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CASE I – 03-12068 (AFIP 2887460)

Signalment: 6.5 years, neutered male, American Staffordshire terrier, (*Canis familiaris)*, canine.

History: This 33 kg dog presented to the University of MN Veterinary Medical Center with a four-day history of vomiting, weakness, and ataxia after ingesting a two-pound (0.9 kg) bag of store-bought raisins [0.97 oz/kg or 27.3 g/kg]. During this time, the dog was anorexic and oliguric. The dog had no previous medical problems. On presentation, the dog was quiet, alert, and responsive. The dog was slightly ataxic, but the remainder of the neurologic examination was within normal limits. The dog remained oliguric and continued to vomit while in the ICU. Therapy included placing a urinary catheter, intravenous fluids, famotidine, furosemide, and dopamine. Due to the lack of response to diuretics and fluid therapy, the dog was euthanized.

Gross Pathology: At necropsy, the major findings were restricted to the stomach. The gastric mucosa was diffusely reddened and there was moderate submucosal edema at the level of the cardia, extending along the most proximal (orad) 1/3 of the body of the stomach. There were no significant macroscopic lesions noted in the other organs.

Laboratory Results:
Complete blood count (CBC): within normal limits.
Serum chemistry: blood urea nitrogen (BUN): 209 mg/dl; creatinine: 16.6 mg/dl; phosphorus: 18.1 mg/dl; calcium: 13.0 mg/dl.
Ocular fluid (aqueous): "BUN" ocular fluid equivalent: 204 mg/dl; creatinine: 7.3 mg/dl.

Contributor's Morphologic Diagnosis: Kidney, [renal cortex], bilateral, nephrosis with acute tubular necrosis, extensive, moderate to marked, and renal tubular epithelial regeneration, multifocal, moderate.

Contributor's Comment: There have been several publications in the past 3 years that discuss cases of acute renal failure in dogs, associated with ingestion of grapes and raisins. The exact mechanism and pathogenesis of this putative association is not currently known. To date, diagnostic screening for mycotoxins, heavy metals, pesticides, and other contaminants has been negative.

Since 1989, the Animal Poison Control Center (APCC) of the ASPCA (American Society for the Prevention of Cruelty to Animals) has documented a number of cases of renal failure in dogs that had eaten raisins or grapes[1]. In several recent publications, the amount of raisins or grapes ingested was estimated to be between 0.41 and 1.1 oz/kg [1,4,5]. There was a wide variety in the types of grapes ingested in these cases, including fresh, store-bought grapes, fresh grapes from private vineyards, and fermented grapes from wineries[1]. In the cases of raisin ingestion, the majority of the raisins were commercial, sun-dried raisins[1].

As more data were collected from these cases, it was noted that most of these dogs typically vomited within a few hours of ingesting the grapes or raisins. Signs of anorexia and diarrhea were also noted in many of these cases. Most of the cases had abnormalities in the serum chemistry profile, including hypercalcemia, hyperphosphatemia, increased Ca X PO_4 product, and elevated blood urea nitrogen (BUN) and serum creatinine concentrations [1,2,4].

Histopathologic findings from one necropsied dog were similar to those seen in this case, including mild renal tubular damage. The published case also had metastatic mineralization of numerous tissues. There has been some discussion as to whether the renal lesions were sufficient to cause the severity of the dog's clinical signs[1]. The history, clinical signs, and histologic lesions in this dog are at least suggestive of an association between the ingestion of raisins and acute renal failure. Additional screening for contaminants in several of the reported cases is currently being conducted at a number of institutions.

The exact cause of the renal damage in these cases is not known. Possible etiologies include nephrotoxins, fruit contaminants, mycotoxins, high levels of vitamin D, interference of sugar metabolism, or an idiosyncratic or anaphylactic reaction possibly leading to hypovolemic shock and subsequent renal ischemia[1,2,3,4].

AFIP Diagnosis: Kidney, tubules: Necrosis, acute, multifocal, with regeneration, American Staffordshire terrier, canine.

