The Armed Forces Institute of Pathology
Department of Veterinary Pathology

Conference Coordinator
Matthew Wegner, DVM

WEDNESDAY SLIDE CONFERENCE 2010-2011

Conference 11

17 November 2010

Conference Moderator:
Terrell Blanchard, DVM, Diplomate ACVP

CASE I: SP-09-2326 (AFIP 3162243).

Signalment: 12-year-old, spayed female, miniature schnauzer (*Canis familiaris*).

History: This dog had a recent history of inappetence and upon abdominal palpation a multinodular mass was noted in the cranial abdomen. Radiographs confirmed a multinodular mass affecting the liver. Abdominal surgery was performed, and biopsy samples were collected from the nodular masses present on the liver.

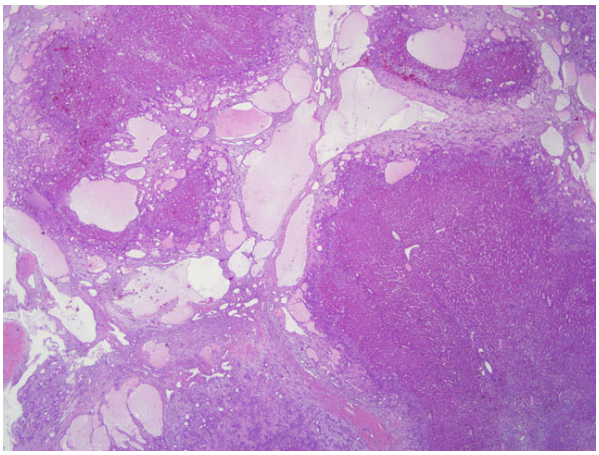
Laboratory Results: Moderate elevation in alkaline phosphatase levels were reported by the referring veterinarian.

Histopathologic Description: Slides contain representative sections of one of the liver masses. Sections consist of a poorly demarcated, non-encapsulated, pseudolobulated hepatocellular neoplasm. Multifocally, neoplastic hepatocytes are arranged in cords and trabeculae (2-6 cells thick) supported by a fine fibrovascular stroma. These regions transition into areas of architectural collapse, composed of small nests and clusters of anaplastic cells suspended within edematous fibrous stroma and separated by large, cystically dilated sinusoids and lymphatics. Neoplastic cells are polygonal and have a moderate amount of eosinophilic cytoplasm with distinct cell borders. Nuclei are round with euchromatic, stippled chromatin, and 1-2 prominent nucleoli. Mitoses range from 0 to 4 per high power field. There is marked anisocytosis and anisokaryosis, and occasional neoplastic hepatocytes contain large clear cytoplasmic vacuoles. Portal triads are absent.

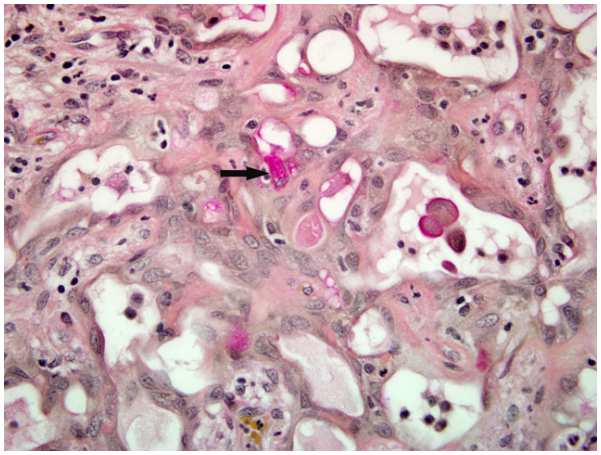
Immunohistochemistry was performed. Regions of well differentiated neoplastic hepatocytes have strong stippled cytoplasmic expression of Hepatocyte Paraffin 1 (Hep Par 1) with loss of expression of this antigen along the periphery of pseudolobules as neoplastic cells become anaplastic. Anaplastic cells have strong cytoplasmic expression of Cytokeratin 7.

Contributor's Morphologic Diagnosis: Liver: Multinodular combined hepatocellular cholangiocarcinoma.

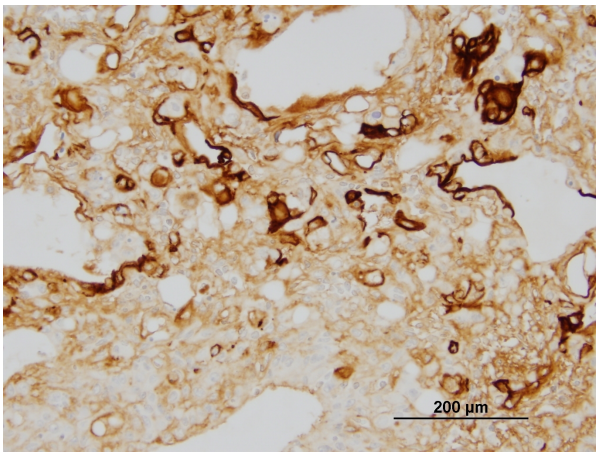
Contributor's Comment: The incidence of malignant hepatocellular neoplasms is less than 1 percent of all canine neoplasms, with fewer reported cases of biliary tumors.[3] The subset, hepatocellular cholangiocarcinoma (HCCC) or hepatocholangiocarcinoma, is extremely rare with only three cases reported in dogs[7] and one case reported in a horse.[4] Established classification systems of HCCCs describe 3 main histologic types: 1) Type I- occurrence of both histologically distinct hepatocellular carcinoma and cholangiocarcinoma that can present either as distinctly separate masses or as coalescing masses (collision tumor); 2) Type II- combined tumor with commingling and often transitional elements of both hepatocellular carcinoma and cholangiocarcinoma (transitional tumor), and 3)



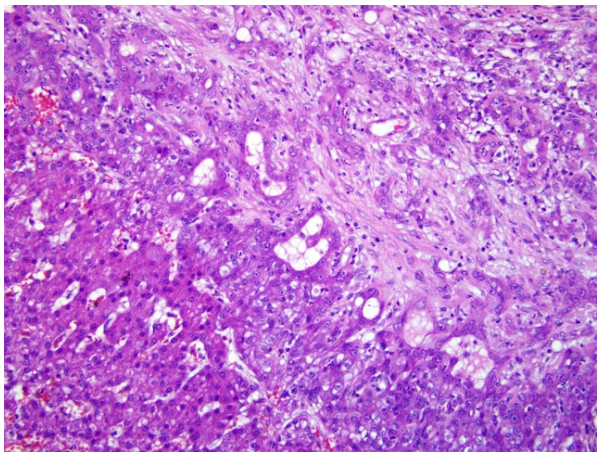
1-1. Liver, hepatocholangiocarcinoma, dog. The liver is infiltrated by a densely cellular, lobulated neoplasm composed of polygonal cells arranged in cords and trabeculae and containing variably-sized cystic structures filled with eosinophilic proteinaceous material. (HE 20X)



