

The Armed Forces Institute of Pathology  
Department of Veterinary Pathology  
WEDNESDAY SLIDE CONFERENCE  
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CONFERENCE 8  
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CASE I – TAMU 01-1 (AFIP 2788636)

**Signalment:** 8-year-old, Jersey cow

**History:** The animal was off feed with weight loss for five days and treated with antibiotics. Hematochezia was present on the day of presentation, and the animal was sent to a University Hospital. The animal on arrival was unable to stand in the trailer and had piles of bloody feces on rectal palpation. In addition, copious amounts of bloody liquid were passed. The animal was euthanized without work-up.

**Gross Pathology:** The colonic content was essentially fresh blood. There were numerous, agonal hemorrhages throughout the body, but unusual, "dry" hemorrhagic plaques were in the mesentery, particularly along the mesenteric attachment to the small intestine and colon. The spleen was enlarged and meaty with scattered dry areas interpreted as necrosis. A large (6 cm) mediastinal lymph node was granular to dry and red. That specimen is presented.

**Laboratory Results:** Serum neutralizing titers: none.

**Contributor's Morphologic Diagnosis:** Mediastinal lymph node: Acute necrosis and hemorrhagic edema with lymphadenitis and acute pericapsulitis; numerous trypanosomes. *Trypanosoma theileri*

**Contributor's Comment:** A similar necrotic, edematous and hemorrhagic reaction containing numerous Trypanosomes was noted in the spleen and in plaques of "dry" hemorrhage on the mesenteric attachments. Visualizing trypanosomes in formalin fixed specimens in cattle is rare. In African trypanosomiasis, trypanosomes are usually seen only in blood films and impression smears of lymph nodes at necropsy. Although most diagnoses of trypanosome species are done on

morphometrics of smears, because these organisms are so large and because they are from a cow in the United States, it can be assumed they are *T. theileri*, the type species of the subgenus, *Megatrypanum*. This cosmopolitan organism's distribution is widespread and it is transmitted by tabanid flies. This organism commonly subclinically infects cows and is readily isolated from blood cultures of ten to eighty percent of cattle in the United States and Canada. *T. theileri* has been associated with some (albeit mild) clinical illness and lymphocytosis in cattle, neonatal bovine disease, and abortion. Severe illness is uncommon. No other lesions were noted in this cow. Despite colonic hemorrhage, there was no colitis and no viral or bacterial pathogens identified in the colon. Systemic bacterial infection was not identifiable via aerobic bacterial culture of lung, liver, and spleen. Thus, the association of the organisms with severe lesions compelled us to diagnose bovine trypanosomiasis and a presumed terminal hemorrhagic diathesis due to DIC. Other similar cases have not been reported on the farm or in the same area of the state.

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**AFIP Diagnoses:** Lymph node: Lymphadenitis, necrotizing and fibrinous, subacute, diffuse, severe, with hemorrhage and numerous flagellated protozoa, etiology consistent with *Trypanosoma* sp., Jersey, bovine.

**Conference Comment:** *Trypanosoma theileri* is a flagellated protozoan whose host range appears to be restricted to Artiodactyla. Transmission can occur by mechanical transmission, via infected tabanids and, as there are several reports in bovine fetuses, transplacentally.

Flagellated protozoans fall within the class Zoomastigophorea and can be further divided according to their location in host tissues and life cycle. Hemoflagellates, which include the order Kinetoplastida, live in the blood, lymph, and tissue spaces. Mucosoflagellates live in the alimentary or genital tract and include the orders Diplomonadida (Genera *Giardia* and *Spironucleus*) and Trichomonadida (Genera *Trichomonas* and *Histomonas*).

The order Kinetoplastida is characterized by the presence of a kinetoplast, a small accumulation of extranuclear DNA, and includes the genera *Trypanosoma* and *Leishmania*. With respect to *Trypanosoma* sp., four morphological forms are described. Three forms are distinguished based upon the position of the kinetoplast relative to the nucleus; promastigote (the kinetoplast is at the anterior end of the cell), epimastigote (the kinetoplast is immediately anterior to the nucleus), and trypomastigote (the kinetoplast is posterior to the nucleus). The fourth form, the amastigote, is defined by its lack of a flagellum.

Arthropod hosts become infected by ingesting the blood of infected mammals. Depending on the trypanosome involved, infection of new mammalian hosts is either salivarian or stercorarian. Salivarian transmission is by the bite of an

infected fly, introducing the organism into the circulation (*T. brucei*). Stercorarian transmission is through contamination of abraded skin or mucous membranes by the feces of infected flies; most often while they are feeding (*T. theileri*, *T. cruzi*).

