Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2015-2016

C o n f e r e n c e 19

30 March 2016

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CASE I: 11-966 (JPC 4002877).

Signalment: 12-year-old male neutered Chihuahua, canine (*Canis familiaris*).

History: Initially, the animal was presented to the referring veterinarian (rDVM) for abdominal enlargement due to ascites. Exploratory laparotomy was performed and the liver was described as having numerous yellow nodules over the entire surface. Clinically, the rDVM was concerned about neoplasia. After the biopsies were reviewed, the rDVM was contacted and some additional information was obtained. The animal had crusty lesions on all four footpads and multifocally on all four limbs. The dog's owners had recently moved to a new residence and they thought the skin issues were due to flea infestation. The animal was euthanized three weeks after surgery and was submitted for necropsy. Samples submitted for the slide conference originated from the original surgical biopsy and necropsy tissue.

Gross Pathology: Footpads on all four limbs are covered by flaky golden-brown crusts. Crusts are either firmly adhered or easily peel away from the digital, metacarpal and metatarsal pads. The skin over the distal left lateral forelimb and left cubital region have similar areas of crusting and mild alopecia which measure 7 mm in diameter and 18 x 4 x 1 mm, respectively. Crusts are also present bilaterally over both hocks and measure 20 x 10 x 1 mm (left) and 22 x 12 x 1 mm on the (right). The subcutaneous tissues are expanded and ooze clear fluid (edema). The abdomen contains 1220 mL of golden serous fluid. The liver is 3.7% of total body weight (after removal of abdominal fluid). The liver contains innumerable widespread variably sized brown-red nodules separated by depressed tan areas (parenchymal collapse). The nodules range in size from 3 mm diameter up to 2.5 cm diameter.

Laboratory Results: No laboratory diagnostic results were provided by referring veterinarian.

Liver, dog. The liver represented 3.7% of the dog's body weight, and grossly, was covered with numerous 3mm-2.5cm hepatocellular nodules. The intervening parenchyma is yellow-orange and collapsed. (Photo courtesy of: University of Tennessee Colle College of Veterinary Medicine, Department of Pathobiology, 2407 River Drive, Room A201, Knoxville, TN 37996 http://www.vet.utk.edu/)

Histopathologic Description: Liver: At low power, there is multifocal to coalescing collapse and loss of hepatic parenchyma. The areas of collapse separate variably sized unencapsulated nodules of well diff-erentiated hepatocytes (regenerative hyperplasia). In areas of collapse, there are heavily vacuolated and swollen hepatocytes, increased bile duct profiles, plugs of golden material (bile) in canaliculi (canalicular cholestasis), and low numbers of lymphocytes, plasma cells, and neutrophils. Multifocally, within the areas of collapse, there are occasional areas containing mildly increased fibrous tissue (confirmed with trichrome). There are multifocal clusters of macrophages containing golden brown pigment.

There is some variation among slides because some samples were from the original biopsy while others were obtained during necropsy 3 weeks later.

Contributor's Morphologic Diagnosis:

Liver: Severe chronic multifocal to coalescing parenchymal loss and nodular regeneration with fatty change, biliary hyperplasia and canalicular cholestasis

Footpad: Marked chronic basal cell hyperplasia, intracellular edema (stratum spinosum), and parakeratotic hyperkeratosis (*slide not included*)

Contributor's Comment: Gross and histologic changes in the liver and skin are consistent with hepatocutaneous syndrome. Most animals present clinically for the skin lesions; however, in this case the animal presented for the hepatic disease because the owners mistakenly associated the cutaneous lesions with fleas.

Hepatocutaneous syndrome has been reported in humans, dogs, cats, and the black rhinoceros.[1,2,5-7] Older small breed dogs are primarily affected. The long-term prognosis for animals with hepatocutaneous syndrome is very poor. In cases of hepatocutaneous syndrome, the liver has a gross appearance resembling cirrhosis. Histologically, there

Footpads, dog. Digital, metacarpal, and metatarsal footpads are covered by flaky golden-brown crusts. (Photo courtesy of: University of Tennessee College of Veterinary Medicine, Department of Pathobiology, 2407 River Drive, Room A201, Knoxville, TN 37996 http://www.vet.utk.edu/
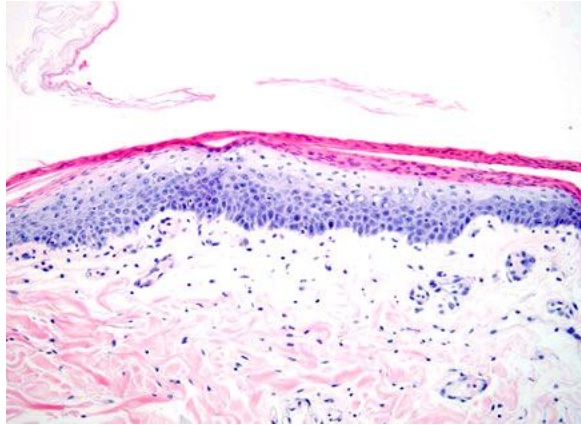
Footpads, dog. Histologic examination of the footpad lesion should a superficial "red" zone of parakeratotic hyperkeratosis, a "white" zone of underlying intracellular edema of the cells within the stratum spinosum, and at bottom, a "blue" zone of basal cell hyperplasia (HE, 200X) Photo courtesy of: University of Tennessee College of Veterinary Medicine, Department of Pathobiology, 2407 River Drive, Room A201, Knoxville, TN 37996 *http://www.vet.utk.edu/*

are foci of regenerative nodular hyperplasia separated by areas of parenchymal collapse containing heavily vacuolated hepatocytes. Hepatic changes are considered idiopathic, but have been suggested to be due to an underlying metabolic, hormonal, or toxic etiology. The pathogenesis of the associated skin lesions is unknown, but hepatic dysfunction with derangement of glucose and amino acid metabolism have been suggested.[1,2,7]

This disorder produces characteristic cutaneous changes that typically occur on the footpads, mucocutaneous junctions, and pressure points. Gross changes may include crusting, erosion/ulceration, erythema, alopecia, and exudation. The histologic appearance is often referred to as "red, white and blue." (The "red" corresponds to superficial parakeratotic hyperkeratosis; "white" corresponds to pallor in the stratum spinosum which is due to intracellular edema; "blue" corresponds to basal cell hyperplasia.[2] Depending on the duration of the disease, all of the classical components of the histologic lesion may or may not be

seen and could also be obscured by secondary changes (e.g. erosion, ulceration, infection). Superficial necrolytic dermatitis (SNE), necrolytic migratory erythema (NME), and metabolic epidermal necrosis (MEN) have been terms used to refer to the cutaneous syndrome in humans.[1] The majority of canine cases are associated with liver disease, whereas human cases are typically associated with a functional pancreatic glucagonomas. However, in animals, the cutaneous lesions have also been associated with glucagon secreting tumors, diabetes mellitus, pancreatic carcinoma, gastric carcinoma and thymic amyloidosis.[5,7]

Other differential diagnoses to consider for parakeratotic skin diseases include zinc-responsive dermatosis, generic dog food dermatosis, lethal acrodermatitis of bull terriers, irritant contact dermatitis, and thallium toxicosis.[1,2] The signalment, clinical history, and ancillary diagnostic tests, in combination with histologic findings, should easily differentiate between these potential differentials.
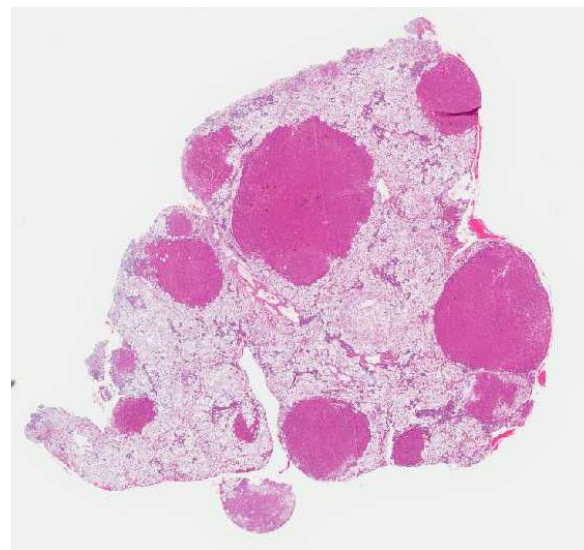
Liver, dog. Subgross of the submitted tissue exhibits nodules of hyperplasic hepatocytes with few to a single portal area. The intervening parenchyma is collapsed, with loss of lobular architecture. (HE, 5X)
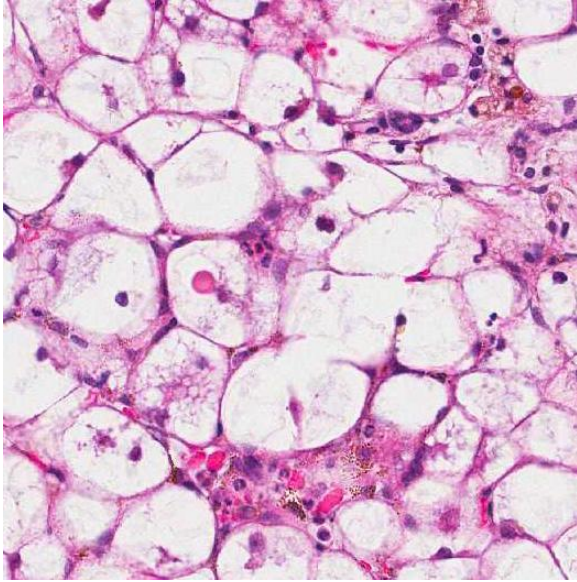
Liver, dog. Hepatocytes within the majority of the stroma are swollen with abundant hepatocellular glycogen and small amounts of fat (HE, 400X)

JPC Diagnosis: Liver: Hepatocellular glycogenosis and lipidosis diffuse, severe with foci of regeneration.

