

**Joint Pathology Center
Veterinary Pathology Services**



WEDNESDAY SLIDE CONFERENCE 2015-2016

C o n f e r e n c e 1 7

10 February 2016

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CASE I: TAMU-1-2013 (JPC 4033379).

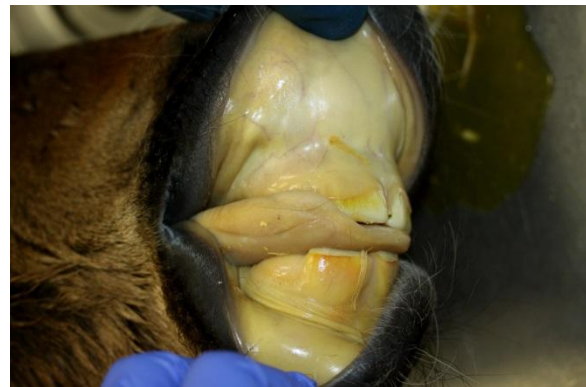
Signalment: 18-day-old thoroughbred colt
(*Equus caballus*)

History: A healthy newborn foal nursed and the next day, became lethargic and had hemoglobinuria. With progressive stupor, icterus, hyperbilirubinemia and anemia, the foal was presented to the clinic at 2 days of age.

Gross Pathology: Yellow mucous membranes and tissues (icterus); ~200ml abdominal transudate with fibrin strands; myocardial mottling grey and red (hemorrhage and necrosis) a small Thebsian vein in the noncoronary aortic sinus; urachus patent.

Laboratory Results:

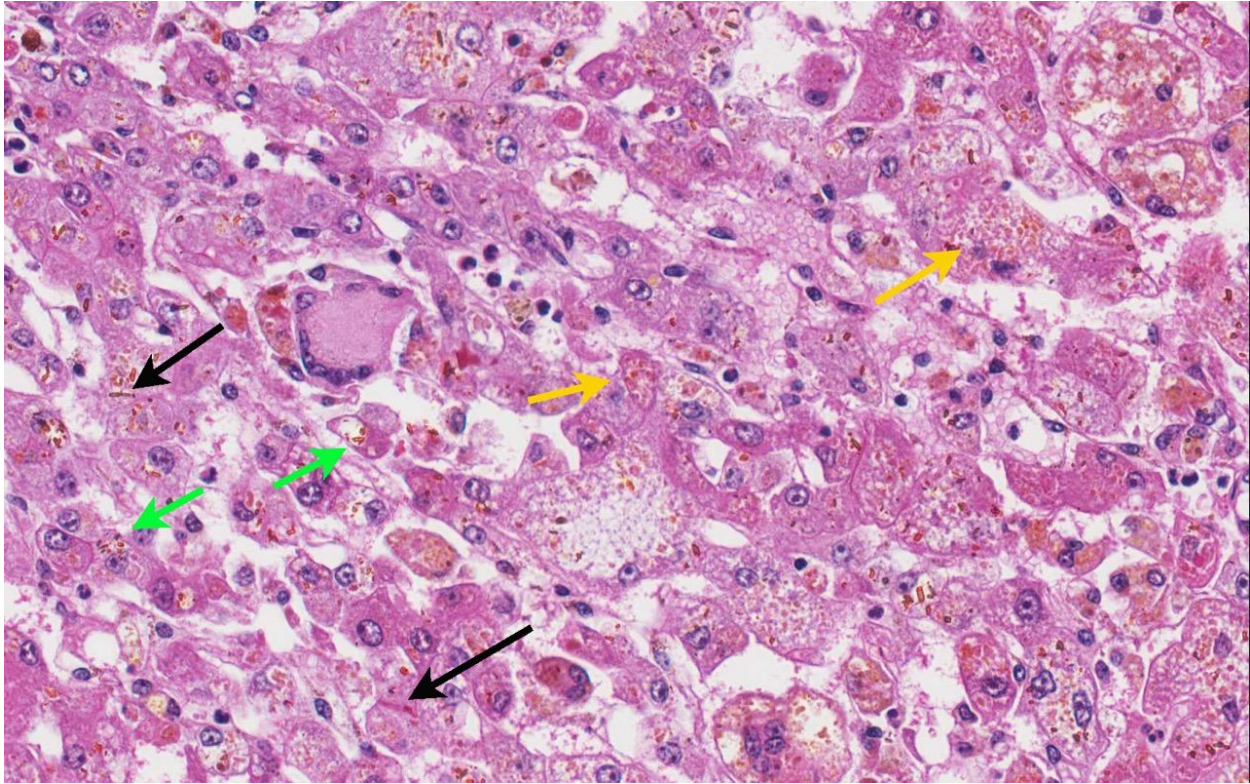
PCV=12 (32-53) no Nrbc's
Platelets 470,000/ul (100-350,000)
TP 4.7g/dl (5.3-7.3)



Mucous membranes, foal. The mucous membranes of this foal are extremely yellow, indicating icterus. (Photo courtesy of: Dept Vet Pathobiology, College Vet Med Texas A&M University, College Station, TX 77843-4467 <http://vetmed.tamu.edu/vtpb>)

Total bilirubin 14.5 mg/dl (0-1.9)
GGT 124U/L (0-53)
Alkaline phosphatase 553U/L (128-512)
ALT 850U/L (134-643)
Saline agglutination test positive
Mare serum Anti-Qab positive

Histopathologic Description: The section of liver has widespread atrophy of hepatocytes with hypertrophied and



Liver, foal. There is extensive pigment deposition throughout the section, to include brown granules in hepatocytes (lipofuscin- yellow arrows), brown spicular material (acid hematin – green arrows) and distended bile canaliculi (cholestasis – black arrows). Syncytial hepatocytes, often with peripheralized nuclei are scattered throughout the section. (HE, 40X)

occasionally binucleate nuclei. Some hepatocyte syncytia contain 6-8 nuclei. Often, the hepatocytes exhibit feathery degeneration, but centrilobular hepatocytes are degenerating or have individual cell necrosis. The spaces of Disse are expanded (edema), and sinus leukocytosis is common with leucocytes often concentrated in areas of hepatocyte loss. Scattered lipofuscin-laden macrophages are often near the pale staining triads, and bile casts and mild bile duct proliferation are noted. Throughout the section are syncytia similar to Langhan's type multinucleate giant cells with nuclei surrounding a tan, granular cytoplasm (lipofuscin). Erythrophagocytosis by Kupffer cells is noted, and iron-staining Kupffer cells are in sinusoids. Hepatic cords are disassociated (shock).

Contributor's Morphologic Diagnosis:

Liver: Subacute hepatopathy with centrilobular hepatocellular degeneration, necrosis and collapse; edema, lipofuscinosis, bile casts and mild bile duct proliferation; hepatocyte syncytia; erythrophagocytosis and hemosiderosis; multifocal neutrophilic hepatitis.

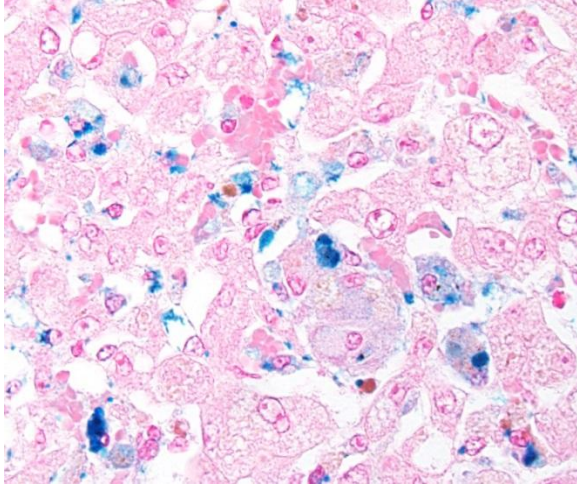
Contributor's Comment: This is a classic case of equine neonatal isoerythrolysis /isoimmune hemolytic anemia (NI). The animal was treated with dexamethasone, banamine, and antibiotics; clinical and post-mortem blood cultures were sterile. No organisms were noted with Gram or GMS stains. While there is inflammation in the lesion, it was felt to reflect secondary, cholestasis-induced hepatitis.