Conference Comment: This is a classic example of acute tubular necrosis, although raisin toxicity is not widely reported in the literature. Although reference intervals were not given, conference attendees discussed clinical pathology data related to azotemia.

An increased serum blood urea nitrogen (BUN) and creatinine indicate azotemia and must be interpreted in light of other clinicopathologic parameters to determine if it is prerenal, renal, or postrenal. Prerenal azotemia is caused by a reduction in the glomerular filtration rate (GFR), and there is no decline in urine concentrating ability. This is an insensitive measure of renal disease since shock, dehydration, or cardiovascular disease may cause decreased renal perfusion, and thus decrease GFR. Renal azotemia occurs when 75% of the nephrons are nonfunctional and the kidneys lose the ability to concentrate urine. Postrenal azotemia is generally due to obstruction or postrenal leakage, and concentration abnormalities may or may not occur.[6]

The contributor notes that ocular fluid was obtained for evaluation. It is reported that postmortem urea nitrogen and creatinine concentrations in ocular fluid correlate closely with antemortem serum concentrations, and although it varies among species, are generally valid within 24 hours after death at a body temperature of 37 degrees Celsius.[7]

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References:

1. Gwaltney-Brant S, Holding JK, Donaldson CW, Eubig PA, Khan SA: Renal failure associated with ingestion of grapes or raisins in dogs. J Am Vet Med Assoc **218**(10):1555-1556, 2001
2. Means C: The wrath of grapes. ASPCA Animal Watch, Summer **22**(2), 2002
3. Singleton VL: More information on grape or raisin toxicosis. J Am Vet Med Assoc **219**(4):434-436, 2001
4. Penny D, Henderson SM, Brown PJ: Raisin poisoning in a dog. Vet Rec **152**(10):308, 2003
5. Campbell A, Bates N: Raisin poisoning in dogs. Vet Rec **152**(12):376 2003
6. Gregory CR: Urinary system. *In*: Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse KW, 4th ed., pp. 250-253. Iowa State Press, Ames, Iowa, 2003
7. Purdue Animal Disease Diagnostic Laboratory, 1995 Newsletter: http://www.addl.purdue.edu/newsletters/1995/occular.shtml.

CASE II - 03N156 (AFIP 2888669)

Signalment: Five year-old, female, spayed, Dalmatian, *Canis familiaris*, dog.

History: This five year-old Dalmatian had a history of intermittent vomiting which occurred within 10 minutes to 8 hours after eating, every 2-3 days for one week. Stool appeared normal. CBC and serum chemistries were submitted. All values were within normal limits. Metoclopramide and a bland diet were begun. Two days later, vomiting

continued and plain and barium contrast films of GI tract were taken. No obstructions or masses were noted. Intermittent vomiting was still occurring ten days later, so endoscopy of the stomach was performed revealing mild "granularity" to the mucosa. A mucosal biopsy was taken with histopathological findings of lymphoplasmacytic gastritis. Carafate and prednisone were initiated. Intermittent vomiting was still occurring two weeks after the biopsy was performed. The dog has lost four pounds and now appeared depressed. Another CBC and serum chemistries were submitted; results showed an increase in liver enzymes and an elevated WBC. At this time the decision was made to wean the dog off of the prednisone. Three days later the dog's appetite improved. Another CBC and serum chemistries were submitted. Liver enzymes had improved, however the WBC was still elevated. Antibiotics (cephalexin) were initiated. Six days later, the dog was vomiting every 3-4 days. This time a mass was palpated in the left upper cranial abdominal quadrant. Another CBC and serum chemistries were submitted. The WBC count was within normal and the liver enzymes had improved. An exploratory was performed. A large firm lobulated mass was found cranial to the left kidney, adhered to the dorsal body wall and vena cava and wrapped around the renal vessels. The tumor was hemorrhagic, friable and unresectable due to its proximity to major vessels. The abdomen and skin were closed and the dog was removed from anesthesia and placed in ICU where it died shortly thereafter.

Gross Pathology: There are hemorrhagic sections of small intestine as well as colon. The liver has two yellow masses each two cm in one direction. There is a mottled, partially encapsulated left adrenal mass (9 x 6 x 2 cm) adhered to the left renal vessels and vena cava extending cranially to the liver and attached to the parietal peritoneum.