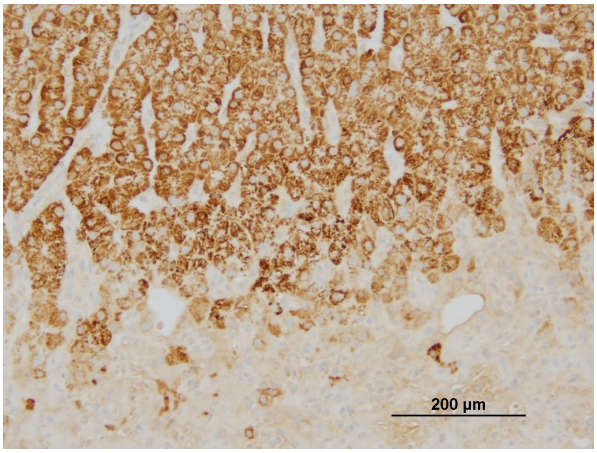
1-3 Liver, hepatocholangiocarcinoma, dog. Occasionally, the cytoplasm of neoplastic biliary epithelial cells contains carminophilic material (arrow). The lumens of ductular structures contain variable amounts of similar carminophilic material. (Mucicarmine 400X)



1-5. Liver, hepatocholangiocarcinoma, dog. Anaplastic cells of the biliary component demonstrate strong cytoplasmic immunopositivity for cytokeratin 7. (Cytokeratin 7). Photograph courtesy of Diagnostic Center for Population & Animal Health, 4125 Beaumont Road, Lansing, MI 48910, Fitzgerald@dcpah.msu.edu



1-2. Liver, hepatocholangiocarcinoma, dog. Cords of neoplastic hepatocytes with abundant eosinophilic cytoplasm transition with neoplastic biliary epithelial cells arranged in irregular ductular structures separated by variable amounts of reactive fibrous stroma. (HE 200X)



1-4. Liver, hepatocholangiocarcinoma, dog. The neoplastic hepatocellular component demonstrates strong cytoplasmic immunoreactivity for hepatocyte paraffin 1, with loss of expression of this antigen along the periphery of the pseudolobules as neoplastic cells become anaplastic. (Hep Par-1). Photograph courtesy of Diagnostic Center for Population & Animal Health, 4125 Beaumont Road, Lansing, MI 48910, Fitzgerald@dcpah.msu.edu

Type III-fibrolamellar variant, resembling fibrolamellar hepatocellular carcinomas but with pseudoglands containing mucin.[7] Based on the published criteria, this neoplasm would be classified as type II.

Hep Par-1 can be used to demonstrate hepatocellular origin, whereas Cytokeratin 7 can be used to demonstrate biliary origin.[6] Other immunohistochemical markers used to differentiate primary liver neoplasms, including hepatoblastomas, are α-fetoprotein (AFP) and carcinoembryonic antigen (CEA).[6,7]

Survival or behavioral data has not been established specifically for HCCCs. Metastasis of hepatocellular carcinomas occurs earliest and most frequently to the

hepatic lymph nodes and lungs.[3] However, one year after making the diagnosis we have no further information on this animal's survival or disease progression. Surgical resection of affected liver lobes can prolong the animal's life by approximately one year on average;[3] however, in this case, only portions of the neoplasm were removed, and not the entire affected lobe.

AFIP Diagnosis: Liver: Hepatocholangiocarcinoma.

Conference Comment: Based on evaluation of the hematoxylin & eosin stained sections, participants readily agreed on the presence of a biliary component to the specimen, but were divided as to the presence of a hepatocellular component. Slide variation likely contributed to this divergence in histologic interpretation, as some slides contain markedly more cystic sections with minimal parenchyma. The presence of a biliary component was confirmed by carminophilic staining of the neoplastic biliary epithelial cells with the mucicarmine histochemical stain at the AFIP. Further evidence supporting a hepatocellular cholangiocarcinoma (HCCC) is the histomorphologic appearance of gradual transition from the hepatocellular component to a biliary component, which is quite evident when comparing the immunohistochemical staining results for Hep Par-1 and cytokeratin 7 provided by the contributor.

Within the canals of Hering reside a population of bipotential progenitor cells referred to in the literature as oval cells. With proper stimulation, these cells can differentiate into hepatic cells or biliary epithelial cells. In response to chronic hepatitis, fulminant liver failure or severe, end-stage cirrhosis, oval cells begin proliferating. This is seen histologically as ductular reaction with reduplication of biliary epithelium and occurs prior to hepatocyte or cholangiocyte differentiation.[1] Several molecular pathways appear to play a role in oval cell regulation. The Wnt/β catenin and Sonic Hedgehog signaling pathways both regulate oval cell renewal; deregulation (i.e. increased signaling) allowing oval cell proliferation of these pathways is reported in hepatocellular carcinomas.[1,5] Conversely, Notch signaling results in decreased oval cell proliferation and increased apoptosis; this pathway is frequently down-regulated in HCC.[5]

There is unresolved debate regarding the histogenesis of hepatic neoplasia including HCC, cholangiocarcinoma (CC) and combined HCC-CC (i.e. HCCC). The two current, popular theories propose either de-differentation of malignant hepatocytes or cholangiocytes to a hepatic stem cell; or neoplastic transformation of oval cells resulting in neoplasia involving one or both cell types.[1,5,8,10] An interesting feature of hepatic tumorigenesis supporting the hepatic stem cell theory is the histologic observation of a ductular reaction prior to tumor development.[2] Likewise, Tang et al. induced hepatic stem cells to transform into liver cancer through IL-6 stimulation with concurrent TGF-β signaling inactivation.[8]

A thorough discussion of the stem cell theory of cancer is beyond the scope of this paper, and readers are invited to read the recent review paper by Trosko for an overview of the two theories of cancer histogenesis.[9] During the conference discussion the moderator did reference a recent symposium of the National Toxicology Program which discussed HCCC in B6C3F1mice.[2] The overall incidence of HCCC in mice, based on the NTP database, is less than 1% with a greater number of males affected than females; overall, metastasis is high at 84%.

We would like to thank the staff pathologists in the Department of Hepatic Pathology for reviewing this case.

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References:

1. Alison MR, Islam S, Lim S. Stem cells in liver regeneration, fibrosis and cancer: the good, the bad and the ugly. *J Pathol*. 2009;217:282-298.
2. Bach U, Hailey JR, Hill GD, et al. Proceedings of the 2009 National Toxicology Program Satellite Symposium. *Toxicol Pathol*. 2010;38(1):9-36.
3. Cullen JM, Popp JA. Tumors of the liver and gall bladder. In: Meuten DJ, ed. *Tumors in Domestic Animals*. 4th ed. Ames, IA: Wiley-Blackwell; 2002:486-492.
4. Kato M, Higuchi T, Orita Y, Ishikawa Y, Kadota K. Combined hepatocellular carcinoma and cholangiocarcinoma in a mare. *J Comp Pathol*. 1997;116(4):409-413.
5. Lee TKW, Castilho A, Ma S, Ng IOL. Liver cancer stem cells: implications for a new therapeutic target. *Liver Int*. 2009;DOI:10.1111/j.1478-3231.2009.02040.x: 955-965.
6. Ramos-Vara JA, Miller MA, Johnson GC. Immunohistochemical characterization of canine hyperplastic hepatic lesions and hepatocellular and biliary neoplasms with monoclonal antibody hepatocyte paraffin 1 and a monoclonal antibody to cytokeratin 7. *Vet Pathol*. 2001;38(6):636-643.
7. Shiga A, Shirota K, Enomoto M. Combined hepatocellular and cholangiocellular carcinoma in a dog. *J Vet Med Sci*. 2001;63(4):483-486.
8. Tang Y, Kitsin K, Jogunoori W, et al. Progenitor/ stem cells give rise to liver cancer due to aberrant TGF-β and IL-6 signaling. *Proc Natl Acad Sci U S A*. 2008;105(7):2445-2450.

