



WEDNESDAY SLIDE CONFERENCE 2013-2014

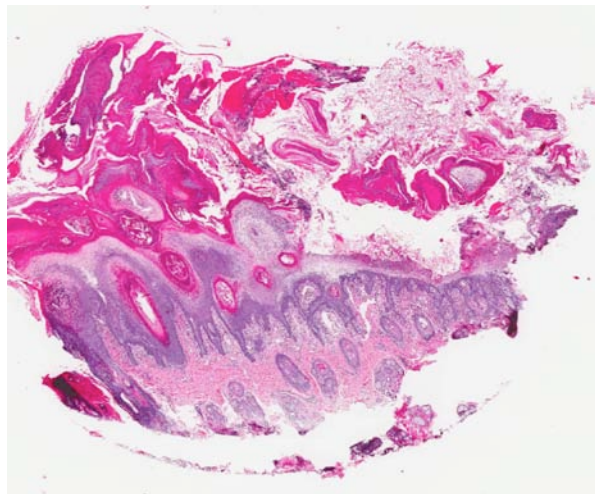
Conference 13

29 January 2014

CASE I: AVC C3670-13 (JPC 4032590).

Signalment: 10-year-old male castrated Shetland Sheepdog (*Canis familiaris*).

History: This dog was diagnosed with dermatomyositis as a puppy; it had footpad lesions characterized by hyperkeratosis, erythema, scaling and crusting. The dog was treated first with novalexin and then prednisone; he did well



1-1. Haired skin, dog: This section of skin is covered by a thick parakeratotic serocellular crust (extending down into hair follicles), with marked acanthosis and hyperplasia of basal epithelium. (HE 0.63X)

for 10-12 days and then stopped eating. Most recently, the dog developed diarrhea, non-regenerative anemia and glucosuria. An abdominal ultrasound revealed a severe and diffuse mottled appearance of the liver with a honeycomb pattern. Liver aspirates were taken for cytology assessment and the footpads were biopsied. The animal continued to deteriorate and was euthanized at the owner's request. The veterinarian obtained permission to perform a postmortem examination. The liver was diffusely nodular; the rest of the abdominal viscera were unremarkable. Samples of liver and skin were submitted for histopathology.

Gross Pathology: Two punch skin biopsies (0.7 cm in greatest diameter) with ulcerated surfaces and two fragments of liver (3.5 cm and 2.5 cm in their largest dimension) were submitted for histopathology. The liver samples had a nodular, irregular, capsular surface.

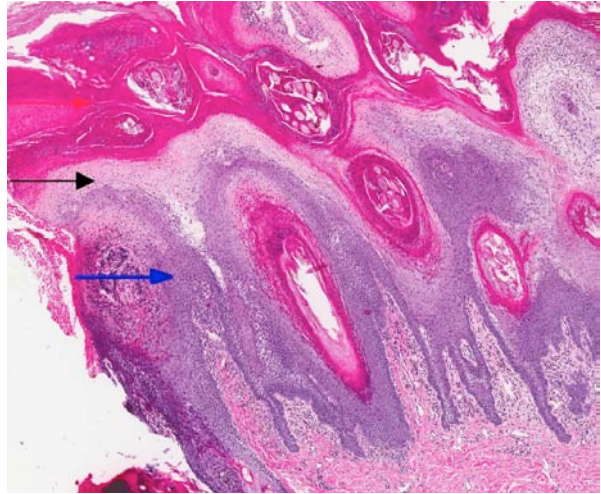
Laboratory Results:

Parameter	Value	Reference Interval
Albumin	23 G/L	24-55
ALP	1632 U/L	20-150

ALT	442 U/L	10-118
Glucose	9.2 mmol/L	3.3-6.1
WBC	19.1 x 10 ³ / mm ³	6.0-17.0
segmented	94%	
lymphocytes	3%	
monocytes	2%	
bands	1%	
RBC	4.12 x 10 ⁶ / mm ³	5.50-8.50
urine glucose	3+	

Liver cytology performed approximately one week before euthanasia revealed cholestasis, low numbers of attenuated and rarefied hepatocytes and possible neutrophilic inflammation.

Histopathologic Description: Two samples taken from the edges of the metatarsal pad and including the adjacent haired skin are examined. The epidermis is markedly, irregularly acanthotic

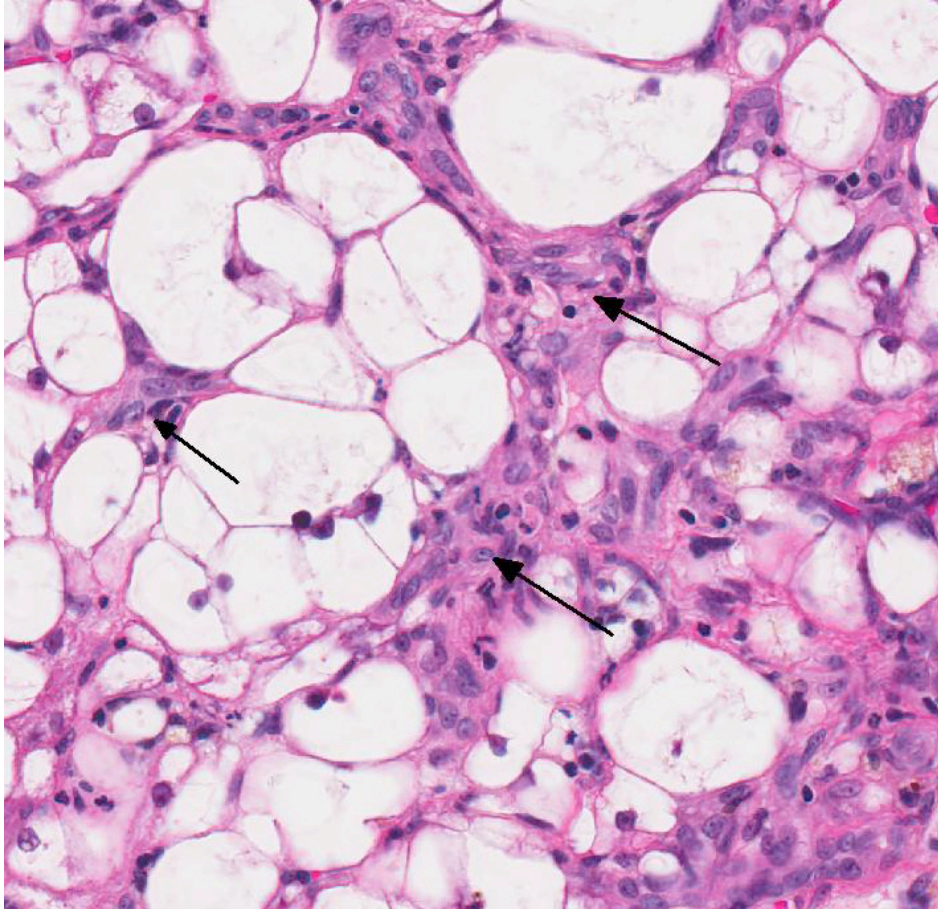


1-2. Haired skin, dog: Superficial necrolytic dermatitis exhibits what is known as the "red, white and blue sign", with superficial hyperkeratosis (red arrow), marked spongiosis of the stratum spinosum (black arrow), and marked hyperplasia of the basal layer (blue arrow). (HE 42X)

and covered with many layers of parakeratotic keratin admixed with multifocal dense aggregates of necrotic cell debris and hemorrhage. There are foci of erosion with multiple foci of epidermal neutrophilic and lymphocytic infiltration. Keratinocytes in the stratum spinosum are



1-3. Liver, dog: This section of liver contains numerous regenerative nodules of hepatocytes, which are easily seen against the pallor of remaining glycogen-distended hepatocytes. (HE 0.63X)



1-4. Liver; dog: Hepatocytes are swollen up to 5 times normal with numerous coalescing clear vacuoles (glycogen) surrounded by pale pink cytoplasmic strands ("spider cells"). Throughout the section there is marked ductular reaction (arrows). (HE 400X)

