Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2013-2014

Conference 18

12 March 2014

CASE I: N13-46 (JPC 4032911).

Signalment: 13-year-old Bay Warmblood mare (*Equus caballus*).

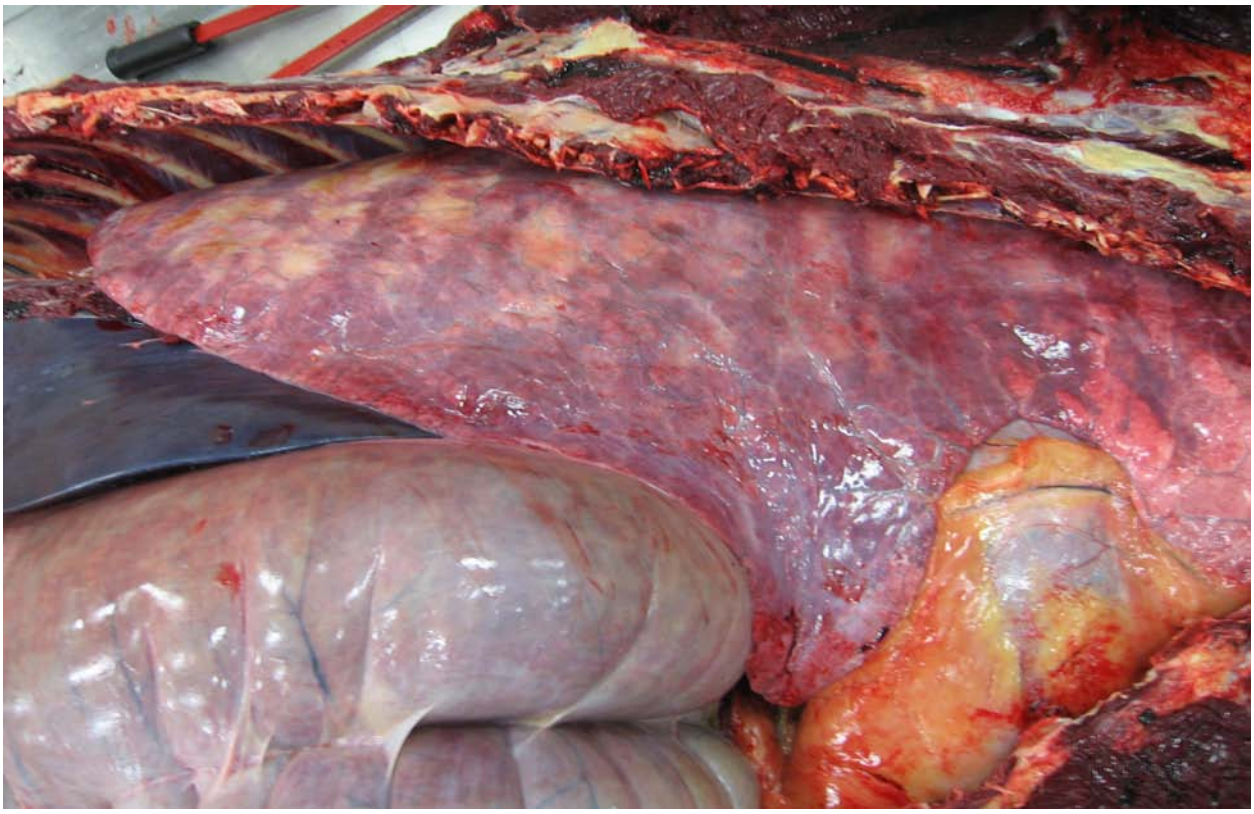
History: Since December 2012 the horse has had fever and increased respiratory rate. The horse started being treated by Baytril and Naxcel, but did not improve. At this time, the animal was presented to Tufts University Cummings School of Veterinary Medicine. Transtracheal aspirate (TTA) fluid was cultured, but no pathogen was found. Thoracic radiographs show that the lung has a diffuse, severe, interstitial, and nodular pattern. The TTA fluid was positive for equine herpesvirus 5 by PCR test. The biopsy of lung tissue reveals fibrotic changes. Later, the mare developed laminitis and was humanely euthanized.