Laboratory Results:
CBC/chemistries 4/9/03: All values within normal limits

After prednisone: 5/5/03: Bilirubin 4.2 mg/dl, Alk phos 1154 mg/dl, ALT 160 u/l, AST 79 u/l, GGTP 71 u/l. Sample was icteric. WBC 32.6 x 10^3/cmm, monocytes 2608 (8%).

Weaning off prednisone: 5/8/03: Bilirubin 1.6 mg/dl, Alk phos 2130 mg/dl, ALT 167 u/l, GGTP 97u/l. Sample was icteric. WBC 33.4 x 10^3/cmm, monocytes 3674 (11%).

Steroids discontinued post antibiotics: 5/14/03: Bilirubin 0.4 mg/dl, Alk phos 1282 mg/dl, ALT170 u/l, GGTP 51 u/l. WBC 15.8 x 10^3 /cmm

Contributor's Morphologic Diagnoses:
1. Pheochromocytoma, left adrenal gland.
2. Moderate diffuse hepatocellular swelling with mild lobar hyperplasia.
3. Minimal to mild acute hemorrhage, mucosa of the colon.
4. Mild acute congestion, mucosa of small intestine.

Contributor's Comment: The most common adrenocortical tumors in dogs are adenomas and carcinomas, and the most common tumors in the adrenal medulla of dogs are pheochromocytomas. All of these neoplasms may be functional.

Functional pheochromocytomas occur infrequently in animals. Norepinephrine is the catecholamine most commonly secreted from these tumors in dogs and may be secreted sporadically or continuously. Clinical signs reported are related to the release of catecholamines and include congestive heart failure, pulmonary edema, and ventricular fibrillation as a result of hypertension.[4] Pheochromocytomas may be large and invade the posterior vena cava, as seen in this case. Grossly, they are often light brown to yellow-red because of areas of hemorrhage and necrosis.[5]

Pheochromocytomas are tumors of chromaffin cells, which vary from small cuboidal or polyhedral cells to large pleomorphic cells often subdivided into small lobules by fine connective tissue septa and capillaries.[5] Immunohistochemically, neoplastic cells express chromogranin and synaptophysin. Neurosecretory granules are positive using Churukian-Schenk.

In bulls, pheochromocytomas may develop along with calcitonin-secreting C-cell tumors of the thyroid gland, which is part of a syndrome of multiple endocrine neoplasia (MEN) in which there is neoplastic transformation of numerous cells of neuroectodermal origin in the same animal.[1,5]

Functional tumors of the adrenal cortex secrete cortisol, however only about 50% of cases in one study had historical clinical signs, morphologic signs, or clinicopathological evidence of hyperadrenocorticism.[1]

Adrenal cortical carcinomas occur less frequently than adenomas. They have been reported most often in cattle, sporadically in old dogs and rarely in other species. Carcinomas develop in adult to older animals, and there is no particular breed or sex prevelance.[2]

Adrenal cortical carcinomas are generally larger than adenomas and may be more likely to develop in both glands. In dogs they are composed of a variegated, yellow to brownish red, friable tissue that incorporates most or all of the affected adrenal gland. They are often fixed in location because of extensive invasion of surrounding tissues and the posterior vena cava, forming a large tumor cell thrombus.[1]

Adrenal cortical carcinomas are composed of more pleomorphic cells when compared to adenomas, which are subdivided into groups by a fibrovascular stroma of varying thickness. The pattern of growth varies between individual tumors, and within the same carcinoma, resulting in the formation of trabeculae, lobules, or nests of tumors cells.[1]

Surgical resection of adrenal neoplasms can be achieved in dogs that do not have tumor invasion of the vena cava. Although long-term outcome in these patients is often good, adrenalectomy is technically difficult surgery, and perioperative complications are not uncommon. Perioperative complications include thromboembolism, pancreatitis, adrenal insufficiency, and cardiac arrest.[3]

AFIP Diagnosis: Adrenal gland: Pheochromocytoma, Dalmatian, canine.