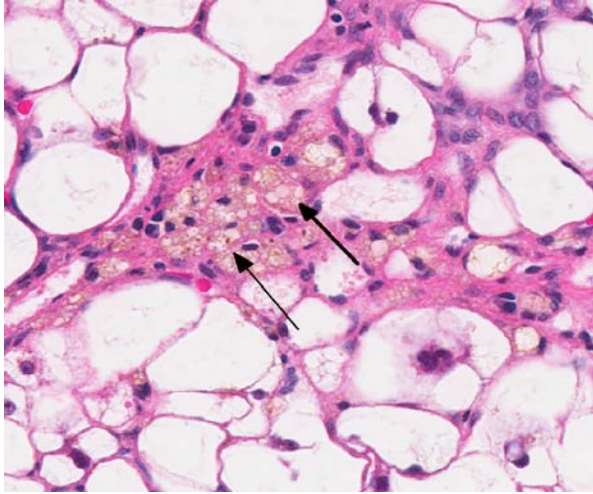
The liver sections show extensive, interconnecting areas of parenchymal collapse and hepatocellular vacuolation, usually surrounding and isolating variably sized nodules of hepatocytes (nodular regeneration). The vacuolated hepatocytes are often markedly enlarged with large amounts of clear to occasionally granular cytoplasm and peripheralized dense nuclei. The areas of vacuolar change also contain multifocal clusters of bile ducts and scant bundles of fibrous tissue. Small collections of band and segmented neutrophils (consistent with extramedullary myelopoiesis) and small numbers of

markedly vacuolated, swollen and pale (intracellular edema) with plump nuclei. The stratum basale is markedly hyperplastic with numerous mitotic figures and increased nuclear to cytoplasmic ratio (in general, giving the epidermis a "red, white and blue" layered appearance). Occasional apoptotic bodies and migrating leukocytes are noted within the epidermis. Numerous bacterial colonies and 7 x 2 micron, PAS positive, budding yeast (consistent with *Malassezia* sp.) are present within the surface keratin (not present in all slides). Multiple hair follicles are dilated and filled with fragmented keratin. Perifollicular, periadnexal and perivascular infiltrates of lymphocytes, plasma cells, macrophages and neutrophils, often associated with fibrous tissue, are present within the dermis. In rare instances, the infiltrates appear to invade the dermoepidermal junction. Gram staining of bacterial colonies reveals gram positive cocci and gram negative rods.

plasma cells and lipid-laden macrophages infiltrate the portal spaces in these areas. Small aggregates of pigment (ceroid and/or iron)-laden macrophages are often seen within the nodules of parenchymal cells (pigment granulomas); scant neutrophils and plasma cells also infiltrate these nodules.

Contributor's Morphologic Diagnosis:

1. Haired skin, bilateral, metatarsal areas: Lamellar epidermal edema, degeneration and necrosis, severe, with marked basal cell hyperplasia, parakeratotic hyperkeratosis (ie. superficial necrolytic dermatitis), and pustules/neutrophilic crusts containing bacterial colonies and budding yeast (*Malassezia* sp.).
2. Liver: Hepatocellular vacuolar change (vacuolar hepatopathy), severe, diffuse, with parenchymal collapse, moderate bile duct proliferation and nodular regeneration.



1-5. Liver, dog: Scattered throughout the section are aggregates of macrophages contain lipid and lipofuscin, resulting from rupture of hepatocytes (lipogranulomas). (HE 400X)

Contributor's Comment: The histomorphologic appearance of the skin and liver samples are consistent with hepatocutaneous syndrome (also called superficial necrolytic dermatitis, metabolic epidermal necrosis, and necrolytic erythema) with secondary pyoderma and cutaneous yeast infection.

Hepatocutaneous syndrome is a rare necrotizing skin disorder of dogs that is most often associated with metabolic hepatic disease (often idiopathic vacuolar hepatopathy),^{2,8} although it has also been described in diabetes mellitus, glucagon-secreting tumors (glucagonomas)^{2-4,9} and prolonged phenobarbital administration.⁵ It is thought that hepatic dysfunction may result in hypoaminoacidemia, preventing essential amino acids from reaching the skin, leading to nutritional deprivation and subsequent necrosis. The proposed pathogenesis involves increased hepatic catabolism of amino acids (from increased gluconeogenesis),^{2,7} elevated glucagon levels (due to decreased hepatic metabolism or glucagon-secreting tumors), or disturbance of zinc metabolism (possibly a result of malabsorption).^{4,9} A report of superficial necrolytic dermatitis (SND) in dogs receiving phenobarbital suggested that drug induction of hepatic microsomal enzymes may result in excessive utilization of amino acids by the liver leading to deficiency.⁵

Affected animals typically develop alopecia, erythema, crusting, exudation, and ulceration of the skin. The lesions are generally distributed over the ventral aspect of the abdomen,

mucocutaneous junctions, ears, periorbital region, and distal portions of the extremities. Probably the most common clinical dermatologic lesion is hyperkeratosis and deep fissuring of the footpads. Secondary skin infections with bacteria, yeasts, and dermatophytes are common. Pruritus and signs of pain are often apparent.^{2-4,9} Anorexia, weight loss and lethargy may also be present.^{3,4,9} Circulating liver enzyme activities are frequently high and plasma amino acid concentrations are severely low.^{2,7} Results of a study of 36 dogs with histologically confirmed SND indicated that older small-breed dogs (including Shetland Sheepdogs) were primarily affected. The median age was 10 years, and 27 (75%) dogs were male.⁷ Superficial necrolytic dermatitis has also been reported in cats⁴ and captive black rhinoceroses.⁶

In skin sections, the histopathologic finding of parakeratosis with crusting, hydropic degeneration of keratinocytes in the stratum spinosum, and hyperplasia of the basal cell layer, imparts the characteristic red, white, and blue appearance (referred to by some as the “French flag”) that is diagnostic for the disease.² Livers of dogs with hepatocutaneous syndrome are grossly nodular. Histologically, there is severe vacuolation of hepatocytes, with parenchymal collapse and condensation of the reticulin fiber network accompanied by nodular regeneration.⁸ This combination of liver features has been confused with hepatic cirrhosis by many authors. Ultrasonographic evaluation of the liver of affected dogs reveals an almost pathognomonic “honeycomb” pattern or Swiss cheese-like appearance consisting of a hyperechoic network surrounding hypoechoic areas of parenchyma.^{2,3} The underlying cause of these hepatic changes is often undetermined; however, they have been suggested to support an underlying metabolic, hormonal, or toxic cause.³ The cause for the hepatopathy in this dog was not determined.

Hepatocutaneous syndrome in dogs often has a poor prognosis, and survival times are often less than one year. Treatment generally includes parenteral and oral administration of amino acids, zinc, and essential fatty acids.⁷ If the disease is caused by a glucagon-secreting pancreatic neoplasm, surgical removal of the neoplasm can lead to resolution of skin lesions.²

Differential diagnosis for canine SND includes other parakeratotic diseases such as zinc-

responsive dermatosis and generic dog food-associated dermatosis. Clinical differential diagnoses include chronic erythema multiforme of older dogs, drug eruption, pemphigus foliaceus and systemic lupus erythematosus. Most of these can be ruled out by appropriate clinical history, physical examination, clinical laboratory, and histopathologic findings.²

JPC Diagnosis: 1. Liver: Hepatocellular vacuolar degeneration, diffuse, marked with hepatocellular loss, nodular regeneration and ductular reaction.
2. Haired skin: Epidermal edema, degeneration, and necrosis, superficial, diffuse, marked, with basal cell hyperplasia, parakeratotic hyperkeratosis, and subacute dermatitis.

Conference Comment: We thank the contributor for providing this excellent example and thorough analysis of the entity. Some conference participants reported difficulty in distinguishing regenerative hepatic nodules from islands of pre-existing, normal hepatic parenchyma. Following an animated discussion regarding hepatic histopathology, participants eventually agreed that hepatocytes within regenerative areas tend to be larger than those in the surrounding parenchyma and nodules are often delineated by variable amounts of fibrosis.¹⁰ Additionally, while foci of hepatic regeneration may contain rudimentary portal tracts and terminal venules, hepatic cords are usually more than two cells thick with reduced sinusoidal space, resulting in a somewhat disorganized nodule that may compress adjacent tissue.^{1,9}

The distinctive gross and histological appearance of nodular regeneration occurs as a result of its contrast with adjacent expanses of parenchymal atrophy and loss, which typically occur due to reduced blood flow and bile drainage. Macro/micronodular hepatic regeneration is classically associated with cirrhosis, where bridging fibrosis is also a characteristic lesion; however, the liver in this case is striking in its relative lack of fibrosis. Instead, foci of regeneration are bound by extensive areas of parenchymal collapse, hepatocellular vacuolation and bile ductular proliferation, which is a consistent finding in superficial necrolytic dermatitis.⁹ Participants further noted the prominent hepatocellular vacuolar degeneration within

submitted sections of liver, which prompted a focused exploration of the distinction between hydropic, glycogen and lipid-type vacuolar degeneration. Hydropic degeneration often follows hypoxic incidents, toxic/metabolic insults or cholestasis, while intracellular accumulation of lipid is a response to physiologic or pathologic increases in lipid mobilization or derangements in lipid metabolism. Hepatocellular glycogen accumulation, otherwise known as steroid-induced hepatopathy or hepatic glycogenosis, is typically induced by exogenous or endogenous corticosteroids. Histologically, lipid-type vacuolar degeneration produces hepatocytes with discrete globules which may coalesce into a single large vacuole that peripheralizes the nucleus, while glycogen or hydropic-type vacuolar degeneration causes significant cell swelling with indistinct vacuolar boundaries and fine, feathery cytoplasm. In severe cases of glycogenosis, nuclei and organelles may be displaced to the periphery of the cell, which complicates differentiation from lipid-vacuoles.⁹ In this situation, histochemical stains may be helpful; glycogen stains strongly with PAS, while lipid stains with oil-red-O.

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CASE II: 4378 (JPC 4006293).

