The Armed Forces Institute of Pathology
Department of Veterinary Pathology
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2200 Bergquist Drive, Bldg 4430
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CASE I – 05-17970 (AFIP 2988330)

Signalment: 2 year old spayed female American Cocker Spaniel.

History: This dog was presented to the referring veterinarian 48 hours after the onset of diarrhea with occasional vomition while at a boarding kennel. Physical examination identified icterus and melena. The dog's condition deteriorated rapidly while hospitalized for blood work, and she died 4 hours after presentation.

Gross Pathology: Mucous membranes were icteric and melenic feces were adherent to the perineum. The liver was diffusely yellow and atrophic, and normal architecture was effaced due to micronodular hyperplasia. The gallbladder was distended and the bile duct was patent. Vascular (venous) shunts were present between the vena cava and ovarian veins (right and left), and within the mesentery adjacent to the pancreas. Approximately 60 ml of clear yellow fluid was free in the abdominal cavity, and 100 ml of similar fluid was present in the thoracic cavity. Lungs were diffusely congested and edematous.

Laboratory Results:
WBC 38.47 x 10 9 / L (6.0-17.0)
Lymph 13.46 x 10 9/L (0.6-6.8)
Mono 1.84 x 10 9 /L (0.1-1.7)
Granulocytes 23.17 x 10 9/L (3.0-13.6)

RBC 3.15 x 10 12/L (5.5-8.5)
MCV 62.0 fl (58.0-73.0)
Hct 19.5% (35.0-55.0)
MCH 21.2 pg (19.5-24.5)
MCHC 34.3 g/dl (28.0-40.0)
RDW 12.1 (8.0-12.0)
Hb 6.7 g/dl (10.0-18.0)

Plt 109 x 10 9 /L (120-600)

Albumin 8 g /dl (25-44)
ALP 267 U/L (20-150)
ALT 81 U/L (10-118)
Amylase 851 U/L (200-1200)
Total bilirubin 198 umol/L (2-10)
BUN 18 mmol / L (2.5-8.9)
Calcium 2.15 mmol/L (2.15-2.95)
Phosphorus 3.14 mmol/L (0.93-2.13)
Creatinine 247 umol/L (27-124)
Glucose 1.3 mmol/L (3.3-6.1)
Sodium 136 mmol/L (138-160)
Potassium 3.6 mmol/L (3.7-5.8)
Total protein 25 g/L (54-82)
Globulins 0 g / L (23-52)
Na:K 37.8

PT, APTT elevated to twice normal values

Abdominal fluid (harvested at necropsy):
Cellularity 2.3 x 10 9 / L
Protein T.S. <20 g/L
Segmented neutrophils 73%
Macrophages 27%
Sheets of mesothelial cells present with moderate numbers of neutrophils and a few mononuclear cells. No bacteria seen.

Leptospira serology:
Suspicious titers on thoracic fluid (1:20) & abdominal fluid (1:40) for *L. bratislava*.
Leptospira PCR negative of liver and kidney.

Histopathologic Description: In the liver, there was marked, generalized dissecting fibrosis with hepatocellular nodular hyperplasia, and piecemeal and single cell necrosis to total loss of hepatocytes. Moderate to marked cholangiolar proliferation

and canalicular cholestasis were generalized. Most hepatocytes were vacuolated or contained red-brown granular cytoplasmic pigment. Hepatocellular anisokaryosis and anisocytosis were moderate. A mild infiltrate of primarily plasma cells was present in the large tracts of periportal fibrous tissue.

Lesions were also present in kidney and brain, although these tissues are not included in the slide set. Cortical tubular degeneration in kidney was characterized by epithelial loss, flattening, and attenuation. Amorphous to slightly granular eosinophilic debris is present in lumina of affected tubules, and the mesangial matrix was mildly increased in some glomeruli. In brain, neuronal vacuolation with occasional neuronal necrosis was present in mesencephalon, particularly in the rostral colliculus and reticular formation. Occasional Alzheimer type II astrocytes, with abundant pale cytoplasm and vesicular nuclei, were present in cortex.