9. Trosko JE. Cancer stem cells and cancer nonstem cells: From adult stem cells or from reprogramming of differentiated somatic cells. *Vet Pathol*. 2009;46(2): 176-193.
10. Yeh MM. Pathology of combined hepatocellular-cholangiocarcinoma. *J Gastroenterol Hepatol*. 2010;25:1485-1492.

CASE II: N10-41-1 (AFIP 3165181).

Signalment: 11.5-year-old, spayed female, Chihuahua dog (*Canis familiaris*).

History: This dog was presented to Tufts emergency clinic for acute onset of worsening dyspnea. Pulmonary computed tomography (CT) revealed diffuse patchy interstitial, alveolar and airway changes affecting all lung lobes and mild lobar bronchi traction bronchiectasis. An echocardiogram demonstrated moderate pulmonary hypertension. Due to poor prognosis, euthanasia was elected.

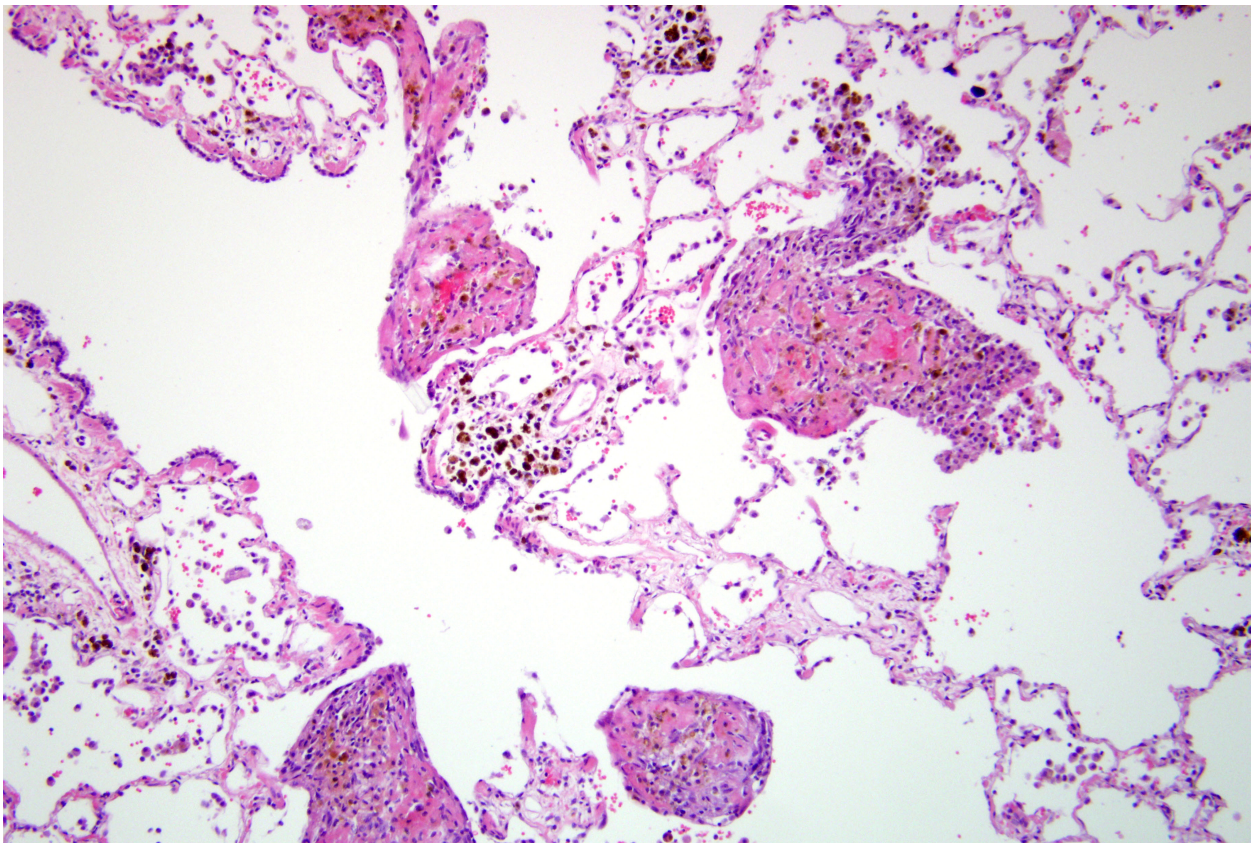
Gross Pathology: At necropsy, 80 % of all lung lobes are mottled dark red to dark brown, slightly firm and wet on cut surface.

Histopathologic Description: Most terminal bronchioles are obliterated by variably sized (25-200 µm diameter) aggregates of fibrin, collagen, fibroblasts, macrophages (often containing intracytoplasmic yellow-brown pigments, hemosiderophages) and few lymphocytes and plasma cells. Moderate numbers of macrophages/ hemosiderophages and erythrocytes are present within the alveoli. There is multifocal hyperplasia of type II pneumocytes. Alveolar septa are variably thickened by the previously described inflammatory cells and small amounts of fibrous tissue. Moderate amounts of fibrous tissue expand the subpleural space segmentally.

Contributor's Morphologic Diagnosis: Lung: Chronic, severe, intra-bronchiolar and intra-alveolar organizing fibrosis with chronic hemorrhage (bronchiolitis obliterans with organizing pneumonia)

Contributor's Comments: The histopathologic changes in the lungs are consistent with bronchiolitis obliterans with organizing pneumonia (BOOP), a well-documented entity in humans.[1,7] BOOP, synonymous with cryptogenic organizing pneumonia (COP), is a distinct clinicopathological entity with clinical, imaging and prognostic features different from those of

2-1. Lung, dog. Multifocally, terminal bronchioles are partially to completely filled with variably sized aggregates of immature collagen, reactive fibroblasts, and hemosiderin-laden macrophages. (HE 100X)



obliterative bronchiolitis and usual interstitial pneumonia/idiopathic pulmonary fibrosis (UIP/IPF). The disease is an inflammatory reaction to lung injury and has been associated with a wide variety of causes, including infections, drugs, inhalants, collagen-vascular disorders, and graft-versus-host disease in heart and bone marrow transplants. A substantial percentage of cases are idiopathic. Clinically, the patients are presented with acute to subacute (weeks to a few months) onset of dyspnea, coughing, malaise, and fever (flulike symptoms). Thoracic radiographs reveal patchy bilateral airspace opacification that may be difficult to distinguish from other conditions, such as usual interstitial pneumonitis and small airway disease. Lung biopsy is the preferred method for the definitive diagnosis of this entity. In humans, the prognosis is excellent, with complete recovery occurring within weeks or months. The lesions usually respond to treatment with corticosteroids.[1,7]