Gross Pathology: All lung lobes contain multifocal to coalescing, firm, white to tan, variably-sized, slightly-raised nodules, ranging from 0.5 cm x 0.3 cm x 15 cm to 15 cm x 9 cm x 7 cm. The cut surface of the nodules is tan and firm. Lung parenchyma between the nodules is dark red and markedly congested. The tracheobrochial lymph nodes are enlarged ranging from 5 cm x 3 cm x 2.5cm to 9 cm x 4 cm x 3 cm. The midsagittal section of hoof of both forelimbs revealed the dorsal surface of third phalanx is widely separated (up to 0.5 cm) from the epidermal laminae of the inner surface of hoof wall with ventral rotation of the tip of the third phalanx. The space between them is filled with tan-white firm tissue.

Laboratory Results: Transtracheal aspirate (TTA)

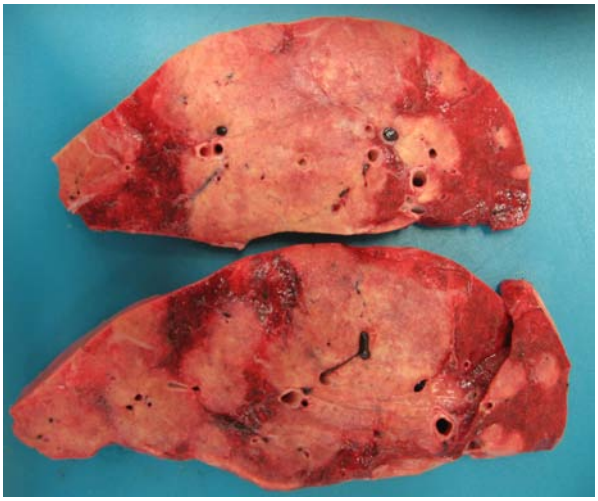
1. PCR test: Equine herpesvirus 5 positive
2. Bacterial culture: Negative

Histopathologic Description: In a multifocal to regionally extensive area, the alveolar septa are variably thickened up to 3 times by fibrous connective tissue composed of well differentiated to plump fibroblasts admixed with moderate to high numbers of foamy macrophages, neutrophils, lymphocytes, plasma cells, and necrotic cellular and karyorrhectic debris. The alveoli are lined by plump cuboidal cells (type II pneumocyte hyperplasia) and filled with moderate to marked numbers of giant foamy macrophages, intact and degenerate neutrophils, and necrotic epithelial cell debris with karyorrhexis and karyolysis. Occasionally, macrophages contain up to 5 µm diameter eosinophilic to amphophilic intranuclear inclusion bodies that marginate chromatin.



1-1. Lung, horse: All lung lobes contain multifocal to coalescing, firm, white to tan, variably-sized, slightly-raised nodules. (Photo courtesy of: Section of Pathology, Department of Biomedical Science, Tufts Cummings School of Veterinary Medicine, http://vet.tufts.edu/dbs/pathology.html

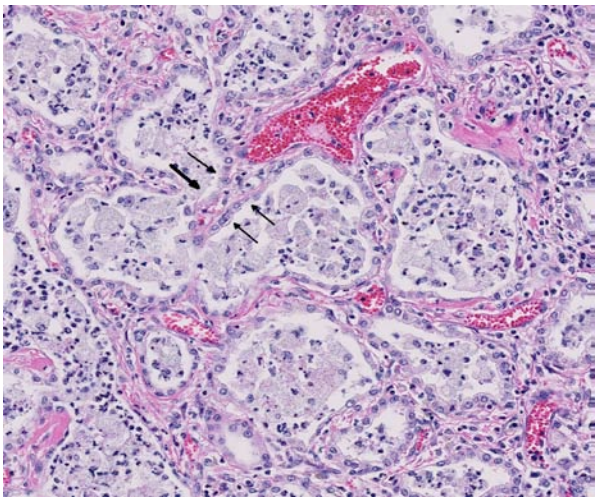
Bronchi and bronchioles within the affected areas contain a minimal to mild amount of cellular and karyorrhectic debris sometimes with few degenerate neutrophils. Occasionally, bronchi have mild epithelial hyperplasia. Multifocally, there is moderate perivascular, peribronchial and peribronchiolar fibrosis admixed with moderate number of macrophages, lymphocytes, plasma cells, and few neutrophils. Multifocally, the adjacent relatively normal lung parenchyma has mild amounts of eosinophilic, homogenous, acellular material in the alveoli and the interstitia is diffusely thickened by increased clear spaces (edema).