Conference Comment: Hepatic lesions in canine hepatocutaneous syndrome tend to diffusely affect the liver and are non-inflammatory in nature. Lesions are often described as a degenerative hepatopathy with formation of cytoplasmic vacuoles, eventually leading to parenchymal collapse. Histologically, affected hepatocytes de-monstrate severe ballooning (both glycogen and/or lipid, micro- and macro-vesicular, vacuolar degeneration). Hepatic nodular regeneration is also an important component of the histologic lesions and the pattern is usually multifocal to coalescing and random. Bile duct proliferation may also be seen. A "honeycomb" appearance to the liver is described ultrasonographically.[3] Common clinicopathologic abnormalities include elevated hepatic enzymes and non-regenerative anemia which may be microcytic. Other less common but reported abnormalities include hyperglycemia, thrombocytosis and elevated total bilirubin.

Low plasma amino acid concentrations are also reported.[3] Therapy is generally not effective and most dogs succumb to the disease within months of developing skin lesions.[1,3] Although many dogs are pr-esented initially due to cutaneous lesions, some may only have hepatic lesions at the time of initial diagnosis and present with non-specific signs of lethargy and in appetence, have elevated hepatic enzymes and/or signs of hepatic encephalopathy.[3]

The moderator commented that this syndrome is very difficult to diagnose correctly based solely on the histologic hepatic lesions. The conference histologic description was very similar to the contributor's description above. There is a sharp line of demarcation between de-generative and regenerative areas; sinusoids are compressed and largely obscured in the areas of vacuolar degeneration. Low numbers of binucleate hepatocytes, as well as rare mitoses within the foci of hepatocellular regeneration.

The moderator commented on the atypical appearance of the regenerative nodules,
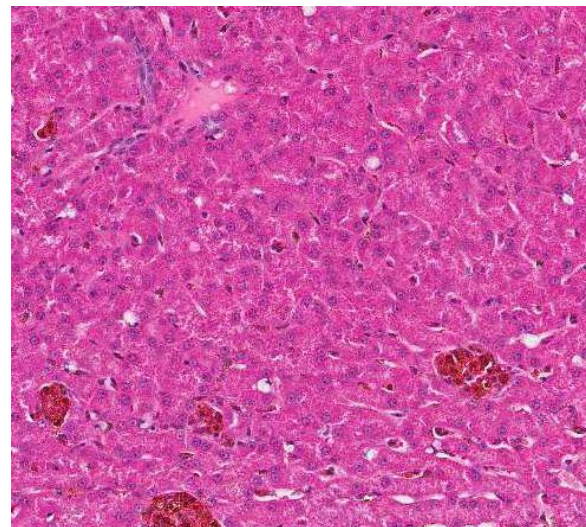
Liver, dog. Nodules of hyperplastic hepatocytes contain aggregates of siderophages, few portal areas, and lack the fibrosis often seen in regenerative nodules. (HE, 75X)

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noting that in regeneration, hepatic cords are most often arranged in double rows, while in this case they are singly arranged. The presence of one portal area per regenerative nodule is typical and is seen in some of the regenerative foci in this case. Conference participants also commented on the relative absence of fibrosis in the H&E stained section. The excellent gross image provided by the contributor was also discussed and the moderator noted that the gross "cluster of grapes" appearance imparted by the regenerative nodules is classic for this condition.

Similar cutaneous lesions may be seen (without liver lesions), with glucagon-secreting pancreatic tumors. There is also a single case report of superficial necrolytic dermatitis associated with an insulin producing tumor in a dog.[4]

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References:

1. Byrne KP. Metabolic epidermal necrosis-hepatocutaneous syndrome. *Vet Clin North Am Sm Anim Pract.* 1999; 29(6):1337-1355.

2. Gross TL, Ihrke PJ, Walder EJ, Affolter VK. *Skin Diseases of the Dog and Cat.* 2nd ed. Ames, IA: Blackwell Publishing Professional; 2005:86-91.

3. Hall-Fonte DL, Center SA, McDonough SP, Peters-Kennedy J et al. Hepatocutaneous syndrome in Shih Tzus: 31 cases (1996-2014). *J Am Vet Med Assoc*. 2016; 248(7):802-813.

4. Isidoro-Ayza M, Lloret A, Bardagi M, Ferrer L, Martinez J. Superficial necrolytic dermatitis in a dog with an insulin-producing pancreatic islet cell carcinoma. *Vet Pathol*. 2014; 51(4):805-808.

5. Kimmel SE, Christiansen W, Byrne KP. Clinicopathological, ultrasonographic, and histopathological findings of superficial necrolytic dermatitis with hepatopathy in a cat. *J Am Anim Hosp Assoc*. 2003; 39:23-27.

6. Munson L, Koehler JW, Wilkinson JE, Miller RE. Vesicular and ulcerative dermatopathy resembling superficial necrolytic dermatitis in captive black rhinoceroses (*Diceros bicornis*). *Vet Pathol*. 1998; 35:31-42.

7. Stalker MJ, Hayes MA. Liver and biliary system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 5th ed. Philadelphia, PA: Elsevier Saunders; 2007:332.

CASE II: H06-002960 (JPC 3174957).

Signalment: 11 year old, female, thoroughbred, equine (*Equus caballus*).

History: Mare with recurring anemia, edema of the ventrum, weight loss and lethargy. The animal previously had a positive Coggin's test.
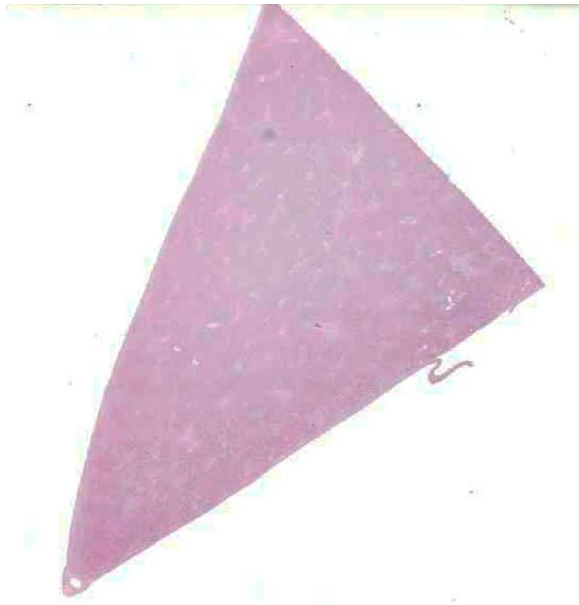
Liver, horse. A triangular, pale-staining section of liver is presented for evaluation. (HE,5X)

Gross Pathology: Subcutaneous and visceral adipose tissue reserves were reasonable. Approximately 10 ml of clear fluid was recovered from the pericardial sac. The liver was enlarged and dark red. Splenomegaly was evident with petechiae evident over the capsule. Similar petechiation was noted over the small intestinal serosa and kidney capsules. The medullary bone marrow is diffusely red and translucent.

Laboratory Results: Positive Coggin's test.

Histopathologic Description: Periacinar necrosis of the liver with attendant, predominantly periportal, infiltration by admixed lymphocytes and macrophages, including hemosiderophages. Hemosiderin-containing Kuppfer cells and erythrophagocytosis evident.

Additionally, there was lymphohistiocytic interstitial nephritis and similar infiltrates noted within the capsular and sub-capsular regions of the adrenal gland. Congestion, focal hemorrhage, lymphoid depletion and medullary histiocytic infiltration (including hemosiderophages) were evident in lymph nodes. Hemosiderophages present in alveolar septa.