When reviewing the case with the resident, the pathologist commented that the giant cells were classically seen with NI; however, the resident noted that this lesion is not mentioned in textbooks as a lesion associated with NI. Young horse hepatocytes often form syncytia, and giant cell hepatopathies/ hepatidides are described; especially associated with leptospiriosis,^{13,20} but “idiopathic” hepatocyte syncytia have been described in 5-7 month-old, equine abortuses³ and have been seen by this contributor in leptospira PCR-negative cases. In human infants with jaundice, it was described as “giant cell hepatitis”¹⁷ or “syncytial giant cell hepatitis.”¹⁵ Initially, some considered it an expression of viral hepatitis, particularly serum hepatitis, in infancy. Some feel the lesion reflects a specific insult such as blood group incompatibility between mother and infant. It has been considered a nonspecific response of liver regeneration to any injury.^{10,19} Still others consider it a congenital defect in the formation of bile canaliculi or another genetically transmitted abnormality.^{5,14} Popper and Schaffner¹⁴ concluded hepatocyte giant cell formation was a feature of cholestasis in infancy, and that it may be associated with some inflammatory changes. Syncytial cell hepatitis has been reported in humans and is associated with bacterial sepsis (especially toxoplasmosis, syphilis listeriosis, tuberculosis), viral diseases (cytomegalovirus, herpes simplex, varicella, echovirus, parvovirus B19, enterovirus, rubella, human herpesvirus-6, human immunodeficiency virus, hepatitis types A, B and C, Marburg virus and paramyxovirus), liver transplantation and death from severe liver failure.^{8,16} However, in the neonatal period it is considered a non-specific reaction of immature hepatocytes to various forms of “aggression,”¹⁰ and the syncytia stain with nuclear proliferation and

growth factors.⁷ Just as in autoimmune hemolytic anemia in early childhood, syncytial cell hepatopathy has been reported in cases of equine NI.² It was noted in a case of NI presented in the WSC of 5-14-86. How does one explain the lack of the lesion description? Foal livers in fatal cases of NI are “busy,” and the syncytial cells, while typical, may be down-played in face of the catastrophic clinical and autopsy findings. Because our cases are often referral cases and are subacute to chronic, it may also be that syncytia are just more frequent in long-standing, fatal cases. Syncytia are characteristic. Foals seem prone to respond this way. Although neonatal infections may complicate clinical NI, infectious agents are not always documentable. We should remember that only recently a viral agent of equine Theiler’s disease has been identified,⁴ which may play a role (though I doubt it).

Kernicterus is always a concern in cases of prolonged unconjugated hyperbilirubinemia in neonates. This foal had no macroscopic lesions of kernicterus, the cortex did not fluoresce and no neuronal lesions were noted in the cerebral cortex, Purkinje cells or hippocampus, where it has been described in foals with NI.¹² It’s of interest that syncytia were not reported in any of their autopsy cases, even their chronic cases. There is a case report of NI in a three-day-old foal with kernicterus and neuronal necrosis represented macroscopically by yellow-discolored nuclei.⁶

For completeness, it is interesting that this colt had a persistent neutrophilia and developed a thrombocytosis. Alloimmune, neonatal neutropenia has been described in a foal with Ka antibodies in a recent report of NI.²¹ The most complete study of anti-erythrocyte antibodies in mare serum and colostrum was done in thoroughbred and



Liver, foal: There are moderate amounts of iron within Kupffer cells. (Perl's, 40X)

standardbred mares and Qa and Aa seemed to be the most common alloantibodies found at large;¹ however, these alloantibodies may not be the most common seen in clinical cases of NI.^{2,9} Alloantibodies to Qa antigens were found in the mare of our case. The colt of our study did not show a regenerative erythroid response. Over the 2-week hospitalization, no nucleated erythrocytes were noted and the bone marrow had erythrocytic hypoplasia. We wondered if this may have reflected a specific destruction of precursors of the erythroid series.

JPC Diagnosis: Liver: Hepatocellular degeneration and atrophy, diffuse, severe with Kupffer siderosis, cholestasis, and hepatocellular syncytial cell formation.

Conference Comment: Neonatal isoerythrolysis is a type II hypersensitivity reaction that results from antibodies directed against neonatal red blood cells, most frequently IgG or IgM. This leads to activation of complement component C1q and eventual formation of the membrane attack complex which lyses the red blood cell. Red blood cell lysis can also result

from opsonization of red blood cells by complement component C3b, or by antibody, followed by phagocytosis.¹⁸

Lipofuscin is an intracellular pigment thought to accumulate as a result of aging and cellular “wear and tear,” including processing of senescent cellular organelles and other material. It accumulates within lysosomes most significantly in post mitotic or slowly dividing cells as an end product of autophagy and is often seen as a perinuclear golden brown pigment. It looks very similar histologically to ceroid, which is more commonly associated with pathologic conditions such as severe malnutrition and vitamin E deficiency or can be seen in certain inherited conditions such as neuronal ceroid lipofuscinosis (see WSC 2015-16, conference 10, case 1). Ceroid is known to accumulate in both Kupffer cells and hepatocytes as well as other tissues and can be intracellular or extracellular. Lipofuscin is thought to accumulate slowly over the life of the animal, whereas ceroid usually accumulates rapidly depending on the associated condition. Histochemical techniques which can aid in identifying both ceroid and lipofuscin include Sudan black, oil-red-O, PAS and Ziehl-Neelsen stains. Although differences in content of the two pigments have been demonstrated by special histochemical techniques, it is not possible to differentiate the two pigments in standard H&E stained sections.¹¹

The primary histologic features in this case include hepatocyte degeneration and atrophy, with formation of syncytial cells. Extensive pigmentation of hepatocytes and Kupffer cells was present throughout the section prompting extensive discussion. . The brown granular to globular intracellular pigment within hepatocytes was interpreted as lipofuscin and within Kupffer cells as hemosiderin. An iron stain confirmed the presence of moderate amounts of hemo-

siderin within Kupffer cells and occasionally within hepatocytes. Viewing under fluorescence confirmed the presence of small amounts of autofluorescent pigment within hepatocytes consistent with lipofuscin. Abundant birefringent, yellow-brown spiculated material was ultimately identified as acid hematin. Numerous dilated bile canaliculi were also noted, indicating cholestatic disease. A Hall's stain for bile confirmed this finding.

We thank the contributor for providing clinical pathology data with the submission, which greatly adds to the teaching value of the case. One of the best indicators of regenerative anemia is reticulocyte count, but horses do not release reticulocytes. The absence of nucleated red blood cells may suggest a nonregenerative anemia; however, the best way to determine regenerative status would be a bone marrow sample. In this case, a bone marrow aspirate revealed erythrocytic hypoplasia, as indicated above in the contributor's comment. Conference participants briefly discussed bilirubin metabolism and the elevated total bilirubin in this case was interpreted as both pre-hepatic and hepatic, which fits with both the pathogenesis and histologic findings.

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CASE II: A15-9631 (JPC 4065767).

Signalment: 1-year-old, gelding, Arabian horse (*Equus ferus caballus*)

History: Forty days after being gelded, vaccinated (annual vaccines plus tetanus antitoxin), and de-wormed (ivermectin), this horse was acutely anorectic and ataxic in the morning with progression to recumbency by afternoon. On physical examination at the Veterinary Teaching Hospital later that day, the horse was recumbent in the trailer, depressed but responsive, and hypothermic (92.2 F). Other abnormal physical examination findings included hyperpnea (28 breaths per minute), intermittent vertical nystagmus, bilateral inconsistent menace response, mydriasis and delayed pupillary light reflex, prolonged capillary and jugular refill times, icterus, and dehydration.