Contributor's Morphologic Diagnoses:

1. Micronodular hepatic cirrhosis
2. Acute renal tubular necrosis (nephrosis)
3. Mild spongiform encephalopathy

Contributor's Comment: Chronic hepatitis with micronodular cirrhosis in Cocker Spaniels has been recognized since 1993, although documentation in the literature is limited to few descriptive reports (1, 2). In the most complete report, affected dogs ranged in age from 1.5 to 11 years, although the majority of dogs were relatively young, with a mean age of 5.6 years (1). Male dogs were over-represented. Most animals had a short history of clinical disease, as in this case, and demonstrated a variety of clinical signs expected with liver disease and including anorexia, vomiting, lethargy, depression, and weight loss. Ascites was consistently evident on physical examination. Disease progression was rapid in most of these dogs, as in the Cocker Spaniel described here, with survival limited to one to several weeks. Gross and histologic lesions described are similar to those in our dog and included small livers with multiple foci of nodular regeneration, lymphoplasmacytic portal hepatitis with bridging fibrosis, destruction of limiting plates, piecemeal necrosis, biliary proliferation, and hepatocellular cytoplasmic vacuolation. Acquired extrahepatic portosystemic shunts, as seen in this dog, were not reported in other cases. Results of hepatic copper evaluation were based mainly on histochemical staining and were equivocal. Other studies have also identified an increased incidence of chronic hepatitis in Cocker Spaniels, supporting a hereditary basis for this disorder (3).

Cirrhosis is a common end-stage lesion in chronic hepatitis due to a variety of etiologies, including hepatitis associated with genetic factors and hepatic copper accumulation. An excellent review of canine chronic hepatitis, with breed

predispositions and association with hepatic copper levels, is present in Ettinger and Feldman's Textbook of Veterinary Internal Medicine (4).

Spongiform lesions in brain of this dog were consistent with hepatic encephalopathy. Leptospirosis remained a differential diagnosis for renal lesions, although this could not be confirmed, and may have been a major contributing factor in this dog's rapid demise.

AFIP Diagnosis: Liver: Hepatocellular degeneration, necrosis and loss, chronic, diffuse, severe, with parenchymal collapse, bile stasis, nodular regeneration, moderate dissecting fibrosis, biliary hyperplasia, and mild lymphoplasmacytic portal hepatitis (cirrhosis), Cocker Spaniel, canine.

Conference Comment: The histologic changes within this section of liver are profound and all the features of an end-stage, or cirrhotic, liver are identifiable.

In general, hepatic parenchyma responds to chronic injury in three ways: 1) by forming regenerative nodules, 2) by undergoing fibrosis and 3) by biliary duct hyperplasia. Regenerative nodules form under the influence of transforming growth factor – alpha (TGF-A) and hepatocyte growth factor which stimulate hepatocyte stem cells, or oval cells, to replicate. Stimulated oval cells differentiate and undergo rapid replication in an attempt to compensate for lost hepatic mass. The end result to prolonged regeneration is the formation of nodules of hepatic parenchyma. Unfortunately, regenerative nodules lack normal architecture which disrupts the flow of both blood and bile and promotes vascular shunt formation. Fibrosis occurs following hepatocellular and extracellular matrix injury when Ito cells (a.k.a. hepatic stellate cells) undergo a change from their normal role of lipid and vitamin A storage to a myofibroblastic–type cell capable of producing collagen and extracellular matrix proteins. Fibrosis has a detrimental impact on liver function and can occur in several recognized patterns which may help determine the type of injury sustained. Biliary hyperplasia is most prominent in diseases that cause obstruction of bile flow. The mechanism is unknown; however, following hepatic injury proliferation of bile ducts is most profound in portal and periportal areas (5).

The contributor also provides a complete hematology and serum chemistry analysis. With regards to hepatic injury, there are three general categories of parameters frequently measured to diagnose hepatic disease: 1) leakage enzymes, 2) inducible (or cholestatic) enzymes and 3) function tests. Leakage enzymes are found in hepatocyte cytosol and mitochondria and literally "leak out" when cell membranes are damaged. The levels measured in the serum depend on the duration and severity of the injury. In this case, alanine aminotransferase (ALT) is