Histologically, BOOP is characterized by patchy fibrosis filling the lumens of terminal and respiratory bronchioles and extending in a continuous fashion into alveolar ducts and alveoli. Typically, fibrosis is characterized by plugs of young fibroblasts embedded in a myxoid matrix and admixed with scant mononuclear inflammatory cells that adopt a polypoid appearance (granulation tissue) within the lumen of the airspaces ("Masson bodies"). Other histological features include chronic inflammation in the walls of the surrounding alveoli with reactive type II cells, increased foamy macrophages in the alveoli, and preservation of lung architecture. The fibrotic process is confined to the lumen of the airspaces and does not involve the interstitium. A unique feature of the BOOP is its temporal uniformity (i.e. all lesions seem to be in the same stage at any given time). Ultrastructurally, the cellular proliferation is composed of fibroblasts and myofibroblasts. BOOP is differentiated from organizing pneumonia, which is defined by the presence of granulation tissue in the distal air spaces, while obliterative bronchiolitis (or constrictive bronchiolitis obliterans) is characterized by narrowing of the bronchiolar lumens by concentric fibrosis and inflammation. The histologic changes in BOOP may be similar to those of diffuse alveolar damage, except that they are localized to the peribronchiolar parenchyma.[1,7]

This disease entity has been rarely reported clinically in dogs,[6] but has been produced experimentally by infecting dogs with adenovirus[1] or accidental intra-airway exposure with pure oleic acid.[5] In the present case, there is no evidence of infectious agents within the examined tissues and the etiology of the severe pulmonary changes is uncertain. Though BOOP has not been well described in animals, especially its response to corticosteroids, lung biopsy would be helpful to establish a definitive diagnosis.

AFIP Diagnosis: Lung: Bronchiolitis obliterans, multifocal, moderate, chronic with hemosiderosis.

Conference Comment: The contributor provides an excellent overview of bronchiolitis obliterans organizing pneumonia (BOOP). Conference participants briefly discussed ascribing a human disease name to a similar condition in animals. The moderator commented that, while in some instances it is appropriate, there may be instances in which the disease process differs from that of humans; therefore use of human disease nomenclature may not be warranted. This case was reviewed by the AFIP Department of Pulmonary and Mediastinal Pathology. They offered a diagnosis of BOOP with hemosiderin most likely resulting from an infection leading to hemorrhage followed by resolution. They further speculated that the acute process occurred 3-6 weeks prior to this phase of the lesion.

Although the cause of pulmonary fibrosis is not completely understood, recent advancements indicate that an exuberant fibroblastic or myofibroblastic proliferation has a significant impact on its development. As in any wound, fibrinous exudates that are not rapidly removed are replaced by fibrosis.[2] It is currently thought that injury to type I alveolar epithelial cells results in release of transforming growth factor-β1 (TGF-β1). TGF-β1is fibrogenic and stimulates the transformation of fibroblasts into myofibroblasts with the subsequent deposition of collagen and the development of fibrosis. TGF-β1 accomplishes this by negatively regulating telomerase activity and inhibiting caveolin-1. By negatively affecting telomerase activity, epithelial cell apoptosis is facilitated which leads to cell death and repair mechanism activation. By inhibiting caveolin-1, which is an inhibitor of pulmonary fibrosis, TGF-β1 increases pro-fibrogenic mechanisms leading to the development of exuberant pulmonary fibrosis.[4]

We would like to thank Dr. Russell Harley of the Department of Pulmonary and Mediastinal Pathology for reviewing this case.

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References:

1. Castleman WL. Bronchiolitis obliterans and pneumonia induced in young dogs by experimental

adenovirus infection. *Am J Pathol*. 1985;119(3): 495-504.
2. Caswell JL, Williams KJ. Respiratory system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol. 2, 5th ed. Philadelphia, PA: Elsevier Ltd; 2007:559-560.
3. Epler GR. Bronchiolitis Obliterans organizing pneumonia. *Arch Intern Med*. 2001;161(2):158-164.
4. Husain AN. The lung. In: Kumar V, Abbas AK, Fausto N, Aster JC, eds. *Robbins and Cotran Pathologic Basis of Disease*. 8th ed. Philadelphia, PA: Elsevier Saunders; 2009:694-696.
5. Li X, Botts S, Morton D, Knickerbocker MJ, Adler R. Oleic acid-associated bronchiolitis obliterans-organizing pneumonia in beagle dogs. *Vet Pathol*. 2006;43(2):183-185.
6. Phillips S, Barr S, Dykes N, et al. Bronchiolitis obliterans with organizing pneumonia in a dog. *J Vet Intern Med*.2000;14(2):204-207.
7. Weidner N, Cote R, Suster S, Weiss L, eds. *Modern Surgical Pathology.* 2nd ed. Philadelphia, PA: WB Saunders; 2002:409-411, 421.

CASE III: B10-13908 (AFIP 3167237).

Signalment: 12-year-old, male castrated, Bengal cat (hybrid of *Prionailurus bengalensis* and *Felis catus*).

History: This cat had a history of several cutaneous masses on the right flank present for a few weeks and rapid growth rate. The masses were surgically excised and initially diagnosed as a possible variant of eosinophilic granuloma complex. The lesions recurred at the same site approximately 3.5 months later, and were surgically excised and submitted for a second biopsy.