Gross Pathology: Icterus, hemoabdomen, perirenal hemorrhage, subendocardial hemorrhage
Liver—pale yellow-brown, flaccid, 2.3 Kg (0.99% body weight)

Laboratory Results:

Test	Result	Reference Range
Packed cell volume (PCV)	58%	35-50%
Segmented neutrophils	0.4 x 10 ³ /μL	6.0-12.0 x 10 ³ /μL

Total Protein	7.6 g/dL	5.7-8.1 g/dL
Lactate	7.2 mmol/L	<2 mmol/L
Fibrinogen	112 mg/dL	115-289 mg/dL
Glucose	<20 mg/dL	73-124 mg/dL
Blood urea nitrogen (BUN)	3 mg/dL	8-27 mg/dL
Creatinine	2.0 mg/dL	0.6-1.8 mg/dL
TCO ₂	19 mmol/L	23-31 mmol/L
Anion gap	26.4 mmol/L	12-20 mmol/L
Aspartate aminotransferase (AST)	3,563 IU/L	206-810 IU/L
Alkaline phosphatase	933 IU/L	109-331 IU/L
γ-Glutamyl transferase (GGT)	158 IU/L	12-46 IU/L
Creatine kinase (CK)	9811 IU/L	88-453 IU/L
Bilirubin	21.1 mg/dL	0.10-2.60 mg/dL
Unconjugated	15.3 mg/dL	
Conjugated	5.8 mg/dL	
Blood ammonia	254.4 μmol/L	25.0-75.0 μmol/L

Aerobic and anaerobic bacterial culture: no significant growth.

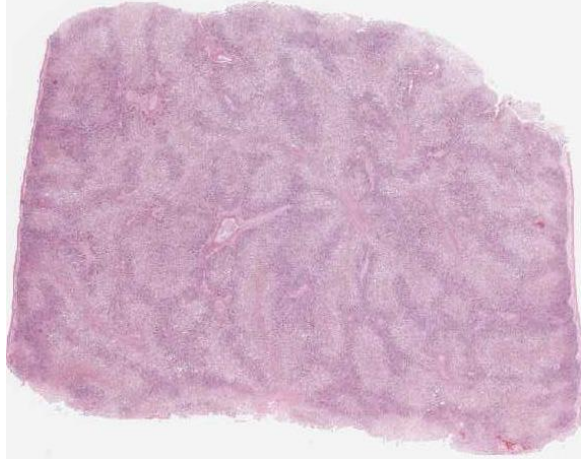
PCR for Theiler's disease-associated flavivirus (TDAV), Cornell University Animal Health Diagnostic Center: nNegative.

Histopathologic Description: Swelling, lysis, and drop-out of hepatocytes affected all hepatic lobules and were centrilobular to massive. Hepatocytes in lobular centers were generally not recognizable. In the lobular periphery, where viable parenchyma remained, hepatic plates were disrupted and hepatocytes had vacuolated cytoplasm. Brown crystals were in the cytoplasm of periportal hepatocytes. Brown granular pigment was in Kupffer cells. Inspissated bile was observed in a few canaliculi. There was patchy increase in fibrous tissue and mononuclear leukocytes (mainly lymphocytes) in portal tracts, but neither inflammation nor fibrosis was severe. Bile duct proliferation was not appreciated.

Changes in the cerebrum (slide not submitted to WSC) included increased space around vessels and cortical neurons with Alzheimer type II astrocytes (hypertrophied and hypochromatic nuclei). These changes were considered consistent with hepatic encephalopathy. In addition, many deep cortical neurons had ischemic change with a shrunken angular profile, intense cytoplasmic eosinophilia, and pyknosis.

Contributor's Morphologic Diagnosis:

Submassive hepatic necrosis



Liver, horse. The retiform pattern noted at subgross results from diffuse centrilobular and midzonal hepatocellular necrosis (pale areas), contrasted with the remaining viable periportal hepatocytes, which stain more intensely. (HE, 4X)

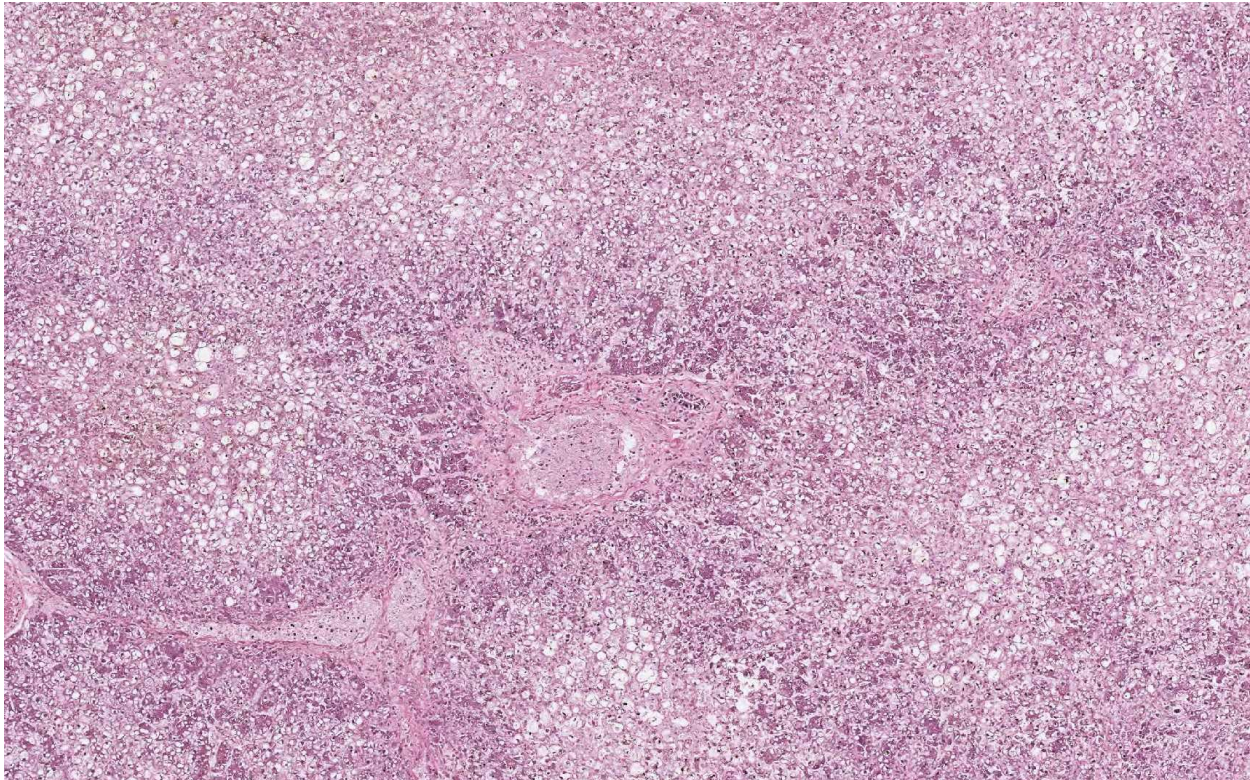
Contributor's Comment: The laboratory results—especially hypofibrinogenemia, decreased BUN, hypoglycemia, elevated hepatic enzyme activity, hyperbilirubinemia, and hyperammonemia—and the history and clinical signs all pointed to liver disease with hepatic encephalopathy as a likely explanation for the neurologic signs. The initial differential diagnosis included Theiler's disease (equine serum hepatitis), pyrrolizidine alkaloid or other hepatotoxicosis, ascending cholangiohepatitis, chronic hepatitis, and hepatic steatosis.

Postmortem gross and histologic lesions were typical of Theiler's disease, hence the PCR testing for Theiler's disease-associated virus (TDAV). This horse was negative by PCR for TDAV, but the serum was positive for a previously unreported non-flavivirus that is under investigation (personal communication, Drs. Bud Tennant, Tom Divers and colleagues at Cornell University and Columbia University).