Signalment: 5-year-old female spayed Shih Tzu (*Canis lupus familiaris*).

History: The animal presented with lethargy and decreased appetite of 5 days duration. No additional information was provided.

Laboratory Results:

Parameter	Value	Reference Interval
HCT	21.9 % ↓	35.0-57.0 %
RBC	3.1 x 10 ⁶ /μL ↓	4.95-7.87 x 10 ⁶ /μL
HgB	8.2 g/dL ↓	11.9-18.9 g/dL
MCV	79.4 fl	69-80 fl
MCHC	31.5 g/dL ↓	32.0-36.3 g/dL
WBC	62.1 x 10 ³ /μL ↑	5.5-13.9 x 10 ³ /μL
Segs	46.6 x 10 ³ /μL ↑	2.9-12.0 x 10 ³ /μL
Bands	9.9 x 10 ³ /μL ↑	0.0-0.45 x 10 ³ /μL
Lymphocytes	1.2 x 10 ³ /μL	0.4-2.9 x 10 ³ /μL
Monocytes	4.3 x 10 ³ /μL ↑	0.1-1.4 x 10 ³ /μL
Eosinophils	0 x 10 ³ /μL	0.0-1.3 x 10 ³ /μL
nRBC	17 (per 100 WBC)	
Reticulocytes	9.4%	
Absolute retics	291 x 10 ³ /μL	
PLT	185 x 10 ³ /μL	235-694 x 10 ³ /μL

Histopathologic Description: Peripheral blood smear, erythron: The red blood cell mass is decreased, consistent with anemia. There is abundant evidence for regeneration, with numerous polychromatophils and many nucleated red blood cells, primarily metarubricytes, with fewer rubricytes. There is moderate to marked anisocytosis, likely due to the presence of large numbers of spherocytes. Occasional schistocytes

and a few red blood cells containing Howell Jolly bodies are observed.

Leukon: There is a moderate to marked leukocytosis, characterized by a neutrophilia with a left shift (including bands and metamyelocytes) and a monocytosis. There is evidence for toxic change in the neutrophils, particularly the early stages, based on the presence of Döhle bodies and intracytoplasmic granules.

Thrombon: The platelet mass appears adequate.

- Contributor’s Morphologic Diagnosis:**
1. Strongly regenerative anemia with evidence for probable immune-mediated destruction.
 2. Inflammatory leukogram (“leukemoid” response).
 3. Adequate platelet mass.

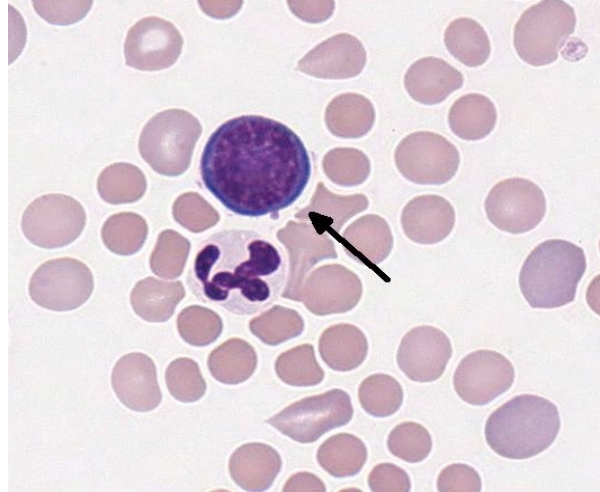
Overall Interpretation: Immune mediated hemolytic anemia (IMHA) with secondary leukemoid response.

Contributor’s Comment: Immune-mediated hemolytic anemia is a disease condition that occurs when erythrocytes or their precursors in the bone marrow are destroyed via a type II hypersensitivity reaction. Initially immunoglobulin attaches to the red blood cell membrane and this can either 1) activate the classical pathway of complement resulting in the formation of membrane attack complexes and subsequent intravascular hemolysis or 2) interact with Fc and complement receptors on phagocytic cells in the liver and spleen, resulting in extravascular hemolysis. IMHA can be primary (with no known underlying cause) or secondary (due to the presence of antibody specific for infectious agents, drugs, or vaccine components). Many argue that only primary IMHA truly results from an autoimmune reaction and therefore should be termed autoimmune hemolytic anemia (AIHA).²

Numerous breed predispositions have been discovered for development of primary IMHA, including Cocker Spaniels, Old English Sheepdogs, Border Collies, Poodles, Irish Setters, and Miniature Schnauzers. One study suggests an association between disease development and the genetic structure of the major histocompatibility complex.⁴ The average age of onset is 6-8 years and, although there is no clear gender



2-1. Peripheral blood smear, dog: Several characteristic findings of autoimmune hemolytic anemia – polychromasia (blue arrows), spherocytes (black arrows), a metarubricyte (red arrow), and Howell-Jolly bodies (green arrows). (Wright's 1000X)



2-2. Peripheral blood smear, dog: Circulating rubricyte. (Wright's 1000X)

predisposition, it can be precipitated by the stress of heat or whelping.²

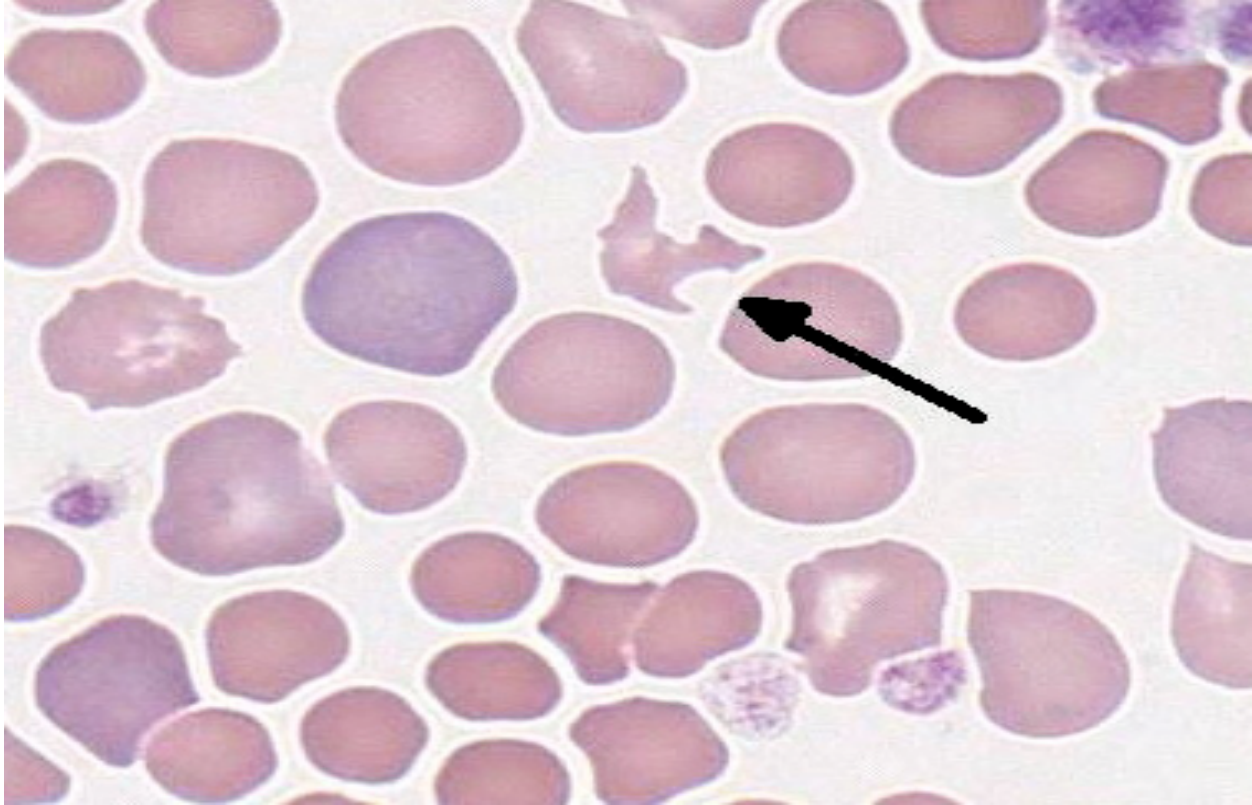
Affected animals can present with either chronic disease with nonspecific signs that have been present for days to weeks or they can present with acute illness, with severe signs of illness that have developed in one to two days. The chronic form is most commonly associated with extravascular hemolysis, while animals with acute disease typically exhibit signs secondary to intravascular hemolysis (e.g., icterus, hemoglobinemia, and hemoglobinuria).²

Diagnosis of IMHA begins with the examination of an EDTA anticoagulated blood sample for autoagglutination. This can be done both grossly and with a saline dilution. Microscopic examination of the blood smear typically shows a decreased red cell mass, usually with marked regeneration. The presence of abundant spherocytes is strongly suggestive for IMHA, although not pathognomonic, as these can be seen with other conditions, including zinc toxicity, DIC, and hemangiosarcoma.³ A pronounced neutrophilia with a left shift is often seen in affected animals and is thought to be due to the effect of pro-inflammatory cytokines (e.g., IL-1, IL-6, and TNF- α), which are produced by activated macrophages. Tissue necrosis due to secondary thromboembolic disease may also play a role.⁸ A Coombs' test using species specific serum that recognizes patient IgM, IgG, and

complement C3 can be of value in further characterizing the underlying mechanism.