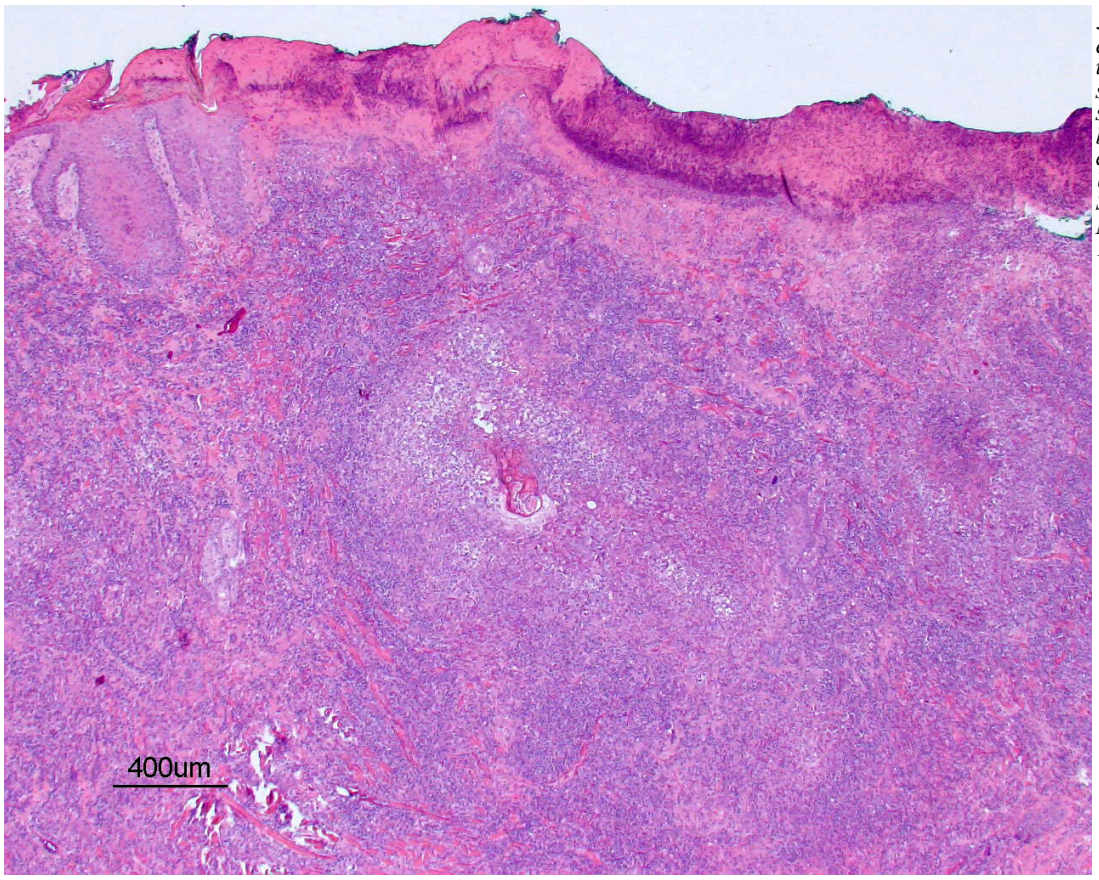
Gross Pathology: The lesion was described by the referring veterinarian as a raised, erythematous, ulcerated mass measuring 2 x 2 x 1 cm.

Histopathologic Description: Haired skin, right flank: The epidermis is extensively ulcerated and covered by thick serocellular crusts consisting of fibrin, cell debris, and numerous necrotic granulocytes. The remaining epidermis is eroded in some regions, with multifocal spongiosis and acanthosis. The necrosis, spongiosis, and acanthosis extend to the adjacent hair follicles with multifocal loss and destruction of adnexal units. The superficial and deep dermis is infiltrated by numerous eosinophils with lesser numbers of macrophages, plasma cells, lymphocytes, neutrophils, and mast cells, as well as variable fibroplasia and fibrosis. Within the dermis, there are multiple foci of necrosis often centered around remaining islands of adnexal epithelial cells. In these regions, there is a similar dense inflammatory cell infiltrate consisting predominantly of eosinophils and macrophages with abundant cell debris and fibrin. Keratinocytes in these regions often have pale eosinophilic cytoplasm, and occasionally contain intranuclear inclusion bodies. The intranuclear inclusions are glassy and amphophilic with marginated chromatin measuring approximately 10-12 µm. In other areas, the intranuclear inclusions are eosinophilic surrounded by a clear halo with peripheralized chromatin measuring 4-7 µm in diameter. In some of the sections, macrophages and eosinophils surround blood vessels with occasional destruction of the vessel wall and adjacent cell debris and fibrin. Immunohistochemistry revealed multifocal predominantly cytoplasmic staining with feline herpesvirus 1 antibodies both in the epidermis and adnexal epithelium adjacent to areas of necrosis.

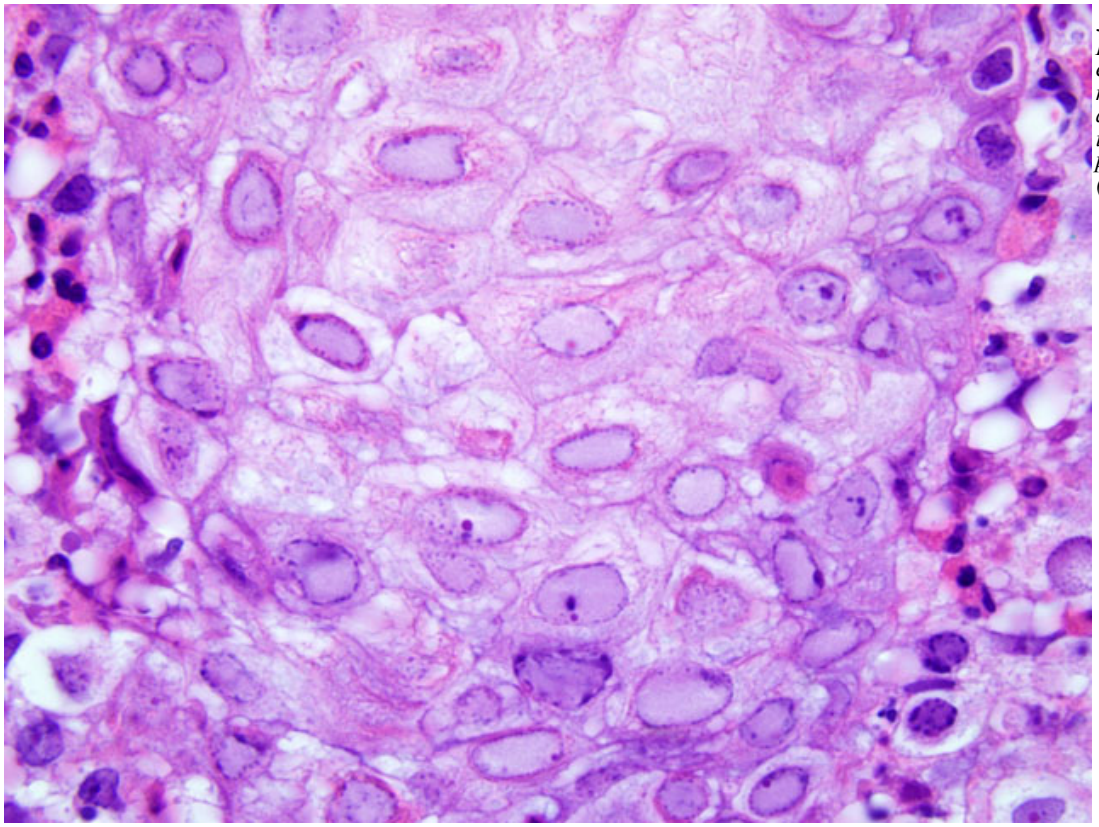
Contributor's Morphologic Diagnosis: Haired skin (right flank): Severe ulcerative necrotizing eosinophilic and histiocytic dermatitis with intranuclear inclusion bodies (consistent with feline herpesvirus 1).

Contributor's Comment: Feline herpesvirus 1 (FeHV-1), an alphaherpesvirus, is a double-stranded DNA enveloped virus causing primarily upper respiratory disease and conjunctivitis in cats.[1] FeHV-1 infection has also been associated with pneumonia, keratitis, ulcerative dermatitis and ulcerative stomatitis. Similar to other alphaherpesviruses, FeHV-1 causes necrosis of epithelial cells and establishes a latent infection in the trigeminal ganglion, optic nerve, olfactory bulb and cornea.[1] Clinical signs are most common in kittens, but may occur in adults following recrudescence of latent infections due to stress, corticosteroid therapy, lactation or change in housing.[1] Systemic disease is uncommon; however, it may occur in young or debilitated animals. Classic respiratory lesions of FeHV-1 include necroulcerative fibrinosuppurative rhinitis and bronchointerstitial pneumonia, often with intranuclear inclusion bodies.