Equine serum hepatitis or Theiler's disease was first described in 1919, when Arnold Theiler reported acute hepatic atrophy and hepatitis in South African horses vaccinated

against African horse sickness with an equine antiserum-containing live virus vaccine.⁶ Today, Theiler's disease is usually recognized in horses 4-10 weeks after injection with a product that contains equine serum or plasma, though some affected horses have no history of injection with equine blood-containing biologic products. Although the incubation period is long (42-90 days), clinical disease (subclinical cases are also recognized) is fulminating with acute hepatic failure and death within 24 hours in up to 90% of clinically ill horses. At autopsy, the carcass is icteric. The liver is generally close to normal size, but flaccid ("dish-rag" liver) due to massive necrosis with loss of so many hepatocytes. The histologic lesion is centrilobular to massive necrosis with mild, mainly lymphocytic, inflammation of portal tracts. Surviving periportal hepatocytes are swollen with vacuolated cytoplasm.

Theiler's disease has long been suspected to be a viral hepatitis, but the first candidate virus, Theiler's disease-associated virus (TDAV, a pegivirus in the Flaviviridae family), was only identified a few years ago in horses treated with equine antiserum to botulinum toxin.¹ Another pegivirus called equine pegivirus has been identified in horses, but has not been documented to cause hepatitis.⁴ The recently discovered non-primate hepacivirus (NPHV, equine hepacivirus) is another member of the Flaviviridae family that is hepatotropic and can result in transient or chronic infection with elevated hepatic enzymes and hepatitis in horses.³⁻⁵ Many horses (30-40%) have serum antibodies to NPHV; fewer horses have detectable viral RNA in the peripheral blood. The horse has been proposed as an animal model of viral hepatitis because NPHV is so closely related to the human hepatitis C virus.



Liver, horse. Higher magnification demonstrating the differential staining between hepatocytes in centrilobular and midzonal areas and those in the periportal areas. (HE 80X)

JPC Diagnosis: Liver: Necrosis, centrilobular to midzonal, diffuse with hepatocellular lipidosis.

Conference Comment: Although the majority of cases of equine serum hepatitis are associated with prior administration of equine biologics, cases have also been documented in horses with no history of prior injection. While Theiler's disease-associated virus has been proposed as an etiology for this condition,¹ additional research is necessary to prove definitive causation and fulfill Koch's postulates.² It is also unclear what percentage of horses develop subclinical hepatic disease after exposure to equine origin biologics as the disease is rarely diagnosed prior to development of hepatic failure; however, some animals have been known to survive after mild disease. Histologic findings often suggest a subacute process, which contrasts with the relatively acute clinical course of

disease. Lesions range from the presence of abundant degenerate and/or necrotic hepatocytes to complete loss of parenchymal cells. Many hepatocytes are often completely lost, leaving behind variable numbers of degenerate and lipid-filled hepatocytes, dilated congested sinusoids, and collapsed stromal remnants. Hemorrhage and acute necrosis are not typical findings. Other common features, albeit varying in severity, include low numbers of inflammatory cells, mild fibrosis in portal areas and an increase in bile duct profiles.²

In this case, conference participants described diffuse loss of hepatic cord architecture with hepatocellular degeneration, necrosis and loss. There is stromal collapse and mild portal bridging fibrosis. The brown pigment present within Kupffer cells and hepatocytes was interpreted as hemosiderin. The section is

moderately autolytic and there is abundant acid hematin present.

We thank the contributor for providing clinical pathology data with the submission, which greatly enhances the teaching and learning value of the case. Severe neutropenia is most commonly associated with endotoxemia, but the precise etiology is difficult to ascertain in this case. Elevated packed cell volume (PCV) is likely secondary to dehydration and the normal total protein is indicative of hypoproteinemia in a dehydrated animal. Low fibrinogen, blood urea nitrogen, elevated ammonia and low glucose are indicators of liver failure. The profoundly low glucose is not compatible with life and may indicate some degree of artifact. The decreased TCO₂ and elevated anion gap are indicative of a metabolic acidosis and the unmeasured anions in this case include lactate and renal acids. Elevated AST is secondary to both myocyte and hepatocyte damage and elevated CK provides additional evidence of muscle damage. Elevated GGT and ALP are both indicators of cholestasis and the most common cause of elevated total bilirubin horses is anorexia. The hyperbilirubinemia in this case is higher than can be attributed to anorexia alone and, therefore; the remarkable hepatic changes likely contribute to the elevation. Elevated bilirubin can also be seen in cases of endotoxemia due to impaired excretion of conjugated bilirubin into the biliary tract.

Contributing Institution:

Purdue University

Animal Disease Diagnostic Laboratory:

<http://www.addl.purdue.edu/>

Department of Comparative Pathobiology:

<http://www.vet.purdue.edu/cpb/>

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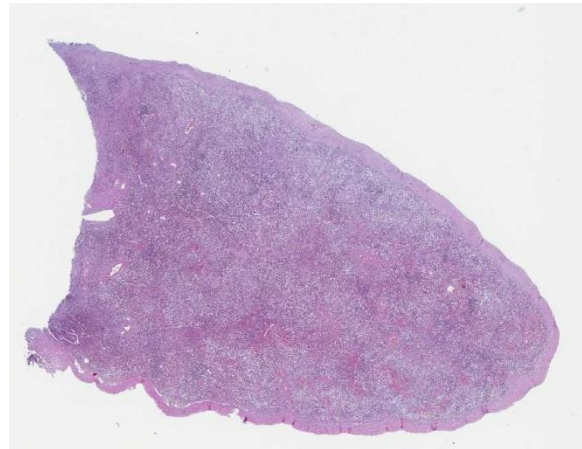
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CASE III: 705-14 (JPC 4066679).

Signalment: 10-year-old, neutered female, dog, Pekingese (*Canis familiaris*)

History: The dog was presented to the veterinary hospital with a history of apathy and severe anemia. Due to a previous

diagnosis of hemolytic anemia, the dog received prednisolone (10 mg BID) and doxycycline (5 mg/kg IV bid), for two weeks, and a blood transfusion was performed 40 days prior to admission. One week after the admission, a blood sample was collected for total blood cell count and serological tests for detecting anti-



Spleen, dog. The spleen parenchyma is expanded by large numbers of macrophages, effacing the normal red and white pulp architecture. (HE, 5X).

Leishmania antibodies. At physical examination, the cornea of the left eye was diffusely opaque, and there was moderate exophthalmos. Ophthalmic examination revealed retinal detachment. The clinical condition of the dog was followed-up for two months, and total blood cell count was performed weekly. During this period, the dog received three blood transfusions. The animal died two and half months after the admission, despite medical treatment.

Gross Pathology: The dog was in good body condition. The cornea of the left eye was diffusely opaque and whitish. There was moderate exophthalmos of the left eye. The upper and lower incisor teeth were absent. The cervical, popliteal and mesenteric lymph nodes were enlarged. On the cut surface, these lymph nodes were diffusely light brown and soft (interpreted as hyperplasia and hemosiderosis). The spleen was markedly enlarged. The splenic capsule

was thickened by fibrosis and with several whitish plaques (1.0 to 3.0 mm in thickness) of chronic active inflammation (perisplenitis with multifocal hyperemia). On the cut surface, the splenic parenchyma protruded and it was firm (carnous) and reddish, with multiple small white foci. The liver was diffusely pale red with multifocal white foci (1.0 mm in diameter) in the parenchyma. The lungs were moderately congested and edematous. Mild myxomatous valvular degeneration was observed in the mitral valve. There were several petechiae in the epicardium of left ventricle and in the peritoneum. Dark red areas (approximately 2.0 cm in diameter), with sharp edge were observed in the parenchyma of the right and left kidney (acute infarction).