Recent studies have evaluated the outcome of dogs with IMHA.^{5,7,10} Around 50% of affected animals die during initial hospitalization, with much worse outcome for patients with the acute versus the chronic form of the disease. The presence of gross autoagglutination, a profound neutrophilia with a left shift, and concurrent thrombocytopenia have been associated with worse prognosis. The average survival time of those patients surviving the initial insult is just over one year. However, approximately 25% of patients have good long-term survival with appropriate immunosuppressive therapy.¹⁰

The patient in this case presented with the chronic form of the disease. While there was no gross or microscopic evidence for autoagglutination, examination of her peripheral blood revealed many of the "classic" features for immune-mediated disease, including abundant spherocytes and a strongly inflammatory leukogram. The presence of schistocytes and the absence of evidence for intravascular hemolysis is suggestive for antibodies to Fc receptors that are removed by splenic and hepatic macrophages, as schistocytes are often seen circulating secondary to membrane phagocytosis by activated macrophages.⁶ To date, this patient has responded well to immunosuppressive therapy.



2-3. Peripheral blood smear, dog: Schistocyte. (Wright's 1000X)

JPC Diagnosis: 1. Peripheral blood smear, erythron: Severe regenerative anemia with spherocytosis, schistocytosis and metarubricytosis, consistent with hemolysis.
2. Peripheral blood smear, leukon: Inflammatory leukogram with significant left shift and toxic neutrophil change.

Conference Comment: The moderator led a detailed analysis of the clinicopathological findings in this case, which include reduced red blood cell mass with prominent anisocytosis, polychromasia, reticulocytosis, metarubricytosis and spherocytosis on the peripheral blood smear, in combination with decreased HCT, hemoglobin and MCHC, high normal MCV and reticulocytosis on the CBC. Mean corpuscular hemoglobin concentration (MCHC), the most accurate RBC index, is used to categorize anemia as normochromic or hypochromic. The most common cause of decreased MCHC is reticulocytosis; however, iron deficiency and lead toxicosis can cause hypochromic anemia as well. Hyperchromasia is not a true finding as erythrocytes do not overproduce hemoglobin; elevations in MCHC are typically secondary to

erythrocyte hemolysis or Oxyglobin[®] administration. Increased erythrocyte mean corpuscular volume (MCV) is another laboratory finding associated with regenerative anemia; in this dog, MCV was at the high end of the reference range. Reticulocytosis is the most common cause of macrocytosis (i.e., elevated MCV), but other causes include folate (B9) or cobalamin (B12) deficiency and feline FeLV infection, as well as several congenital conditions in various species. Additionally, greyhounds normally have a higher MCV due to their shorter red blood cell lifespan. Erythrocyte agglutination can cause a false increase in MCV. Spherocytes are globoid erythrocytes with a lack of central pallor and decreased membrane surface area. In animals, they are strongly associated with immune-mediated hemolytic anemia (IMHA), and form when portions of the erythrocyte membrane bound with autoantibody are phagocytosed by macrophages.¹ Overall, the laboratory findings discussed above indicate a strongly regenerative anemia and support a diagnosis of IMHA.

Interestingly, avian species mount the most intense regenerative erythrocyte response,

followed (in decreasing order) by dogs, cats, cows and horses. The presence of nucleated erythrocytes on a stained blood smear is classified as an appropriate response in animals with a strongly regenerative anemia or increased erythropoiesis; in these cases nucleated red blood cells are accompanied by reticulocytosis. Additionally, moderate numbers of nucleated red blood cells are normal in the peripheral blood of healthy piglets, and of course all erythrocytes in birds and reptiles are nucleated. On the other hand, metarubricytosis associated with iron, lead and copper toxicosis; hemangiosarcoma; leukemia; bone marrow disease; intervertebral disc disease; hereditary macrocytosis of poodles; endotoxemia; and FeLV is considered an inappropriate response.¹

The most common causes of regenerative anemia are hemolysis and blood loss. Reticulocytosis is generally more severe with hemolysis, because iron from hemolyzed erythrocytes is more readily available for erythropoiesis than storage forms of iron, which must be mobilized when there is external loss of erythrocytes. Hemolysis is further classified as extra- or intravascular. Extravascular hemolysis, which is much more common, results from phagocytosis or lysis of erythrocytes within the spleen or liver, whereas intravascular hemolysis is erythrocyte destruction within the circulation. Schistocytes, or fragmented red blood cells, often indicate a microangiopathic hemolytic anemia (MAHA) and intravascular hemolysis¹; however, as noted by the contributor, they may result from membrane phagocytosis, as is likely in this case. Serum chemistry and urinalysis results (not provided in this case) can also be helpful in distinguishing extravascular from intravascular hemolysis; hyperbilirubinemia is typically associated with extravascular hemolysis, whereas hemoglobinemia and bilirubinuria occur subsequent to intravascular hemolysis.

As noted by the contributor, the severe neutrophilia with a left shift and toxic change in neutrophils present in this case is common in IMHA. Four manifestations of toxic change in canine neutrophils are cytoplasmic basophilia, vacuolation, Döhle bodies and toxic granulation.⁹

In conclusion, this case illustrates several distinctive clinicopathological findings that

permit the diagnosis of IMHA, and underscores the importance of performing a manual differential count on all blood smears. Some conference participants detected a lymphopenia based upon their manual differential counts, despite a count within the reference interval on the automated CBC. Participants speculated on possible explanations for this discrepancy, including the possibility that nucleated erythrocytes were erroneously categorized as lymphocytes on the automated counter.

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CASE III: 17796-12 (JPC 4032251).

Signalment: 8-day-old female alpaca (*Vicugna paco*).

History: The cria presented with an abrupt onset of recumbency and illness. Clinically she was lethargic, febrile and has an elevated cardiac and respiratory rate. It was later revealed that she was being fed a powdered goat colostrum supplement.

Gross Pathology: A mildly autolyzed cria weighed 8.4 kg and was in thin body condition. She was mildly autolyzed. Mild, watery subcutaneous edema fluid was present in the fascia outside the ventral thorax. The abdominal cavity contained 300mL watery. Transparent, pale yellow fluid and an additional 30 ml similar fluid was present in the thorax. The lungs were mottled bilaterally, but floated in formalin. The left atrioventricular valve leaflets were diffusely opaque white. The kidneys were paler than expected and several milliliters of edema fluid surrounded each kidney. The abomasal mucosa was an intensely dark red.

Laboratory Results:

Test	6 days of age	7 days of age	Normal values in llamas (Merck Veterinary Manual)
Glucose	160	ND	90-140 mg/dL
Urea nitrogen	180 mmol/L	183	13-32 mg/dL
Creatine	10.5	10.8	1.5-2.9 mg/dL
Sodium	147	149	134-150 mmol/L
Potassium	8.5	9.0	4.3-5.6 mEq/L
Chloride	110	112	106-118mEq/L
Albumin	1.7	2.0	2.6-4.7 mg/dL
Total protein	3.3	ND	5.6-7.3 mg/dL
Calcium	13.1	13.1	2.0-2.6
Phosphorus	13.4	14.6	4.4-8.5
AST	141	ND	10-280 IU/L

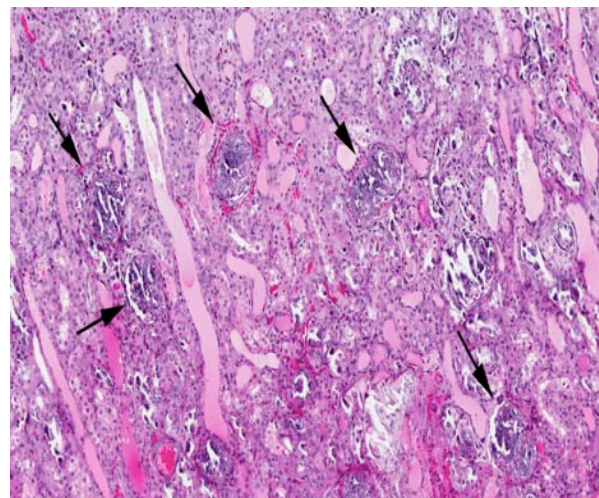
GGT	17	ND	5-29 U/L
CK	65	ND	10-200 IU/L

Histopathologic Description: In both kidneys, glomeruli are diffusely replaced by hard, readily fractured, intensely basophilic granular to homogeneous material consistent with mineral. The nuclei of mesangial cells can occasionally be made out in this material. Cortical tubular basement membranes are also frequently affected, occasionally along with basophilic stippling of tubular epithelial cells. In other parts of the nephron, tubular epithelial cells contain protein-rich casts or cytoplasmic hyaline droplets.