the only leakage enzyme provided and is within the normal reference range. This is most likely due to either decrease liver mass or decreased amounts of enzyme per cell - leading to decreased amounts of the enzyme being leaked which then is measured within the "normal" range. Induced enzymes are membrane bound and require enzymatic induction for elevations to occur in the serum. In this case, alkaline phosphatase (ALP), a sensitive indicator of cholestasis, is elevated to almost twice normal. This elevation is most likely due to cholestasis but could also have been induced by the administration of corticosteroids. In dogs, there is a corticosteroid induced ALP isoenzyme. This isoenzyme can be differentiated from the hepatic isoenzyme by utilizing levamisole, since the corticosteroid isoenzyme is largely resistant to levamisole inhibition. Function tests for the liver include bilirubin measurement and bile acid tests. Hyperbilirubinemia can be either prehepatic (e.g. hemolytic disease), hepatic (e.g. decreased functional mass), or posthepatic (e.g. cholestasis). Total bilirubin in this case is nearly 20 times normal and may be due to a combination of both hepatic and posthepatic causes. Bile acid testing is also another commonly used method of detecting decreased liver function. Bile acids are made in the liver and stored in the gallbladder. Following ingestion of a meal, the gallbladder contracts and expresses the bile acids into the small intestine where they are responsible for solubilizing lipids and fats. Most are reabsorbed from the small intestine via a process known as enterohepatic circulation. Anything that disrupts the cycle can result in increased serum bile acids. The most common causes are portosystemic shunts, loss of functional hepatocellular mass and cholestasis.

Other important serum chemistry findings in this case include azotemia and hyperphosphatemia, possibly due to concurrent renal failure but differentiation is impossible without a urine specific gravity. The hypoproteinemia and hypoalbuminemia could be the result of concurrent renal disease or decreased production by the liver. Likewise, increased clotting times may be due to decreased production of clotting factors by the liver and possibly disseminated intravascular coagulation (DIC). Hypoglycemia may be due to anorexia or prolonged contact of the serum with erythrocytes in vitro (6).

Important hematologic findings include normocytic, normochromic anemia; mild thrombocytopenia; and a leukocytosis characterized by mature neutrophilia, lymphocytosis and mild monocytosis.

Special stains were used to help identify the nature of cytoplasmic granules noted within hepatocytes. Rhodanine identifies most of the hepatocyte cytoplasmic granules as copper; whereas Kupffer cells often contain deposits of iron stained with Perl's Prussian blue. All attendees agreed that the copper accumulation may be the result of the liver's inability to manufacture and export it as ceruloplasmin or to excrete it in the bile, rather than excess copper being the cause of liver damage.

A short list of the most common causes for end–stage liver include:

Long term administration of anticonvulsants

Abnormal metabolism or accumulation of copper or other metals (West Highland White Terriers, Bedlington Terriers)

Chronic aflatoxicosis, especially Aflatoxin B1

In ruminants: ingestion of pyrrolizidine alkaloid containing plants such as *Senecio* spp. (tansy ragwort, groundsel); *Crotalatia* spp.; *Heliotropium* spp.

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CASE II – N2005-310WCS (AFIP 2987445)

Signalment: 3 year old, female brown pelican (*Pelecanus occidentalis*)

History: This was one of twelve brown pelicans moved from an indoor winter holding facility to an outdoor pond. One month later, this bird presented with lethargy, progressive weakness, and anorexia. Despite hospitalization and intensive treatment, the bird died twelve days after presentation. A total of seven of the

twelve birds subsequently died with similar clinical signs, lab results, and necropsy findings.

Gross Pathology: The bird was in thin body condition, with moderate muscle wasting over the keel. There was extensive, multifocal to coalescing, tan discoloration throughout all major skeletal muscles, including the ocular muscles. The heart contained numerous, multifocal to coalescing, pale yellow streaks that were most prevalent within the region of the left ventricle.

Several, 0.3 cm diameter, ulcers were present on the mucosal surface of the stomach.