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## **CASE II – ND01-183 (AFIP 2788617)**

**Signalment:** 11 year-old, Male-Castrate, Dachshund, Canine

**History:** This animal had a history of disease resulting from mitotane toxicity. Clinical signs included ataxia, lethargy, weight loss, anorexia and vomiting. The dog had been given such drugs as Prednisone, Dexamathasone, Cimetidine, others, and intravenous fluids. The animal expired and was submitted for necropsy.

**Gross Pathology:** In the endocrine system, the pituitary gland was judged to be 3-4 times normal, dark red and extended into the dorsal brain parenchyma. The adrenal glands had a circumferential yellow-orange layer involving the cortex at the cortico-medullary junction.

The kidneys had multifocal, depressed areas randomly distributed over the cortex. The skin of the ears was reddened and hardened. The liver had a generalized mottled appearance with pale and dark brown to red areas intermixed.

**Laboratory Results:** None

**Contributor's Morphologic Diagnoses:** Adrenal gland: Zona fasciculata and zona reticularis, cell vacuolation and loss, diffuse, severe, Dachshund, canine.

**Contributor's Comment:** Diffusely, the deep adrenal cortex has loss of cortical cells, vacuolation of remnant cells and prominent blood-containing capillaries. In a few areas, the vacuolation extends into the middle cortical layer as well as into the superficial medulla. The pituitary gland (tissues not submitted) contained a carcinoma and was likely responsible for the overstimulation of the adrenal gland with ACTH. In an effort to limit the clinical effects of hyperadrenocorticism, Mitotane was administered over a period of time resulting in loss of adrenal cortical cells.

Canine hyperadrenocorticism is a disorder that results from excessive cortisol production by the adrenal cortex. Two separate conditions cause canine hyperadrenocorticism; adenoma/carcinoma of the adrenal cortex and pituitary dependent forms. The pituitary dependent forms accounts for about 90% of the cases. The drug used most often to control the pituitary dependent forms is *o,p'*-DDD (mitotane). Mitotane decreases cortisol production by causing selective necrosis and atrophy of the adrenocortical zona fasciculata and zona reticularis. The zona glomerulosa is relatively resistant to the cytotoxic effects of mitotane, so aldosterone levels are usually maintained.

Adverse side effects develop when plasma cortisol levels decrease below normal resting range or hypoadrenocorticism results. Approximately 5% of dogs treated with maintenance mitotane dosages develop iatrogenic hypoadrenocorticism characterized by low basal and post-ACTH cortisol concentrations and electrolyte changes of hyperkalemia and hyponatremia.

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**AFIP Diagnoses:** Adrenal gland, zona fasciculata and zona reticularis: Vacuolar degeneration and loss, diffuse, moderate, with stromal collapse, Dachshund, canine.

**Conference Comment:** In veterinary medicine, hypercortisolism (Cushing's syndrome) has been described as having one of four causes: a primary pituitary lesion with hypersecretion of ACTH (Cushing's disease), hypersecretion of cortisol by a primary adrenal lesion (e.g. adenoma, carcinoma, or hyperplasia), iatrogenic, and idiopathic.

Several clinical tests help to differentiate the underlying causes. The ACTH stimulation test differentiates spontaneous from iatrogenic hypercortisolism. Following ACTH administration, dogs with pituitary or adrenal lesions may have an exaggerated cortisol response while dogs with iatrogenic hypercortisolism will have little or no response. In normal dogs, suppression of cortisol levels occurs following administration of low-dose dexamethasone; absence of suppression, while not specific, suggests a pituitary or adrenal lesion. High-dose dexamethasone suppression tests fail to suppress the cortisol level in dogs with adrenal lesions while often suppressing the cortisol level in pituitary-dependent hypercortisolism. Dogs with pituitary-dependent hypercortisolism have high or normal endogenous ACTH levels while those with adrenal lesions have low or undetectable levels.

Characteristic histomorphology following Mitotane administration includes swelling, vacuolation, and necrosis of adrenocortical cells of the zona fasciculata and zona reticularis. Prolonged administration results in stromal collapse, thinning of the cortex, and dilatation of vascular sinusoids.

**Contributor:** Department of Pathobiology, School of Veterinary Medicine, Tuskegee University, Tuskegee, AL 36088

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### **CASE III – 19228A (AFIP 2787198)**

**Signalment:** 8.5-year-old, male, Sheltie

**History:** Several weeks of seizures treated unsuccessfully with an unspecified anti-seizure medication were reported. Seizures continued and eventual weakness with the inability to stand was reported. Proprioceptive deficits were noted late. Euthanasia was performed immediately before necropsy.