Contributor's Morphologic Diagnosis: Lymphohistiocytic hepatitis, chronic, periportal, moderate-severe with periacinar necrosis and occasional erythrophagocytosis.

Contributor's Comment: Equine infectious anaemia (EIA) virus is a lentivirus of the Retroviridae family with a worldwide distribution. EIA, or 'swamp fever', affects all species of Equidae, causing pyrexia, edema, splenomegaly and tissue petechiation.[5,6] Transmission occurs via the mechanical transfer of blood by contaminated medical equipment or biting insects and the resulting infection can persist with cyclic recurrence.[5,6] Viral replication occurs primarily in tissue macrophages of the liver, spleen and lymph nodes, and to a lesser extent in the kidney, and lung. A persistent, life-long viremia may result in clinical relapses when the viral load is high.[5,6]

The lentivirus causing EIA is considered to have little direct cytopathic effect and with the pathogenesis involving indirect effects of the immune response.[6] Anemia corresponds with the appearance of circulating antibody, which is complement-fixing but not initially virus-neutralizing. C3 appears on erythrocyte membranes, and probably explains the erythrophagocytosis. Antibodies are believed to react with virus adsorbed to erythrocytes which results in complement activation and red cell destruction of the "innocent bystander" type. The lifespan of red blood cells is typically shortened by up to 65% and the hemolysis that occurs is largely intracellular. The bone marrow is initially highly responsive but ultimately becomes hypoproliferative.[6]
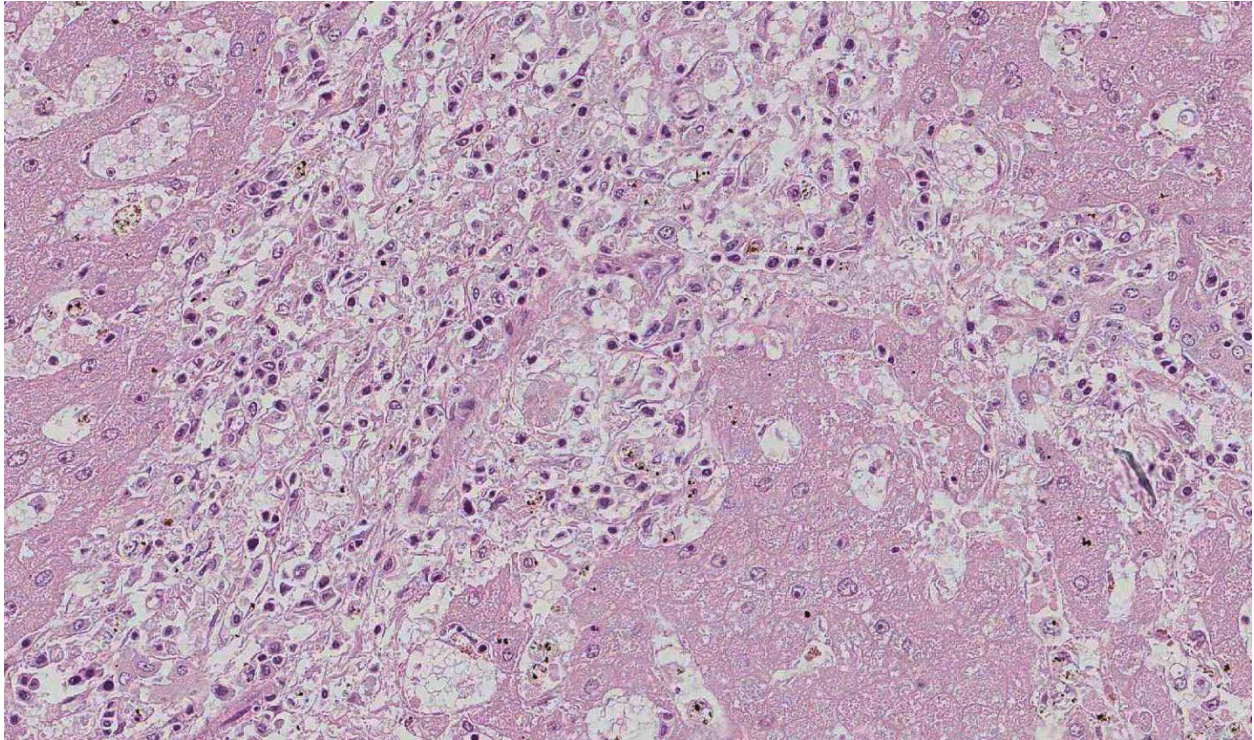
Liver, horse. Portal areas are expanded by abundant fibrosis and infiltration of low to moderate number of lymphocytes and plasma cells. (HE, 320X)

In 2006, an outbreak of EIA occurred in the Republic of Ireland in four foals following administration of contaminated hyperimmune plasma imported from Italy. The disease spread by iatrogenic transmission, resulting in 38 cases.[3] Disease was confirmed by agar gel immunodiffusion, ELISA, immunoblot and using a novel PCR technique.[1] This outbreak highlighted the threat posed to animal populations by the use of unregulated veterinary medicines.

JPC Diagnosis: Liver: Hepatitis, lymphoplasmacytic, portal, diffuse, moderate with centrilobular fibrosis, erythrophagocytosis, hemosiderosis and mild hepatocellular degeneration and loss.

Conference Comment: Equine infections anemia (EIA) evades the immune system by escaping the antibody response, resulting in a chronic infection. This retroviral disease in horses serves as a model to study lentiviral immune escape mechanisms. Recrudescence of infection after a period of inactivity is related to antigenic variation of surface glycoproteins. Studies have shown that selection pressure against the predominant virus strain, namely via virus neutralizing antibody in this case, results in increased antibody escape by viral variants.[4] This seems to indicate a more effective immune response against the primary infecting strain, and increases the likelihood of escape by a viral variant.