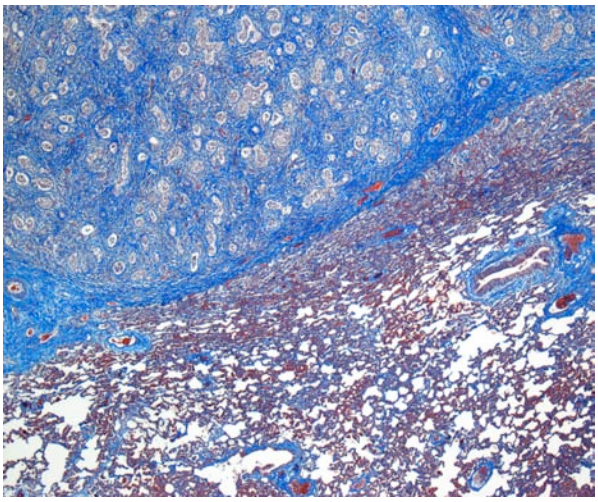
1-2. Lung, horse: The cut surface of the nodules is tan and firm. Lung parenchyma between the nodules is dark red and markedly congested. (Photo courtesy of: Section of Pathology, Department of Biomedical Science, Tufts Cummings School of Veterinary Medicine, http://vet.tufts.edu/dbs/pathology.html

Contributor's Morphologic Diagnosis: Multifocal to regionally extensive moderate pulmonary fibrosis and bronchointerstital pneumonia with intrahistiocytic intranuclear viral inclusion bodies consistent with equine multinodular pulmonary fibrosis.

Contributor's Comment: Equine multinodular pulmonary fibrosis (EMPF) is a recently reported fibrotic interstitial lung disease in adult horses.[5] No breed or sex predilection was determined. The mean age of affected horses was 13 years. The common clinical presentations are weight loss, pyrexia, and tachypnea.[5,6] Thoracic radiographs often demonstrate an interstitial to nodular pulmonary pattern. Grossly, there are two



1-3. Lung, horse: Alveolar septa are markedly expanded by fibrous connective tissue and lined by type II pneumocytes (arrows). (HE 168X)



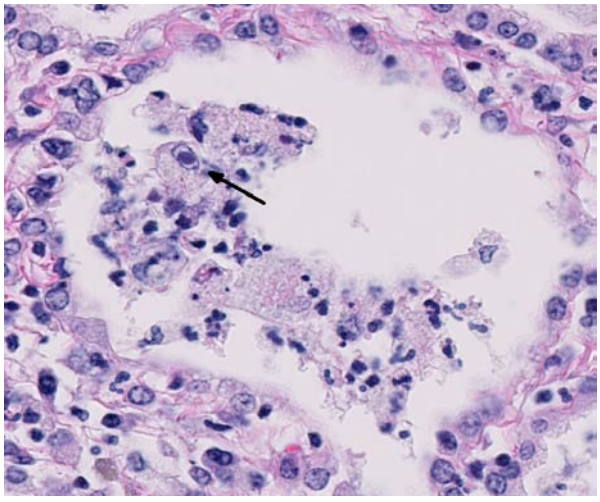
1-4. Lung, horse: A Masson's trichrome stain demonstrates the amount of fibrous connective expanding alveolar septa. (Masson's trichrome, 4X)

major manifestations. The more common lesions are numerous pale tan-white, coalescing, firm nodules, involving all lung lobes. Sometimes, the lesions form masses, which makes it difficult in differentiating from neoplasia. The borders are discrete where they meet unaffected lung. The less common one often only demonstrates multiple discrete nodules separated by grossly normal lung.[5] Histopathologically, there is often marked multifocal and regionally extensive interstitial and alveolar septa fibrosis with often preservation of cuboidal epithelial cells (type II pneumocytes) forming alveolar-like structures. The lumen of the structures is often filled with mild to moderate number of intact and degenerate neutrophils, macrophages and cellular and karyorrhectic debris. Dark eosinophilic intranuclear viral inclusion bodies may be seen in the foamy macrophages.[4-6]