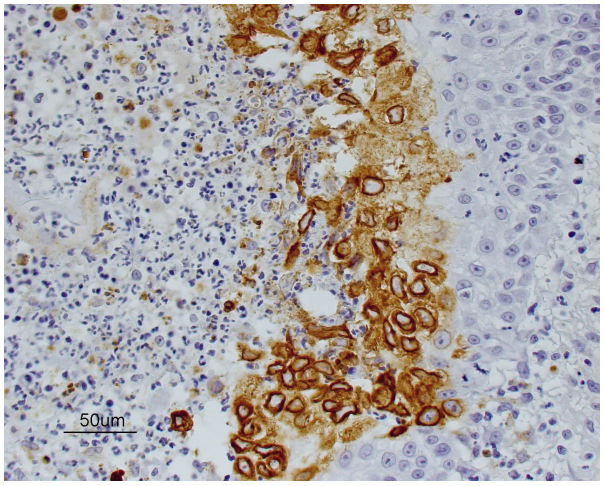
Ulcerative facial and nasal dermatitis with eosinophilic infiltrates associated with FeHV-1 has been reported in cats.[4] Lesions of FeHV-1 dermatitis are predominantly seen in the face and rarely on distal extremities.[3,4] Cats with herpesviral associated dermatitis may have a history of previous or concurrent respiratory disease. Preceding glucocorticoid therapy and environmental stress were suspected triggers of disease in a report.[4] Grossly, the lesions are characterized by erosion and ulceration of the face, with the dorsal and lateral muzzle, nasal planum and periorbital regions most commonly affected.[3,4] Hallmarks of facial herpesvirus dermatitis in cats include intense eosinophilic dermatitis with ulceration and epithelial cell necrosis. Intranuclear inclusion bodies are often present within keratinocytes, both in the epidermis and hair follicles,



3-1. Haired skin, cat. The epidermis is extensively ulcerated and covered by a serocellular crust. The subjacent dermis is expanded by a numerous inflammatory cells. Photograph courtesy of University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA 19104, kolsky@vet.upenn.edu



3-2. Haired skin, cat. Keratinocytes contain pale eosinophilic cytoplasm and nuclei with glassy, amphophilic intranuclear inclusion bodies which peripheralize the chromatin. (HE 1000X)



3-3. Haired skin, cat. Keratinocytes demonstrate cytoplasmic immunoreactivity for feline herpesvirus 1 antibodies. Photograph courtesy of University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA 19104, kolsky@vet.upenn.edu

as well as in sebaceous glands.[3,4] However, inclusion bodies may not be present in all cases or may be rare. Similar lesions associated with FeHV-1 have been described in cheetahs, characterized by dense infiltrates of eosinophils and plasma cells, and pseudoepitheliomatous hyperplasia.[6] Lesions in cheetahs were present predominantly on the face, although lesions also occurred at the top of the head, distal forelegs, flank, tail, and footpads.[6]

Grossly, the differential diagnosis for FeHV-1 facial ulcerative dermatitis includes mosquito bite hypersensitivity, food allergy, pemphigus foliaceus, and neoplasia.[3] Cases of herpesvirus dermatitis may be misdiagnosed histologically as a variant of the eosinophilic granuloma complex (EGC) or allergic dermatitis. Immunohistochemistry and PCR can serve as useful diagnostic tools to differentiate herpesvirus associated dermatitis from other causes of eosinophilic dermatitis.[4,5] Proper diagnosis is important regarding treatment, since glucocorticoid therapy that may be used to treat other eosinophilic skin disorders may cause worsening of skin lesions caused by FeHV-1. Vaccination does not seem to prevent this cutaneous manifestation of herpesvirus infection, either in cats or cheetahs.[4,6] It is imperative to thoroughly search for intranuclear inclusion bodies within the epithelium in feline skin samples showing intense eosinophilic inflammation, especially if accompanied by ulcers and hair follicle involvement/necrosis.

The presence of this lesion in the right flank of this cat is highly unusual. Direct contact of the face and salivary gland secretions during grooming may be the cause of the lesion at this unusual location, similar to what has been previously postulated for lesions in the distal extremities.[3,6] Therefore, FeHV-1 associated dermatitis should not be ruled out based on the location of the lesion.

AFIP Diagnosis: Haired skin: Dermatitis and folliculitis, necrotizing, eosinophilic and lymphohistiocytic, multifocal to coalescing, severe, with ulceration and epithelial intranuclear inclusion bodies.

Conference Comment: As commented by the contributor, detection of the characteristic intranuclear inclusion bodies associated with herpesviral infection is the key to identifying the underlying etiology in this case. Participants were intrigued by the unusual location of this herpesviral lesion; in the absence of the characteristic inclusions, many would not have included herpesviral infection as the primary differential diagnosis. This observation stimulated discussion concerning the differential diagnosis for eosinophilic and ulcerative dermatitis in the cat, including the various forms of feline eosinophilic granuloma complex: feline eosinophilic plaque, feline eosinophilic granuloma and indolent ulcer. The chart below summarizes the often overlapping findings of each of these entities.[2]

The moderator also stressed the importance of knowing the ultrastructural properties of viruses known to infect the skin. Some of the viruses known to infect the skin of domestic cats and their corresponding ultrastructural features include:[7]

- Felid herpesvirus-1: Enveloped, 150 nm in diameter with an icosahedral 100 nm diameter nucleocapsid
- Felis domesticus papillomaviruses 1 and 2: Non-enveloped, 50 nm diameter spherical, with icosahedral symmetry
- Feline leukemia virus and feline immunodeficiency virus: Enveloped, 80-100 nm, with a three layered structure containing an innermost genome-nucleoprotein complex, surrounded by an icosahedral capsid which is further bounded by the envelope with glycoprotein peplomeres

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References:

1. Caswell JL, Williams KJ: Respiratory system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's*

Disease Form	Cause	Anatomic Location	Gross Appearance	Histologic Findings
Feline eosinophilic plaque	Suspected hypersensitivity to food, parasites or related to atopy	Inguinal, axillary, perineal areas or lateral thigh	Red, raised, ulcerated plaques; pruritic	• Epidermal acanthosis, spongiosis, eosinophil exocytosis • Diffuse to perivascular eosinophilic dermatitis
Feline eosinophilic granuloma	Unknown; suspect hypersensitivity or hereditary	• Linear form: caudal or medial thigh • Nodular form: lips, chin, oral cavity, face • Also footpads and mucocutaneous junctions	Raised, pink, alopecic	• Diffuse eosinophilic dermatitis • Flame figures: degranulating eosinophils surrounding collagen •Macrophages and multi-nucleated giant cells
Indolent ulcer	Unknown	Upper lip adjacent to philtrum; uni- or bi-lateral	Non-pruritic and non-painful ulcer	• Acute: diffuse infiltrates of eosinophils with neutrophils, mast cells and macrophages • Chronic: lymphocytes, plasma cells, macrophages, neutrophils, fibrosis