In addition to anemia, thrombocytopenia is also a component of equine infectious anemia, sharing the same mechanism as the anemia. The thrombocytopenia may be reflected in acute cases where petechial hemorrhages are evident under the tongue and on the ocular and vulvar mucosa. The majority of hemolysis is intravascular and during acute disease may be severe enough
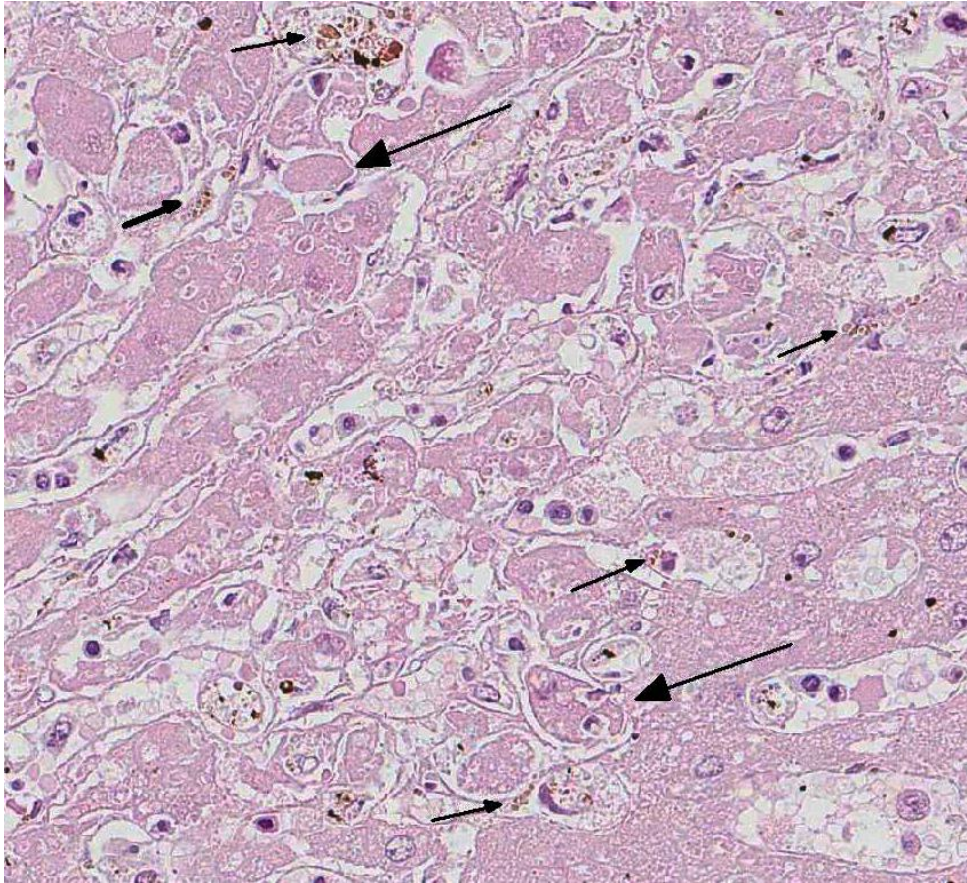
Liver, horse. Throughout the section, individual hepatocytes are rounded up, effacing plate architecture (large arrows). Kupffer cells diffusely contain abundant hemosiderin pigment (small arrows). (HE, 360X)
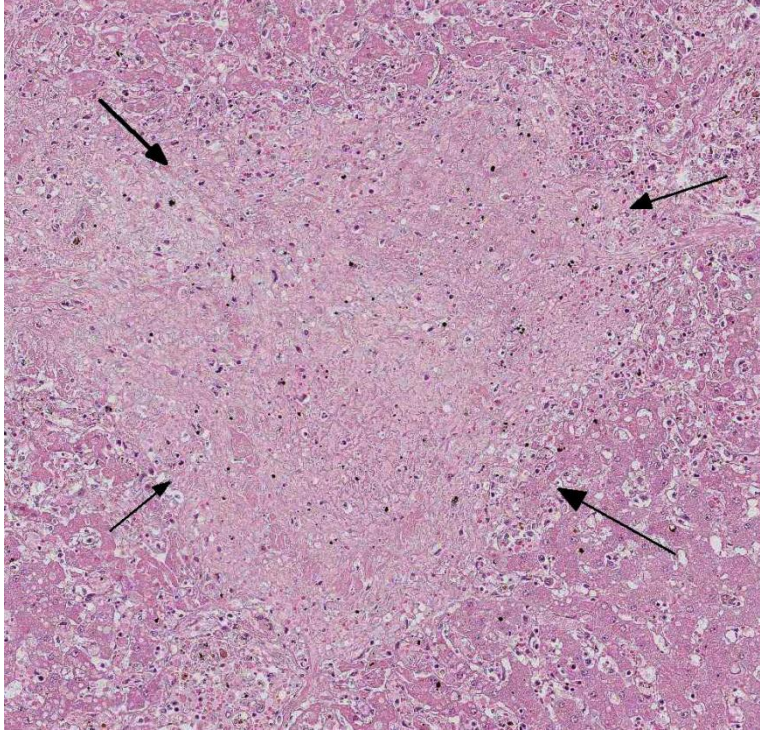
Gross lesions include splenomegaly, ventral edema, petechial hemorrhage in the kidneys, and degree of reddening within the bone marrow noted to be in direct proportion to disease duration.[7] Capsular hemor-rhage may be seen on both the liver and spleen; the liver may also have a fine lobular pattern. Micro-scopic lesions vary with duration but can be seen in most tissues. The heap-tic changes vary in range and severity and include per-iportal infiltrates (as seen in this case), atrophy of hepatic chords and sinusoidal dilation, increased perioportal connective tissue, and Kupffer cell hyperplasia. Hemorrhage and necrosis may be seen in centrilobular areas in acute cases and hepatocellular vacuolar degeneration in subacute cases. Regardless of the stage of infection, whether quiescent or in clinical relapse, the presence of increased hemosiderin laden macrophages provides evidence of infection. Splenic follicles may be enlarged but appear hypocellular and, acutely, the spleen will be congested.[7]

In this case, there is increased pallor of portal areas at subgross histologic examination due to the presence of increased fibrous connective tissue; multifocally,

to result in debilitation and death. During the initial stages of infection, the anemia is regenerative but eventually becomes non-regenerative.[7] The anemia is due to hemolysis from secondary antibody and complement fixation as described above; but is also due to inhibition of erythropoiesis, and impaired bone marrow response is likely due in part to mechanisms associated with anemia of inflammation.[2] Leukopenia and a relative monocytosis may also be seen, as well as erythrophagocytosis, a feature noted in this case. Chronically, the bone marrow is hypoplastic and there is sinusoidal expansion imparting a grossly red appearance to the bone marrow. A plas-macytosis may also be seen within the bone marrow in chronic cases.[7]

Liver, horse: Centrilobular necrosis is present throughout the section (and often bridging) as a result of long-standing anemia. (HE, 140X)

portal areas are also infiltrated by lymphocytes and macrophages. Low to moderate numbers of periportal hepatocytes are necrotic and mild hepatocellular vacuolar degeneration is present at the margin of affected areas. Cholestatic bile canaliculi and occasional erythrophagocytosis are also histologic features noted in this case. Multifocally within centrilobular areas, there is hepatocellular necrosis, as well as fibrosis; these findings reflect a degree of hypoxemia most likely secondary to anemia.

During the conference, the moderator commented on the uncommon nature of active hepatitis in the horse liver; and some conference participants described vasculitis as a prominent component in this section of liver leading some to list equine viral arteritis virus (the causative agent of equine viral arteritis) as their main differential diagnosis. Vasculitis can be seen in cases of

EIA. Other features described and discussed during the conference include mild portal to centrilobular bridging fibrosis and mesothelial hypertrophy.

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References:

1. Cullinane A, Quinlivan M, Nelly M, Patterson H et al. Diagnosis of equine infectious anaemia during the 2006 outbreak in Ireland. *Vet Rec.* 2007; 161: 647-652.

2. Grimes CN, Fry MM. Nonregenerative Anemia: Mechanisms of decreased or ineffective erythropoiesis. *Vet Pathol*. 2015; 52(2):298-311.

3. More SJ, Aznar I, Bailey DC, Larkin JF, et al. An outbreak of equine infectious anaemia in Ireland during 2006: Investigation methodology, initial source of infection, diagnosis and clinical presentation, modes of transmission and spread in the Meath Cluster. *Eq Vet J*. 2008; 40: 706-708.

4. Schwartz EJ, Nanda S, Mealey RH. Antibody escape kinetics of Equine infectious anemia virus infection of horses. *J Virology*. 2015; 89(13):6945-695.

5. Sellon DC, Perry ST, Coggins L, Fuller FJ. Wild-type equine infectious anemia virus replicates in vivo predominantly in

tissue macrophages, not in peripheral blood monocytes. *J Virol*. 1992; 66:5906-5913.

6. Valli VEO: Hematopoietic system. In: Maxie MG, ed. Jubb, Kennedy and Palmer's Pathology of Domestic Animals. 5th ed. Vol 3. Philadelphia, PA: Elsevier; 2007: 235-239.

7. Valli VEO, Kiupel M, Bienzle D. Hematopoietic system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. Vol 3. St. Louis, MO: Elsevier; 2016:114-116.

CASE III: R15/72 (JPC 4071582).

Signalment: Domestic cat, 5 years and 8 months old, male, castrated. (*Felis silvestris catus).*

History: The cat was brought to the clinic with hypotension and icteric mucous membranes. All lymph nodes were slightly prominent. Radiograph showed an enlarged, pendulous abdomen with hepatomegaly and severe splenomegaly. The ultrasound examination revealed that the liver is covered with hypo echoic, nodule-like structures. The visible mesenteric lymph nodes were enlarged. Laboratory tests: Coagulation time was significantly prolonged. Liver-associated values were as well significantly altered. Because of poor prognosis the animal was euthanized.

Gross Pathology: Right eye: Buphthalmos. A brownish, 0.5 cm mass is present laterally on the sclera.

Liver: Diffusely severely enlarged, with disseminated white-green and black, up to

Liver, cat. The liver is diffusely enlarged and covered with raised green and black nodules ranging up to 5mm in diameter. Nodules contain central areas of necrosis. (Photo courtesy of: University of Bern, Vetsuisse faculty, Institute for Animal Pathology (ITPA), Laenggassstrasse 122, CH – 3012, Switzerland, http://www.itpa.vetsuisse.unibe.ch/)

5mm nodules which are multifocally slightly raised and show central necrosis.

Spleen: Diffusely severely enlarged. Multifocally are up to 4 cm nodules present that are dark red and slightly firm.