Equine herpesvirus 5 (EHV-5), a gamma herpesvirus, is consistently detected in the lung tissue of affected horses.[1,4-6] And, compared to other tissues, lung has a remarkably higher viral load than in other organs, especially within the pulmonary fibrotic lesions. These evidences support the high correlation between EHV-5 and EMPF.[4] Interestingly, equine herpesvirus 2 and asinine herpesvirus 5 (AHV5), another two types of gammaherpes viruses, may also be detected in the lesions.[1,4] Although these gammaherpes viruses are detected in the lesions, the pathogenic roles of them are currently still unclear.

JPC Diagnosis: Lung: Pneumonia, interstitial, necrotizing and fibrosing, focally extensive, severe, with marked type II pneumocyte hyperplasia, neutrophilic and histiocytic alveolitis, and rare intrahistiocytic viral intranuclear inclusion bodies.

Conference Comment: Conference participants observed that pleural arteries are often quite prominent, with hypertrophied smooth muscle within the tunica media and abundant collagen; this is likely an indication of severe pulmonary hypertension secondary to diffuse fibrosis. The differential diagnosis for the gross and histopathological lesions in this case includes silicate pneumoconiosis, which generally exhibits variable amounts of granulomatous inflammation in association with pulmonary interstitial fibrosis;[5] idiopathic pulmonary fibrosis, which more commonly affects foals than adults and is attributed to diffuse alveolar damage;[1,5] paraquat/diquat toxicosis, which, although rare, also causes fulminant pulmonary fibrosis;[2] and exercise-induced pulmonary hemorrhage, which has large areas of pulmonary fibrosis admixed with numerous hemosiderophages.[2] Participants also noted that the fibrosis in this case did not appear as nodular as normally described in cases of EMPF; this led to the suggestion of equine herpes virus (EHV)-1, which is most notorious as a cause of abortion in horses, but may also cause respiratory disease and encephalomyelitis, or EHV-4 (equine rhinopneumonitis virus) as possible etiologies.[3]



1-5. Lung, horse: Rarely, alveolar macrophages contain a single, intranuclear viral inclusion body. (HE 400X)

In conference, the moderator led a detailed review of the immune response as it relates to this case, touching upon various general pathology concepts such as the components of innate immunity (see WSC 2013-2014 conference 3, case 1 for an abridged examination of toll-like receptors), the cellular and humoral arms of the adaptive immune response, the MHC molecule, and potential paths of differentiation for T-helper cells (see WSC 2013-2014 conference 2, case 2 for a review of T_H1 versus T_H2 reactions). Additionally, participants briefly discussed an interstitial pneumonia of donkeys which has been reported in association with asinine herpesvirus. This disease differs from EMPF in that it is a diffuse inflammatory disease with syncytial cell formation without viral inclusions; interstitial fibrosis is considered a secondary component.[5]

Contributing Institution: Section of Pathology
Department of Biomedical Science
Tufts Cummings School of Veterinary Medicine

References:

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5. Williams KJ, Maes R, Del Piero F, et al. Equine multinodular pulmonary fibrosis: a newly recognized herpesvirus- associated fibrotic lung disease. *Vet Pathol*. 2007;44:849–862.
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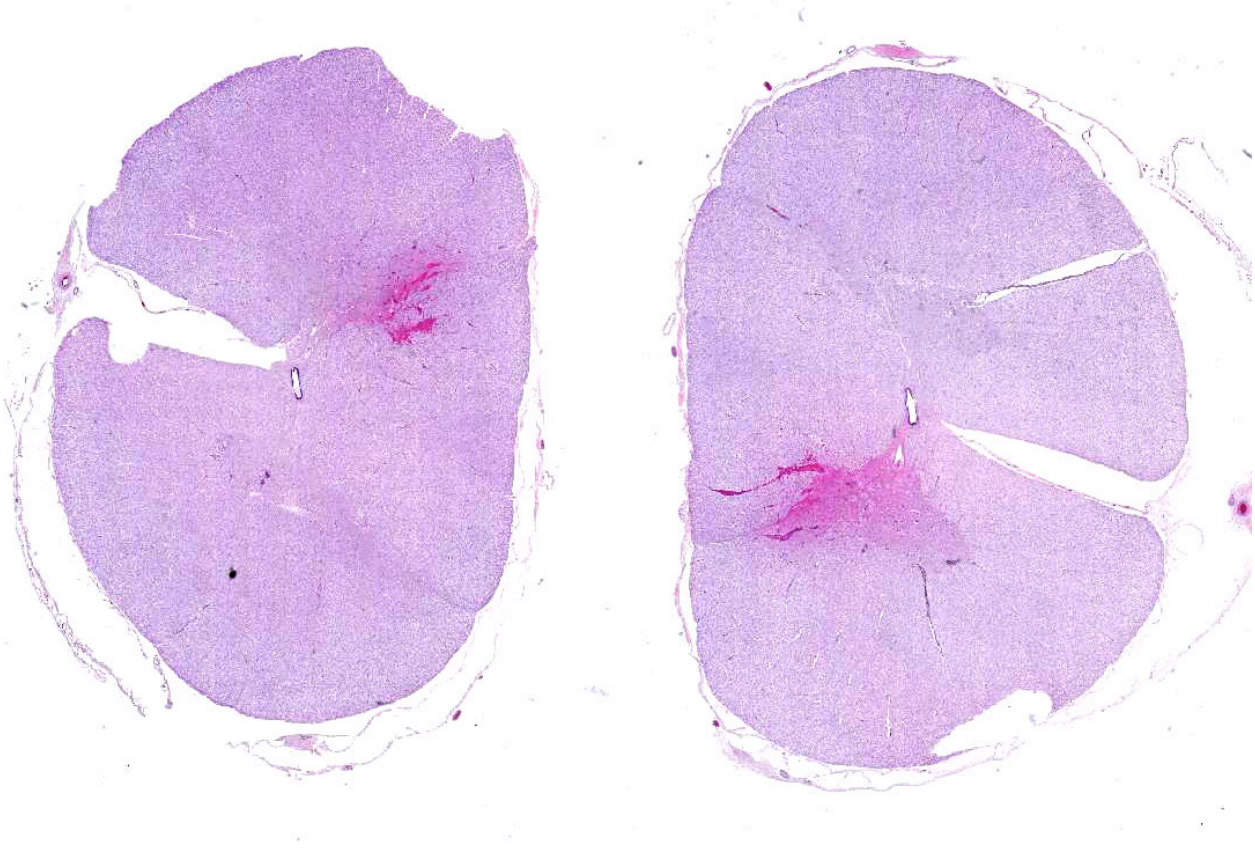
CASE II: KAHDL 8398 (JPC 4035597).