Conference Comment: Conference attendees had some difficulty in tissue identification, but the packeted appearance of the tumor cells helped to classify this as a neuroendocrine tumor.

If an adrenal mass is present in a dog, an adrenocorticotropic hormone (ACTH) stimulation test, low-dose dexamethasone suppression test, and high-dose dexamethasone suppression test may be helpful in ruling out a functional adrenocortical tumor.

The ACTH stimulation test evaluates the ability of the adrenal gland to increase plasma cortisol in response to stimulation by exogenous ACTH. Normal dogs will have a two- to three-fold increase in plasma cortisol levels, compared to baseline values. This is the test of choice for diagnosis of iatrogenic hyperadrenocorticism because dogs will have little to no response to exogenously administered ACTH. Dogs with functional adrenocortical tumors may have a normal response, but at least 50% of dogs will have abnormal ACTH responses.[6]

The low-dose dexamethasone suppression test is used to screen animals for pituitary-dependent and adrenal-dependent hyperadrenocorticism. In healthy dogs, administration of dexamethasone inhibits cortisol secretion. Dogs with either pituitary-dependent or adrenal-dependent hyperadrenocorticism will have inadequate suppression of cortisol levels.[6]

The high-dose dexamethasone suppression test is used to differentiate pituitary-dependent hyperadrenocorticism from dogs with adrenal-dependent hyperadrenocorticism. Dexamethasone inhibits cortisol secretion in healthy dogs, identified at the lowest limit of detection by the cortisol assay when dexamethasone is administered at the high dose. Dogs with pituitary-dependent hyperadrenocorticism have similar suppression. Dogs with functional adrenal tumors will not adequately suppress plasma cortisol, nor will approximately 25% of dogs with pituitary dependent hyperadrenocorticism.[6]

The presence of an adrenal mass with normal dexamethasone suppression test results may suggest the presence of a pheochromocytoma. Hormonal tests for veterinary patients with pheochromocytomas are not specific because they have not been adapted from their use in human patients.[1] The value in clinicopathologic testing and clinical findings remain important noninvasive methods of diagnosis in patients with a functional adrenal mass.

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References:

1. Myers NC: Adrenal incidentalomas: Diagnostic workup of the incidentally discovered adrenal mass. Vet Clin North Am Small Anim Pract **27**(2):381-399, 1997
2. Capen CC: Tumors of the endocrine glands. *In*: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp.629-638. Iowa State Press, Ames, Iowa, 2002
3. Anderson CR, Birchard SJ, Powers BF, Belandria GA, Kuntz CA, Withrow SJ: Surgical treatment of adrenocortical tumors: 21 cases (1990-1996). J Am Anim Hosp Assoc **37**:93-97, 2001
4. Sako T, Kitamura N, Kagawa Y, Hirayama K, Morita M, Kurosawa T, Yoshino T, Taniyama H: Immunohistochemical evaluation of a malignant pheochromocytoma in a wolfdog. Vet Pathol **38**:447-450, 2001
5. Capen CC: The endocrine glands. *In*: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 3, pp. 340-345. Academic Press, San Diego, California, 1993
6. Ferguson DC, Hoenig M: Endocrine system. *In*: Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse KW, 4th ed., pp. 294-299. Iowa State Press, Ames, Iowa, 2003

CASE III – 03-8778 (AFIP 2888891)

Signalment: Black lab X, neutered male; 5 years old, *Canis familiaris*.

History: Dog presented 5 days after hiking in wilderness area with elevated temperature (106F), lethargy, anorexia and pronounced malaise. These signs progressed to ataxia, focal seizures and then over a month to weakness, inability to rise and dysphagia with pronounced weight loss and muscle atrophy. Intermittent fevers were present throughout this time. The dog was treated with a variety of medications including anti-oxidants, anti-inflammatory drugs (meloxicam and prednisolone) and antibiotics including trimethoprim sulfa. Final treatment was with Clindamycin. The dog is recovering.

Gross Pathology: Not applicable.