**Gross Pathology:** Skeletal muscles were tan colored and streaked in one-third to one-half of the muscles examined. Streaking was present in muscles of mastication, neck, front and hind limbs, intercostal regions, and abdominal muscles. Three 1-2 cm slightly raised nodules were present on the surface of the spleen. No gross brain lesions were reported.

**Laboratory Results:** Immunohistochemistry of the brain and skeletal muscle were positive for *Neospora caninum*.

**Contributor's Morphologic Diagnosis:** Brain: Cerebellum, meningoencephalitis, granulomatous and lymphoplasmacytic, severe, with intralesional *Neospora* tissue cysts and tachyzoites and white matter vacuolation.

**Contributor's Comment:** The muscle lesions in this dog were disseminated granulomatous to pyogranulomatous myositis with intralesional *Neospora* tachyzoites and tissue cysts. Unlike other cases where the cerebrum has the most consistent changes, cerebellar folia were the only location of brain lesions in this case. Tissue cysts were present in the cerebellum along with scattered individual and groups of tachyzoites, while tachyzoites but no tissue cysts were detected in skeletal muscle.

*Neosporosis* affects a variety of species but most importantly dogs and cattle. It is reported to be one of the most important causes of bovine abortion in many parts of the world. In dogs, neuromuscular disease is the most important clinically but it is uncommon, particularly given the apparent widespread occurrence of the organism.

Newly described in 1988, *Neospora caninum* was previously misdiagnosed as *Toxoplasma gondii*. The organisms are very similar but have some morphologic differences. Tissue cysts in neosporosis occur typically in central nervous tissue, although they have recently been reported in skeletal muscle of dogs and calves. Tissue cyst walls are thicker in neosporosis, up to 4 micrometers in width, while cyst walls in toxoplasmosis are typically less than 1 micrometer. Ultrastructurally, the tachyzoites are similar, but the rhoptries of *Neospora* are electron dense while those of *Toxoplasma* are honeycombed. Micronemes are similar but are reported to be more numerous in *Neospora*. Because of the morphologic similarity of the organisms, diagnosis in tissue specimens is achieved by immunohistochemistry. Cross-reaction between the organisms is not expected.

The life cycle of *Neospora caninum*, a coccidian parasite, has recently been reported. Dogs are definitive hosts and produce oocysts in the feces. Ingestion of the sporulated oocysts results in infection of intermediate hosts (cattle, sheep, goats, horses, deer, and others, including dogs). Within the intermediate host tachyzoites can be transmitted to the fetus across the placenta. In cattle, transplacental transmission can occur more than once.

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**AFIP Diagnosis:** Cerebellum: Meningoencephalitis, lymphohistiocytic, neutrophilic and eosinophilic, diffuse, moderate, with multifocal necrosis and protozoal cysts and tachyzoites, Shetland sheepdog, canine.

**Conference Comment:** The contributor has provided a concise review of *Neospora caninum* as well as the key features which differentiate it from *Toxoplasma gondii*. As there is variation in sections, some conference participants commented on similar changes in the brain stem.

**Contributor:** Department of Veterinary Pathology and Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames, IA 50011

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#### **CASE IV – 0-52119 (AFIP 2787359)**

**Signalment:** 1.5 year-old male castrated Dalmatian

**History:** Acute onset of lethargy, vomiting, and anorexia for a one-week duration. No known history of exposure to toxins or ingestion of foreign body material. Physical exam (PE) revealed a thin body condition with icteric sclera and mucous membranes. PE was otherwise unremarkable.

Despite therapeutic intervention, clinical signs did not resolve and euthanasia was elected due to a guarded prognosis.

**Gross Pathology:** At necropsy, the oral mucous membranes, sclera, and preputial mucosa were moderately yellow (icterus). The liver was normal to slightly small subjectively (organ weight was not obtained). The liver was diffusely greenish-tan with a generalized accentuated reticular pattern and regionally extensive, random, smooth, depressed, pale tan areas ranging in size from 1 x 2 cm to 5 x 5 cm (subtotal necrosis). These changes were continuous throughout the hepatic parenchyma on cut surface. Additional changes included markedly enlarged pancreatic lymph nodes which measured approximately 5.5 x 1.0 x 1.0cm. These lymph nodes were soft with increased friability, greenish-tan, and on cut surface the medulla was dark green to black.