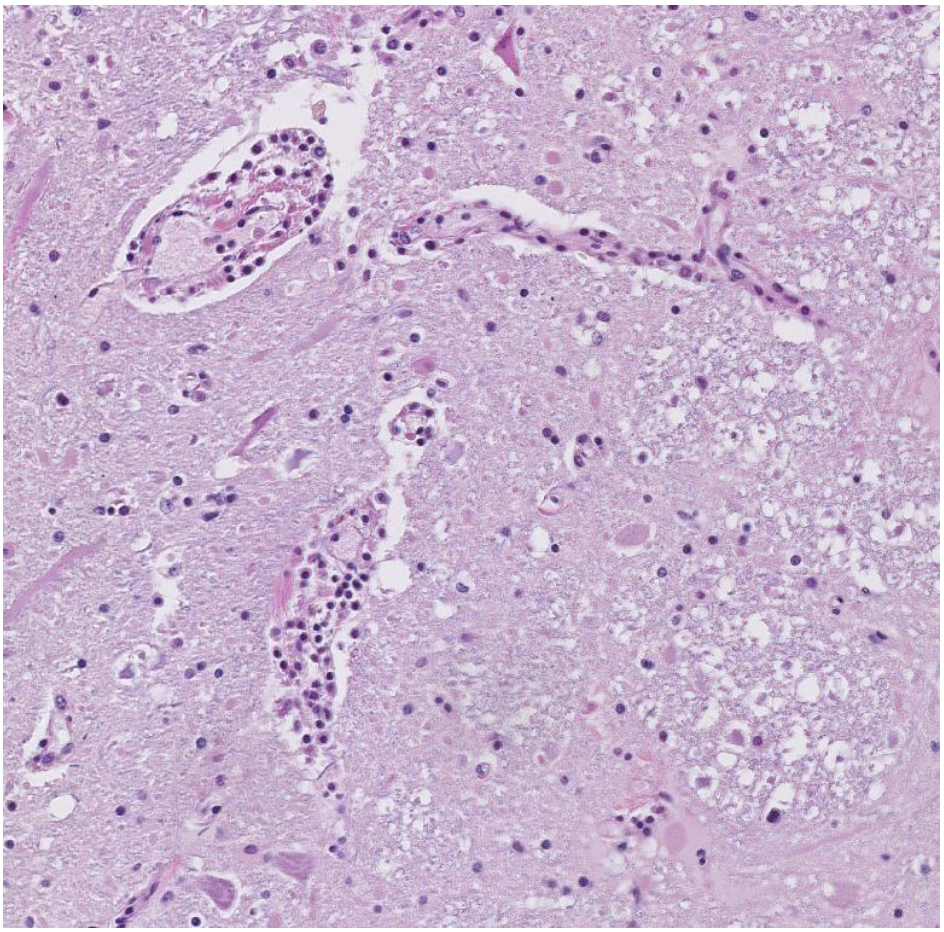
Signalment: 7-year-old gelded male mixed breed pony, (*Equus ferus caballus*).

History: The horse was a seven-year-old mixed breed gelded pony kept in a grassy pasture and fed a diet of hay and grain. Two weeks prior to euthanasia, the horse was in the proximity of strong winds and tornados which leveled several buildings on the adjacent property. For approximately 5-7 days after the storm, the pony avoided its pasture-mate (another horse) and human contact. During this time, he avoided being touched on the left and seemed lethargic. Thereafter he returned to normal behavior but two days prior to euthanasia he was unable to eat and held his head at the water trough without seeming to drink much. He progressively developed a generalized weakness and had difficulty standing. He avoided contact with his head (i.e., became "head-shy") and started to have intermittent trembling of his lips and mouth. The owners tried rinsing his mouth with salt water and thoroughly examined the oral cavity (by touch). Given the progression of clinical signs and poor prognosis, owners elected euthanasia.

Gross Pathology: A seven-year-old gelded mixed breed horse with a body condition score of 2 out of 5 and mild to moderate autolysis (16 hour post-mortem interval) was received for necropsy and rabies testing. On oral exam there were moderate lingual points on the mandibular cheek teeth and buccal points on the maxillary check teeth. The spleen had an approximately 12-15 cm long laceration of the parietal surface opposite the stomach and extending transversely over the middle of the organ. The laceration site had organizing blood clots (red but with visible surrounding contraction and pallor (fibrosis)). Numerous 2-8 mm brown red nodules were scattered throughout the omentum in the area of the ruptured spleen. The cervical spinal cord had a focal area of hemorrhage and swelling at approximately the level of C1-C2.



2-1. Spinal cord, horse: There is a focal area of hemorrhage unilaterally within the dorsal horn. (HE 0.63X)



2-2. Spinal cord, horse: Blood vessel walls and surrounding parenchyma are expanded by edema and are surrounded and rarely infiltrated by moderate numbers of lymphocytes and plasma cells. Rabies inclusions were not seen in this individual. (HE 140X)

Laboratory Results: Rabies virus fluorescent antibody was positive for rabies. Speciation of the virus was interpreted as the North Central United States and California skunk rabies.