Laboratory Results:

Initial laboratory results were:
Marginal leucopenia with degenerative left shift. ALT=3436 iu/l (reference range 0-113); ALP 312 iu/l (reference range 04-113) CK 841 iu/l(reference range 00-314) Bilirubinuria was present and urine SG was 1.032.
The PCV dropped from .051 to .036 l/l (reference range 0.390-0.560) over the first week of treatment. Azotemia developed, BUN 16.8 mmol/l (reference range 2.5-9.20) Creatinine 173 µmol/l (reference range 68-141) and urine SG was 1.020 with both bilirubinuria and hemoglobinuria. ALT dropped to 259 iu/l and ALP to 293 iu/l, GGT

increased to 22 iu/l (reference range 2-20). CK dropped to 410 iu/l Antibody titer for ICH was positive at 1:24 (animal had been vaccinated).
One month later anemia had improved, PCV 0.416; the ALT was 764 iu/l but ALP and GGT were normal, the CK was 13206 iu/l. Mild azotemia persisted BUN 11.0 mmol/l. An ANA test was negative as was an IgG toxoplasmosis titer. IFA for *Neospora caninum* was positive at 1:800. Muscle biopsies submitted from biceps and semitendinosus muscle. Immunoperoxidase procedure on the muscle biopsy was positive for neospora and negative for toxoplasmosis.

Contributor's Morphologic Diagnosis: Pyogranulomatous myositis, severe, chronic, biceps and semitendiosus muscle; with numerous tissue cysts of *Neospora caninum.* Tachyzoites are also present in the areas of inflammation.

Contributor's Comment: The animal presented with a multisystemic disease that initially involved the liver, progressed to CNS signs and then to a profound muscle weakness with dysphagia. It is an unusual clinical presentation for this organism as the dog was 5 years old, and previously healthy with no evidence of immunocompromise. The most severe disease manifestations usually occur in animals <1 year of age. The initial manifestations were of hepatocellular injury, and although no biopsy was obtained, the subsequent findings of muscle tissue cysts and tachyzoites support this organism as the causative agent for the 8-week illness. The late involvement of the muscle tissue is also interesting but may reflect a resurgence of disease as a consequence of therapy that included both corticosteroids and nonsteroidal anti-inflammatory drugs.

AFIP Diagnosis: Skeletal muscle: Myositis, necrotizing, subacute, diffuse, moderate, with regeneration and intrasarcoplasmic protozoal cysts, Labrador Retriever cross, canine.

Conference Comment: This case was reviewed in consultation with Dr. J. P. Dubey. By light microscopy, Dr. Dubey favors a diagnosis of *Sarcocystis* based on the presence of metrocytes within immature cysts and internal septa separating mature organisms into compartments within the cysts, neither of which are features of *Neospora caninum*. Immunohistochemistry performed by Dr. Dubey indicates this is *Sarcocystis*. Dogs are definitive hosts for *Sarcocystis*, where the organism sporulates then is excreted in the feces in an infective form. Since dogs are definitive hosts, it is unusual for tissue cysts of *Sarcocystis* to be present, and especially unusual for the organisms to cause inflammation in tissue. In this case, the presence of tissue cysts and a substantial inflammatory reaction suggests either an aberrant life cycle of *Sarcocystis* or an unusual presentation of *Neospora*.[7,8] Dr. Dubey is working with the contributor to clarify the etiology and describe this case for publication.

Conference attendees discussed differential diagnoses for tissue cysts in skeletal muscle, including neosporosis, toxoplasmosis, and sarcocystosis.