Laboratory results (See Table 1, next page)

Histopathologic Description: Liver: Extending to all cut borders, infiltrating the sinusoids and disrupting the architecture of the hepatocellular cords, there is a diffuse, densely cellular, non-demarcated and non-encapsulated neoplasm consisting of neoplastic cells that are arranged in sheets

Area	Parameter	Unit	Reference	
Hematology	Hematocrit	l/l	0.27-0.47	0.19
	HB total	g/l	82-153	62
	Erythrocytes	10e12/l	5.9-11.2	3.43
	MCV	fl	37.0-55.0	54.7
	MCH	pg	11.3-17.2	18.2
	MCHC	g/l	263-359	332
	RDW	%	13.8-21.1	28.1
	Retikulocytes %	%	0.05-1.17	6.35
	Retikulocytes	10e9/l	3.7-94.1	217.7
	CHr	pg	>14.2	20.7
	MCVr	fl	47.6-72.8	83.2
	Thrombocytes	10e9/l	180-430	52
	MPV	fl	10.2-25.8	25.2
	Leukocytes	10e9/l	6.5-15.4	13.14
	Leukocytes corrected	10e9/l	6.5-15.4	10.81
Chemistry	Hemolitic Index		<15	29
	Icterus Index		<10	10
	Lipemia index		<100	30
	Na	mmol/l	144-159	146
	K	mmol/l	3.11-4.93	4.93
	Cl	mmol/l	110-130	103
	Ca	mmol/l	2.22-2.92	1.98
	P	mmol/l	0.82-1.91	4.05
	Glucose	mmol/l	3.17-5.71	6.96
	Total protein	g/l	55-76	45.3
	Albumin	g/l	30.3-40.5	25.5
	Urea	mmol/l	6.46-12.20	72.79
	Creatinine	µmol/l	52-138	658
	Bilirubin	µmol/l	0-6.2	155.4
	ALAT (GPT)	IU	12-77	4231
	AP	IU	0-93	122
	ASAT (GOT)	IU	12-61	7146
	CK	IU	0-596	4095
	gGT	IU	0-2	4
	GLDH	IU	0-3	2279
	Serum Amyloid A	µg/ml	<5	121.3
Coagulation	PT (Quick)	sec	9.4-13.1	27.8
	PTT	sec	12.0-16.3	58.2
	TT	sec	16.5-25.1	42.3
	Fibrinogen	mg/dl	150-300	19

Liver and spleen, cat. Cross sections of the spleen (left) and liver (right) exhibit poorly defined, pigmented nodules, which range up to 4cm in diameter. (Photo courtesy of: University of Bern, Vetsuisse faculty, Institute for Animal Pathology (ITPA), Laenggassstrasse 122, CH – 3012, Switzerland, http://www.itpa.vetsuisse.unibe.ch/)

and vague packets. The cells are round to polygonal, up to 25 μm in diameter with moderately distinct cell borders and abundant basophilic, finely granular cytoplasm that multifocally contains brownish pigment (melanin). The cells contain a round to oval, central nucleus with finely vesicular chromatin and mostly one prominent, basophilic nucleolus. There are abundant multinucleated, up to 100 μm giant cells with up to 8 nuclei. Anisokaryosis and anisocytosis are severe. Mitoses range from 3 to 6 per 400x HPF. The neoplastic cells are in large numbers present in almost all blood vessels and lymphatics (vascular invasion, Figure 3). Multifocally there are large areas of necrosis, surrounded by hemorrhage, edema and an inflammatory infiltrate consisting mostly of degenerate neutrophils, macrophages and some lymphocytes and plasma cells. The adjacent hepatocytes are rounded, have an eosinophilic and vacuolated cytoplasm (degeneration). Single cell necrosis is present. Multifocally bile plugs are present in the canaliculi and yellow to brown pigment in the hepatocytes (cholestasis). There is diffuse congestion present.

Masson-Fontana: Many of the neoplastic cells contain variable amounts of intra-cytoplasmatic, agyrophilic, granular material (melanin).

Immunohistochemistry: The neoplastic cells stain positive with 'Melan A' and 'PNL-2' (Melanoma).

**Contributor's Morphologic Diagnosis:** Liver: Melanoma, metastatic

Contributor's Comment: The present case describes a rare case of metastatic conjunctival melanoma in the liver and spleen of an adult spayed, domestic shorthair cat, presented because of jaundice and an enlarged, pendulous abdomen. The hematology, blood chemistry and co-agulation values were severely abnormal

(see Table 1). The primary tumor was found in the eye as a small conjunctival, brownish nodule. In the clinic, because of the exclusive conjunctival involvement and no evidence of iridial or corneal involvement, the mass was diagnosed as a conjunctival melanoma. This mass was not clinically relevant and was surgically removed before the clinical symptoms occurred.

Malignant melanomas are generally tumors of older animals; however, they have been reported in juvenile animals of many species.[2] They are common in the dog and uncommon in other domestic species. In cats, they are uncommon and occur mainly in older cats showing no sex predisposition. Malignant melanomas can be composed of a variety of cell morphologies including spindle cells, epithelioid cells, a mixture of spindle cells and epithelioid cells, singet-ring cells or balloon cells. In addition they can be heavily pigmented or amelanotic.[3] The present metastatic mass in liver and spleen is considered as an epithelioid type and is pigmented. Metastasis occurs commonly with spread via lymphatics to regional lymph nodes and lungs. It is not uncommon for malignant melanomas to spread to other body sites, including unusual locations such as the brain, heart and spleen.[3]

Ocular melanoma in cats occurs in a variety of sites. The vast majority of feline ocular melanomas arise diffusely from the anterior uvea (iris, ciliary body).[5] Conjunctival melanomas in dogs and cats are in general rare.[3] One study reported in a comparison study of 19 feline ocular melanomas that 16 were intraocular, while only three were
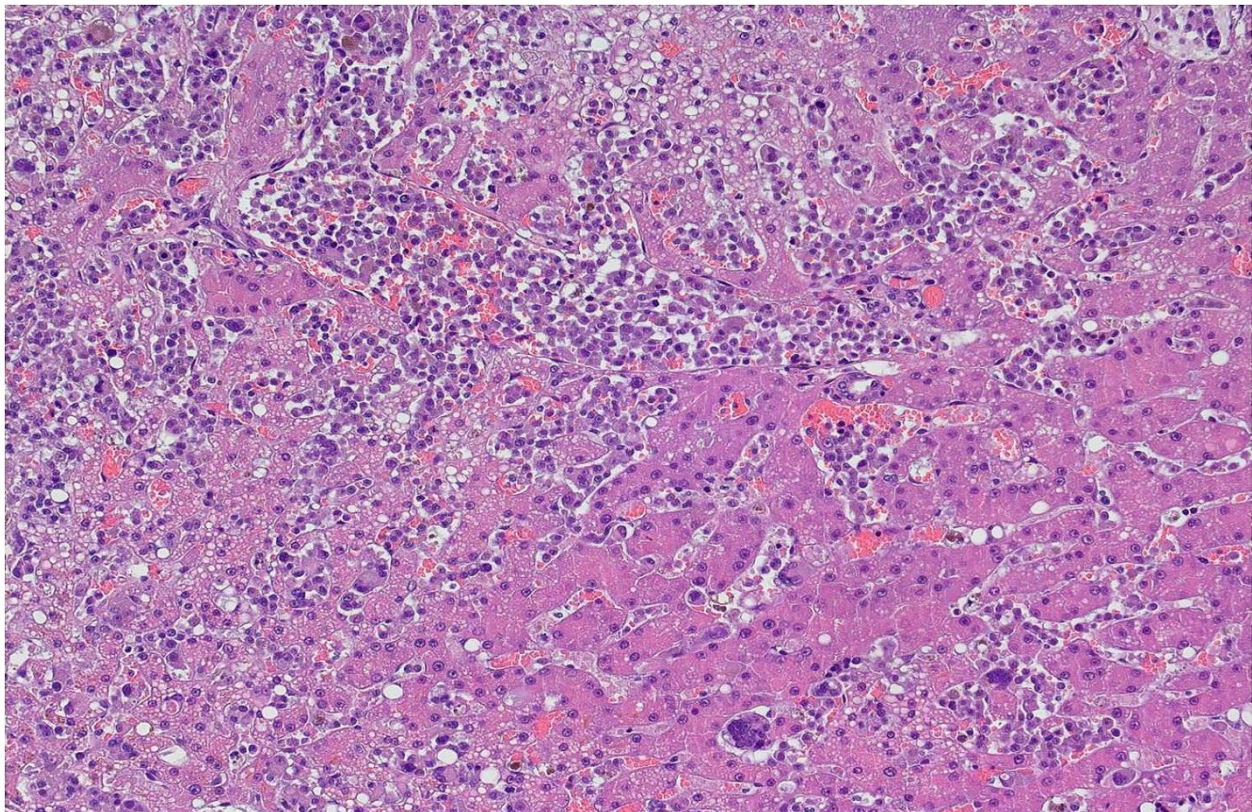
Liver, cat. Sinusoids and hepatic vessels are expanded by numerous neoplastic cells, some multinucleated. Hepatocytes often contain numerous discrete lipid vacuoles. (HE, 200X) (Photo courtesy of: University of Bern, Vetsuisse faculty, Institute for Animal Pathology (ITPA), Laenggassstrasse 122, CH – 3012, Switzerland, http://www.itpa.vetsuisse.unibe.ch/)
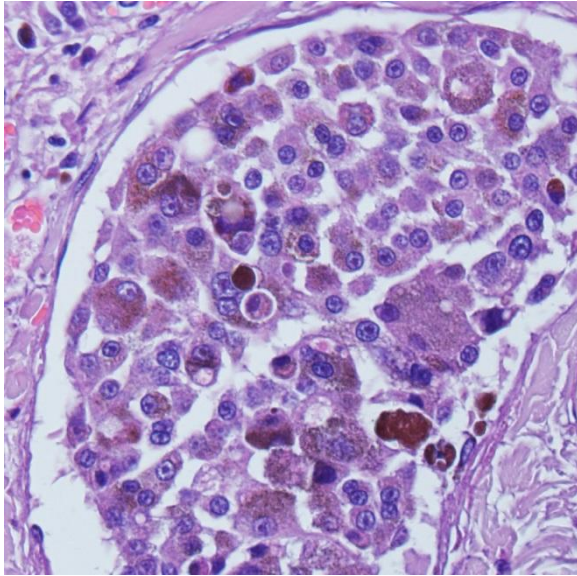
Liver, cat. Neoplastic cells contain abundant intracytoplasmic melanin pigment. (HE, 400X)