**Laboratory Results:** Serum biochemical profile revealed significantly elevated liver enzymes, dominated by an ALT (alanine aminotransferase) of 1,399 (high normal: 120) and an AST (aspartate aminotransferase) of 880 (high normal: 40). ALP (alkaline phosphatase) was 283 (high normal: 141). Additionally, there was a hyperbilirubinemia at 2.6mg/dl (high normal: 0.4), and an elevated CK (creatinine kinase) of 678 (high normal: 250).

The complete blood count (CBC) revealed a neutrophilic leukocytosis at  $15.6 \times 10^3$  (high normal:  $11.5 \times 10^3$ ), but was otherwise unremarkable.

Urinalysis (free catch) revealed glucosuria (4+) despite normal serum glucose levels.

Quantitative hepatic copper levels: 8,390 ppm dry weight (deficient: < 80 ppm, normal: 120 – 400 ppm, toxic levels: > 1,500 ppm)

**Contributor's Morphologic Diagnosis:** Liver: Hepatitis, subacute, necrotizing, random, severe with intracellular copper accumulation and cholestasis.

**Contributor's Comment:** Special stains with Rhodanine, for confirmation of copper accumulation within hepatocytes and macrophages (Kupffer cells), were strongly positive in this case. Copper stains were also positive on sections of pancreatic lymph nodes examined. Hepatic copper accumulation can occur secondary to primary cholestatic disease or as a primary storage disease. Primary copper storage disease is best recognized in the Bedlington terrier.

Copper accumulation occurs secondary to a metabolic defect which inhibits appropriate biliary copper excretion and results in subsequent hepatocellular lysosomal copper accumulation. This defect has been identified as an autosomal recessive inherited trait in the Bedlington terrier and has been compared to genetic defects of copper metabolism in humans, Menkes' disease and Wilson's disease. Although the precise protein/gene abnormality in copper storage disease of the Bedlington terrier remains somewhat debatable in the literature, some believe the



defect involves hepatic metallothionein (MT) which binds to copper within the hepatic lysosome.

Other breeds which have been implicated in hereditary copper storage disease include, the West Highland white terrier, Skye terrier, and Doberman pinscher. In addition to the case presented here, the Colorado State Veterinary Diagnostic Lab has seen three other cases over the last two years of Dalmatians with elevated quantitative hepatic copper levels (> 2000) and associated hepatopathies. Three individual case reports in the literature also discuss copper associated hepatic disease in the Dalmatian. Although no genetic link between any of these Dalmatians has been identified, we suspect that hereditary copper storage disease is an emerging syndrome in the Dalmatian breed. We would like to indicate however, that microscopic hepatic lesions typically reported in the Bedlington terrier include centrilobular (zone 3) hepatocellular copper accumulation and necrosis, while that noted in the Dalmatian appears to be more of a random to periportal distribution. Further investigation of the association between copper accumulation and hepatic disease in the Dalmatian is warranted.

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**AFIP Diagnosis:** Liver: Hepatocellular degeneration and loss, centrolobular, diffuse, moderate, with histiocytic and neutrophilic inflammation and abundant intracellular amphophilic pigment, Dalmatian, canine.

**Conference Comment:** Copper is an essential micronutrient and a required component of many enzymes including: lysyl oxidase, cytochrome c oxidase, and superoxide dismutase. Regulation of copper transport in enterocytes is mediated by metallothionein (a heavy metal induced binding protein) and ATPase7A. ATPase7A, a transmembrane copper transporter, regulates copper entry into the blood. In humans, defective ATPase7A results in copper accumulation within the enterocyte, low blood levels of copper, and a deficient state. The condition is X-linked, referred to as Menkes' disease, and is modeled by the mottled mouse.

Once in the bloodstream, copper is bound to albumin or loosely bound to transcuperin as it is transported to the liver. In the liver, copper is incorporated into enzymes; stored within the liver as copper metallothionein; or tightly bound as ceruloplasmin for transport to extrahepatic tissues. Ceruloplasmin is formed in the cytomembrane system from an apoprotein and copper; ATPase7B facilitates transportation of copper from the hepatocyte cytoplasm into this system. Defective ATPase7B disrupts ceruloplasmin formation and results in hepatic accumulation of copper. In humans this condition is known as Wilson's disease and is modeled by the Long-Evans Cinnamon rat and the toxic milk mutant mouse.

As the major route of copper excretion is through the biliary system, cholestasis can result in toxic hepatic copper accumulations. Regardless of the

mode of copper accumulation either acute hepatic necrosis or cirrhosis due to chronic hepatitis may ensue.

While not clearly evident on H&E, review of the Rhodanine stain suggests a predominantly centrilobular to midzonal pattern for the hepatic changes present in this case.

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