Until recently, tissue cysts of *Neospora caninum* were reportedly only present in central or peripheral neural tissues, and have a characteristic thick (up to 4um) wall.[1] Recently, however, thin-walled (0.3-1um) tissue cysts have been reported in muscles of dogs and cattle that were naturally infected. This, however, has not been reproduced in experimentally infected animals.[6]

Dogs that develop generalized signs related to *Toxoplasma* infection are immunocompromised and young, most often less than one year of age. Older dogs develop specific clinical signs associated with lesions in neural and muscular systems, similar to clinical signs seen with *Neospora caninum* infection. Another similarity between *Neospora caninum* and *Toxoplasma gondii* is that the tissue cysts and tachyzoites of each have a comparable light microscopic appearance. In fact, it is believed that some previous reports of toxoplasmosis in dogs may have been neosporosis.[1,8]

Conference attendees discussed clinicopathologic alterations related to muscle disease. Creatine kinase (CK) and aspartate aminotransferase (AST) are important enzymes of muscle origin that may be altered in diseases when there is disruption of muscle cell membranes and subsequent enzyme leakage. Lactate dehydrogenase (LDH) may also be elevated with muscle damage and, when measured along with other enzymes, can support a diagnosis of muscle damage.

Creatine kinase is the enzyme of choice for detecting muscle disease because most serum CK activity is of muscle origin, although isoenzymes for cardiac muscle and brain also exist (the brain isoenzyme is only present in cerebrospinal fluid). Hemolysis affects CK activity because enzymes and intermediates released from erythrocytes may cause CK to become falsely elevated. Elevations in CK activity occur quickly, within 4-6 hours of muscle injury and return to reference intervals within 24-48 hours if muscle injury is not ongoing. Since CK is very sensitive to minor muscle injury, such as intramuscular injections, it is best evaluated in conjunction with other enzymes of muscle origin.[9]

Aspartate aminotransferase is not tissue specific, but muscle and liver are the major sources of this enzyme. Aspartate aminotransferase activity increases more slowly than CK and may persist for several days after cessation of muscle injury. Skeletal muscle, cardiac muscle, liver, and erythrocytes are sources of LDH activity. Erythrocytes contain very high activity, so even mild hemolysis may significantly alter LDH activity. The half-life of LDH is longer than that of CK or AST (approximately 5 days).[9]

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References:

1. Dubey JP, Lappin MR: Toxoplasmosis and neosporosis. *In*: Infectious Diseases of Dogs and Cats, ed. Greene CE, 2nd ed., pp. 493-507. W.B. Saunders Company, Philadelphia, Pennsylvania, 1998
2. Lindsay DS; Dubey JP: Immunohistochemical diagnosis of *Neospora caninum* in tissue sections. Am J Vet Res **50**(11):1981-1983, 1989
3. Ruehlmann, D. Podell M, Oglesbee M, Dubey JP: Canine neosporosis: A case report and literature review. J Am Anim Hosp Assoc **31**:174-183, 1995
4. Greig B. Rossow KD, Collins JE, Dubey JP: *Neospora caninum* pneumonia in an adult dog. J Am Vet Med Assoc **207**(7):1000-1001, 1995
5. Odin M, Dubey JP: Sudden death associated with *Neospora caninum* myocarditis in a dog. J Am Vet Med Assoc **203**(6):831-833, 1993
6. Dubey JP: Review of *Neospora caninum* and neosporosis in animals. Korean J Parasitol **41**(1):1-16, 2003
7. Dubey JP, Greene CE: Enteric coccidiosis. *In*: Infectious Diseases of the Dog and Cat, ed. Greene CE, 2nd ed., pp. 514-515. W.B. Saunders Company, Philadelphia, Pennsylvania, 1998
8. Podell M: Inflammatory myopathies. Vet Clin North Am Small Anim Pract **32**(1):147-167, 2002
9. Bender HS: Muscle. *In*: Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse KW, 4th ed., pp. 260-265. Iowa State Press, Ames, Iowa, 2003

CASE IV - 03 C 1607 (AFIP 2893688)

Signalment: Five-year old intact female Labrador retriever x German Shepherd cross.

History: A 5-year old Labrador retriever x German shepherd cross bitch was presented for left pelvic limb lameness. She was treated with a non-steroidal anti-inflammatory drug (carprofen; Rimadyl, Pfizer Animal Health)(87.5 mg BID). After 14 days of treatment the bitch was re-examined and a drawer sign detected in the left stifle. The animal was treated with another COX-2 inhibitor (deracoxib; Deramax, Novartis Animal Health); carprofen withheld for 4 days. The veterinarian performed surgery for a ruptured anterior cruciate ligament. The bitch was discharged and given a course of carprofen for 5 days BID. The dog was treated with carprofen for 19 days total.