conjunctival.[4] In another study by Schobert et al. (2010), in 13 cases of conjunctival melanoma in cats with adequate follow-up information, only three reported metastasis (14%). Of those three cases, two had metastasized to the submandibular lymph nodes, while in the third case, an abdominal mass was detected.[5] Interesting about our case is that two abdominal organs (liver, spleen) were severely infiltrated and that the neoplastic cells were present in the lymphatics and blood vessels diffusely within the spleen and liver.

**JPC Diagnosis:** Liver: Metastatic melanoma.

Conference Comment:

The most common location for feline conjunctival melanoma is on the bulbar conjunctiva with extension into the orbital tissues, and abundant pigmentation is seen in the majority of tumors. The presence of multinucleated tumor cells is common and may explain the presence of multinucleated

neoplastic cells in the metastatic disease in the liver in this case. Conjunctival melanoma in cats has a poorer long term prognosis than the same neoplasm in dogs.[5] In general, melanomas are less common in cats than dogs but the eye is the most common site, and melanoma is the most common intraocular neoplasm in cats. Non-ocular melanomas occur in cats as well but are considered rare and metastasis was present in 30% of cases in one study.[1] In general non-ocular melanoma is a disease of older cats, with the exception of auricular melanoma which occurs in a significantly younger population.[1] Non-ocular me-lanomas often occur on the head of cats and have a similar distribution as squamous cell carcinoma. Amelanotic melanomas in cats carry a poorer prognosis and are often negative for Melan A but most are positive for S100.[1]

This was a challenging case for conference participants with the majority diagnosing this lesion as histiocytic sarcoma due in large part to the presence of multinucleated cells. Cholestasis, as seen in this case, is also a common feature of hepatic histiocytic
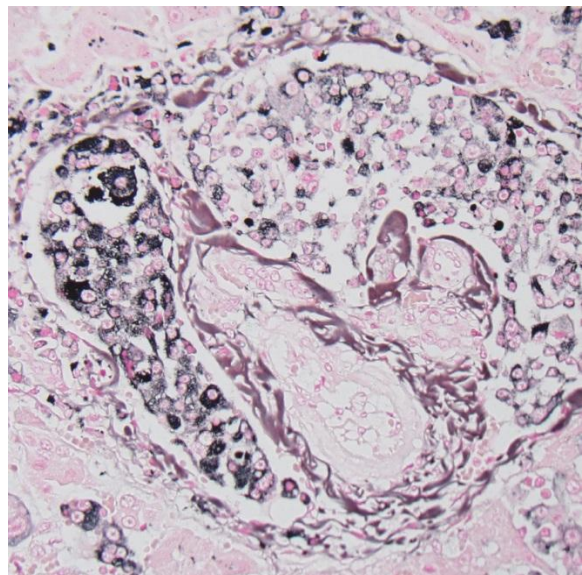
Liver, cat. Neoplastic melanocytes exhibit strong positivity with a Fontana-Masson's stain. (Fontana Masson, 240X)

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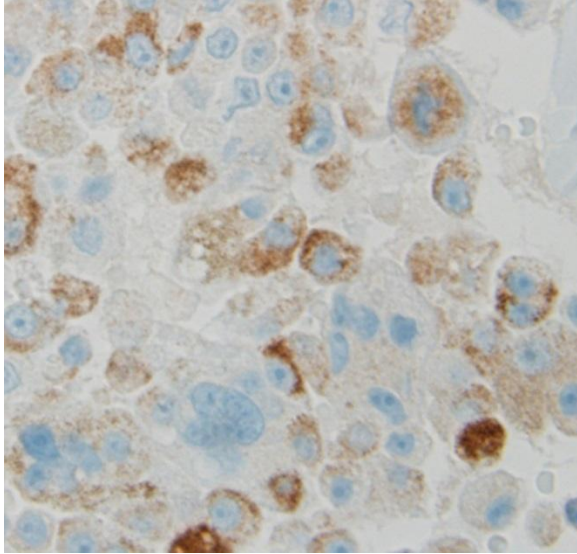
Liver, cat. Neoplastic melanocytes exhibit diffuse strong reactivity against Melan A. (anti-Melan, 360X).

sarcoma. The moderator commented this is an unusual case and he had not previously seen a case like this one. Additional features described and discussed included vascular thrombosis and, interestingly, rare mitoses were noted in hepatocytes in the absence of regenerative nodules. The moderator pointed out the collar of connective tissue surrounding the central vein, which is a normal feature in the feline liver that distinguishes it from other species. The areas of hepatocyte necrosis and loss surrounding the central vein are presumed secondary to hypoxia from obstruction of sinusoids by tumor emboli. The excellent gross image and the laboratory data provided by the contributor greatly enhanced the learning / teaching value of this case.

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References:

1. Chamel G, Abadie J, Albaric O, Labrut S. Non-ocular melanomas in cats: a retrospective study of 30 cases. *J Feline Med Surg*. 2016; Jan 14(E pub):1-7.

2. Ginn PE, Mansell JEKL, Rakich PM. Skin and appendages. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 5th ed. Vol 1. Philadelphia, PA: Elsevier Saunders; 2007:760.

3. Meuten DJ. *Tumors in Domestic Animals*. 4th ed. Iowa State Press; 2002.

4. Patnaik AK, Mooney S. Feline melanoma: a comparative study of ocular, oral, and dermal neoplasms. *Vet Pathol.* 1988; 25: 105-112.

5. Schobert CS, Labelle P, Dubielzig RR. Feline conjunctival melanoma: histo-pathological characteristics and clinical outcomes. *Veterinary Ophthalmology.* 2010;

CASE IV: 12-0011 (JPC 4019862).

Signalment: 12-year-old, female spayed, domestic shorthair cat, feline *(Felis domesticus).*

History: The cat was presented to an animal shelter with a history of vomiting and anorexia. Upon physical examination, she was thin with pale mucous membranes. The abdomen was distended, and hepatomegaly was palpated. The cat underwent exploratory laparotomy, and representative samples of liver, as well as the lesions in the greater omentum and parietal peritoneum were collected for histopathologic evaluation. However, the cat was euthanized due to poor prognosis.

Liver, cat. An infiltrative, partially necrotic neoplasm replaces 50% of the section (HE, 5X)

Gross Pathology: The abdominal cavity contained blood, and clotted blood and fibrin tags covered the surface of the liver. Hepatic architecture was also extensively effaced and expanded by multiple, coalescing, umbilicated, firm, pale tan nodules (5-20mm diameter) that extended across the surface and throughout the parenchyma. Firm, pale tan, coalescing nodular lesions (3-10mm diameter) also extended to involve the greater omentum and parietal peritoneum.

Laboratory Results: Serum biochemistry abnormalities: Total protein 8.9 g/dL (5.8-8.1); Albumin 1.8 g/dL (2.6-4.0); Alanine aminotransferase 240 U/l (15-52); Aspartate aminotransferase 112 U/l (14-42); Bilirubin 4.5 mg/dL (0-0.6).

Hematologic abnormalities: Hematocrit 18% (24-45); Hemoglobin 6.5 g/dL (8.0-15.0); Red blood cell count 4.37 M/μL (5.0-10.0).