Laboratory Results:

WBC: 27.2 x 10^3/UL (Mean: 12.55; SD 5.114)
CPK: 65,000 U/L
AST: 934 IU/L (Mean: 266; SD 256)
ALT: 106 IU/L (Mean: 45; SD 43)
Vitamin A, liver: 111.1 ug/g DRY
Vitamin E, liver: 39.91 ug/g DRY
West Nile Virus PCR and viral isolation, heart, kidney, brain: Negative
Heavy metal screen, liver (another bird from affected group): Acceptable levels of all heavy metals
Gas chromatography-mass spectrometry organic chemical screen, liver (another bird from affected group): No toxic compounds detected

Histopathologic Description: Skeletal muscle: The majority of the submitted tissue is affected by various degrees of myofiber degeneration and necrosis, with hypereosinophilia, loss of striations, variations in fiber size, sarcoplasmic vacuolation and/or floccular degeneration, and rare mineralization. A mild mononuclear inflammatory infiltrate is present within the more acutely affected areas, and fatty infiltration and fibrosis characterize the more chronically affected regions. A few aggregates of macrophages contain fine granular yellow pigment (lipofuscin, presumptive) within their cytoplasm.

Heart: There is moderate multifocal myocyte degeneration, necrosis, and replacement by fibrous connective tissue. A mild mononuclear inflammatory infiltrate is present. There is mild multifocal fatty infiltration. Mild multifocal hemorrhage is present within the more acutely affected areas.

Contributor's Morphologic Diagnoses: Skeletal muscle: Myofiber degeneration and necrosis, subacute to chronic, multifocal, moderate, with myofiber drop-out, replacement by fibrous connective tissue and fatty infiltration

Heart: Myocyte degeneration and necrosis, subacute to chronic, multifocal, moderate, with myofiber drop-out, replacement by fibrous connective tissue, mild multifocal fatty infiltration, and minimal hemorrhage

Contributor's Comment: The morphologic findings within the heart and skeletal muscle of these brown pelicans are consistent with a degenerative myopathy. Differentials based on morphology include hypovitaminosis E, ionophore toxicity, and exertional myopathy. Although these pelicans were supplemented with a paste that purportedly contained vitamin E, nutritional analysis of the paste at two laboratories indicated that none of the submitted samples contained vitamin E. Additionally, nutritional analysis was performed on fish normally fed to the pelicans. Vitamin E levels within herring and spearing were at the low end of the normal range, and those in capelin were low. Combined, these findings were highly suggestive of hypovitaminosis E as the cause of the myopathy in these birds. Although vitamin E levels in the liver were within the normal range reported for most adult animals (between 20 and 40 ug/g dry wt.), this is most likely due to the fact that this bird had been on vitamin E injections from the time it was admitted to the hospital (twelve days prior to death). There was no exposure to ionophores in these birds.

The histories of these birds suggest that increased activity (recent release to an outdoor enclosure) may have led to the acute onset of clinical signs within multiple birds.

Vitamin E deficiency results in oxidative damage to actively contracting muscle fibers due to a decrease in free radical scavenging[1]. Similar findings are also present in cases of selenium deficiency. Selenium is a vital component of the antioxidant enzyme glutathione peroxidase, a lack of which also results in oxidative damage. Myopathies due to vitamin E and selenium deficiencies are most often seen in young herbivores fed poor-quality grass hay with little to no access to pasture[2]. Affected animals often have generalized weakness, with preferential involvement of muscles involved in suckling and mastication, as well as locomotory musculature. Myopathies in birds, including brown pelicans, have also been reported[3]. In the case of pelicans, fish diets are frequently high in polyunsaturated fatty acids, which when stored over time have an increased propensity to form lipid-derived oxidation products, increasing the need for vitamin E.

It is interesting to note that although other piscivorous birds in the collection (including white pelicans) were receiving the same vitamin paste and diet, none have presented to date with similar clinical signs or lesions. It could therefore be hypothesized that brown pelicans are more sensitive to hypovitaminosis E or have higher vitamin E requirements than other birds, and further investigation may be warranted.

AFIP Diagnoses: Heart; skeletal muscle: Myofiber degeneration and necrosis, multifocal, mild to severe, with replacement fibrosis and fatty infiltration, brown pelican (*Pelecanus occidentalis*), avian.

Conference Comment: The contributor provides an insightful glimpse at one of the many challenges faced when working with wildlife species as well as a concise review of vitamin E and selenium deficiency.

