerve. The resulting meningoencephalitis is characterized by focal gliosis, neutrophilic infiltrates (microabscesses), and central necrosis (liquefaction) with a distribution that is restricted to the brainstem, specifically the pons and medulla. The thalamus and cervical spinal cord can also be affected. Signs include depression, head pressing and paralysis of one or more medullary centers with contralateral facial paralysis and unidirectional circling.

AFIP Diagnosis: Spinal cord: Myelitis, suppurative, subacute, focally extensive, severe, with multifocal subacute meningitis, and few Gram-positive bacilli, etiology consistent with *Listeria monocytogenes,* Merino sheep (*Ovis aries*), ovine.

Conference Comment: Listeriosis is most common in ruminants, and sporadically occurs in swine, horses, dogs, rabbits, guinea pigs, chinchillas and birds. In humans, meningitis is the most common form of listeriosis, and infection is often traced to post-processing contamination of foodstuffs.

Poor quality silage is often the source of infection for ruminants. It provides a suitable aerobic environment with a pH higher than five in which *Listeria monocytogenes* proliferates. Asymptomatic carriers are a source of environmental contamination through fecal shedding. Listeriosis is most prevalent in the winter and spring. The bacterium secretes a hemolysin virulence factor, listeriolysin-O, which is important for intracellular multiplication within macrophages and monocytes.

The three common disease patterns of listeriosis are septicemia, meningoencephalitis and abortion. Septicemia occurs in ruminant neonates subsequent to intrauterine infection and in monogastric animals; hepatic and splenic necrosis are common findings. The contributor concisely described meningoencephalitis (circling disease). Abortion occurs late in gestation and may be combined with placentitis and metritis.

Some species develop monocytosis when experimentally infected with *L. monocytogenes*; rabbits, guinea pigs, and dogs, however; infected ruminants and swine do not.

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

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CASE IV - 02B1433 (AFIP 2841968)

Signalment: 3-day-old female Gelbvieh-Red Angus calf

History: A heifer calf in a large beef herd was born with hyperesthesia. When examined at three days of age she was laterally recumbent with the head tilted to one side. Nystagmus and strabismus were absent. Retinas were ophthalmoscopically unremarkable. Oculomotor function was normal and papillary dilation was absent. The blind calf appeared cognizant of its surroundings. The owner of the stabilized 250 animal Gelbvieh-Red Angus herd in western Nebraska recognized other calves with similar congenital neurological signs. There were 3 cases in 1998, 0 in 1999, 2 in 2000, 2 in 2001 and 1 in 2002. Affected calves comprised 5 heifers and 3 bulls. They were born full term following an uneventful birth, at which time clinical signs were present. Calves were unable to stand and shook constantly when disturbed. Muscular fasciculations ceased only during sleep. Calves remained in lateral recumbency, occasionally attempting to rise. With nursing care, they survived to 10 days *post-partum*. Affected calves died. Dams were normal. Examination of pedigrees of 8 affected calves indicated a high degree of inbreeding (inbreeding coefficient: 0313 - .2578) and, in most cases, a common ancestor on both sides of the pedigree. A field necropsy was done and various tissues, including brain, were submitted to the laboratory.

Gross Pathology: There were no gross abnormalities in the calf, including in the brain.

Laboratory Results: No viruses were isolated from tissues including brain. Sections of brain were negative for BVDV antigen on immunohistochemistry.

Contributor's Morphologic Diagnosis: Status spongiosus, severe, diffuse, white matter.

Contributor's Comment: Lesions of this disease are indistinguishable from bovine maple syrup urine disease (MSUD; formally a component of the "neuraxial edema" disease group). Mutations of the E1 alpha subunit of bovine branched-chain alpha-keto acid dehydrogenase are responsible for maple syrup urine disease in both Poll Hereford (248C→T) and Poll Shorthorn breeds (1380C→T). Calves that are homozygous for either mutation or that are compound heterozygous for both are essentially normal at birth, and then exhibit signs of progressive neurological dysfunction culminating in death within 5 or 6 days. Calves rarely survive for longer than a week post-partum. The disease is characterized biochemically by elevated concentrations of branched chain keto- and amino acids in body fluids and tissues.

DNA was extracted from unfixed frozen spleen of the affected calf, from peripheral blood leukocytes (PBL) of 7 dams that gave birth to affected calves, and of a grand-dam of three affected calves, and examined by polymerase chain reaction for 1380C→T and 248C→T mutations. All 9 animals were homozygous wild-type at both sites ((J. Dennis, Elizabeth Macarthur Agricultural Institute, Australia, *personal communication*). Amino acid analysis of blood from an affected calf revealed normal concentrations of the three branched-chain amino acids, valine, isoleucine and leucine ((T. Bottiglieri, Baylor Institute of Metabolic Disease, *personal communication*). This precludes the possibility that the condition in the herd was caused by a novel mutation in the branch-chain keto acid dehydrogenase complex. The presence of the disease at birth, and survival of calves for as long as 10 days also distinguishes the disease from bovine MSUD.

On the basis of preliminary data, although the disease is morphologically indistinguishable from MSUD, it is clinically, genetically and biochemically distinct. Light microscopic changes are consistent with intramyelinic vacuolation, although electron microscopic examination is required for confirmation. A variety of congenital diseases characterized by spongy degeneration of white matter are recognized in children, such as Canavan disease due to aspartoacylase gene mutations, and in various canine and feline breeds.

AFIP Diagnoses: Cerebellum and cerebrum, white matter; and brainstem: Spongiosis, diffuse, moderate to severe, Gelbvieh-Red Angus, bovine.

Conference Comment: This case was reviewed in consultation with the AFIP's Department of Neuropathology. The spongiform degeneration of the white matter in this case is similar to both maple syrup urine disease and Canavan disease in humans. Also in this case, staining with Luxol fast blue indicates there is myelin preservation, which is not typical of Canavan disease. A characteristic electron microscopic change of branched-chain ketoacid decarboxylase deficiency (BCKAD) is separation of the myelin lamellae at the intraperiod line. BCKAD is most often reported in polled or horned Herefords and polled Shorthorn calves.

Conference participants discussed the differences between this case and other conditions that cause spongiform change in the brains of cattle. Congenital cerebral edema occurs in horned Herefords; signs are present from birth and histological changes include pronounced vacuolation of the white matter, hypomyelinogenesis, and

hydropic degeneration of perineuronal astrocytes in the gray matter. Citrullinemia is a rare autosomal recessive metabolic disease reported in Holstein-Friesian calves that results in a deficiency of arginosuccinate synthetase. The deficiency causes hypercitrullinemia and hyperammonemia; the brain lesion includes astrocytic swelling and spongy vacuolation of deep laminae in the cerebral cortex. Cattle with bovine spongiform encephalopathy exhibit spongiform change in the gray matter neuropil and neuronal vacuolation in various brainstem nuclei. Several participants noted the similarity between the spongiosis in this case and the toxin-induced spongiosis of hexachlorophene and triethyltin.

White matter spongiform degenerative lesions have also been reported in Samoyed, Labrador Retriever and Silky Terrier pups, Egyptian mau kittens, and silver foxes from Norway.

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