Histopathologic Description: Liver: The hepatic parenchyma is extensively necrotic and effaced by focal to coalescing proliferations of neoplastic cells that differentiate toward biliary epithelium and form acini, tubules, and packets. Cells are low cuboidal to columnar with indiscrete borders, and pale amphophilic, vacuolated cytoplasm. Nuclei are large, round to oval, and contain one or two prominent nucleoli. There are 2-4 mitotic figures per ten 40x objective fields. Neoplastic ducts and acini contain luminal neutrophils, proteinaceous fluid, and cell debris, and are embedded within an extensive background collagenous stroma (scirrhous response). Neoplastic cells are present in lymphatics, and along the capsular surface in some regions (not present in all sections). Blood vessels and sinusoids contain fibrin thrombi. Scattered neutrophils are present throughout the necrotic tissue. Mesothelial cell proliferation is also seen along the capsular surface.

Within the adjacent remaining parenchyma, portal regions are expanded by moderate proliferations of lymphocytes & plasma cells, with bile duct proliferation, and periductular fibrosis. Some bile ducts are lined by attenuated epithelium and contain luminal or periductal neutrophils.
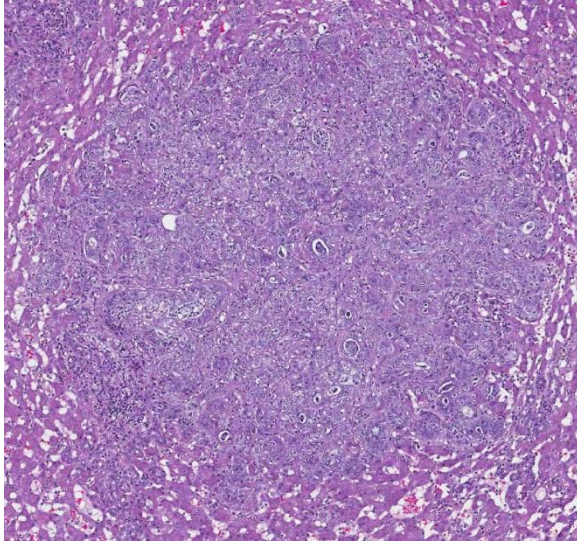
Liver, cat. Well-demarcated, often coalescing neoplastic nodules are distributed throughout the section. (HE, 70X)

Contributor's Morphologic Diagnosis: Liver: Cholangiocellular carcinoma with lymphatic invasion; Chronic fibrosing, lymphoplasmacytic portal hepatitis, domestic shorthaired cat, feline.

Contributor's Comment: Cholangio-cellular carcinomas are malignant neoplasms that can occur in all species. They arise from bile duct epithelium, predominantly from intrahepatic ducts, although extrahepatic ducts can be involved also. These neoplasms can present either in the form of a mass or as coalescing, umbilicated, nodular proliferations on the surface of the liver and throughout the parenchyma.[7,11]

Although primary hepatobiliary tumors are rare in cats, cholangiocellular carcinoma represents the most common non-hematopoietic, hepatic malignancy in this species.[1] This is a locally aggressive neoplasm with a high metastatic potential, and metastases are documented in up to 80% of necropsy cases.[8] Extrahepatic metastasis is common, especially to cranial abdominal lymph nodes and lung, and seeding of tumor into the abdominal cavity is not uncommon, with lesions extending throughout the
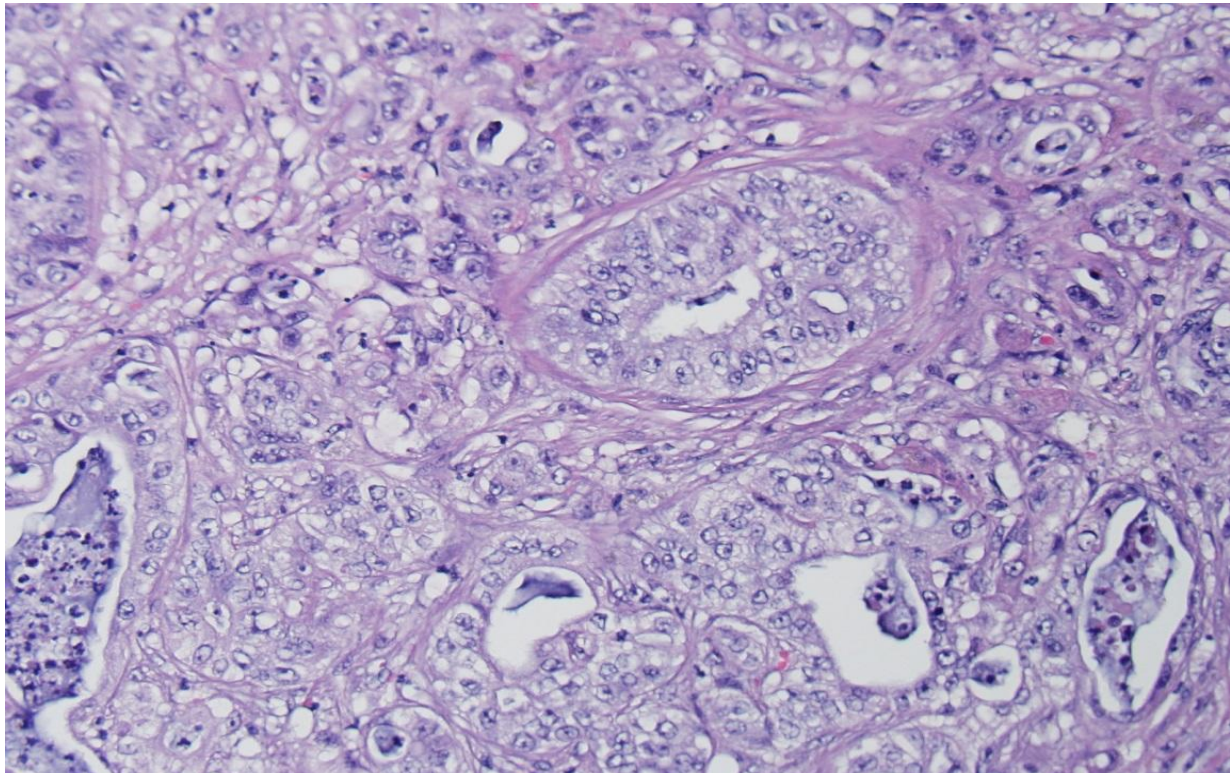
Liver, cat. The neoplasm is composed of epithelial cells forming cell differentiated tubules and acini. (HE, 200X)

mesentery and visceral peritoneal surfaces.[11]

In cats, cholangiocellular carcinomas are typically found in animals of 9 years of age and older.[1] There are no breed predilections, and although there are some suggestions that this tumor is more commonly diagnosed in male cats,[1] other studies do not demonstrate such a gender difference.[6] The prognosis is poor, with a life expectancy of less than 6 months.[6]
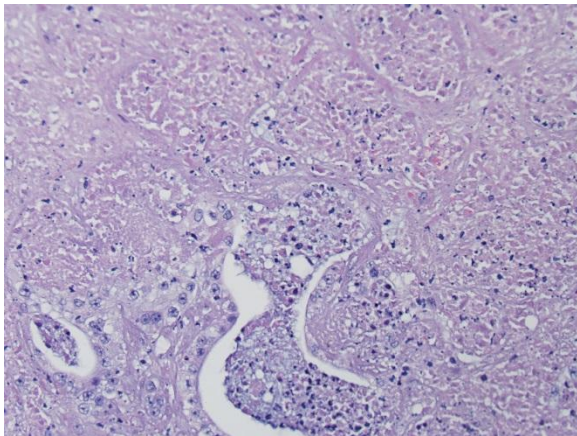
Liver, cat. Large areas of the neoplasm are necrotic. (HE, 200X)

As compared with cats with benign tumors of the biliary tract, cats with cholangiocellular carcinoma are more likely to exhibit clinical signs, and lethargy, anorexia, and vomiting are most commonly reported.[1] Upon physical examination, the most common finding is the presence of a cranial abdominal mass or hepatomegaly, while ascites and icterus are less common.[1]

Hematologic and biochemical profiles are often nonspecific.[1] Leukocytosis is sometimes reported, and alanine aminotransferase, aspartate aminotransferase, and total bilirubin levels may be elevated.[6]

Microscopically, these tumors are usually distinctly adenocarcinomatous, comprising proliferations of cells that differentiate toward biliary epithelial cells, and form acini, tubules, and papillary projections.