In general, vitamin E is a fat soluble vitamin and the alpha-tocopherol isomer is the most available and active form. It is found in vegetables, grains, nuts, fish, meat and dairy products. After absorption, vitamin E is transported via chylomicrons through the blood and accumulates in fat depots, liver, and muscle. Vitamin E is an important antioxidant that sequesters free radicals before they can initiate peroxidation of the polyunsaturated fatty acids of cell membranes. Fish often have decreased levels of vitamin E and increased levels of polyunsaturated fat which antagonizes the absorption of vitamin E. Selenium is found in varying amounts in the soil and is an essential component of the enzyme glutathione peroxidase which prevents peroxides from causing membrane damage.

A simplified pathogenesis for vitamin E/selenium deficiency follows:
Deficiency of vitamin E and/or selenium results in increased levels of oxygen free radicals which disrupt cellular metabolism and cause extensive membrane damage. Membrane damage results in an influx of calcium ions from the extracellular compartment into damaged cells greatly increasing cellular demand for energy to move calcium into mitochondria and away from calcium-sensitive myofilaments. Once mitochondria become overloaded with calcium, the end result is energy depletion. Excess calcium still in the cytosol causes myofibril hypercontraction, degeneration and subsequent cellular destruction.

In a production situation, deficiency may be severely affected by the climate and housing conditions, poor quality hay, grain, or rancid food sources such as fish or cod liver oil. Additionally, iron injections given to neonatal pigs can cause cell membrane lipid peroxidation and depletion of vitamin E/selenium.

Below is a helpful comparative pathology list of conditions associated with vitamin E and/or selenium deficiency in a variety of species:

- Muscle necrosis (skeletal, cardiac): most species, including cattle, sheep, goats, horses, pigs, dogs, mink, rats, mice, rabbits, guinea pigs, nonhuman primates, birds, reptiles and humans
- Dietetic microangiopathy (Mulberry Heart Disease), hepatic necrosis (hepatosis dietetica), encephalomalacia - conditions of vitamin E deficiency noted in swine and reported in elephants
- Hepatic necrosis (hepatosis dietetica): pigs, experimentally induced in rats, mice

- Glossal necrosis: young, suckling ruminants
- Myelin sheath and spinal cord degeneration, Equine Motor Neuron Disease: horses and other equids
- Encephalomalacia, neuronal necrosis, axonal degeneration: pigs, birds, dogs, rats, monkeys, humans
- Steatitis (yellow fat disease): may result from a combination of membrane damage and production of ceroid, an acid fast, PAS-positive, yellow pigment which may act as an irritant foreign material, inducing inflammation; most strikingly affects subcutaneous adipose tissue, but also intracavitary fat; cats, mink, pigs, rabbits, birds, reptiles; not noted in ruminants
- Intestinal lipofuscinosis (brown dog gut): dogs
- Testicular degeneration and aspermatogenesis: rats, guinea pigs
- Fetal resorption: rats
- Hemolytic anemia: pigs, cattle, nonhuman primates, humans
- Pigmentary retinal degeneration: dogs, nonhuman primates, humans
- Cataracts: rabbits
- Exudative diathesis: poultry, pigs
- Cherry cerebellum: turkeys
- Decreased hatchability due to inadequate pipping musculature: birds

Vitamin E and selenium deficiency affects multiple species and is probably the prevailing nutritional myopathy recognized in veterinary medicine. Anytime myodegeneration, necrosis and regeneration are present vitamin E/selenium deficiency should be considered in the differential diagnosis (2).

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CASE III – CP96-322 (AFIP 2594648)

Signalment: 7 month old, male, BXH-2 mouse

History: This animal died on the 13th day of quarantine

Gross Pathology: The spleen, liver and mesenteric lymph nodes were enlarged. The liver had gray mottling and the spleen was uniformly enlarged.

Contributor's Morphologic Diagnoses: Liver: myeloid leukemia
Lymph node, mesenteric: myeloid leukemia
Spleen: myeloid leukemia
Bone marrow: myeloid leukemia

Contributor's Comment: This strain of mice has a high incidence of myeloid leukemia which results in poor production of offspring and early death of adult mice. Transmission of the virus occurs not via the germ line but in utero or by the milk.

AFIP Diagnosis: Liver: Leukemia, favor myeloid origin, BXH-2 mouse, rodent.