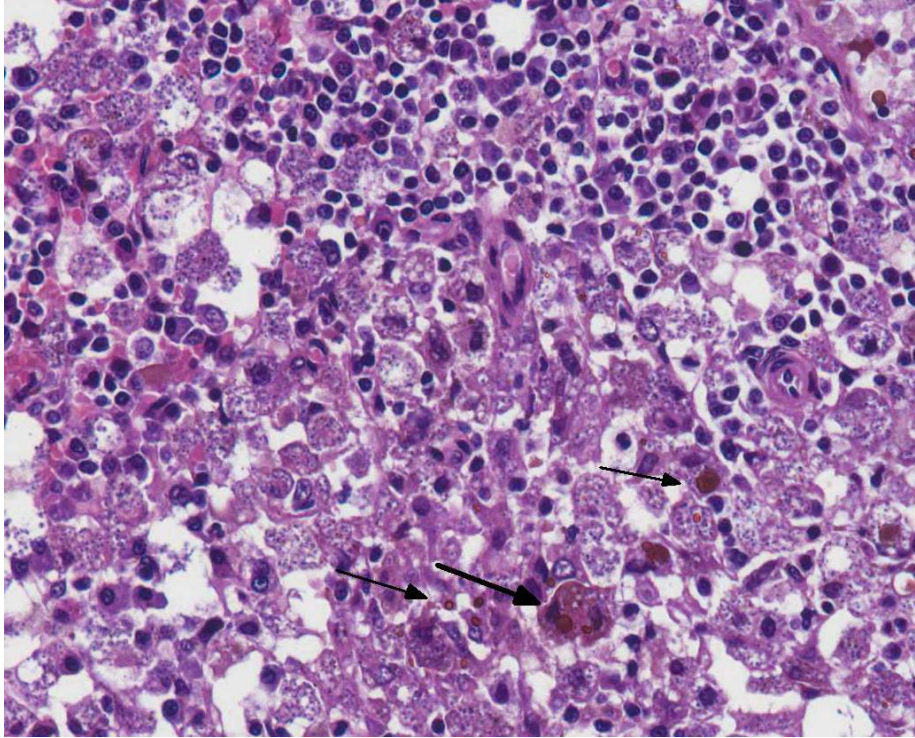
Laboratory Results: The initial serological tests resulted in positive indirect immunofluorescence reaction (RIFI) (1:40 dilution), and negative by ELISA, whereas the second serological tests were positive by both techniques (i.e. RIFI and ELISA).

The dog was observed for two months and total blood cell count analyses were performed weekly. Overall, the dog had marked anemia, leukopenia, and thrombocytopenia. Clinical pathology results are presented in Table 1.

After the last transfusion, i.e. 62 days after the first hematological evaluation, the RBC, HCT, and hemoglobin values were within the reference range.

Concomitantly with the fourth hematological evaluation, a bone marrow aspiration was performed. It was noticed the paucity of hematopoietic precursors, mainly from the erythroid lineage. Serological tests at this time resulted in positive ELISA and negative RIFI.

All hematological analyses revealed reduced numbers of platelets and lymphocytes. The numbers of monocytes remained below the



Spleen, red pulp. The red pulp is replaced by large numbers of amastigote-laden macrophages admixed with numerous plasma cells and fewer lymphocytes. Moderate numbers of macrophages (arrows) contain phagocytized erythrocytes.

Occasional areas of neovascularization are within the capsule. There are scattered areas of karyorrhectic and cellular debris (lytic necrosis) containing occasional viable and degenerate neutrophils admixed with variable amounts of an eosinophilic beaded to fibrillar material (fibrin). There is multifocal histiocytic erythrophagocytosis, rare megakaryocytes, and numerous macrophages containing intracytoplasmic granular and brown pigment (hemosiderosis).

reference range in most of the hematological exams.

Histopathologic Description: Spleen: The normal splenic architecture is markedly distorted due to complete replacement of the red and white pulp by numerous macrophages, moderate numbers of plasma cells, fewer lymphocytes, and occasional neutrophils, that expand the cords, fill the sinuses and invade the splenic trabeculae. Macrophages are enlarged and contain myriad of 2.0-4.0 μm , ovoid, intracytoplasmic amastigotes (Fig. 3) with a 1.0 μm nucleus, surrounded by a 1.0-2.0 μm clear zone and a kinetoplast perpendicular to the nucleus (compatible with amastigotes of *Leishmania* spp.). Multifocal areas of the capsule are expanded due to deposition of variable amounts of fibrous connective tissue and infiltration of scattered lymphocytes, plasma cells, and macrophages.

Bone marrow (proximal femur): The marrow parenchyma is completely replaced (myelophthisis) by numerous macrophages, moderate plasma cells and fewer lymphocytes. Macrophages contain myriad of basophilic structures similar to those described in the spleen (Fig. 4). Multifocally, are areas of karyorrhectic and cellular debris (lytic necrosis) containing occasional viable and degenerate neutrophils admixed with variable amounts of an eosinophilic beaded to fibrillar material (fibrin). There is multifocal hemosiderosis.

Tissues not submitted:

Lymph nodes present changes similar to those described in the spleen. Additionally, moderate lymphoid hyperplasia and increased plasma cell differentiation are observed. Macrophages containing myriad of amastigotes of *Leishmania* spp. are seen within lymphatic sinus, medullary cords,

cortical and paracortical regions. In the liver there are multiple areas with loss of hepatocytes and infiltration of macrophages, plasma cells and lymphocytes. Some macrophages are loaded with amastigotes of *Leishmania* spp. In both organs there is moderate multifocal hemosiderosis.

The kidneys presented with moderate membranous glomerulonephropathy. The Lumina of several tubules are ectatic, and contain aggregates of eosinophilic material (protein casts).

Amastigotes of *Leishmania* spp. were detected by immunohistochemistry (Streptavidin-biotin-peroxidase) in the spleen (Fig. 5) and in the bone marrow (Fig. 6 and 7).

Contributor's Morphologic Diagnosis:

Spleen: Splenitis and perisplenitis, histiocytic and plasmacytic, diffuse, severe, with myriad of intrahistiocytic amastigotes, etiology consistent with *Leishmania* spp.

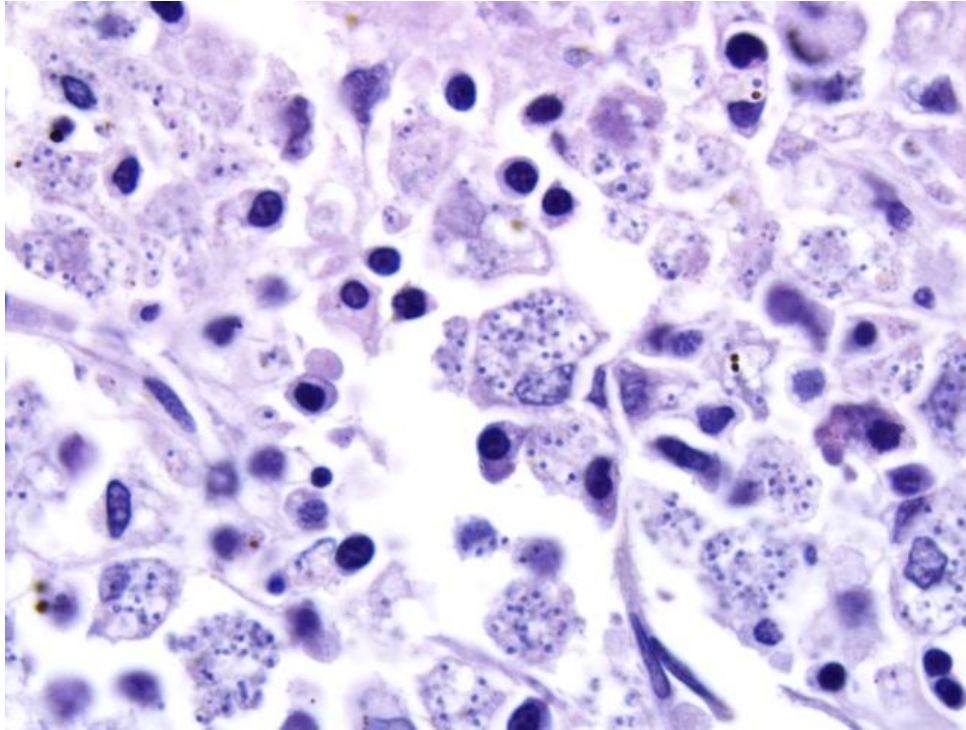
Bone marrow: Myelitis, histiocytic and lymphoplasmacytic, diffuse, severe, with myriad of intrahistiocytic amastigotes, etiology consistent with *Leishmania* spp.