At the end of the second course of carprofen, the bitch was presented by the owner. The bitch was unwell, constipated, icteric, and vomiting. A seroma developed at the surgery site. The veterinarian hospitalized the animal and administered amoxycillin, prednisolone, and cimetidine. After four days the bitch appeared improved and the veterinarian planned to send her home. At that time she was vomiting but less icteric. The following day icterus was marked and petechial hemorrhages developed in mucous membranes. The veterinarian gave her half a unit of blood, the bitch arrested and was revived, but never regained consciousness.

Gross Pathology: The submitting veterinarian reported the presence of nutmeg liver, and blood in the peritoneal cavity, omental bursa, mesentery and bowel. Internal hemorrhage was the presumed cause of death.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Hepatocellular necrosis, subacute, multifocal, periportal and centrilobular, with hemorrhage and lymphocytic-histiocytic periportal hepatitis.

Contributor's Comment: The presumed cause of subacute hepatopathy in this dog is idiosyncratic carprofen-associated hepatocellular toxicosis. The basis of the diagnosis is a temporal association following recent administration of carprofen and histological lesions consistent with toxic hepatopathy. The dog's breed (Labrador retriever cross) may be significant. Thirteen of 21 cases of carprofen hepatocellular toxicosis in one report occurred in the Labrador breed.[1]

Histological changes of variable severity were reported in liver in association with carprofen administration.[1] They included multifocal to extensive hepatocellular necrosis, periportal neutrophilic and lymphocytic inflammation, bridging fibrosis, biliary hyperplasia, intracanalicular and hepatocellular bile pigment accumulation, and extramedullary hematopoiesis. Four of 21 dogs in that study died 3 - 5 days after presentation. In addition to hepatocellular necrosis, one of two dogs examined post-mortem had multifocal renal tubular necrosis with regeneration and a perforating jejunal ulcer. Only liver was available from this dog, so the presence or absence of lesions in other tissues could not be documented histologically.

Carprofen (Rimadyl) is a non-steroidal anti-inflammatory drug (NSAID) in the propionic acid class. It is widely used of osteoarthritic and post-operative pain. The compound is a substituted carbazole, 6-chloro-a-methyl-9H-carbazole-2-acetic acid ($C_{15}H_{12}ClNO_2$) that inhibits cyclooxygenase activity, particularly the inducible cyclooxygenase COX-2. It is rapidly and nearly completely absorbed (>90% bioavailable) when administered orally, with peak blood plasma concentrations 1-3 hours after oral administration. It has a half-life of ~8 hours. It is eliminated primarily by biotransformation in liver followed by rapid excretion of metabolites in feces (70-80%) and urine (10-20%). Some enterohepatic circulation of the drug occurs. The manufacturer (Pfizer) has reported animal safety studies and adverse reactions.[2] The most common adverse clinical reactions are vomiting (4%), diarrhea (4%), changes in appetite (3%), lethargy (1.4%), behavioral changes (1%), and constipation (0.3%). Hepatopathy was not consistently reproduced in safety studies.

This dog was also treated with another NSAID, deracoxib. Hepatotoxicity is a rare adverse reaction associated with this drug.[3] The manufacturers recommend that deracoxib should not be given at the same time as other NSAIDs, including carprofen. Concurrent administration was not done in this instance.

The basis for hepatotoxicity in a small proportion of dogs given carprofen is unknown. The most likely explanation is an idiosyncratic toxic reaction. The drug was sold on the European continent for 10 years before an association was detected in North America between use of the drug and hepatic disease. A genetic feature unique to some Labrador bloodlines in North America might be an explanation, but that possibility was not pursued due to limited pedigree data.

AFIP Diagnosis: Liver: Necrosis, Rappaport zone 3, diffuse, with mild subacute hepatitis and intrahistiocytic hemosiderin, German shepherd x Labrador retriever cross, canine.

Conference Comment: This case was reviewed in consultation with the Armed Forces Institute of Pathology's Hepatic Pathology department. We recognize that most of the necrosis is in the centrilobular area, but prefer to categorize it as zone 3 because the pathologic processes in this case correspond better to acinar landmarks based on the three-dimensional physiologic unit rather than the landmarks of the classic lobule, which are an artifact of two-dimensional microscopic sections. Based on the acinus described by Rappaport, zone 3 is mostly centrilobular but in some planes of the two-dimensional section it can also be periportal.[4]

Conference attendees noted the prominent cholestasis, as verified by a Hall's stain. Perl's iron stain also revealed abundant intracytoplasmic hemosiderin.

Although serum chemistries were not reported in this case, conference attendees discussed possible laboratory abnormalities with liver disease. The hepatocellular leakage enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and sorbitol dehydrogenase (SDH) are released with damage to the hepatocyte. The serum activity of these enzymes is dependent upon the number of hepatocytes injured, the severity of the injury, and the half-life of the enzyme. The inducible enzymes alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) are membrane-bound and increased activity is seen with cholestasis, or when induced by certain drugs or hormones.[5]

The cytosolic enzyme ALT may be elevated with liver or muscle damage, or may be induced by drugs. This enzyme is not useful in horses, ruminants, pigs, or birds due to low activity in these species. Aspartate aminotransferase is both a cytosolic and mitochondrial enzyme, although more severe cellular injury is necessary for release of the mitochondrial isoenzyme. Like ALT, AST is not liver specific. It may be elevated in muscle disease or with in vivo or in vitro hemolysis since it is present in erythrocytes. Sorbitol dehydrogenase is liver specific in all animals studied, and is generally the enzyme of choice in horses, sheep, goats, and cattle.[5]

Alkaline phosphatase is bound to the plasma membrane of hepatocytes and biliary epithelium and is present in several isoenzyme forms, the most clinically important of

which are hepatic, bone, intestinal, placental, and corticosteroid. The intestinal and placental isoenzymes do not contribute significantly to serum ALP activity because of their short half-life (less than 6 minutes in the dog). The bone isoenzyme has increased activity in young growing animals, animals with lytic or proliferative bone lesions, or those with active bone resorption. The liver isoenzyme is specific for liver disease and is a sensitive indicator of cholestasis. Cats have a lower hepatic ALP activity and shorter half-life than dogs. Increases in ALP activity with cholestasis in cats are less dramatic than increases in GGT, except with hepatic lipidosis where ALP increases more dramatically than GGT. The corticosteroid isoenzyme is only present in the dog and has increased activity with exogenous and endogenous corticosteroids. Initially, the hepatic isoenzyme increases with corticosteroid treatment but eventually the corticosteroid isoenzyme, which increases more gradually, becomes the predominant form of ALP. ALP is not a sensitive indicator of liver disease in large animals because it has a wide reference interval in these species.[5]

Gamma glutamyl transferase activity is induced in cholestasis, and is the preferred indicator of cholestasis in large animals and birds. It is generally a more specific indicator of cholestasis than ALP, but can also be induced by corticosteroids in dogs. Its activity is also high in the colostrum of dogs, sheep, and cattle so neonates have a very high serum GGT, which may be a useful indicator of passive transfer.[5]

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http://wyovet.uwyo.edu/

References:

1. MacPhail CM, Lappin MR, Meyer DJ, Smith SG, Webster CR, Armstrong PJ: Hepatocellular toxicosis associated with administration of carprofen in 21 dogs. J Am Vet Med Assoc **212**(12):1895-1901, 1998
2. Pfizer Animal Health prescribing information on carprofen (Rimadyl®): http://www.rimadyl.com/about/pi.html
3. Novartis Animal Health prescribing information on Deracoxib (Deramaxx®): http://www.petwellness.com/deramaxx/content/label.asp
4. Fawcett DW: A Textbook of Histology, 11th ed., pp. 683-684. W.B. Saunders Company, Philadelphia, Pennsylvania, 1986
5. Bain PJ: Liver. *In*: Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse KW, 4th ed., pp. 195-199. Iowa State Press, Ames, Iowa, 2003

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