Histopathologic Description: The section of spinal cord has multifocal perivascular cuffing by 2-4 layers of mononuclear cells dominated by lymphocytes with fewer histiocytes and occasional plasma cells. Most vessels are lined by plump endothelial cells and a focus of vessels near the tip of a dorsal funiculus is surrounded by amorphous eosinophilic material (high-protein edema fluid or an autolyzed area of hemorrhage). Axon sheaths in this area frequently contain macrophages (axonophagia) or dilated nerve processes (spheroids). Within the gray matter, neurons frequently have loss of nissl substance and nuclear material (chromatolysis) and there are multifocal small aggregates of glial cells (Babe's nodules).

Contributor's Morphologic Diagnosis: Diffuse non-suppurative and hemorrhagic vasocentric encephalomyelitis with neuronolysis with focal myelomalacia and hemorrhage.

Contributor's Comment: This case was challenging clinically because of the seemingly waxing and waning history. Nevertheless, the referring veterinarian had some concern about rabies at the time of submission. The lesion on the spleen and the history immediately after the local tornado activity are thought to represent the effects of blunt trauma from an airborne projectile. It was not until the three days preceding euthanasia that the horse was thought to be showing signs attributable to rabies.

Antemortem diagnosis of rabies remains problematic, but the disease should be considered in horses whenever there are rapidly progressing and/or diffuse neurologic signs. Differentials for rabies include hepatoencephalopathy, Eastern equine encephalitis, herpesviral encephalomyelopathy, protozoal encephalomyelitis, nigropallidal encephalomalacia, botulism, lead poisoning, cauda equine neuritis, meningitis, space-occupying masses, CNS trauma, or esophageal obstruction.[4]

Equine rabies can also be challenging to diagnose on necropsy. Numerous cases of disease confined to the spinal cord in horses have been reported making it advisable to include spinal cord in routine rabies testing in horses.[1] Horses are also

unique in that their lesions often are associated with significant hemorrhage making it a consideration for focal spinal lesions associated with hemorrhage. Finally, as is demonstrated in this case, greater than half of the rabies cases in horses do not have identifiable Negri bodies.[2]

JPC Diagnosis: Spinal cord, gray matter: Neuronal degeneration, multifocal, moderate, with mild gliosis and lymphoplasmacytic meningitis.

Conference Comment: Although the contributor identified multifocal small nodules of glial cells, known as Babe's nodules,[4] most conference participants did not appreciate this feature. The anatomic location as well as the subtle microscopic findings and lack of Negri bodies in this case engendered some difficulty in arriving at a diagnosis of rabies; many participants suspected an alternate viral etiology, such as Eastern equine encephalitis (alphavirus) or West Nile virus (flavivirus).

Rabies virus is an enveloped RNA virus of the family *Rhabdoviridae* and the genus *Lyssavirus*; it causes meningoencephalomyelitis, ganglionitis and parotid adenitis, is almost invariably fatal, and is capable of affecting any mammalian species. Following infection with the virus, herbivores, unlike carnivores, are typically dead-end hosts. Reservoir hosts may vary temporally and regionally; among the most common are foxes, skunks, raccoons, feral dogs, wolves, jackals and mongoose. Fructivorous, insectivorous and vampire bats are also capable of transmitting rabies virus. Rabies viral neurotropism is due to a viral coat protein known as rabies virus glycoprotein (RVG), which binds several neural tissue receptors, including neuronal cell adhesion molecule (NCAM) and the p75 neurotrophin receptor (p75NTR). Virus inoculation typically occurs through contaminated saliva entering bite wounds inflicted by rabid animals. Initial viral replication occurs in myocytes adjacent to the bite wound, with subsequent invasion of the local neuromuscular junction and, eventually, the CNS and paravertebral ganglia via axoplasmic flow. Following viral replication in the CNS, there is centrifugal spread to salivary glands, nasal mucosa and adrenal glands.[3,4]

Three clinical manifestations of rabies are described: dumb, furious, and paralytic forms. The two most common clinical signs in affected mammals are progressive paralysis and aberrant behavioral patterns. In addition, horses in particular can have clinical signs associated with spinal cord injury, such as pelvic limb lameness, proprioceptive defects, ataxia, paralysis, and colic. