

**The Armed Forces Institute of Pathology  
Department of Veterinary Pathology**

*Conference Coordinator*  
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**WEDNESDAY SLIDE CONFERENCE 2010-2011**

**C o n f e r e n c e 2**

**18 August 2010**

**Conference Moderator:**  
**COL (Ret.) Michael J. Topper, DVM, PhD, Diplomate ACVP**

**CASE I: UW-N09-295 (AFIP 3 134360).**

**Signalment:** 6-year-old, male, neutered, domestic short hair cat (*Felis catus*).

**History:** Six-year-old, male neutered domestic shorthair who presented to the University of Wisconsin emergency room (ER) service 2/21/09 for severe anemia- PCV 12%. Anemia was non-regenerative, but had signs of autoagglutination, mild elevation in bilirubin, rubriblasts in peripheral blood smear, and a left shift. Mycoplasma PCR positive. Started treatment with steroids and doxycycline during visit. Came back through the ER 3/12/09 for pale white gums, murmur, collapse. Current physical exam findings: Temperature=96.1°F; Pulse=150; Respiratory rate=30; Mucous membranes=pale/white; murmur auscultated; abdomen soft.

**Gross Pathology:**

1. General body condition
  - i. Moderate, diffuse icterus of the subcutaneous fat
2. Abdominal cavity
  - i. Moderate diffuse icterus of the abdominal fat
  - ii. Mild abdominal

effusion

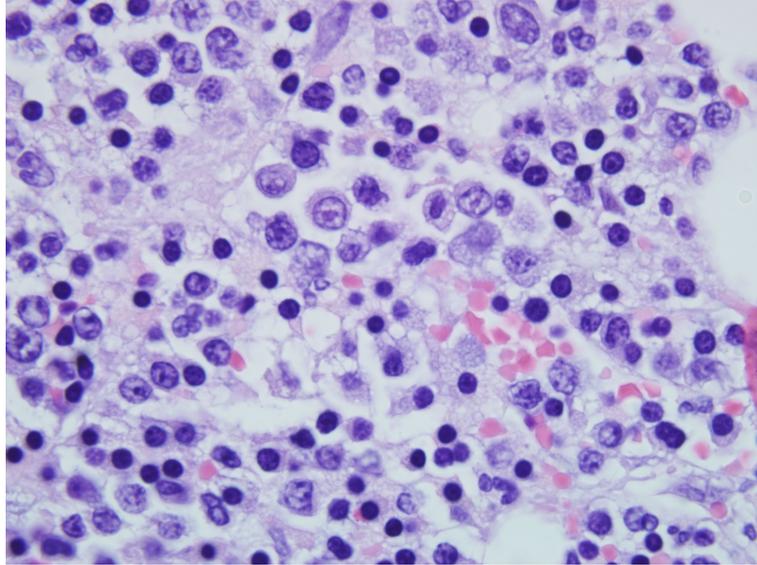
- iii. Moderate, diffuse hepatic fibrosis (presumptive)
  - iv. Moderate splenomegaly
  - v. Moderate, diffuse renal icterus
3. Thoracic cavity
- i. Moderate, diffuse lung collapse
  - ii. Moderate, focal, right cranial lung lobe emphysema

**Laboratory Results:**

Test	Results	REF INT	Units	Test	Results	REF INT	Units
RBC	2.67	5.8-10.7	x10 <sup>6</sup> /μl	WBC	23.2	5-19.5	x10 <sup>3</sup> /μl
PCV	12	-	%	Segments	17.8	-	x10 <sup>3</sup> /μl
MCV	47.4	39-55	fL	Monos	2.3	0-0.85	x10 <sup>3</sup> /μl
MCH	16	13-17	pg	Lymp	2.6	0-0.75	x10 <sup>3</sup> /μl
MCHC	34.4	30-36	g/dl	Atypical nuclear cells	1.07	-	x10 <sup>3</sup> /μl
Platelet	260	175-600	x10 <sup>6</sup> /μl				
Platelet	Clumped, > than reported value						

**Histopathologic Description:** Bone marrow:

The bone marrow is highly cellular with more than 85% of the cells being nucleated cells of the erythroid type. More than 50% of the cells have high nuclear:cytoplasmic ratios with basophilic cytoplasm, round, sometimes irregular, nuclei, finely stippled chromatin and one to two nucleoli. Mitotic figures are rare and atypical cells are also present. Based on the morphology, these cells resemble rubriblasts. Occasionally, the nuclei have moderately large magenta nucleoli. More mature cells of the erythroid type, including metarubricytes and rubricytes, are found in clusters and distributed throughout the section. The granulocytic lines are severely decreased in number. Moderate numbers of megakaryocytes are diffusely distributed throughout the bone marrow. A few, rare scattered iron stores are observed in the sections examined. Small islands of red blood cells are also seen.



*1-1. Bone marrow, cat. The highly cellular bone marrow is composed primarily of nucleated erythroid cells (greater than 85%). Over 50% of the cells are rubriblasts; metarubricytes and rubricytes are found in clusters within the field. (HE 1000X)*

The neoplastic cells are also observed in the sinusoids of liver, alveolar septae of lungs and in the vascular lumen in brain, kidney, heart and urinary bladder (sections not provided).

**Contributor's Morphologic Diagnosis:** Bone marrow – Erythremic myelosis, feline.

**Contributor's Comment:** Myeloproliferative disorders are defined as medullary and extramedullary proliferation involving marrow myeloid cell lines.<sup>1,4</sup> Erythremic myelosis (EM) is a proliferative disorder of early erythrocyte precursors (Di Guglielmo's disease).<sup>4</sup> The disease is categorized as an acute myeloid leukemia (M6b) containing only erythroid cells, with malignant erythroblasts being the predominant cell type. Erythroblasts along with prorubricytes constitute as much as 80% of all nucleated marrow cells. This is different from erythroleukemia (M6a), which constitutes proliferation of both erythroid and myeloid cells. Under veterinary adapted guidelines of French-American-British (FAB), EM is currently categorized as either a myelodysplastic syndrome with erythroid predominance (MDS-Er) or an acute myelogenous leukemia (M6-Er erythroleukemia with erythroid predominance). There are several reports of transition from M6-Er to M6 or M6a (erythroleukemia) in cats, which is described as lineage switching. M6 or M6a is rare in animals and more so in humans. M6b/M6-Er/EM are also rare in animals, but are more commonly seen in cats.<sup>4</sup>

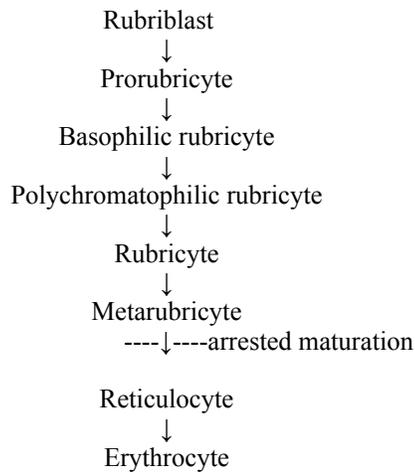
Erythremic myelosis is characterized by severe non-regenerative anemia with peripheral blood smears revealing variable numbers of metarubricytes, rubricytes, and rubriblasts with markedly decreased mature erythrocytes. In a healthy animal that is not anemic, metarubricytes are a rare

occurrence; further, in response to hypoxia or regenerative anemia, increase in metarubricytosis is usually transient. However, lack of significant numbers of circulating polychromatophilic erythrocytes (in Romanowsky- stained blood smears) or reticulocytes (in new methylene blue-stained blood smears) classifies the anemia as nonregenerative. In regenerative anemia, metarubricytosis may be present in acute splenic trauma, post splenectomy, bone fracture or following blood loss.

The pathogenesis of EM has not been completely elucidated, but a positive association is present with infection by Feline leukemia virus (FeLV) type C retrovirus. FeLV is a transmissible retrovirus responsible for or associated with a variety of disease processes. Three subgroups are present, A, B and C. Subgroup A is the infective, horizontally transmissible form of the virus, and subgroup B and C result from viral recombination within individual cats. Subgroup B is primarily responsible for the development of lymphoma and subgroup C is responsible for severe anemia and erythremic myelosis. Though the pathogenesis has not been identified, the prominent finding in EM is a maturation arrest of erythrocytes.<sup>2</sup> The erythrocyte arrest, a key component of EM and other disease, occurs before the reticulocyte stage (Diagram 1) resulting in severe anemia.

As indicated above, absence of reticulocytes in the blood in anemic animals is indicative of a disease process at the bone marrow level. A consensus exists that the site of action of the disease in the marrow is at the level of the burst-forming and colony-forming units of the erythroid line (Diagram 2).<sup>2</sup>

**Diagram 1.**



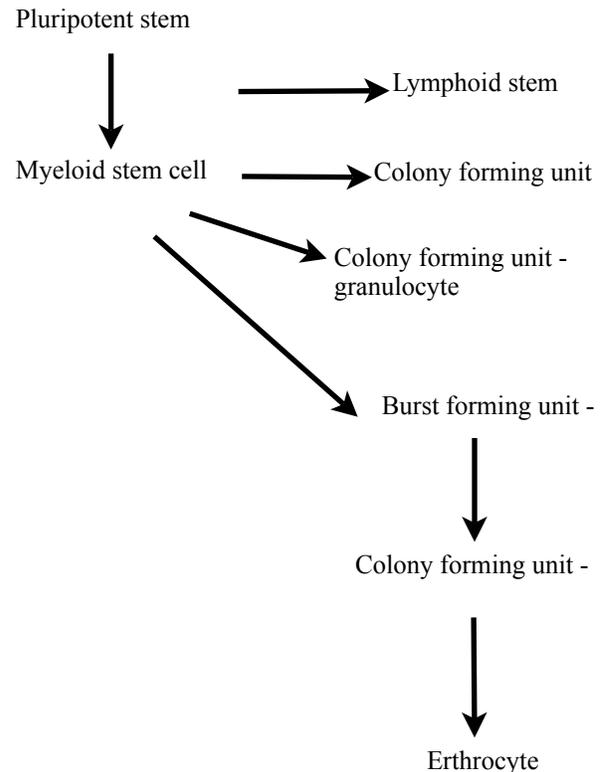
Clinically, the cats with EM present with depression of days to months, anorexia and mild icterus. The most consistent finding in cats with EM is a low hematocrit value of 12 to 15%. In addition to the severe anemia, the reticulocytes are either in the low-normal range or are not present. As EM is associated with FeLV infection, which is shown to have effects of all three bone marrow cell lines to varying degrees, a complete blood count along with evaluation of peripheral blood smear are indicated.

The numerous circulating nucleated red cells in M6-Er/EM/M6b can superficially mimic the finding in acute hemolytic anemia. In hemolytic anemia, the absence of rubriblasts or very immature erythroid cells in the peripheral blood and an increased reticulocyte count are characteristic of acute hemolysis with regeneration and can aid in differentiation from EM. Also the marked anemia seen with erythremic myelosis is nonregenerative and rubricytes are in maturation arrest. Feline infectious anemia caused by *Mycoplasma haemofelis* (previously *Haemobartonella felis*) is often suspected and may be present but should be recognized as an incidental finding because of the lack of polychromasia and presence of blasts in the blood of cats with EM.<sup>4</sup>

**AFIP Diagnosis:** Bone marrow: Myelodysplasia, diffuse with erythroblastosis and maturational arrest.

**Conference Comment:** Tissue identification was one of the diagnostic challenges experienced by some conference participants for this case, but most correctly identified the tissue as bone marrow despite the absence of bone spicules. Many conference participants also identified and described a proliferative lesion of erythroid cell lineage in the examined sections of bone marrow; but in the absence of the comprehensive clinical history and extensive laboratory data provided by the contributor, most favored a more general histologic diagnosis of myelodysplasia with erythroblastosis, and hence the diagnosis indicated above. Once the specific

**Diagram 2.**



clinicopathologic data were revealed during conference by the moderator, participants concurred with the contributor's more specific diagnosis.

The moderator and conference participants also discussed the variety of classification schemes for proliferative bone marrow lesions in the published literature, including the WHO classification system and the French-American-British system. The differential diagnosis for hypercellular bone marrow with erythroblastosis and reduced/absent myeloid elements discussed by participants included: 1) myelodysplastic syndromes; 2) myelodysplastic/myeloproliferative diseases; 3) acute myeloid leukemia (e.g. erythremic myelosis, erythroleukemia); and 4) chronic myeloproliferative diseases.<sup>3</sup> Some overlap exists with specific disease entities within each group of disorders among the various current classification systems.

Myelodysplastic syndromes (MDS) are characterized by maturation defects in clonal stem cells associated with ineffective hematopoiesis and qualitative and quantitative marrow cell dysplasia, and can evolve into acute leukemias.<sup>3,4</sup> Clinically, animals with MDS present with poor body condition, lethargy, pale mucous membranes, and a history of recurring infections, often involving the respiratory tract. Histologically, the marrow is hypercellular with morphologic change(s) in one or more cell lineages (i.e. erythrocytic, leukocytic, and megakaryocytic).<sup>4</sup> In addition to the

contributor's concise discussion of erythremic myelosis in the cat, more information concerning myelodysplastic and myeloproliferative disorders of domestic animals is available within the references cited below.

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[http://www.vetmed.wisc.edu/Pathobiological\\_Sciences.15.6.html](http://www.vetmed.wisc.edu/Pathobiological_Sciences.15.6.html)

**References:**

1. Comazzi S, Paltrinieri S, Caniatt M and De Dominic. Erythremic myelosis (AML6er) in a cat. *Journal of Feline Medicine and Surgery*. 2000; 2:213-215.
2. Morrison, JA. Erythremic myelosis. *Compen Contin Edu Pract Vet*. 2001;23:880-886.
3. Stockham SL, Scott MA. Bone marrow and lymph node. In: *Fundamentals of Veterinary Clinical Pathology*. 2<sup>nd</sup> ed. Ames, IA: Blackwell Publishing; 2008:324-358.
4. Valli VEO. Hematopoietic system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 5<sup>th</sup> ed., vol. 3. Philadelphia, PA: Elsevier Ltd.; 2007:125-147.

**CASE II: E18091 (AFIP 3135176).**

**Signalment:** 8-year-old, male, neutered, domestic-mixed breed cat (*Felis silvestris*).

**History:** The animal presented with anorexia and in poor general condition. Blood biochemical examination revealed increased enzyme activities of aspartate aminotransferase (AST) and alkaline phosphatase (ALP). One month later, exploratory laparotomy was performed because of further worsening of body condition. The surface of the liver was irregular, and multiple small nodules were observed on the small intestine. Wedge biopsy of the liver and small intestine was performed.

**Gross Pathology:** The surface of the biopsied liver tissue was irregular and multiple small nodules were scattered in the small intestine.

**Laboratory Results:** On day of first presentation: ALT 300 IU/L; ALP 130 IU/L; and Total bile acids 12.5µmol/L. One month later: ALT 600 IU/L; ALP 300 IU/L; and Total-bilirubin 2.8 mg/dL.

**Histopathologic Description:** Liver: Neoplastic lymphocytes were observed in the cytoplasm of hepatocytes, sinusoids, interlobular veins, and the interstitium of Glisson's sheath. The number of neoplastic cells in the hepatocytes was one to several. Mitotic figures were often observed among the engulfed neoplastic cells. Neoplastic cells invaded the epithelial layer and lumen of the bile duct. Some hepatocytes engulfing the neoplastic cells had lipofuscin

pigments or clear vacuoles in the cytoplasm. However, apparent morphological changes suggesting cell death were not detected in these hepatocytes.

A large number of neoplastic lymphocytes had round or ovoid nuclei which were elongated or cleaved in some cells. The nuclei of neoplastic cells had darkly-stained coarse chromatin and were easily distinguishable from those of hepatocytes. The neoplastic cells had a scant amount of eosinophilic cytoplasm.

In addition, varying amounts of yellow pigment were engulfed in Kupffer cells. Neutrophils and other segmented nuclear granulocytes were increased in the sinusoids and emigrated into some hepatocytes and bile ducts.

Immunohistochemically, large number of neoplastic cells showed positive reaction for CD3. Positive reaction for CD20, CD56, and CD79 was not observed. Immunopositive reaction for cleaved-caspase 3 was not observed in the hepatocytes with or without infiltrated neoplastic cells. Immunohistochemical examination for proliferating cell markers, such as Ki-67 and PCNA, brought equivocal results due to the intense positive reaction of infiltrating neoplastic cells in these hepatocytes.

**Contributor's Morphologic Diagnosis:** Liver: T-cell chronic lymphocytic leukemia.

**Contributor's Comment:** Malignant lymphoma, including the leukemic type, is the most common neoplasm of cats and accounts for more than half of all feline hemolymphatic tumors. Among the liver tumors of hematopoietic cell origin, malignant lymphoma/leukemia is also most common in the cat; however, infiltration of tumor cells into hepatocytes is rare.<sup>2</sup> Epithelial invasion by neoplastic cells is a characteristic feature of some special types of malignant lymphoma, such as epitheliotropic cutaneous lymphoma and primary intestinal lymphoma in dogs and cats. In addition to epithelial invasion by neoplastic cells in these types of malignant lymphomas, emperipolesis may also occur. Emperipolesis is a phenomenon in which some kind of viable cell, for example a lymphocyte, is engulfed by a large host cell without damage to either cell. This phenomenon is usually observed among cells in tissue cultures or isolated human cell smears. The phenomenon of emperipolesis has been reported to occur under various physiological and pathologic conditions. Host cells recorded to engulf lymphocytes, granulocytes, or other blood cells include mesenchymal cells, fibroblasts, thyroid epithelial cells, endothelial cells of high endothelial venule, megakaryocytes, monocytes, macrophages, and cancer cells. Normal lymphocytes, neoplastic cells obtained from leukemias, or lymphomas were also reported to be involved in emperipolesis when cultured with macrophages. The occurrence of *in vivo* emperipolesis in humans is very rare. In animals, reports of emperipolesis *in vivo* are also very rare.

The present case of feline malignant lymphoma involving the liver is considered to be the leukemic type due to the appearance of neoplastic cells in the sinusoids. Immunohistochemical findings suggest that the neoplastic lymphocytes are T-cell origin. According to the histologic criteria established by the World Health Organization (WHO), this case is T-cell chronic lymphocytic leukemia judging from the small cells with a dense chromatin distribution.

As stated above, neoplastic lymphocytes of T-cell origin occasionally have a character of infiltrating into the epidermis, the epithelium in adnexal tissues, or mucosal epithelium. In addition, as in the present case, the fact that CD3 positive neoplastic T-cell lymphocytes invaded the epithelium of a relatively large bile duct along with hepatocytes suggests that infiltration into hepatocytes reflects a common mechanism of neoplastic cells of T-cell origin.

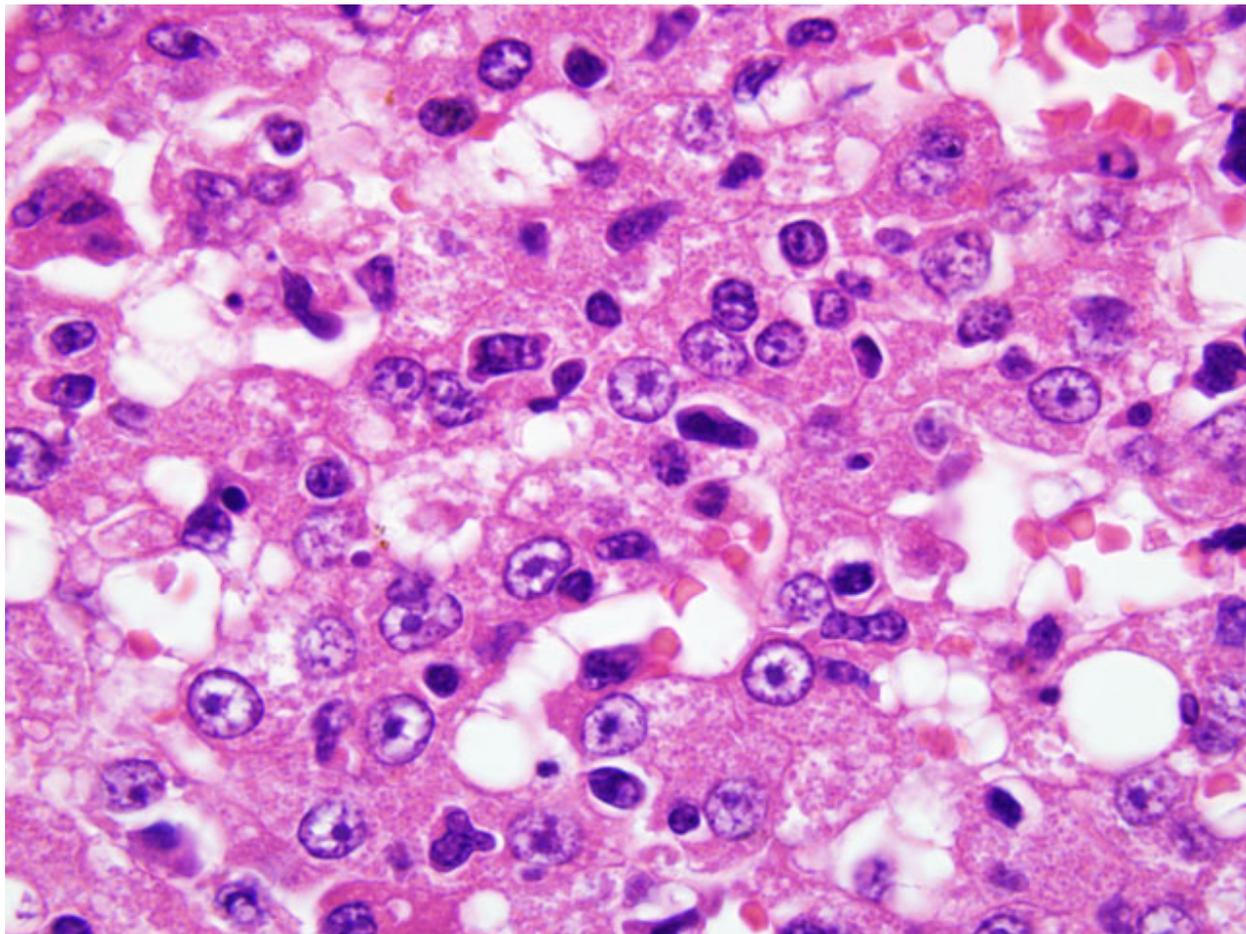
In the present case hepatocellular damage due to intracellular invasion by neoplastic lymphocytes was considered possible;

*2-1. Liver; malignant lymphoma, T-cell, large granular lymphocyte type, cat. Neoplastic lymphocytes occur within sinusoids and within the cytoplasm of hepatocytes (emperipolesis). (HE 1000X)*

however, necrotic and apoptotic changes of hepatocytes were not detected morphologically and hepatocytes had a negative reaction for one of the enzymes concerning apoptosis, cleaved-caspase 3, by immunohistochemical examination. From these results, cytotoxic effect of infiltrating lymphocytes on hepatocytes was not evident.

**AFIP Diagnosis:** Liver: Malignant lymphoma, T-cell, favor mature large granular lymphocyte lymphoma with emperipolesis.

**Conference Comment:** This very intriguing and diagnostically challenging case stimulated a vibrant discussion during the conference, and participants were evenly divided as to whether the lesion represented an inflammatory process or malignant lymphoid neoplasia. All participants identified an infiltrate of small lymphoid cells in the liver, with frequent occurrence of the cells in the cytoplasm of hepatocytes (emperipolesis), as described by the contributor. Close visualization of the lymphoid infiltrate under oil immersion reveals that the cells have irregularly round to ovoid, often indented nuclei with coarse chromatin and inapparent nucleoli. In some areas, eosinophilic granules are present in the cytoplasm, often within an indentation in the nucleus. Occasional mitoses are present. Hepatocytes



are swollen and occasionally contain lipid vacuoles. Based on the interpretation of morphologically abnormal lymphocytes, participants ultimately favored a neoplastic process and preferred the diagnosis of malignant lymphoma. While the infiltration of malignant lymphocytes into the liver may well represent an underlying leukemic condition, bone marrow and peripheral blood evaluation are required to document the definitive diagnosis of leukemia, and hence the diagnosis indicated above. For participants favoring an inflammatory process, an autoimmune condition was suspected as the underlying cause of the lesion.

Humans have a distinct form of hepatic lymphoma termed sinusoidal T-cell lymphoma, and its histopathologic features share similarities with the case of this cat. The primary histologic finding in the human disease is diffuse infiltration of malignant T-cells into the hepatic sinusoids in the absence of a mass effect. The histologic observation of low to moderate numbers of sinusoidal lymphocytes in the liver biopsies and the clinical presentation of affected human patients often lead to the misdiagnosis of acute or chronic inflammatory liver disease.<sup>1</sup> The sinusoidal form of hepatic malignant lymphoma in humans demonstrates the difficulty sometimes encountered when attempting to differentiate an inflammatory process from neoplasia by histopathology.

Among the various lymphoid neoplasms affecting cats, several features in this case are suggestive for large granular lymphocytic (LGL) lymphoma, including the contributor's gross report of nodular lesions in the small intestine, cytomorphology of neoplastic cells containing eosinophilic cytoplasmic granules, and prominent emperipolesis. The most common primary site for LGL lymphoma in the cat is the small intestine;<sup>3</sup> additional information concerning the gross lesions found in the small intestine, including histopathologic findings, might have been useful in this case.

This case was studied in consultation with Dr. Peter Moore, a recognized expert in the field of veterinary hematopoietic neoplasia. Dr. Moore has observed a number of similar cases in which the neoplastic lymphoid cells in feline LGL lymphoma express CD3; based on this finding and other evidence, he suspects LGL type tumors arise from intestinal epithelial lymphocytes (IEL). Dr. Moore also has observed a similar hepatic infiltration pattern for LGL lymphoma in dogs, albeit without the intestinal association; publication of the canine form of the disease is forthcoming. An IEL origin for the malignant lymphoid cells might well explain the unique histologic infiltration pattern observed in the liver of this cat, as most cases of LGL lymphoma in cats have intestinal involvement with frequent involvement of the liver, and disseminating lymphomas frequently metastasize to the liver along hepatic sinusoids.<sup>1,3</sup>

We thank Dr. Moore for his informative consultation with this case.

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<http://www.setsunan.ac.jp/~p-byori/>

#### References:

1. Ishak KG, Goodman ZD, Stocker JT. Primary hepatic lymphomas and suspected lymphomas. In: *Atlas of tumor pathology: Tumors of the liver and intrahepatic bile ducts 3rd series, fascicle 31*. Washington, DC: Armed Forces Institute of Pathology; 2001:335-338., , 2001
2. Ossent P, Stockli RM, Pospischil A. Emperipolesis of lymphoid neoplastic cells in feline hepatocytes. *Vet Pathol.* 1989;26:279-280, 1989
3. Valli VE. T-cell and NK-cell neoplasms. In: *Veterinary Comparative Hematopathology*. Ames, IA: Blackwell Publishing; 2007:304-306.

#### CASE III: AP07-3021 (AFIP 3142118).

**Signalment:** 27-year-old, male, Buckskin gelding (*Equus caballus*).

**History:** This horse presented to the North Carolina State University-Veterinary Teaching Hospital's Equine Emergency Service on 12/16/07 for evaluation of choke. He had been choking for approximately 24 hours. The referring veterinarian had attempted to relieve the choke, but was only able to extract a few leaves out of the trachea. The initial physical examination revealed a heart rate of 96 beats per minute, respiratory rate of 48 beats per minute, severe dyspnea and depression. Thoracic radiographs revealed evidence of aspiration pneumonia. The owner elected euthanasia due to poor prognosis and on humane grounds to relieve suffering. The final clinical diagnosis was aspiration pneumonia.

**Gross Pathology:** The necropsy findings are consistent with aspiration pneumonia. The dentition of this horse is severely worn. The esophagus is dilated and contains a 20 cm long clump of solid feed.

The right thyroid gland is markedly enlarged, measuring 12 x 7 x 5 cm. Its capsule is intact and the gland is freely moveable. On cut surface, almost all of the parenchyma is replaced by several tan, fleshy, expansile, multi-lobulated masses, the largest measuring 5 cm in diameter. One mass is very soft, friable and is mottled yellow and black. The left thyroid gland is moderately enlarged, freely movable, and measures 5 x 2 x 1.5 cm. On cut sections there are greater than twenty 0.2 - 1.0 cm soft, white, well-circumscribed spherical foci scattered throughout its parenchyma.

Bilaterally, affecting 80% of the left and 50% of the right adrenal gland, the medulla is replaced and expanded by red, fleshy, shiny, multifocal to coalescing, spherical masses that

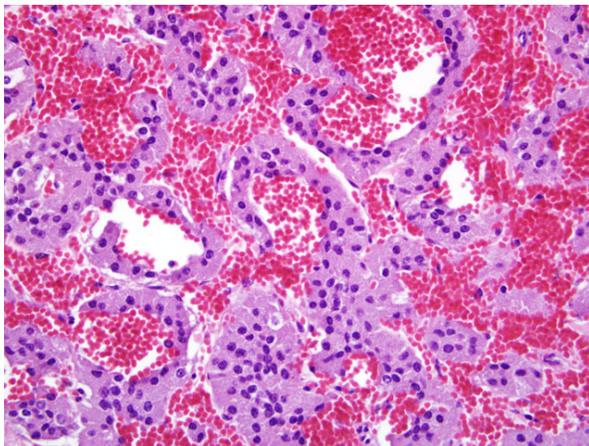


3-1. Adrenal gland and thyroid gland, horse. Both adrenal medullas (bottom) are expanded and replaced by red, fleshy, multifocal to coalescing, spherical masses that vary in size. The right thyroid gland is markedly enlarged, measuring 12x7x5 cm; the cut surface is replaced by several tan, fleshy, expansile, multilobulated masses, the largest measuring 5 cm in diameter. The left thyroid gland is enlarged measuring up to 5x2x1.5 cm; on cut section there are more than twenty (0.2-1.0 cm) white, well circumscribed spherical foci scattered throughout the parenchyma. Photograph courtesy of Mark Simpson, 37 Convent Drive Building 37 Bethesda, Maryland 20892, [websterjd@mail.nih.gov](mailto:websterjd@mail.nih.gov)

vary in size from 0.5-3.5 cm in diameter. The cortices, although slightly compressed, are intact.

**Histopathologic Description:** Adrenal glands, bilateral : Replacing much of the adrenal medulla and multifocally compressing the cortical parenchyma are multiple densely cellular, well circumscribed, and partially encapsulated masses composed of neoplastic polygonal cells arranged in

3-2. Adrenal gland, pheochromocytoma, horse. Neoplastic adrenal medullary cells are arranged in nests, packets, and trabeculae supported by a fine fibrovascular stroma. Neoplastic cells have variably distinct borders, moderate amphophilic granular cytoplasm, and an oval nucleus. (HE 400X)

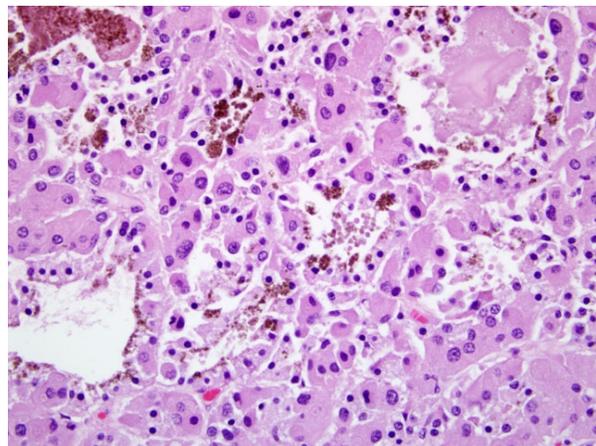


nests and cords supported by a fine fibrovascular stroma. The neoplastic cells have oval nuclei, often with a single, dark, basophilic nucleolus, amphophilic, granular cytoplasm, and variably distinct cell borders. The mitotic rate averages 1 per high power field. Multifocally throughout the neoplastic masses, there are prominent blood filled sinuses.

Right Thyroid Lobe (top tissue section): This section includes a portion of the 5 cm diameter mass described grossly and an adjacent, smaller multilobulated mass. The larger mass is a partially encapsulated, well circumscribed, expansile neoplasm composed of polygonal cells arranged in nests and cords. The neoplastic cells have moderate amounts of eosinophilic granular cytoplasm, round to oval nuclei that have vesicular to hyperchromatic chromatin, occasional eosinophilic intranuclear pseudoinclusions, and variably distinct cell borders. These cells demonstrate moderate to marked anisokaryosis and anisocytosis. The mitoses are less than 1 per high power field. Scattered randomly throughout the mass, accounting for approximately 40% of its composition, are entrapped and frequently compressed thyroid follicles, which are lined by low cuboidal cells with vacuolated cytoplasm and often pyknotic nuclei. A thin strip of thyroid parenchyma is compressed between the previously described mass and the smaller adjacent mass which is composed of disorganized aggregates of more uniform yet similarly shaped polygonal cells to those described in the larger mass. These cells demonstrate minimal anisocytosis and anisokaryosis. Entrapped follicles are also visible, accounting for 60-70% of this mass.

Left Thyroid Lobe (central tissue section): This section includes three of the masses seen grossly. These masses are partially encapsulated and compress the thyroid parenchyma. Otherwise these foci are similar to the smaller mass

3-3. Thyroid gland, C-cell carcinoma, horse. Neoplastic thyroid parafollicular cells are arranged in nests and packets along a fine fibrovascular stroma. Neoplastic cells have moderate to abundant eosinophilic granular cytoplasm and irregularly round to oval nuclei. Cells demonstrate anisocytosis, anisokaryosis, and atypia. (HE 400X)



described in the right thyroid gland. Throughout the remainder of this tissue, there are aggregates of uniform yet similarly shaped polygonal cells to those already described that separate the thyroid follicles but lack any larger mass effect, encapsulation or compression affect of adjacent tissue. These aggregates of cells are interpreted to be hyperplastic foci.

**Contributor's Morphologic Diagnoses:** 1. Adrenals, bilateral: Pheochromocytomas, multifocal to coalescing.  
2. Right thyroid lobe: C cell carcinoma and C cell adenomas.  
3. Left thyroid lobe: C cell adenomas and multifocal C cell hyperplasia.

**Contributor's Comment:** The changes in the adrenals are consistent with pheochromocytomas. No evidence of vascular invasion is seen at necropsy or during histological examination of tissue sections. If these tumors were functionally active, this horse may have had hypertension.

The masses in the thyroid are consistent with a transition from C cell hyperplasia to malignant neoplasia. The determination of carcinoma for the one mass is based on degree of cellular atypia rather than any overt evidence of invasion or metastasis. Initially, our top differential for these tumors was thyroid follicular cell carcinoma. However, immunohistochemical analysis of the tissue revealed that the neoplastic cells that form aggregates and sheets stain variably positive for calcitonin indicating that the tumor is of thyroid C (parafollicular) cell origin. The cells lining the follicles that are scattered multifocally within the masses are calcitonin negative. The foci of hyperplastic cells are positive for calcitonin as are the medullary cells within the remnants of normal thyroid tissue. The latter are interpreted as normal C-cells.

Multiple endocrine neoplastic (MEN) syndrome is extensively described in humans and has multiple subtypes, many with defined genetic bases. This horse has both pheochromocytomas and C cell tumors and is therefore most consistent with MEN 2A wherein there is medullary thyroid carcinoma (C-cell neoplasia) and/or C-cell hyperplasia in nearly all cases as well as pheochromocytoma in approximately 50% of cases.<sup>4</sup> The cell of origin for medullary thyroid carcinoma in humans is derived from neural crest cells and is part of the amine precursor uptake and decarboxylation system (APUD). It is capable of calcitonin secretion, which is a marker for this tumor. Calcitonin levels were not measured in this horse. More common in bulls, C-cell tumors are also called ultimobranchial gland tumors due to their suggested origin from remnants of the ultimobranchial body, which is composed of cells that can differentiate into both C cells and follicular cells. The etiology of these tumors in bulls is unknown but there is a possible link with excessive long term dietary intake of calcium.<sup>5</sup> As in humans, bulls with C cell tumors often have pheochromocytomas and pituitary

adenomas. This equine had multiple pheochromocytomas but no histologic evidence of a pituitary adenoma. De Cock et al. reported a case as well as retrospective data supporting the existence of MEN in horses. In their study, 4/72 horses had C-cell tumors only and 6/72 horses had both C-cell tumors and pheochromocytomas.<sup>2</sup> However, there is no mention that any of these C-cell tumors are carcinoma and their specific case is defined as a C-cell adenoma. Ueki et al. surveyed thyroid glands of aged horses and through use of immunohistochemistry found that the discrete white nodules found in 12/38 thyroid glands were consistent with thyroid C-cell adenomas. These nodules were only apparent when the thyroid was sectioned and involved only a small portion of the parenchyma. Histologically, the cells are described as mature with minimal atypia, unlike the neoplastic thyroid cells in our submission.<sup>6</sup>

**AFIP Diagnoses:** 1. Adrenal gland: Pheochromocytoma; and multifocal medullary hyperplasia.  
2. Thyroid gland, right lobe (per contributor): Parafollicular (C-cell) carcinoma; and parafollicular (C-cell) adenoma.  
3. Thyroid gland, left lobe (per contributor): Parafollicular (C-cell) adenoma; and multifocal parafollicular (C-cell) hyperplasia.

**Conference Comment:** Some conference discussion centered on the histologic distinctions among proliferative thyroid parafollicular (C-cell) lesions, and the following summarizes the general histologic features for each:<sup>1</sup>

- Nodular hyperplasia – multiple small foci of well-demarcated, unencapsulated cells with similar histologic appearance to normal cells
- Adenoma – solitary, well-demarcated, encapsulated, expansile mass which compresses adjacent tissue
- Carcinoma – typically larger than adenomas, with evidence of capsular invasion, secondary foci of growth within the fibroadipose tissue surrounding the gland, intravascular tumor cells, metastasis, and cellular atypia

Thyroid parafollicular neoplasms are most common in aged bulls, rats and adult horses; these tumors are often functional in older bulls, though serum calcium may be within normal limits to mildly decreased owing to the relatively slow metabolic turn-over of bone and compensatory parathyroid gland hyperplasia.<sup>1</sup> It has been proposed that since calcitonin is released in response to hypercalcemia, the lesion in bulls could be due to prolonged ingestion of high calcium diets. Occasionally amyloid, which is believed to be derived from calcitonin, can be found within C-cell tumors in bulls. The typical immunohistochemical staining pattern for C-cell hyperplasia, adenoma and carcinoma is as follows:<sup>3</sup>

- C-cell hyperplasia – consistently positive for calcitonin; variably positive for chromogranin A +B, synaptophysin, and neuron specific enolase

(NSE); may be focally positive for somatostatin and bombesin

- C-cell adenoma – consistently positive for calcitonin; variably positive for chromogranin A +B, synaptophysin, NSE and protein gene product 9.5 (PGP9.5); may be focally positive for somatostatin and bombesin
- C-cell carcinoma – consistently positive for calcitonin; variably positive for chromogranin A +B, synaptophysin, and protein gene product 9.5 (PGP9.5); may be immunopositive for thyroid transcription factor 1 (TTF-1)

Among domestic animals pheochromocytomas occur most frequently in the ox and dog. Metastasis occurs in approximately half of all canine pheochromocytomas, with spread to the liver, regional lymph nodes, spleen and lungs. These malignant adrenal gland tumors are generally non-functional; when functional, clinical signs attributed to catecholamine excess include tachycardia, edema, cardiac hypertrophy, hyperthermia, arteriolar sclerosis and arteriolar medial hyperplasia.<sup>1</sup>

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#### References:

1. Capen CC. Endocrine glands. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 5<sup>th</sup> ed. vol. 3. Philadelphia, PA: Elsevier Ltd.;2007:327-328, 402-405, 419-422.
2. De Cock HEV, MacLachlan NJ. Simultaneous occurrence of multiple neoplasms and hyperplasias in the adrenal and thyroid gland of the horse resembling multiple endocrine neoplasia syndrome: case report and retrospective identification of additional cases. *Vet Pathol*. 1999;36:633-636.
3. Kiupel M, Capen C, Miller M, Smedley R. Histological classification of tumors of the endocrine system of domestic animals, 2nd series, vol. XII, pp. 37-39, 46-47. Armed Forces Institute of Pathology, Washington, DC, 2008.
4. Peczkowska M, Januszewicz A. Multiple endocrine neoplasia type 2. *Familial Cancer*. 2005;4:25-36.
5. Seimiya YM, Takahashi M, Furukawa T, Mizutani K, Kimura K, Haritani M. An aged bull with concurrent thyroid C cell carcinoma, adrenal pheochromocytoma and pituitary chromophobe adenoma. *J Vet Med Sci*. 2009;71:225-228.
6. Ueki H, Koatari T, Oyamanda T, Oikawa M, Yoshikawa H. Non-functional C-cell adenoma in aged horses. *J Comp Pathol*. 2004;131:157-165.

#### CASE IV: 09-129 (AFIP 3162470).

**Signalment:** 3-month-old, male, castrated, Finn x Dorset lamb (*Ovis aries*).

**History:** On October 6, the animal was sedated with ketamine and dexmedetomidine and maintained with isoflurane anesthesia to debride a preputial laceration (presumed shearing injury). The animal was treated with intramuscular enrofloxacin once daily and flunixin meglumine twice daily until October 9, when the animal was noted to be passing pink urine (positive for blood and protein by dipstick). Diminished body condition was noted at this time. Flunixin was discontinued, and animal was started on parenteral ceftiofur and daily subcutaneous fluids. Pink urine was again noted on October 11. Due to declining clinical course, the animal was euthanized on October 12.

**Gross Pathology:** There was mild muscle wasting, although visceral fat reserves were adequate. In both the left and right kidneys there were focally extensive and severe hemorrhages in the renal medulla and crest with pallor of the medulla. The renal cortices were grossly normal.

*4-1. Kidney; lamb: In both the left and right kidneys there were focally extensive and severe hemorrhages in the renal medulla and crest with pallor of the medulla. The renal cortices were grossly normal. Photograph courtesy of Department of Comparative Medicine, Penn State Milton S Hershey Medical Center, Penn State College of Medicine, 500 University Dr, Hershey, PA 17033-0850, [tcoper@hmc.psu.edu](mailto:tcoper@hmc.psu.edu)*



**Laboratory Results** (clinical pathology, microbiology, PCR, ELISA, etc.):

WBC  $17,100 \times 10^3/\mu\text{L}$  (4,000-12,000)  
Neutrophils  $10,431 \times 10^3/\mu\text{L}$  (61%; 400-6000)  
Lymphocytes  $6156 \times 10^3/\mu\text{L}$  (36%; 1,600-9,000)  
Monocytes  $171 \times 10^3/\mu\text{L}$  (1%; 0-750)  
Eosinophils  $342 \times 10^3/\mu\text{L}$  (2%; 0-1200)  
HCT 34% (27-45)  
Hb 10.8 g/dL (9-15)  
Adequate platelets  
Fibrinogen 640 mg/dL  
Serum Chemistry:  
Na 146 mmol/L (139-152)

K 5.3 mmol/L (3.9-5.4)  
Cl 87 mmol/L (95-103)  
Tbili 0.4 mg/dL (0.14-0.32)  
Creatinine 3.8 mg/dL (1.0-2.7)  
BUN 86 mg/dL (8-20)  
Protein 5.2 g/dL (6.0-7.9)  
Albumin 3.1 g/dL (2.4-3.0)  
CPK 273 U/L (42-62)  
LDH 481 U/L (83.1-475.6)

Urinalysis: Clear, straw colored, SG 1.008, pH 8.5, protein 3+, glucose negative, ketones negative, small blood, WBC 0-1/HPF, RBC 10-20/HPF.

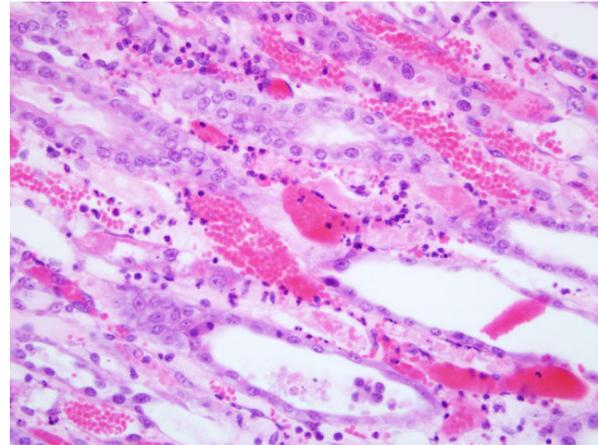
Aerobic blood cultures obtained from the jugular vein were negative for bacterial growth.

**Histopathologic Description:** Kidney: Sections submitted include renal medulla, crest and pelvis with variable amounts of cortex (taken from both kidneys). Within the deep medulla and crest there is focally extensive and severe subacute coagulative necrosis of tubules and interstitium with edema. Surrounding this is a zone of tubular epithelial degeneration and necrosis. Epithelial regeneration in this zone is intense, with intact basement membranes and interstitium. Numerous tubules contain luminal casts of erythrocytes or necrotic cellular debris. Low to moderate numbers of viable and occasionally degenerate neutrophils are present within small vessels as well as the interstitial matrix. The cortex is largely unaffected, although in a few sections there are remote, non-occlusive, adherent fibrin thromboemboli in cortical radial veins. A small amount of proteinaceous exudate is present within the uriniferous space of some glomeruli.

**Contributor's Morphologic Diagnosis:** Kidneys, bilateral, medulla and crest, necrosis, focally extensive, subacute, severe, with tubular erythrocyte casts and epithelial regeneration.

**Contributor's Comment:** Clinical signs and gross and histologic lesions are consistent with ischemic necrosis of the renal medulla and crest due to non-steroidal anti-inflammatory drugs (NSAIDs).<sup>3,6</sup> Anatomically, this lesion is appropriately termed renal medullary crest necrosis in sheep and horses, and renal papillary necrosis in rodents, man, and dogs.<sup>7</sup> Prostanoids are produced by cyclooxygenase-1 (COX-1) and COX-2 in the kidneys and exert a number of autocrine and paracrine effects. Most significantly, the prostanoids prostaglandin E<sub>2</sub> and Pgl<sub>2</sub> modulate renal blood flow and glomerular filtration rate.<sup>6</sup> Additional COX products play roles in renal handling of sodium and release of renin. Renal distribution of the COX-1 and particularly COX-2 isoforms and susceptibility to NSAID nephrotoxicity is species-dependent.<sup>2</sup> The medulla and papilla/crest predominantly express the COX-1 isoform in most species, where prostanoids products modulate urine concentrating ability, antagonize vasopressin-

mediated water and solute reabsorption, alter distal tubule potassium secretion, and promote dilation of the vasa recta to maintain medullary blood flow. Rats and dogs also express COX-2 in the papillary interstitial cells and are relatively sensitive to developing renal papillary necrosis,<sup>2</sup> suggesting that NSAIDs may also be directly toxic to the interstitial cells.<sup>3</sup> Humans are relatively resistant to NSAID nephropathy, and there is typically intercurrent disease or overdose (analgesic abuse).<sup>2</sup> Flunixin meglumine is a potent and non-specific COX inhibitor. Recently, the NSAID diclofenac has been associated with vulture population declines in southern Asia as a result of relay toxicosis and acute renal failure.<sup>4</sup>



4-2. Kidney, lamb: Multifocally within the deep medulla and renal crest, there is extensive tubular degeneration, necrosis, and regeneration; in the adjacent interstitium there is hemorrhage, congestion, and mild acute inflammation. (HE 400X)

In addition to NSAIDs, renal papillary necrosis can be induced by tyrosine kinase inhibitors and can be observed spontaneously in diabetes mellitus, sickle cell disease, and pyelonephritis.<sup>5</sup> Papillary necrosis has also been documented in dehydrated racing greyhounds and with amyloidosis in cats.<sup>3</sup> Renal papillary antigen-1 has been proposed as a urinary biomarker of papillary necrosis.<sup>5</sup> Although fluoroquinolones and cephalosporins can be associated with renal toxicity, this classically presents as an acute interstitial nephritis (AIN) with mononuclear and occasionally eosinophilic infiltrates suggestive of a hypersensitivity reaction.<sup>1</sup> Certain members of both classes of drugs have also been associated with tubular crystal development.

**AFIP Diagnosis:** 1. Kidney, medulla: Tubular degeneration, necrosis and regeneration, diffuse, moderate to severe, with few cellular casts.  
2. Kidney, medulla: Coagulative necrosis, acute, focally extensive (infarct).

**Conference Comment:** As indicated by the contributor, based on the clinical history the tubular changes are most likely due to NSAID administration. Participants reviewed the mechanisms of toxicity elegantly outlined by the contributor. Conference participants interpreted the acute renal crest infarct as most likely a perimortem event resulting from thrombosis of vessels.

In general, the differential diagnosis for renal papillary/crest necrosis across species includes hypoxic and toxic insults (which may occur in concert), dehydration, and urinary obstruction with or without pyelonephritis.<sup>3</sup> The contributor provides an excellent explanation of the pathogenesis of toxic insults. Dehydration contributes to papillary necrosis in dogs (primarily racing greyhounds) and lambs and kids treated with phenothiazines.<sup>3</sup> Urinary obstruction and pyelonephritis was high on the differential list for some participants due to the observation of moderate numbers of neutrophils within the renal crest. However, in this case most of the neutrophils occur in the medullary interstitium, and not in the tubules. The moderator emphasized that ascending pyelonephritis from the lower urinary tract typically produces a neutrophilic tubulitis, which is not evident in the case of this animal.

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#### **References:**

1. John R, Herzenberg AM. Renal toxicity of therapeutic drugs. *J Clin Pathol.* 2009;62:505-515.
2. Khan KN, Venturini CM, Bunch RT, Brassard JA, Koki AT, Morris DL, Trump BF, Maziasz TJ, Alden CL. Interspecies differences in renal localization of cyclooxygenase isoforms: implications in nonsteroidal antiinflammatory drug-related nephrotoxicity. *Toxicol Pathol.* 1998;26:612-620.
3. Maxie MG, Newman SJ. Urinary system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals.* 5<sup>th</sup> ed., vol 3. Philadelphia, PA; Saunders Elsevier; 2007:425-522.
4. Oaks JL, Gilbert M, Virani MZ, Watson RT, Meteyer CU, Rideout BA, Shivaprasad HL, Ahmed S, Chaudhry MJ, Arshad M, Mahmood S, Ali A, Khan AA. Diclofenac residues as the cause of vulture population decline in Pakistan. *Nature.* 2004;427:630-633.
5. Price SA, Davies D, Rowlinson R, Copley CG, Roche A, Falkenberg FW, Riccardi D, Betton GR. Characterization of renal papillary antigen 1 (RPA-1), a biomarker of renal papillary necrosis. *Toxicol Pathol.* 2010;38:346-358.
6. Radi ZA. Pathophysiology of cyclooxygenase inhibition in animal models. *Toxicol Pathol.* 2009; 37:34-46, 2009.
7. Read WK. Renal medullary crest necrosis associated with phenylbutazone therapy in horses. *Vet Pathol.* 1983;20:662-669.