More poorly differentiated forms may be composed of solid epithelial proliferations, with or without the formation of islands, cords, or packets of cells, and foci of squamous differentiation. Numerous mitotic figures are present. A variable connective tissue stroma is present, often with marked collagen deposition, the so-called scirrhous response. Areas of necrosis are also common.[7,11]
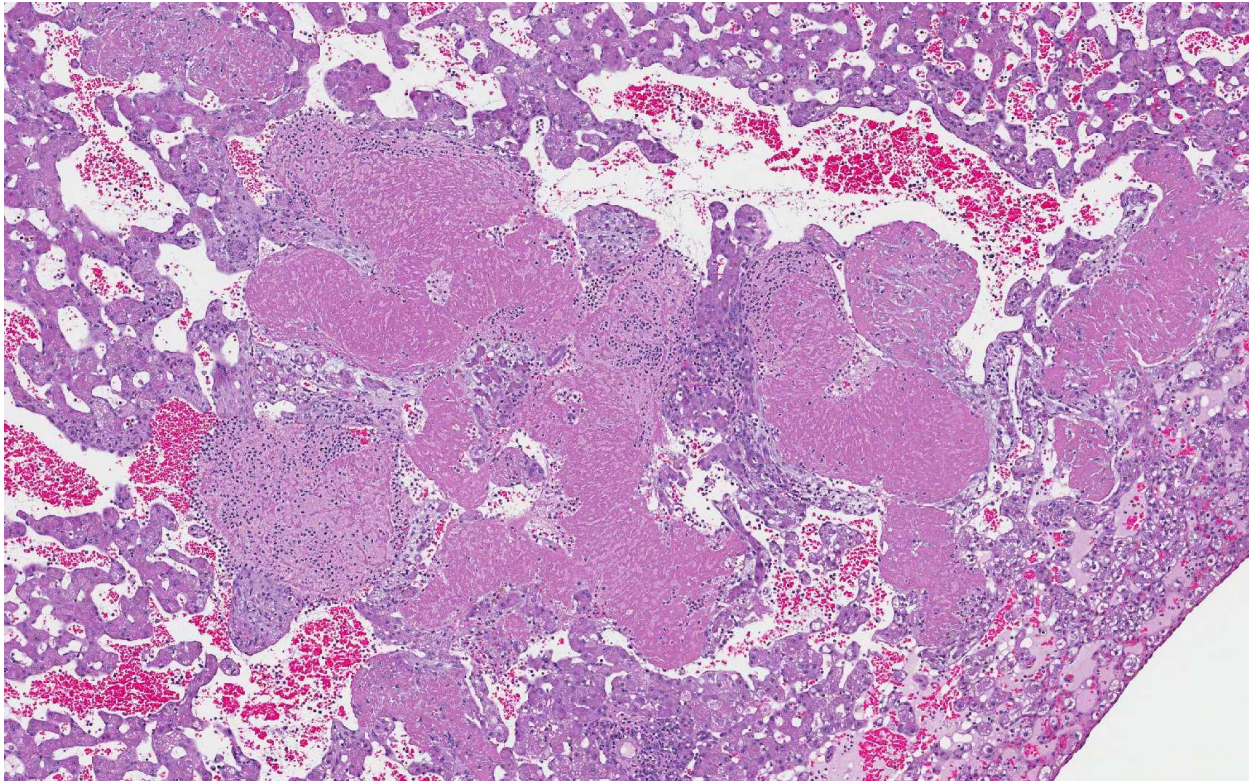
Paraneoplastic alopecia has also been associated with cholangiocellular carcinoma, as well as pancreatic adenocarcinoma in cats. This presents as bilaterally symmetrical alopecia of the ventrum and limbs, sometimes with a shiny appearance to the alopecic skin. Histologically, skin changes comprise follicular and adnexal atrophy with hypoplasia of the stratum corneum.[9]

In people, cholangiocellular carcinoma is the second most common hepatic malignancy after hepatocellular carcinoma, accounting for more than 7% of cancer deaths throughout the world. In the United States, it accounts for 3% of all cancer deaths, and its prevalence is highest in Hispanics. Although most cholangiocellular carcinomas in the western world arise without evidence of antecedent disease, chronic hepatobiliary inflammatory conditions can predispose patients to development of these tumors. The incidence rates of this malignancy are highest in Southeast Asia, where a major risk factor is chronic infection of the biliary tract with the liver fluke *Opisthorchis sinensis*. Additional predisposing risk factors for development of cholangiocellular carcinoma in people, include: primary sclerosing cholangitis; infection with hepatitis B or C; and congenital diseases of the biliary tract, such as choledochal cysts or Caroli's syndrome.[4,5] However, no definitive association between inflammatory hepatobiliary disease, or other antecedent conditions, has been established in cats.

JPC Diagnosis: 1. Liver: Cholangiocellular carcinoma.

2. Liver: Cholangiohepatitis, suppurative, multifocal, severe, with septic thrombi and telangiectasis.

Liver cat: Large sinusoidal fibrin thrombi are present in one edge of the section; adjacent sinusoids are massively dilated due to altered blood flow. (HE, 70X)

Conference Comment: As mentioned above, in certain regions of Southeast Asia there is a link between cholangiocellular carcinoma in people and infection with liver flukes, including *Opisthorchis viverrini*. The fluke is classified as a group 1 carcinogen, and the infective stage can be transmitted by consumption of undercooked fish. Once in the digestive tract of the definitive host, the parasites migrate to the bile ducts where they feed on biliary epithelium. The carcinogenicity of fluke infection is driven by multiple factors, including mechanical damage to biliary epithelium, inflammatory processes driven by cytokines such as IL-6, and parasite excretory products found within host cholangiocytes which have the ability to drive cell proliferation. The fluke *O. viverrini* is capable of secreting extracellular vesicles, which are small membrane bound structures released from different types of helminths. The vesicles are taken up by cholangiocytes resulting in both inflammatory and protumorigenic changes providing a mechanism by which the parasite is able to drive tumorigenesis. Vesicle uptake is documented to result in dysregulation of protein expression involved in wound healing, tumor cell invasion and the proteasome complex.[2] Liver fluke carcinogenesis has not been documented in domestic food animals, such as sheep and cattle; this may be due to interspecies differences in immunomodulatory mechanisms or uptake of procarcinogenic secretory products, or perhaps because of differences in lifespan. Infection with the biliary fluke

Platynosomum fastosum in cats, which results in varying degrees of inflammation, fibrosis and hyperplasia within the biliary tract, has been found concurrently with cholangiocarcinoma in some animals.[3]

In this slide, the lobular hepatic architecture is approximately 75% effaced. Concentric fibrosis surrounding bile ducts is a prominent feature, and the moderator commented this feature is secondary to cholestasis, and neutrophilic cholangitis is, in turn, the result of biliary stasis.

Fibrin thrombi within sinusoids and blood vessels contain enmeshed epithelioid macrophages and other inflammatory cells. In this slide, large areas of sinusoidal dilation border fibrin thrombi. These areas of dilation may represent local blood flow change or, in the normal liver, would often be interpreted as foci of telangiectasis, a common histologic finding in the liver of the cat.

Additionally, two important diagnostic features in this case include the scirrhous reaction and mucous within ducts / tubules, which are not seen in hepatocellular carcinoma. In domestic animal species, metastatic neoplasia in the liver is more common than primary neoplasia.[10]

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References:

1. Balkman C. Hepatobiliary neoplasia in dogs and cats. *Vet Clin North Am Small Anim Pract.* 2009; 39:617-25.

2. Chaiyadet S, Sotillo J, Smout M, Cantacessi C, et al. Carcinogenic liver fluke secretes extracellular vesicles that promote cholangiocytes to adopt a tumorigenic phenotype. *J Infect Dis.* 2015; 212(10):1636-45.

3. Cullen JM, Stalker MJ. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. Vol 2. St. Louis, MO: Elsevier; 2016:322.

4. Friman S. Cholangiocarcinoma – current treatment options. *Scand J Surg.* 2011; 100:30-34.

5. Kumar V, Abbas AK, Fausto N, Aster JC. *Robbins and Cotran, Pathologic Basis of Disease.* 8th ed. Elsevier; 2010:880-881.

6. Lawrence HJ, Erb HN, Harvey HJ. Nonlymphomatous hepatobiliary masses in cats: 41 cases (1972 to 1991). *Vet Surg.* 1994; 23:365–368.

7. Maxie MG. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. 5th ed. Vol 2. Philadelphia, PA: Elsevier; 2007:385-386.

8. Patnaik AK. A morphological and immunocytochemical study of hepatic neoplasms in cats. *Vet Pathol.* 1992; 29:405–415.

9. Tasker S, Griffon DJ, Nuttall TJ, et al. Resolution of paraneoplastic alopecia following surgical removal of a pancreatic carcinoma in a cat. *J Small Anim Pract.* 1999; 40:16-19.

10. World Small Animal Veterinary Association. *Standards for the Clinical and Histologic Diagnosis of Canine and Feline Liver Diseases*. Philadelphia, PA: Saunders Elsevier; 2006.

11. Zachary JF, McGavin MD. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis; Mosby Elsevier; 2012:444.