Pathology of Domestic Animals. Vol. 2, 5th ed. Philadelphia, PA: Elsevier Ltd; 2007:648-649.
2. Ginn PE, Mansell JEKL, Rakich PM. Skin and appendages. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol. 3, 5th ed. Philadelphia, PA: Elsevier Ltd; 2007:738-739.
3. Gross TL, Ihrke PJ, Walder EJ, Affolter VK. Skin diseases of the dog and cat: clinical and histopathologic diagnosis. 2nd ed. Ames, IA: Blackwell Science Ltd.; 2005:124-126.
4. Hargis AM, Ginn PE, Mansell JEKL, Garber RL. Ulcerative facial and nasal dermatitis and stomatitis in cats associated with feline herpesvirus-1. *Vet Dermatol*. 1999;10:267-274.
5. Lee M, Bosward KL, Norris JM: Immunohistological evaluation of feline herpesvirus-1 infection in feline eosinophilic dermatoses or stomatitis. *J Feline Med Surg*. 2010;12:72-79.
6. Munson L, Wack R, Duncan M, et al. Chronic eosinophilic dermatitis associated with persistent feline herpes virus infection in cheetahs (*Acinonyx jubatus*). *Vet Pathol*. 2004;41:170-176.
7. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ. In: *Veterinary Virology*. 3rd ed. San Diego, CA: Elsevier Academic Press; 1999:308,339,373.

CASE IV: HN2598 (AFIP 3167484).

Signalment: Approximately 3-year-old, adult, male, sheep (*Ovis aries*).

History: This sheep suddenly died in the early Spring without any noticeable clinical signs. The animal was fed a hay diet and was not grazed on the pasture.

Gross Pathology: The liver showed marked atrophy (17 × 10 × 10 cm), pale brownish to yellowish color and a round shape. The gallbladder was slightly enlarged containing yellow bile. On the liver's diaphragmatic surface, thin firm whitish regions of fibrosis were noted. Thoracic and abdominal cavities contained 1,000 and 4,000 ml of clear and yellowish fluids, respectively. The carcass showed evidence of emaciation with serous atrophy of subcutaneous and bone marrow adipose tissue.

Histopathologic Description: Liver: Moderate to severe fatty degeneration of hepatocytes in centrilobular regions coalesced throughout the liver. Necrosis of hepatocytes and fatty cyst formation were also prominent. Fibrosis appeared mainly around Glisson's sheath and sometimes bridged adjacent hepatic lobules. Proliferation of bile ducts was also marked in Glisson's sheath and subcapsular area. Small regenerative nodules of hepatocytes were sometimes observed. Accumulation of ceroid-like yellow to pale brownish material occasionally appeared in the cytoplasm of macrophages and hepatocytes. Infiltrations of lymphocytes, plasma cells and neutrophils were noted in fibrotic areas and around necrotic hepatocytes.

Contributor's Morphologic Diagnosis: Fatty degeneration and necrosis of hepatocytes, severe, diffuse, with fibrosis and bile ducts hyperplasia.

Contributor's Comment: Fatty liver has been reported in animals affected with various conditions, such as hypoxia (anemia and passive venous congestion), diabetes, intoxication, and nutritional deficiencies.[6] In ruminants, pregnancy and heavy lactation also contribute to fatty liver. Hepatic lipidosis is a common and sensitive response to hepatocellular injury. This lesion occurs following interruption of the normally high throughput of fatty acids and triglycerides and secretion of lipoproteins at various points in the hepatic lipid metabolism pathway. The microscopic appearance of triglyceride globules in hepatocytes ranges from small discrete microvesicles to large coalescing macrovesicles.

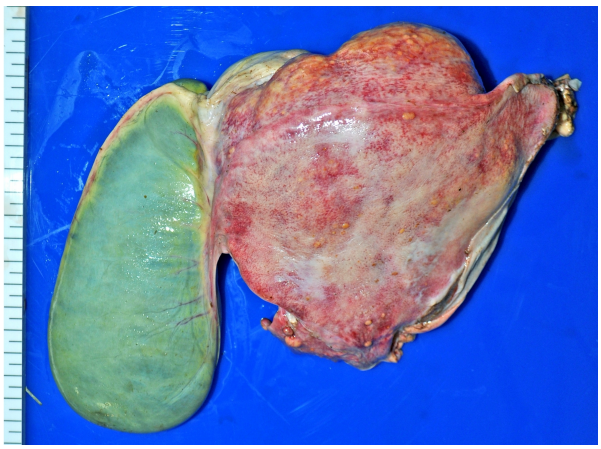
Hepatic fibrosis is a complicated spectrum of reactions that increase the deposition of extracellular matrix in injured areas.[6] The distribution of fibrosis in the liver reflects the pathogenesis of the necroinflammatory response, (i.e., biliary fibrosis, post-necrotic scarring, diffuse hepatic fibrosis and periacinar fibrosis). Cirrhosis, the end stage of several pathogenic processes resulting in hepatocytic death, chronic inflammation and fibrosis, is characterized by nodular regeneration, fibrovascular bridging scars and pseudolobular formations. Veterinary pathologists have been reluctant to use the term "hepatic cirrhosis" since ongoing regeneration and organization are rarely observed in animals.

Hepatic fatty cirrhosis, or "hard yellow-liver disease", is a progressive and chronic disease of sheep, goats, cattle, deer and antelope, which shows similar hepatic lesions to nutritional hepatic injury.[2] Grossly, the liver lesions in affected sheep begin in the subcapsular hepatic parenchyma as pale yellow firm areas that spread peripherally to involve approximately 80% of the liver in the final stages of the disease. Microscopically, periacinar hepatocytic fatty degeneration involving the entire lobule, with rupture and formation of fatty cysts are observed in the later stages. Periacinar fibrosis accompanies the ruptured fatty cysts, progressing to widespread bridging periacinar fibrosis, with islands of regenerating hepatocytes. The etiology of the disease is unknown, but unidentified hepatotoxins (mycotoxins and plant toxicosis)[6] and nutritional stress (a soil cobalt deficiency and low vitamin B_{12})[7] have all been postulated.

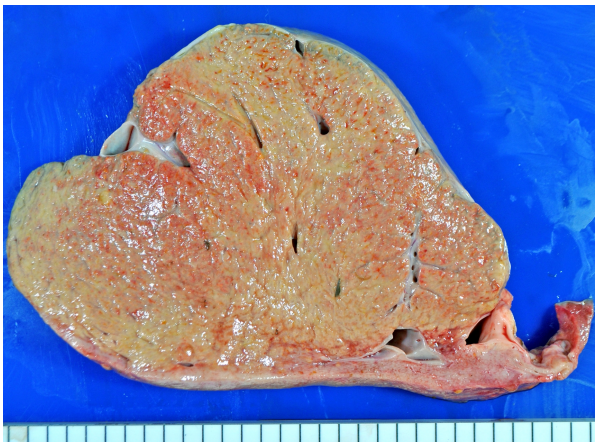
The histopathologic changes in the submitted liver are similar to changes seen in hepatic fatty cirrhosis. The cause of the liver injury in this case was undefined, but similar mechanisms including chronic nutritional deficiency or intoxicosis might have contributed to the development of the hepatic lesion.

AFIP Diagnosis: Liver: Hepatocyte fatty degeneration and necrosis, centrilobular to midzonal, diffuse, moderate with marked bridging portal fibrosis, biliary hyperplasia, and mild lymphoplasmacytic portal hepatitis.

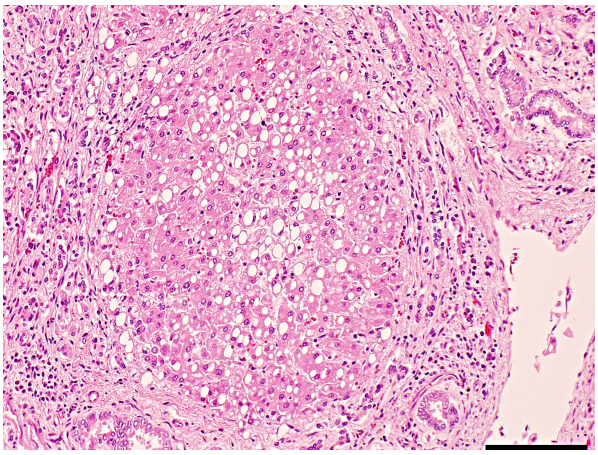
Conference Comment: This challenging case led to a lively discussion of the microscopic anatomy of the hepatic sinusoids. The sinusoids are lined by endothelial cells with thin fenestrations which rest on a thin network of reticulin fibers that support hepatocytes; resident macrophages, i.e. Kupffer cells, are also present within the sinusoids. A narrow space between endothelial cells and hepatocytes is known as the "space of Disse". Within this space are the hepatic stellate cells (HSC, also known as Ito cells or lipocytes); these mesenchymal cells store and release retinoids, assist in the production and turnover of



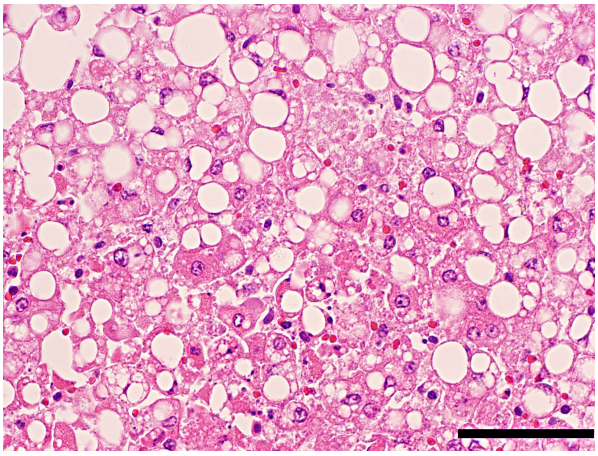
4-1. Liver, sheep. The liver is markedly atrophied, rounded, and pale brown to yellow. The gallbladder is slightly enlarged. Photograph courtesy of Graduate School of Veterinary Medicine, Hokkaido University, Department of Veterinary Clinical Sciences, Laboratory of Comparative Pathology, Sapparo Japan, umemura@vetmed.hokudai.ac.jp



4-2. Liver, sheep. On cut surface, the liver contains thin white regions of fibrosis. Photograph courtesy of Graduate School of Veterinary Medicine, Hokkaido University, Department of Veterinary Clinical Sciences, Laboratory of Comparative Pathology, Sapparo Japan, umemura@vetmed.hokudai.ac.jp



4-3. Liver, sheep. Multifocally, the liver contains regenerative hepatocellular nodules surrounded by areas of fibrosis. Photograph courtesy of Graduate School of Veterinary Medicine, Hokkaido University, Department of Veterinary Clinical Sciences, Laboratory of Comparative Pathology, Sapparo, Japan, umemura@vetmed.hokudai.ac.jp



4-4. Liver, sheep. Multifocally within centrilobular regions there is moderate to severe hepatocellular lipid vacuolar degeneration. Photograph courtesy of Graduate School of Veterinary Medicine, Hokkaido University, Department of Veterinary Clinical Sciences, Laboratory of Comparative Pathology, Sapparo, Japan, umemura@vetmed.hokudai.ac.jp

extracellular matrix, and regulate sinusoidal blood flow.[6] In response to hepatic injury, Kupffer cells secrete transforming growth factor-β (TGF-β), stimulating fibrogenesis.[2] Additionally, quiescent HSC can be directly activated by lipopolysaccharide binding to Toll-like receptor-4 (TLR4),[7] resulting in chemokine release and Kupffer cell recruitment as well as down-regulation of the inhibitory TGF-β pseudoreceptor Bambi.[7] Together, these two signals result in unrestricted Kupffer cell activation of HSC. Hepatic stellate cell migration, survival, and proliferation are maintained by platelet-derived growth factor (PDGF).[2]

There is a niche at the junction of the biliary ductular system and parenchymal hepatocytes within the canals of Hering which contains a specialized microenvironment for mesenchymal, endothelial and other cell types. At this site during chronic hepatic disease, these cell types give rise to bipotential progenitor cells which in the literature are referred to as "oval cells".[2] Chronic hepatic disease results in a concurrent reduction in hepatocyte proliferation as well as the development of an "oval cell reaction".[1] Prior to differentiation of the oval cells into hepatocytes or cholangiocytes, the oval cell reaction amplifies a biliary-derived cell population resulting in "ductular reaction".[1] Oval cells generally do not play a role in hepatic regeneration unless there is fulminant hepatic failure, chronic hepatitis, advanced liver cirrhosis, or hepatic tumors.[2]

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References:

1. Alison MR, Islam S, Lim S. Stem cells in liver regeneration, fibrosis and cancer: the good, the bad and the ugly. *J Pathol*. 2009;217:282-298.
2. Crawford JM, Liu C. Liver and biliary tract. In: Kumar V, Abbas AK, Fausto N, Aster JC, eds. *Robbins and Cotran Pathologic Basis of Disease*. 8th ed. Philadelphia, PA: Elsevier Saunders; 2009:837-838.
3. Helman RG, Adams LG, Bridges CH. The lesions of hepatic fatty cirrhosis in sheep. *Vet Pathol*. 1995;32:635-640.
4. Kierszenbaum AL. Digestive glands. In: *Histology and Cell Biology: An Introduction to Pathology*. 2nd ed. New York, NY: Mosby Inc.; 2007:504-505.
5. Pradere J-P, Troeger JS, Dapito DH, MencinAA, Schwabe RF. Toll-like receptor 4 and hepatic fibrogenesis. *Semin Liver Dis*. 2010;30:232-244.
6. Stalker MJ, Hayes MA. Liver and biliary system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol. 2, 5th ed. Philadelphia, PA: Elsevier Ltd; 2007:305-329, 368-382.
7. Sutherland RJ, Cordes DO, Carthew GC. Ovine white liver disease: a hepatic dysfunction associated with vitamin B12 deficiency. *NZ Vet J*. 1979;27:227-232.