Conference Comment: This case once again illustrates the importance of understanding and researching the specifics of the animal model you are working with, especially when it involves a specialized strain. Of interest in this case is the two-step relationship between a recessive mutation which causes myeloproliferation and a retroviral induced clonal expansion of leukemic cells resulting in myeloid leukemia (5). Although the strain of mouse supports a diagnosis of myeloid leukemia, the interpretation of cellular morphology in this case is equivocal. Immunophenotyping, including use of CD79a and CD3 antibodies, may prove especially helpful in such cases to help rule out lymphoid neoplasia.

More importantly, we used this case as a stepping-off point for a discussion on hematopoietic neoplasia and, more specifically, the two broad categories of lymphoid neoplasia and myeloproliferative disorders and the plethora of classifications and subtypes of each. While it is beyond the scope of this conference report (and this pathology resident) to recreate what Duncan and Prasse have so expertly done, a table (a.k.a. breakdown list or cheat sheet) created directly from the Duncan and Prasse text outline has been included below to help residents remember the separate categories and a few key features of each of these neoplasms (6).

HEMATOPOIETIC NEOPLASIA	
LYMPHOPROLIFERATIVE DISORDERS -lymphoid neoplasms originate from clonal transformation of lymphocytes with various degrees of differentiation and function	MYELOPROLIFERATIVE DISORDERS -all leukemias that originate in the bone marrow and involve cells **other than lymphocytes. Leukemia** is a clonal disorder where a single neoplastic cell gives rise to a homogenous progeny which can replace normal hematopoietic cells and result in myelophthisic disease.
Lymphoma; solid tissue mass, in organs other than bone marrow -**Multicentric**, multiple lymph nodes and organs -**Alimentary**, g.i. tract and regional lymph nodes -**Mediastinal**, young animals, T-cell origin -**Cutaneous**, epitheliotropic or dermal, solitary or generalized	**Acute myeloid leukemia** (AML) "blast cells' Subgroups: -*Acute undifferentiated leukemia* (AUL) -large numbers of blast cells, can be labeled with anti CD-34 -*Acute myeloblastic leukemia* (AML) **M1** If >90% of nonerythroid cells in BM are myeloblasts **M2** If 30-90% of nonerythroid cells are myeloblasts -*Promyelocytic leukemia* (**M3**) Only in pigs; Neoplastic cells have few azurophilic cytoplasmic gran. -*Myelomonocytic leukemia* (**M4**) both neutrophilic and monocytic cell lines; most common type of AML in dogs, cats, horses -*Monocytic leukemia* (**M5**) >80% of the nonerythroid nucleated cells in the bone marrow -*Erythroleukemia* (**M6**) coproduction of erythroblasts and myeloblasts, only reported in cats and poultry -*Megakaryocytic leukemia* (**M7**) >30% of the nucleated BM cells are megakaryoblasts
Acute lymphocytic leukemia (ALL), arises from undifferentiated lymphocytes in the bone marrow -neoplastic lymphocytes are large, round, indented nucleus, dispersed chromatin, one+ nucleoli or nucleolar rings, thin dark blue granular cytoplasm	**Myelodysplastic syndrome** (MDS) a preleukemic condition characterized by: **<30% blast cells in the BM,** **abnormal erythrocyte morphology** and **cytopenia affecting more than one cell line**
Chronic lymphocytic leukemia (CLL), differentiated lymphocytes that "home" to secondary lymphoid organs e.g. spleen -neoplastic cells resemble benign small Lymphocytes	**Chronic myeloid leukemia** (CML) "mature cells" Subgroups: -*Chronic granulocytic leukemia* (CGL) -*Chronic eosinophilic leukemia* -*Chronic basophilic leukemia* -*Polycythemia vera*; an unregulated proliferation of mature erythrocytes

	-*Essential thrombocythemia* -*Chronic myelomonocytic leukemia*; unregulated production of neuts and monos -*Chronic monocytic leukemia*
Large granular lymphocyte (LGL) **lymphoma and leukemia**, arise from bone marrow and "home" to epithelial sites, especially intestine. **LGL lymphoma**-arise in abdominal l.n., intestine, liver and spleen **LGL leukemia**-may be the primary abnormality in the dog -Both have variably sized cells with chunky, pink cytoplasmic granules often near the site of nuclear indentation	**Mast cell leukemia** Rare form of leukemia characterized by circulating mast cells which must be differentiated from a solid mast cell tumor with a leukemic blood profile.
Plasma cell tumors (Plasmacytoma and Plasma Cell Myeloma a.k.a. multiple myeloma) both are clonal proliferations of **B-lymphocytes** **Plasmacytoma**-solitary or multiple masses in older dogs, benign, skin and mucous memb. **Plasma Cell Myeloma**-clonal neoplasm of plasma cells, arises in bone marrow, often associated with monoclonal gammopathy, osteolysis in dogs, rare in cats Must satisfy two of the four diagnostic criteria for a diagnosis: 1) Radiographic evidence of osteolysis 2) Large clusters of plasma cells in the bone marrow 3) Monoclonal gammopathy on serum electrophoresis 4) Bence-Jones proteinuria (free immunoglobulin light chains which readily pass the glomerulus)	

Individuals are encouraged to review WSC case 1 (slide 04-113), conference 5, 2004-2005 for an interesting case of large granular lymphocyte leukemia in a mixed breed dog.

Additionally, a review article has recently been published which outlines the myelodysplastic syndromes: Weiss DJ. Recognition and classification of dysmyelopoiesis in the dog: a review. *J Vet Intern Med.* 2005;19:147-154.

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CASE IV – TAMU-2 (AFIP 2984013)

Signalment: Nine-year-old, castrated male, Maltese *Canis familiaris*

History: The dog was taken to the groomer who called later to tell the owner that the dog "was not doing well". He was depressed, possibly seizuring, and salivating in his cage. The dog had been left in a drying cage with hot air blowers for 3 hours. On presentation, the dog had tachycardia (220 bpm) and had hyperthermia (107.3 F) with widespread cutaneous petechiae and ecchymoses. He began to improve, but then began to vomit and had abdominal pain. Although he was on fluids, diuretics, and mannitol, he became anuric. He developed a bloody, mucoid diarrhea and was euthanized in multiple system failure and DIC.

Gross Pathology: At necropsy, it was noted the dog weighed 7.15 kg while at presentation he weighed 5.21 kg. This obese dog had petechiae and ecchymosis widespread on skin and over viscera with melena as well. Approximately 500 ml and 400 ml of effusion were in the thorax and abdomen respectively. Two, 3 mm calculi were in the urinary bladder. The liver was slightly pale and friable.

Laboratory Results: On presentation, HCT = 59%, corrected to 30% before euthanasia (reference range 37-55%); The dog had 42 nucleated erythrocytes per 100 nucleated cells in the hemogram and circulating leucocytes were degenerating; [Na] on presentation 164 mmole/L corrected to 154 before euthanasia (reference range 139-147 mmole/L). On presentation: Alkaline phosphatase 510 u/L, Alanine aminotransferase 459 u/L (reference ranges 24-147 u/L and 10-130 u/L, respectively). Prothrombin time 10.1 seconds, partial thromboplastin time 31.4 seconds (reference ranges 6.0 – 7.5 seconds and 7.1 – 10.0 seconds, respectively); AT III 73.4% (control >114%). Fibrin degradation products + + and platelets were clumped. Brain cholinesterase 0.074 pH/hr (normal 0.15 – 0.25). Anticoagulant screening and pesticide screening were negative.

Histopathologic Description: The liver presented has submassive, often bridging, periacinar (zone 3) hepatocytic necrosis, hemorrhage and dissociation of cords. Many apoptotic hepatocytes and acidophilic bodies are in the affected area. Edema and scattered megakaryocytes are noted.

Contributor's Morphologic Diagnosis: Submassive periacinar hepatic necrosis.

Contributor's Comment: The impressive liver lesion while typical of exertional heat stroke (EHS) or ambiental heat stroke remains unexplained as to mechanism. In humans, the lesion development varies between individuals and heat "dose", and lesions often occur with a delay from the heat insult. The liver lesion is severe enough to have prompted the practice of liver transplantation in cases of EHS.(5,9,13,14) The periacinar pattern of necrosis has resulted in the hypothesis that this is hypoxic necrosis, and the term, "ischemic hepatitis", has been applied to the lesion.(13) Indeed, shunting of blood from viscera, the dehydration, and the hypovolemia associated with heat stress all would lead to hypoxia; however, the contributor feels it is more than this. The disassociation of cords would support a diagnosis of shock, but the apoptotic features make me want to invoke a reperfusion hypothesis (see below). Interestingly, the lesion is redolent of severe cases of rabbit calicivirus infection.