Contributor's Comment: Canine leishmaniasis (CanL) is a major global zoonosis, potentially fatal to humans and dogs. Dogs are the main reservoir of the infection to humans.³ The disease is endemic in more than 70 countries in the world and occurs predominantly in the southern Europe, Africa, South and Central America.¹¹ While *Leishmania infantum* (synonym *L. chagasi*) occurs in Latin America, as well as in the Mediterranean climate regions of the Old World, *Leishmania donovani* infections are restricted to the (sub-) tropics of Asia and

Africa. In these former regions, transmission is mostly anthroponotic.⁹ In Latin America, approximately 90% of the cases of human visceral leishmaniasis are reported in Brazil, and occur in almost all of the states of the country. The higher incidence of the disease in humans takes place in the northeast region of the country (65% of the cases), followed by the southeast (14%) north (14%) and centerwest (14%).^{3,6} Currently, the disease is gradually spreading to the south and west regions of the country.^{3,10} Studies comparing the incidence or prevalence of the disease in dogs in different regions of the country are scarce. In Belo Horizonte, capital of the state of Minas Gerais, CanL is considered endemic with increasing seroprevalence in the canine population. From 1994 to 2000, this prevalence was estimated in 3.6% of all dogs of the county. In 2007, this index increased to 9.3%.^{3,8}

The life cycle of *Leishmania* spp. involves two hosts – a phlebotomine sand fly vector (genus *Lutzomyia* in the New World) and a mammal (including rodents, canids, or humans). *Leishmania* spp. occurs as flagellated, extracellular promastigotes in the gut of sand fly vectors. Infection occurs when a feeding sand fly deposits metacyclic promastigotes into the dermis of the host. In mammalian hosts, *Leishmania* spp. occur as amastigotes (2.0 to 3.0 µm in diameter) within mononuclear phagocytes in the skin, bone marrow, and visceral organs.¹¹ Although vector-borne is the most important route of transmission, CanL can also be transmitted in the absence of the invertebrate vector.¹³

CanL is manifested by a broad spectrum of clinical signs and degrees of severity. In the typical CanL case, history and physical examination include skin lesions, local or generalized lymphadenomegaly, emaciation,



Bone marrow. Macrophages, which largely replace immature erythropoietic elements, contain numerous 2-3µm amastigotes with a central nucleus and a rod-shaped kinetoplast. (HE, 400X)

cachexia, splenomegaly, anorexia or increased appetite, lethargy, temporal muscle atrophy, exercise intolerance, polyuria/polydipsia, ocular lesions, epistaxis, onychogryphosis, lameness, vomiting, and diarrhea.⁴ Serum biochemistry findings in dogs with clinical leishmaniosis include, most commonly, serum hypoproteinemia with hyperglobulinemia and hypoalbuminemia, resulting in a decreased albumin/globulin ratio. Mild increases of liver enzyme activities are frequent; however, grossly elevated liver enzyme activities, severe azotemia, or both, are found in only a minority of dogs with leishmaniosis. Proteinuria and some renal abnormalities develop in most dogs with this disease.¹

Hematological parameters and the serum biochemical profile in *L. infantum*-infected dogs have limited diagnostic value. However, they can be useful biomarkers for

evaluating the clinical progress of infected animals and may also contribute to the understanding of CanL pathogenesis. One the most remarkable characteristics of CanL-associated hematological disorders is anemia, characterized as non-regenerative normocytic or normochromic. The leucocyte alteration in the blood of symptomatic dogs

includes leucopenia characterized by monocytopenia, lymphopenia, and eosinopenia.¹ The pathogenesis of hematological changes in both red and white blood cells is often related to bone marrow disorders associated with diminished erythropoiesis due to intense bone marrow parasitism. In addition, anemia can be related to reduced plasma iron due to abnormal iron retention by macrophages and increased levels of hepcidin, typical of anemia of chronic diseases. Anemia could also be related to increased hemolysis in enlarged spleen and liver associated with inflammatory response to *L. infantum* and to decreased production of erythropoietin by damaged kidneys.⁷

Severely affected dogs are usually cachectic and suffer from muscle atrophy. The skin and hemolymphatic organs are primarily affected. Generalized lymphadenomegaly and splenomegaly are usually present. Hepatomegaly may be present, but is less

Hematology and biochemical parameters	First haematological evaluation	Second haematological evaluation (post transfusion)	Third haematological evaluation	Average of 11 haematological evaluations (4 th to 14 th) -mean(min-max)-	15 th haematological evaluation - 62 days after the first evaluation (post transfusion)	Reference range
Erythrocytes (million/mm ³)	2.16	2.35	1.57	3.06(1.45-4.13)	8.0	5.5-8.5
Hemoglobin (g%)	4.2	4.8	3.3	7.24(3.7-9.1)	19.3	12-18
Hematocrite (HCT) (%)	15	16	11	22.63(14-29)	53	37-55
VCM (fl)	69.44	68.09	70.06	75.47(62.11-96.55)	66.25	60-77
CHCM (%)	28.00	30.00	30	31.85(26.43-35.5)	36.42	31-36
HCM (pg)	19.44	20.43	21.02	23.88(22.03-26.67)	24.13	19.0-24.5
RDW (%)	17.8	16.8	17.9	15.12(13.6-17.6)	13.2	12-15
Metarubricytes (%)	16	16	18	20.81(4-47)	100	Absent
Total leukocytes (mm ³)	2672	2465	2347	5365(1544-11308)	1645	6000-17000
Bastonetes (mm ³)	106.88	49.30	140.82	560.58(33.53-2398)	Not performed	0-300
Neutrophils (mm ³)	1175.68	1972.00	1595.96	4121.39(218-10064.1)	1348.90	3000-11500
Lymphocytes (mm ³)	1336.0	345.10	563.28	553.29(54.5-1733.34)	197.40	1000-4800
Monocytes (mm ³)	53.44	98.60	46.94	312.31(30.88-1017.72)	65.80	150-1350
Eosinophils (mm ³)	Not performed	Not performed	Not performed	78.25(33.53-104.3)	32.90	
Platelets (mm ³)	38000	16000	44000	59272(16000-131000)	60000	175000-500000
Urea (mg/dL)	120.5	41.1	27.1	31.26(21.1-46.4)	24.7	20-56
Creatinin (mg/dL)	1.5	0.82	0.53	0.79(0.74-0.9)	0.6	0.5-1.5
Alanin aminotransferase (U/L)	124.5	136.5	151.7	75.88(40.85-130)	727.8	0-110
Aspartate aminotransferase (U/L)	168	90.3	130.7	59.30(41.6-75.5)	4.98	0-100
Alkaline Fosfatase (U/L)	315	403.5	264.5	214.6(85-529)	725.5	20-156
Gama-glutamil transpeptidase (U/L)	Not performed	Not performed	Not performed	6.26(1.2-20.1)	187.9	0-25
Glucose (mg/dL)	59.5	110.35	78.49	83.60(76.78-91.8)	81.54	76-119
Total proteins (g/dL)	6.9	6.5	6.8	6.77(6.2-7.3)	7.3	5.4-7.5

Table 1.

common. Small nodular foci of inflammation may develop in various organs, including the skin and the kidneys. Mucosal ulcerations in the nasal cavity, stomach, intestine, and colon are occasionally observed. The typical histopathologic finding in the majority of affected tissues is an inflammatory reaction associated with macrophages in the presence or absence of *Leishmania* amastigotes. Amastigote numbers may vary from very few organisms within macrophages to large numbers in rarer events. Lymphoplasmacytic inflammation is also common in dogs with leishmaniasis.¹ Additionally, renal disease due to glomerulonephritis and interstitial nephritis is commonly associated with CanL due to *Leishmania infantum*.

Diagnosis of canine leishmaniasis is based on the presence of clinical signs together with positive specific antibody assay. Infection can be confirmed by demonstration of the parasites (amastigotes) on

touch prep stained (Wright-Giemsa) slides or in cultures of tissue aspirates or biopsy specimens of the spleen, liver, bone marrow, or lymph nodes.¹ Immunohistochemistry is a useful tool that significantly increases the sensitivity of histopathology for detecting amastigotes, and it is a genus-specific assay to confirm the diagnosis.¹²

JPC Diagnosis: 1. Spleen, red pulp: Splenitis, histiocytic and plasmacytic, diffuse, marked with numerous intracellular amastigotes.

2. Spleen: Reticuloendothelial hyperplasia with erythrophagocytosis and hemosiderosis.

3. Spleen: Extramedullary hematopoiesis.

4. Bone marrow: Myelitis, histiocytic and plasmacytic, diffuse, marked with numerous intracellular amastigotes.

Conference Comment: *Leishmania* sp. organisms primarily infect the monocyte-

macrophage system and the visceral manifestation bears many similarities to histoplasmosis. *Leishmania* amastigotes are able to survive and reproduce in macrophage phagolysosomes due in part to an increase in pH, and macrophage destruction occurs secondary to proliferation of the protozoa.¹⁴ The immune response includes both T helper 1 (Th1) and T helper 2 (Th2) mediated mechanisms and tissue damage occurs via a variety of methods including granulomatous inflammation, immune complex deposition and the formation of autoantibodies. Inefficient killing of the organism by macrophages leads to the various immune responses, eventually resulting in damage to a variety of tissues and an array of clinical manifestations discussed above.² Resistance to infection is based on a strong Th1 response, whereas a strong Th2 response can be deleterious⁵ due to immune complex deposition, particularly in renal glomeruli. The level of parasite burden also appears to play a role in the immune response effectiveness against the invading organisms. In dogs with a high parasite load CD8+ T Cells are less effective at lysing infected macrophages. Genetic susceptibility also plays a role in outcome of infection with certain breeds such as the German Shepherd, cocker spaniel, and boxer being more susceptible.⁴ The most common clinical manifestations of disease include skin, renal and/or ocular disease as well as epistaxis, and the disease is broadly divided into cutaneous, mucocutaneous and visceral forms. Enlargement of the spleen is very common and nearly always occurs in cases of visceral leishmaniasis.¹⁴ While leishmaniasis is more common in Mediterranean countries and South America, endemic foci are also present in North America including in Texas, Oklahoma, Ohio and Michigan.⁵

The conference histologic description was aligned very closely with the contributor's

description above. Conference participants were struck by the marked degree of effacement of both the splenic red pulp and bone marrow.

We thank the contributor for providing clinical pathology data with the submission, which greatly enhances the teaching value of the case. Participants discussed the third hematological evaluation and whether the anemia is regenerative or nonregenerative, but without a reticulocyte count it is difficult to determine with certainty. The presence of nucleated red blood cells (metarubricytosis) can be indicative of regeneration with concurrent reticulocytosis, but nucleated red blood cells can also be present in other conditions such as bone marrow damage from inflammation or necrosis, lead poisoning and due to splenectomy among other things. A normal MCV would support a nonregenerative anemia; additionally, the pancytopenia and histologic changes in the bone marrow also provide support for a nonregenerative anemia. Elevated ALT and AST are indicative of mild hepatocellular damage, which correlates with the histopathologic description mentioned above by the contributor in the tissues not submitted. The total protein is normal but a decrease in albumin and increase in globulin is likely present in this case, based on the degree of infection and ongoing inflammation, which is a common pattern in dogs afflicted with leishmaniasis, as mentioned above by the contributor.

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CASE IV: 21966 (JPC 4066536).

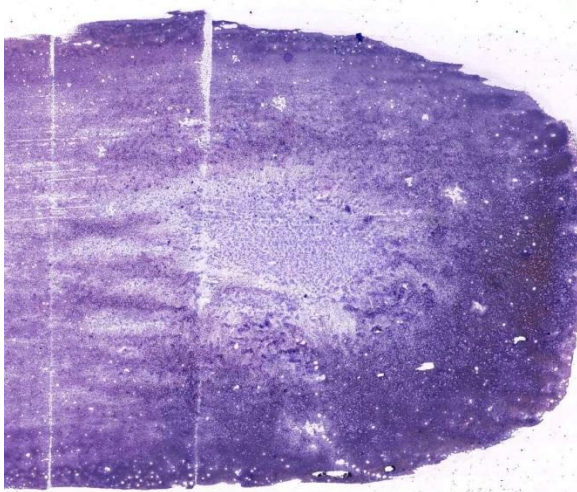
Signalment: 10-year-old spayed female Miniature Dachshund dog, (*Canis familiaris*)

History: The dog was presented with a gingival mass on right maxilla, which had been enlarging for 2 to 3 weeks. Both right and left mandibular lymph nodes were enlarged by palpation. Thoracic CT scan revealed no metastasis in the lung.

Gross Pathology: The mass measured 3.7 x 2.4 x 1.1 cm and was partly red and black.

Laboratory Results: None

Cytologic description: Fine-needle aspiration of the mass was performed. The smear was highly cellular and consisted of monomorphic cells, most of which are in clusters. The cells had a round nucleus in pale abundant cytoplasm. The cytoplasm was mild to moderately vacuolated and small black pigment noted in a few cells. Cells showed mild to moderate anisocytosis and anisokaryosis, and mitotic figures were rarely seen.



Fine needle aspirate, oral mass, dog. A densely cellular, fine needle aspirate was submitted digitally for examination. (Wright's-Giemsa, 4X).

From the left mandibular lymph node, similar morphologic cells were mainly acquired, and several small lymphocytes, plasma cells, and eosinophils were noted. On the smear of right mandibular lymph node, lymphoid cells, predominantly small mature lymphocytes with some lymphoblasts and plasma cells were seen. Scattered melanophages were also observed.

Contributor's Interpretation:

1. Gingival mass: Malignant melanoma with metastasis to the left mandibular lymph node was suspected.
2. Right mandibular lymph node: Reactive lymph node.

Histologic result: The mass was partly resected for histopathology. On histologic examination, the neoplastic cells invaded almost all of the mass and were arranged in nests supported by a fine fibrovascular stroma. The neoplastic cells had clear abundant cytoplasm and round to oval nucleus. The cytoplasm was vacuolated and small amount of brown pigment were observed in several cells. A few mitotic figures are noted. Both left and right mandibular lymph nodes were removed and submitted for the histopathology. Neoplastic cells infiltrated both lymph nodes, and especially in the left lymph node, they spread across a wide area.

Contributor's Comment: Tumors of melanocytic origin are relatively common in the dog. The malignancy of melanoma greatly depends on anatomic location, and oral melanoma is highly aggressive. Cytologic morphology of melanoma is various, showing the feature of epithelial cells, mesenchymal cells or discrete round cells. Balloon cell melanoma, which is a rare variant of melanocytic tumor, has been

reported in human, dog and cat.^{1, 3,8,9,10,11} Microscopically, the neoplastic cells are amelanotic and cytoplasm is vacuolated. Sebaceous carcinoma, liposarcoma, other clear cell neoplasm and granulomatous inflammation are listed as differentials. In this case, sebaceous carcinoma and liposarcoma were unlikely due to the location. The malignant neoplasia was more suspicious than inflammatory disease because of the morphologic atypia. Due to the small amount of granules that can be melanin pigments, melanoma was the primary rule-out; immunohistochemistry such as Melan-A is helpful for further diagnosis.