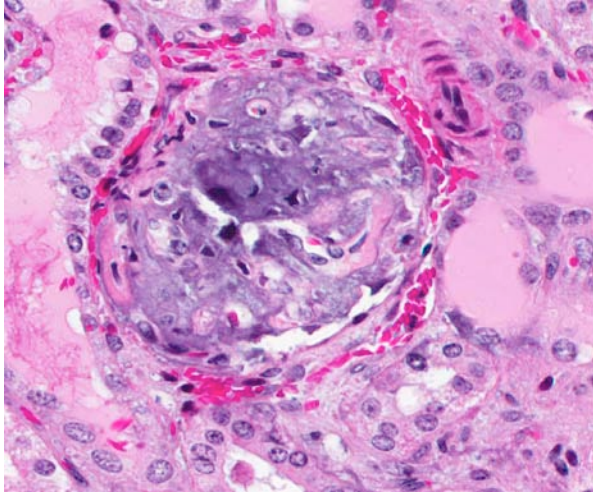
Additional mineralized areas were found in elastic arteries near the heart, trachea, compartment 3, spleen and adrenal.

Contributor’s Morphologic Diagnosis: Kidney: Metastatic calcification, renal glomeruli and tubules, severe.

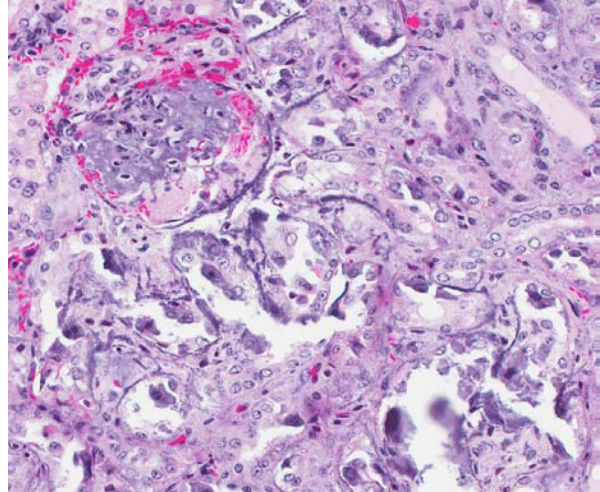
Contributor’s Comment: Rickets is an important nutritional metabolic disease of young animals, attributed to deficiencies of vitamin D, calcium, phosphorus, or combinations of those elements. In camelids, low serum phosphorus may be associated with vitamin D insufficiency. Vitamin D bioavailability is considered low in these species.⁶ The submitter estimated that the cria was fed supplement 3 times a day for a total of 6 days. If she fed one dose/ feeding, that would be a total of 49,500 IU total Vitamin D or



3-1. Kidney, cria: There is diffuse global mineralization of glomeruli (arrows). (HE 84X)



3-2. Kidney, cria: There is diffuse mineralization of basement membranes and mesangium, with pyknosis of mesangial and endothelial cells. Basement membranes are also markedly thickened. (HE 260X)



3-3. Kidney, cria: Adjacent to a mineralized, largely necrotic glomerulus, the basement membranes and epithelial cells are likewise mineralized (and the epithelium is necrotic). (HE 230X)

1,375 IU/kg/day (297,000 total IU or 8250 IU/kg).

Rickets is considered a common clinical problem in camelids and is particularly frequent in crias born in September to March period particularly in the high latitudes of the northern and southern hemispheres. Prolonged inclement weather and conditions of reduced sunlight can be contributory to vitamin D deficiency under these conditions.⁴ This animal was born in late August. Thus many owners supplement.

This cria was given a goat colostrum supplement fortified with vitamin D due to the owner's concern that rickets was a possibility.³ This supplement has been reported as a cause of hypervitaminosis D in alpaca crias previously. Among the signs found in other over-supplemented goats are hypercalcemia, hyperphosphatemia and renal dysfunction as seen in this case.

Metastatic calcification occurs in otherwise normal tissue due to hypercalcemia secondary to some disturbance in calcium metabolism. Entry of large amounts of calcium into cells results in its precipitation in organelles. Common causes are renal, vitamin D intoxication [commonly affects aorta, atrial and left ventricular endocardium, lungs], elevated PTH or PTH-related protein, and neoplastic destruction of bone.⁷ Common target tissues are gastric mucosa, kidney, lung, systemic arteries and pulmonary veins. Many of these cells lose acid and therefore have an alkaline internal

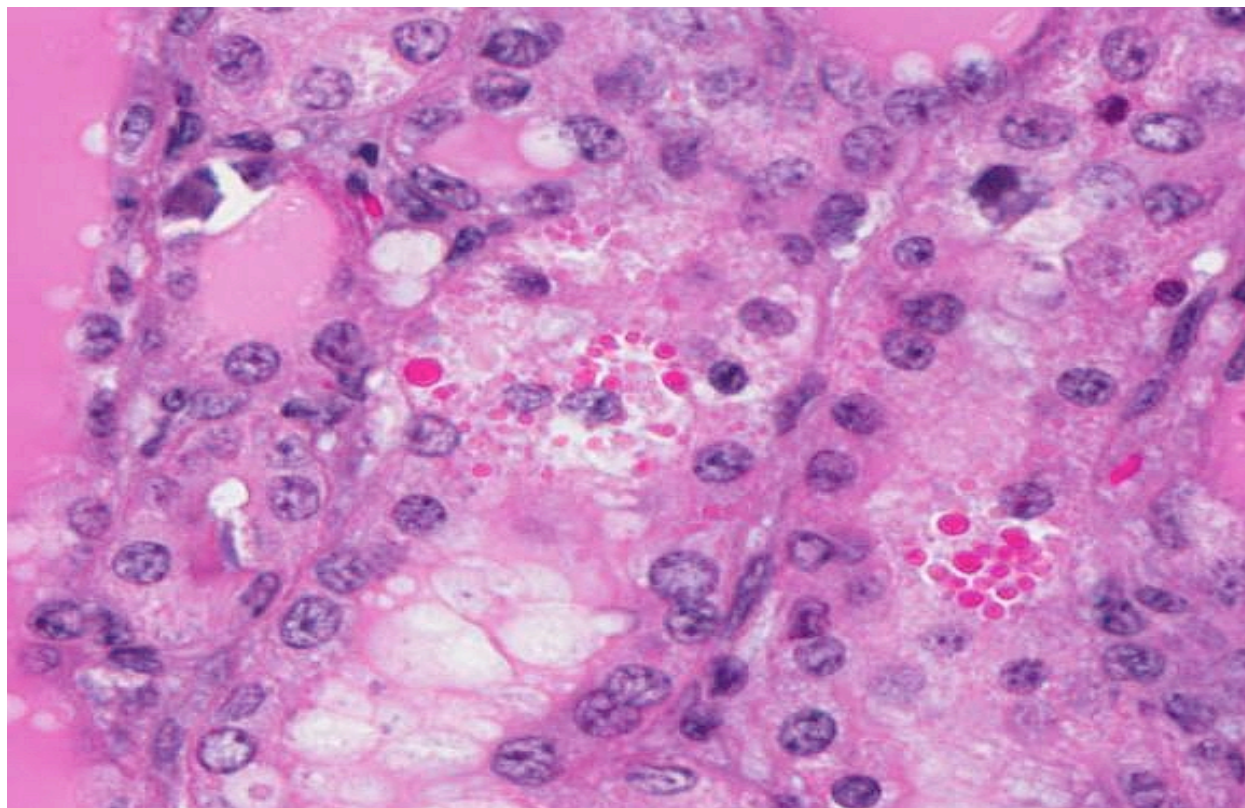
compartment predisposing to deposition of mineral salts.

Calciferol and D3 localize in nucleus as do other steroids, turning on genes for increased calcium transport. In addition to being found in young animals due to vitamin overdose, as in this instance, pets ingesting "Rampage" rodent poison have similar lesions.

JPC Diagnosis: Kidney, glomeruli and tubules: Mineralization, diffuse, severe, with marked intratubular protein casts.

Conference Comment: The contributor provides an illustrative case of vitamin D-induced metastatic mineralization in the kidney of a cria, pairing it with a succinct synopsis of the entity. In conference, participants further explored the pathogenesis of this condition. As noted by the contributor, the renal mineralization in this case is an example of metastatic calcification, which is typically associated with hypercalcemia and/or hyperphosphatemia. In dogs, metastatic mineralization occurs if the calcium-phosphate solubility product, expressed in mg/dL, persistently exceeds 70. Conversely, dystrophic calcification occurs in normocalcemic animals in association with tissue damage, while calcinosis cutis is an example of idiopathic ectopic mineralization.⁵

Common causes of hypercalcemia include hyperparathyroidism, hypoadrenocorticism, acidosis, renal disease (in horses and some dog



3-4. Kidney, cria: Multifocally, degenerate proximal tubular epithelium occasionally contains large brightly eosinophilic intracytoplasmic hyaline protein droplets. (HE 340X)

breeds), vitamin D toxicity, prolonged immobilization, osteolytic lesions, neoplasia (lymphoma, canine adenocarcinoma of the anal sac apocrine glands, plasma cell myeloma, some carcinomas), thiazide diuretics and granulomatous inflammation. Hyperproteinemia and hemoconcentration will also falsely elevate serum calcium.² Common causes of hyperphosphatemia include hemolysis, nutritional 2° hyperparathyroidism, hyperthyroidism, hypervitaminosis D, osteolytic bone lesions, hypoadrenocorticism, renal failure (in most species except for horses), hypoparathyroidism, tumor lysis and administration of phosphate-containing fluids or enemas. Relatively high phosphorus is normal in young animals.² In this case, serum chemistry revealed both hypercalcemia and hyperphosphatemia, narrowing the differential diagnosis down to hypervitaminosis D, osteolysis or hypoadrenocorticism. The history of repeated vitamin D supplementation supports a diagnosis of renal mineralization secondary to vitamin D toxicity. The marked azotemia is secondary to renal failure, while hyperkalemia is attributed to renal failure or acidosis.