The liver lesion of heat stroke in dogs is not a well-characterized.(1,3,4,6,8) There are no good descriptions of the hepatic lesion in dogs with lethal exertional or ambiental heat stroke. The delayed and progressive failure is an important feature because in dogs found acutely dead in cars from heat stroke (e.g. when the owner

puts dog in the trunk of the car for a long ride on a hot day (10), the liver lesion is not present; rather, the lesions are restricted grossly to a "parboiled" carcass, with typical agonal hemorrhages, visceral congestion, and without hemorrhagic diathesis. The veterinary literature comments on renal complications, hemoconcentration, increased circulating nucleated RBCs, hypoglycemia, and hemorrhagic diathesis in animals that die. Many animals that die with a delay from outset of signs have not been completely examined but one case refers to "centrolobular necrosis(4)." The bloody mucoid stool may not be specific and is typically seen in severe shock due to a variety of causes in dogs. The dog of this report also had clinical and histologic renal involvement. Regarding the apoptosis, hyperthermia has been shown to cause hepatic apoptosis in humans, rats, and dogs (7,11,15); such that, liver blocks from dogs treated with one hour of hyperthermia have been used for apoptosis controls (7), cool or what! Experimental hyperthermia in dogs reflects much of what we see in this dog.(2,12) No doubt the obesity and maybe the temperament (excitability) of this Maltese may have exacerbated the hyperthermic event of this dog. Unfortunately, no clinical chemistry was performed in the end stages of this dog's process. It is presumed the liver enzymes would have been much more severely affected just prior to death.

Other interesting features added spice to this case. The owner's lawyer insisted we were negligent in our weighings (the Maltese weighed almost 2kg more at necropsy). However, anuria during correction of dehydration, ascites, and hydrothorax are presumed to explain the weight gain. (Veterinarians are warned to exercise care in hydration of these patients.) Seizuring, perhaps related to hypoglycemia or brain edema, is reported in severe, canine heat stroke, but the groomer's comments of salivation and seizuring urged us to pursue a diagnosis of organophosphate intoxication. No organophosphates were found. It must be concluded that other processes besides organophosphate exposure must be able to lower brain cholinesterase. Because of pending litigation, we tested to be able to say that the hemorrhagic diathesis was not due to anticoagulant exposure.

AFIP Diagnosis: Liver: Necrosis, centrilobular and midzonal, acute, diffuse, Maltese, canine.

Conference Comment: A great lesion with an interesting story to accompany it. The contributor provides thoughtful insight into heatstroke, a commonly encountered condition occurring more frequently in summer when dogs are negligently kept locked in sweltering cars while their owners run errands. As the contributor alluded to, perhaps this dog was kept alive with supportive therapy longer than most, allowing this rarely observed lesion to develop.

Conference attendees discussed the high number of nucleated erythrocytes in the peripheral blood. Although commonly associated with lead toxicosis, chronic hypoxia and extramedullary hematopoiesis; in this case, the extremely high numbers were attributed to an acute breakdown of the blood-bone marrow barrier with systemic dissemination of nucleated red blood cells. Other important laboratory findings include elevated levels of alkaline phosphatase (ALP) and alanine aminotransferase (ALT), inducible and leakage enzymes associated with hepatocellular damage; prolonged prothrombin time (OSPT), partial thromboplastin time (APTT) and elevated fibrin degradation products (FDPs), all supportive of disseminated intravascular coagulation (DIC). Additionally, antithrombin III levels are reduced suggesting either consumption due to DIC or loss via a compromise in glomerular integrity and subsequent proteinuria.

There is some variation in slides which prompted discussion of the pattern of hepatocellular necrosis. Robbins and Cotran defines necrosis of entire lobules as submassive and of most of the liver as massive; whereas, Thomson's defines massive necrosis as that of an entire hepatic lobule or contiguous lobules without reference to a submassive pattern (16,17).

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