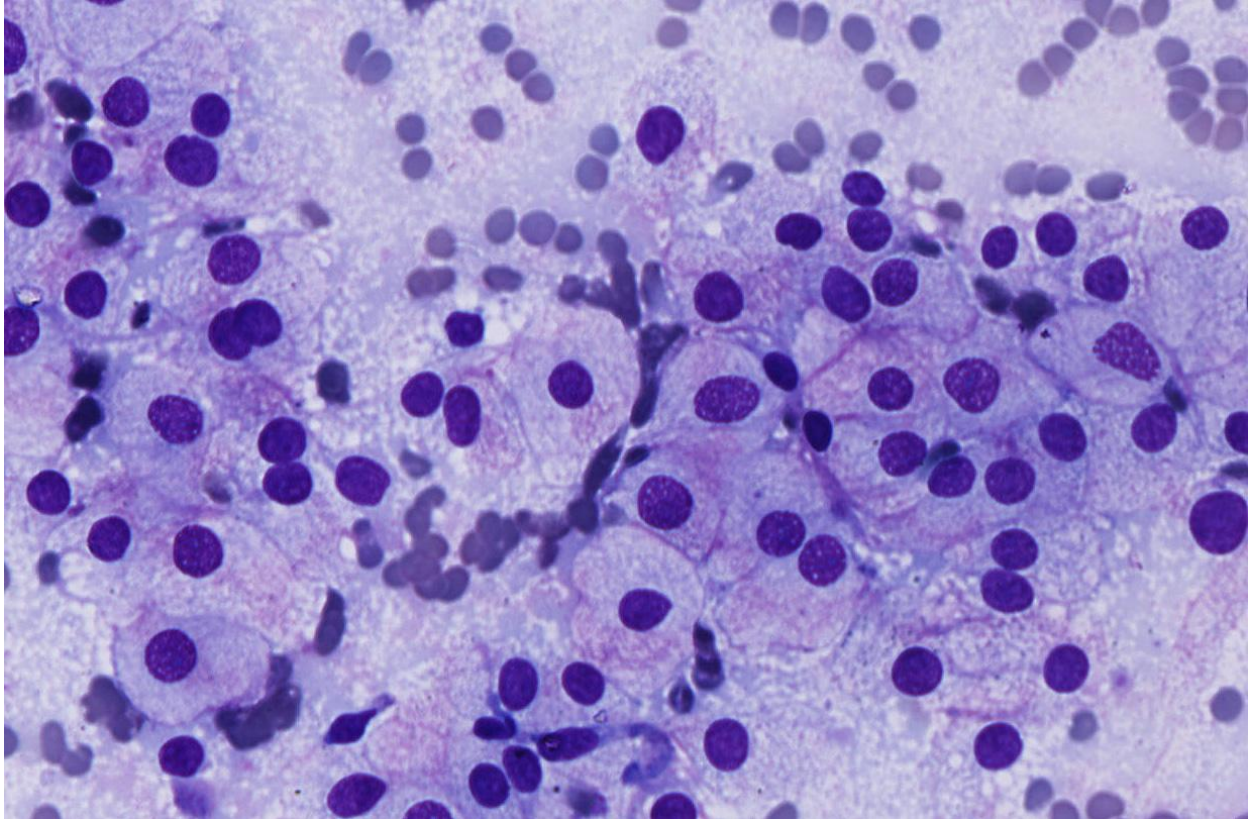
JPC Diagnosis: Fine needle aspirate, oral mass: Malignant neoplasm; differentials include amelanotic melanoma (balloon cell), rhabdomyoma, granular cell tumor, and oncocytoma.

Conference Comment: The neoplastic cells are round to spindled and arranged individually and in small aggregates; the cytoplasm is amphophilic and indistinctly vacuolated and a low to moderate subset of cells contain a pale, granular eosinophilic material in the cytoplasm, the origin of which is unclear. The cells have a single, central, round to oval nucleus with finely stippled chromatin, and 1-2 variably distinct nucleoli. Anisocytosis is mild and anisokaryosis is moderate, and the N:C is low; rare mitoses are observed. Other salient features include nuclear molding, a central fold in the nuclear membrane of few cells, and rare multinucleate cells. Features of malignancy are not striking in this sample, but the anisokaryosis, nuclear molding and presence of mitoses warrants a malignant interpretation. Many conference participants commented melanomas are usually more aggressive looking, particularly

in the oral cavity. A search for melanin was largely unrewarding, particularly with the digital slide only being scanned to 20X, but small amounts are seen in images of the histologic section provided by the contributor. Aside from the differential diagnoses listed above, conference participants remarked the cells resemble hepatocytes in animals with steroid hepatopathy, suggesting glycogen or another similar substrate may be present within the cytoplasm.

Differential diagnoses discussed include balloon cell melanoma, rhabdomyoma, granular cell tumor and oncocytoma. A definitive diagnosis is not possible without the use of immunohistochemistry, which was not performed by the contributor.

Balloon cell melanoma is an uncommon variant of melanoma most commonly described in the skin in both domestic animals and humans, and in cats is often localized to the head. The cells are described as epithelioid, large and round, with abundant finely vacuolated cytoplasm that generally lacks pigment.^{6,11} Glycogen (PAS positive) and lipid rich variants have been described, primarily in humans. Although the balloon cells may contain substances other than glycogen and other similar appearing non-melanocytic tumors may contain glycogen,¹¹ warranting a cautious interpretation of cytoplasmic features. The background of glycogen rich neoplasms is occasionally described as "tigroid," referring to a vague tiger striping pattern imparted by background constituents.⁴ Ultrastructural findings in balloon cell melanomas indicate the cytoplasmic vacuoles represent enlarged melanosomes. In addition to Melan A, balloon cell melanomas may stain for S-100 and neuron-specific enolase.^{1,11}



Fine needle aspirate, oral mass, dog. Neoplastic cells are polygonal with distinct cell borders, abundant vacuolated amphophilic cytoplasm with adherent pink fibrillar matrix. Nuclei exhibit rosy chromatin with mild anisokaryosis, and binucleated cells are frequent. (HE, 120X)

Rhabdomyomas, oncocytomas and granular cell tumors have a similar cytologic appearance with eosinophilic granular material in the cytoplasm, comparable to what is seen in a subset of cells in this case. The similar cytoplasmic appearance in oncocytomas and rhabdomyomas is related to the presence of large numbers of mitochondria, and in granular cell tumors is postulated to be due to metabolic abnormalities and accumulation of lysosomes.⁵ Rhabdomyomas are benign tumors of striated muscle and may occur in the larynx and tongue of domestic animals. Cells may have abundant cytoplasmic glycogen, suggested by PAS positivity.⁷ Oncocytomas are epithelial or neuroendocrine origin neoplasms which arise from oncocytes or oxyphil cells, and are described in the larynx of young dogs. The exact origin of granular cell tumors is unclear but they primarily occur in the oral

cavity and have been conjectured to be of Schwann cell origin. Rhabdomyomas and oncocytomas have benign behavior, and the behavior of granular cell tumors is not well defined. Both rhabdomyoma and oncocytoma have large pale cells with granular or foamy cytoplasm. The nucleus of oncocytoma is central, while the nucleus of rhabdomyoma may be central or peripheral and both have finely clumped chromatin and a single indistinct nucleolus. Multinucleate cells may be seen in rhabdomyoma. The indistinct nucleolus is a feature which may help distinguish them from balloon cell melanoma. Granular cell tumors are variably sized but typically have abundant granular eosinophilic cytoplasm and an eccentric nucleus. Pleomorphism may be moderate in each of the three tumors. Additional diagnostics which may help differentiate the tumors include desmin positivity in

rhabdomyoma, cytokeratin positivity in oncocytoma and PAS positivity (diastase resistance) in granular cell tumor.²

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