Although excessive dietary supplementation of vitamin D is the most frequent cause, ingestion of cholecalciferol-containing rodenticides or plants containing vitamin D glycosides (*Cestrum dirunum*, *Solanum malacoxylon*, *Trisetum flavescens* and *Medicago sativa*) have also been implicated in cases of vitamin D toxicity.⁵ Vitamin D maintains plasma levels of calcium and phosphorus by acting on the small intestine, bone, and kidneys. Specifically, it promotes active uptake and transcellular transport of calcium by increasing calbindin synthesis; it stimulates renal calcium absorption in distal tubules; and it stimulates mobilization of calcium and phosphorus from bones. The latter occurs upon binding of osteoblast RANKL (receptor activator for NF- κ B ligand) to preosteoclast RANK, which induces differentiation into mature osteoclasts and initiates bone resorption via secretion of HCl and proteases such as cathepsin K. Additionally, vitamin D contributes directly to mineralization of epiphyseal cartilage and osteoid matrix by stimulating osteoblasts to synthesize the calcium-binding protein osteocalcin, which is involved in mineralization of these matrices.¹

Vitamin D is obtained directly from dietary sources or synthesized endogenously from a precursor (7-dehydrocholesterol) that is present in the skin. Irradiation of 7-dehydrocholesterol with ultraviolet light induces the formation of cholecalciferol (vitamin D₃). The precursor in plants is ergosterol, which is converted to vitamin D₂ by ultraviolet light and then converted to vitamin D₃ in the body.¹ Inactive cholecalciferol (vitamin D₃) binds to plasma α 1-globulin and is transported to the liver. There, it is converted by hepatic 25-hydroxylases to 25-hydroxycholecalciferol (25-OH-D). Finally, renal α 1-hydroxylase converts 25-OH-D to active 1,25-dihydroxycholecalciferol. Regulation of renal vitamin D production occurs through three major mechanisms. Hypocalcemia upregulates parathyroid hormone production, which induces activation of α 1-hydroxylase and thus increases 1,25-dihydroxycholecalciferol production. Hypophosphatemia directly activates α 1-hydroxylase, resulting in a similar increase in 1,25-dihydroxycholecalciferol production. Conversely, increased levels of 1,25-dihydroxycholecalciferol provoke negative feedback inhibition of α 1-hydroxylase.¹ New world monkeys are entirely dependent upon dietary sources of vitamin D since it cannot be synthesized in their skin and, as noted by the contributor, vitamin D availability is considered low in camelids, a condition which is exacerbated during the winter at high latitudes when ultraviolet radiation is decreased.¹

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CASE IV: 33786/2-26 (JPC 4006472).

Signalment: 14-year-old male Holsteiner horse (*Equus caballus*).

History: The animal presented with a 10-day history of severe and rapid weight loss with anorexia, weakness, malaise, intermittent bilateral epistaxis and ascites. On physical exam, there was pallor of mucous membranes, splenomegaly and mild fever. On the labial, nasal, sublingual pharyngeal and anal mucosae there were numerous multifocal to coalescing, irregularly round, yellow to gray, plaque-like lesions ranging from 3 to 15 mm in diameter, with a thin hemorrhagic halo. After three days of corticosteroid and antibiotic therapy the horse became recumbent and the owner elected euthanasia.

Gross Pathology: On gross necropsy the following findings were noted:

- Severe, diffuse splenomegaly
- Severe, diffuse hepatomegaly; liver appeared yellow to pink in color with rounded margins
- Multifocal white, 2 cm to 4 cm, well demarcated, 1 mm thick plaque-like lesions on pulmonary and diaphragmatic pleura

- Numerous irregularly round, yellow to grey, 0.5 to 1.5 cm, plaque-like lesions on the mucosa of the lip, oral cavity, esophagus, small intestine and large intestine
- Severe distention of large intestine with liquid contents
- Plaque-like lesions were also evident on respiratory mucosa of the nasal cavity, larynx, trachea and bronchi
- Marked (up to 5 L) serosanguinous effusion in the abdomen
- Marked (up to 500 mL) serosanguinous effusion in the thorax
- Replacement of femoral bone marrow by a proliferative lesion

Laboratory Results:

Special stains:

- Giemsa: no cytoplasmic granules were evident in neoplastic cells.
- Toluidine blue: no cytoplasmic metachromatic granules were evident in neoplastic cells

Immunohistochemistry:

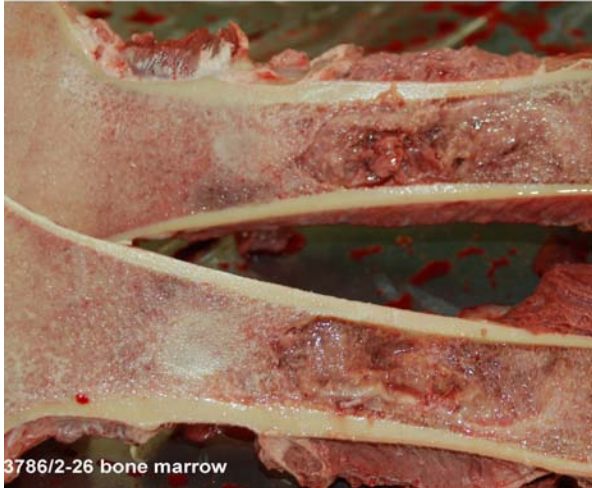
- Myeloperoxidase (M3/38 antibody clone from Cedarlanes): at least 20% of neoplastic cells in the liver and bone marrow were strongly positive.



4-1. Oral mucosa, horse: On the labial mucosa, numerous multifocal to coalescing irregularly round, yellow to gray, plaque-like lesions with a thin hemorrhagic halo were evident. (Photo courtesy of: Department of Public Health, Comparative Pathology and Veterinary Hygiene – Faculty of Veterinary Medicine – University of Padova – Viale dell'Università, 16 - Agripolis - Legnaro - 35020 - PADOVA – ITALY. <http://www.veterinaria.unipd.it>)



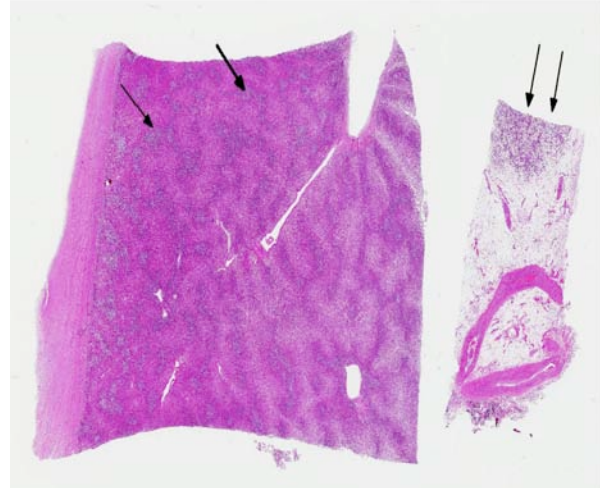
4-2. Liver, horse: The liver was mottled yellow-pink to gray and was markedly enlarged with rounded margins. (Photo courtesy of: Department of Public Health, Comparative Pathology and Veterinary Hygiene – Faculty of Veterinary Medicine – University of Padova – Viale dell'Università, 16 - Agripolis - Legnaro - 35020 - PADOVA – ITALY. <http://www.veterinaria.unipd.it>)



4-3. Femur, horse: Within the marrow of the diaphysis, there is a focal proliferative lesion which effaces both marrow and trabecular bone. (Photo courtesy of: Department of Public Health, Comparative Pathology and Veterinary Hygiene – Faculty of Veterinary Medicine – University of Padova - Viale dell'Università, 16 - Agripolis - Legnaro - 35020 - PADOVA – ITALY. <http://www.veterinaria.unipd.it>)

- Lysozyme: at least 40% of neoplastic cells in liver and bone marrow were positive.
- CD79 and CD5: neoplastic cells in liver and bone marrow were negative. Occasional CD79 positive lymphocytes were associated with the neoplastic population.

Histopathologic Description: Liver: Severely and diffuse expanding the portal areas, diffusely infiltrating and effacing the periportal hepatic parenchyma, and diffusely within the sinusoids and centrilobular veins there is a neoplastic population composed of round cells with distinct cell borders, ranging from 20 to 25 μm in size. Up to 70% of the neoplastic cells have a moderate amount of cytoplasm, an irregularly round to oval, occasionally indented 15 to 20 μm nucleus, with clumped or marginated chromatin and an occasional prominent nucleolus (blasts). Up to 10% of the cells are more condensed, with an eccentrically located, and round to kidney-shaped nucleus and numerous eosinophilic cytoplasmic granules. Approximately 15% of the cells are well differentiated eosinophils and there are scattered lymphocytes. There are up to 3 mitotic figures per HPF. There is marked dissociation of hepatocytes in the periportal areas with occasional fragmentation of hepatocytes (necrosis). The centrilobular hepatocytes are characterized by severe cytoplasmic vacuolar degeneration. The capsule is diffusely and severely thickened by



4-4. Liver and bone marrow, horse: Both organs are infiltrated by neoplasms composed of round cells (arrows). (HE 0.63X)

collagen deposition and fibroblast hyperplasia (fibrosis). Within the lumen of the vessels in the capsule occasional neoplastic cells are evident.

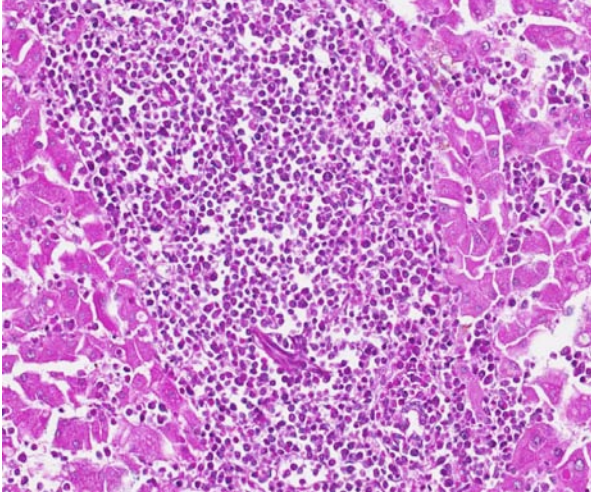
Bone marrow: Bone marrow is effaced by the same neoplastic population described in the liver and there is an absence of erythroid precursors and megakaryocytes (myelophthisis). Blast cells compose up to 80% of neoplastic cells.

Spleen (tissue not submitted): A similar neoplastic population markedly expands the red pulp, filling the sinuses, expanding the splenic cords and multifocally replacing splenic trabeculae. There is diffuse atrophy of the white pulp.

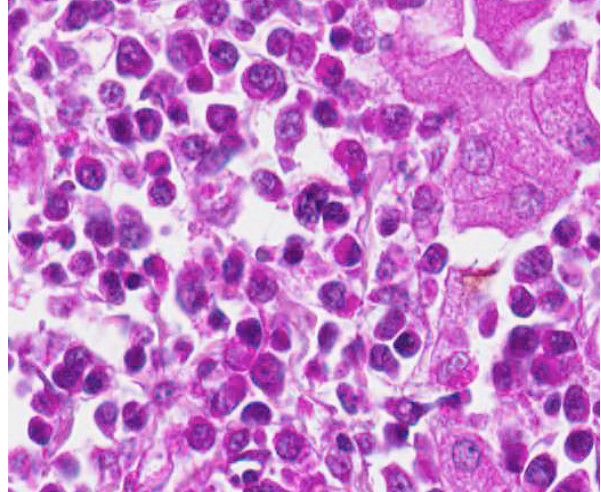
Small intestine (tissue not submitted): The neoplastic cells multifocally infiltrate the mucosa and submucosa, forming flattened nodular lesions. The mucosal epithelium is severely and diffusely necrotic.

Lung (tissue not submitted): Neoplastic cells diffusely expand the alveolar walls and are present within the vessels.

Cytology: Imprint with blood coagulum show numerous irregularly round, 20 to 25 μm neoplastic cells, characterized by moderate amount of blue cytoplasm and a round, often indented 15 to 20 μm nucleus, with clumped chromatin and an occasionally distinct nucleolus.



4-5. Liver, horse: Within the liver, the neoplasm effaces portal areas and extends into the adjacent hepatic plates. (HE 228X)



4-6. Liver, horse: Neoplastic round cells have distinct cell borders, round nuclei, and numerous brightly eosinophilic cytoplasmic granules. (HE 400X)

Contributor's Morphologic Diagnosis: Liver and bone marrow: Acute myeloid leukemia, *Equus caballus*, horse.

Contributor's Comment: Leukemia is a neoplasia of one or more cell lines of the bone marrow with distorted proliferation and development of leukocytes and their precursors.³ Although more common in other domestic animal species, leukemia is also reported in horses. It is typically classified according to the affected cells (myeloproliferative or lymphoproliferative disorders), evolution of clinical signs (acute or chronic) and the presence or lack of abnormal cells in peripheral blood (leukemic, subleukemic and aleukemic leukemia).^{3,4}

The most common lymphoproliferative disorders in horses are lymphoid leukemia, plasma cell or multiple myeloma and lymphoma.³ Lymphoma is the most common hematopoietic neoplasia in horses and usually involves lymphoid organs, without leukemia, although bone marrow may be affected after metastasis.³

The following outline summarizes the classification scheme of acute myeloid leukemia according to World Health Organization (WHO) criteria:

AML M0: Acute myeloid leukemia/undifferentiated leukemia

- Greater than or equal to 90% of myeloid cells are blasts

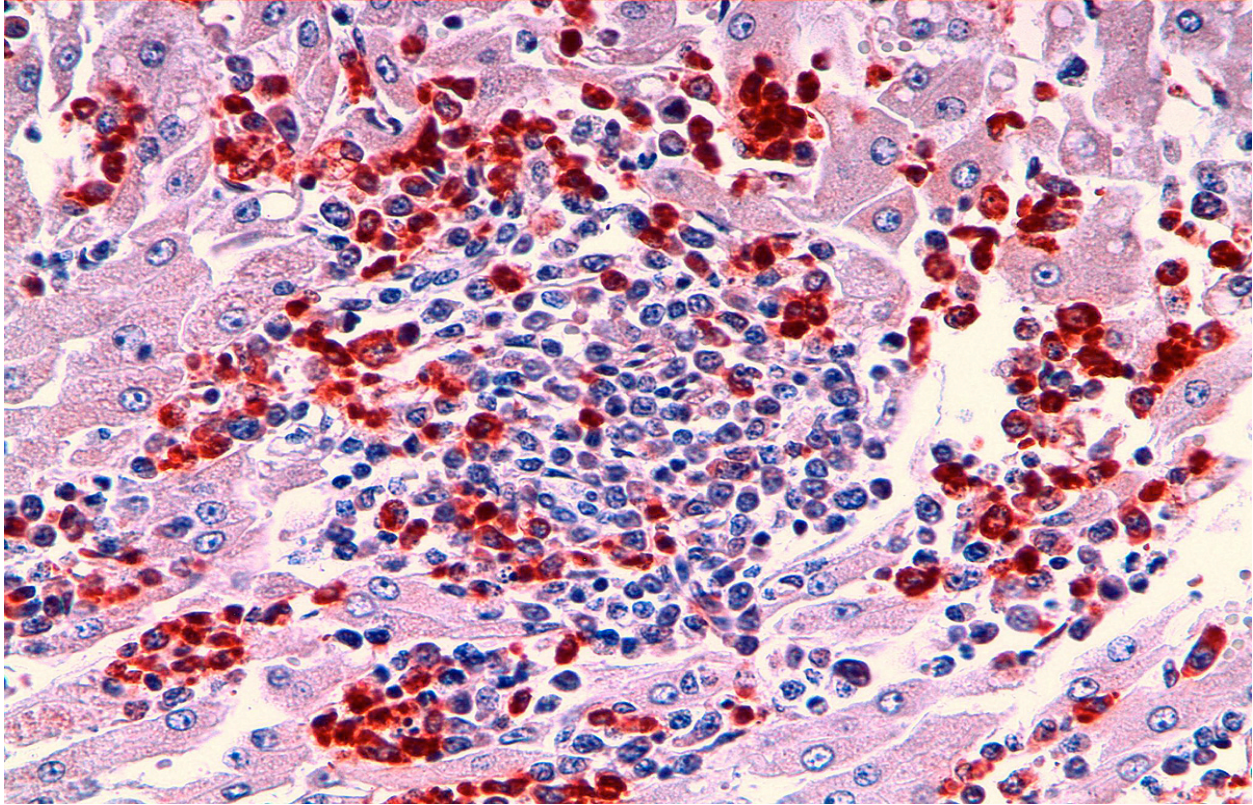
- Less than or equal to 5% of blasts in circulating blood stain with myeloperoxidase
- No Auer rods are seen

AML M1: Acute myeloid leukemia without maturation

- There is a predominance of blasts in circulating blood and bone marrow with less than 10% having cytoplasmic granulation
- At least 5% of the malignant blast population stains with Sudan Black and myeloperoxidase
- Reported in young mature dogs and cats, with increased frequency in males
- Swine are occasionally affected; rarely other domestic animal species
- In humans, at least 3% of bone marrow blasts label with myeloperoxidase
- Auer rods may be present

AML M2: Acute myeloid leukemia with maturation

- Approximately 30–80% of the myeloid cells are blasts, with at least 10% of neoplastic cells showing maturation (promyelocytes or beyond)
- At least 50% stain with myeloperoxidase
- May have Auer rods



4-7. Liver, horse: Neoplastic cells multifocally exhibit strong cytoplasmic immunoreactivity for lysozyme. (400X) (Photo courtesy of: Department of Public Health, Comparative Pathology and Veterinary Hygiene – Faculty of Veterinary Medicine – University of Padova - Viale dell'Università, 16 - Agripolis - Legnaro - 35020 - PADOVA – ITALY. <http://www.veterinaria.unipd.it>)

AML M3: Acute promyelocytic leukemia

- There is a predominance of promyelocytes in both in the circulating blood and bone marrow
- There is strong cytochemical staining for myeloperoxidase
- Rare disease of young mature animals
- Commonly recognized in dogs, cats and swine

AML M4: Acute myelomonocytic leukemia

- Both granulocytic and monocytoid differentiation occurs
- Rare disease recognized in dogs, cat, horses
- At least 20% of both tumor cell lines stain for the neutrophil series or for the monocytic series
- At least 20% of blast cells in blood or marrow and at least 20% of the bone marrow cells must be of the monocytic lineage to distinguish M2 from M4

AML M5: Acute monocytic leukemia

M5a: poorly differentiated

- At least 20% blast cells in blood or bone marrow
- Poorly differentiated cells (blasts), monoblasts predominate
- In contrast to M4 less than 20% of cells stain with myeloperoxidase

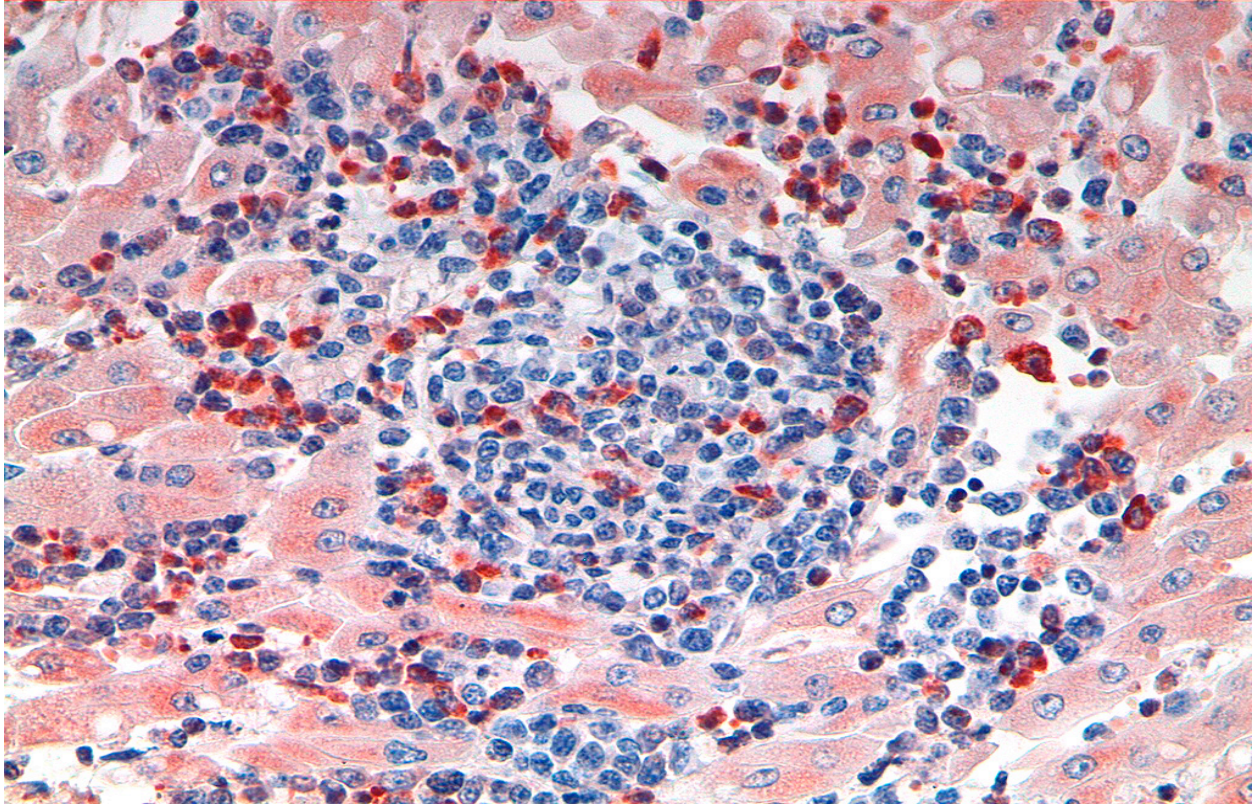
M5b: well differentiated

- At least 20% blast cells in blood or bone marrow
- Promonocytes predominate
- In contrast to M4 less than 20% of cells stain with myeloperoxidase

AML M6: Erythroleukemia

M6a

- More than 50% of the bone marrow is composed of red blood cell precursors



4-8. Liver, horse: Neoplastic cells multifocally exhibit strong cytoplasmic immunoreactivity for myeloperoxidase. (400X) (Photo courtesy of: Department of Public Health, Comparative Pathology and Veterinary Hygiene – Faculty of Veterinary Medicine – University of Padova - Viale dell'Università, 16 - Agripolis - Legnaro - 35020 - PADOVA – ITALY. <http://www.veterinaria.unipd.it>)

- More than 20% of the non-erythroid cells are myeloblasts
- M6b
- Up to 80% of the bone marrow is composed of red blood cell precursors
 - Less than 20% of the non-erythroid cells are myeloblasts

AML M7: Megakaryoblastic leukemia

- Rare disease, mainly in dogs
- Greater than or equal to 20% blasts in circulating blood or bone marrow and at least 50% of the marrow cells must be of megakaryocytic lineage
- Circulating blasts with abnormal megakaryocytes and fibrosis in the marrow
- Immunoreactivity for CD41, CD42 and CD61

In the present case, no lymphadenomegaly or splenic nodules were evident at necropsy. Histopathologic evaluation of the lung, spleen, small and large intestine, esophagus, oral mucosa

and nasal mucosa (slides not submitted) revealed a similar neoplastic population to the one in the liver and bone marrow.

Impression smears from the intracardiac coagulum demonstrated the presence of numerous neoplastic cells consistent with myeloblasts/monoblasts.

Immunohistochemical staining of the neoplastic cells in liver and bone marrow for CD3 was negative. Occasional lymphocytes admixed with the neoplastic population were positive for CD79. Almost 25% of the neoplastic cells in the liver were positive for myeloperoxidase and almost 40% were positive for lysozyme.

Based on the immunohistochemical results, a lymphocytic origin of the neoplastic cells can be ruled out. No laboratory tests for the evaluation of alpha naphthyl acetate esterase activity or naphthol AS-D-chloroacetate esterase activity were available to assess the proportion of neoplastic cells with monocytic origin. Severe, diffuse infiltration of neoplastic cells in portal

areas of the liver is not reported in AML-M2 but is characteristic of AML-M4.

Two different types of acute myeloid leukemia must be considered as differential diagnosis: acute myeloid leukemia with eosinophilic differentiation (AML-M2) and acute myelomonocytic leukemia (AML-M4).

JPC Diagnosis: Bone marrow; liver: Acute myeloid leukemia, with eosinophilic differentiation.

Conference Comment: The contributor provides a comprehensive review of the WHO classification of leukemia, and the notes on differentiating various subtypes of acute myeloid leukemia are especially relevant. In conference, there was some difficulty in the histological identification of sections of bone marrow, however once the tissue type was confirmed, participants explored the immunohistochemical staining characteristics of this case. Myeloperoxidase (MPO) is a lysosomal enzyme found in myeloblasts, immature myeloid cells and the primary granules of mature neutrophils (see 2013-2014 WSC 10, case 3). Approximately 25% of the neoplastic cells were MPO positive, supporting myeloid origin. Additionally, 40% of neoplastic cells were positive for lysozyme, suggesting monocytic origin. Staining for alpha naphthyl acetate esterase activity or naphthol AS-D-chloroacetate esterase may have been helpful in confirming monocytic origin;² however, due to lack of availability of these stains, we are unable to reach a definitive diagnosis and concur with the contributor's differential diagnosis of AML-M2 and AML-M4.

In addition to the microscopic lesions described by the contributor, some participants noted scattered cytosegrosomes and occasional intracytoplasmic or extracellular blue to purple granular material. Histochemical staining with Von Kossa identified the granular material as mineral; however, we are unsure of the origin or clinical significance of this material.

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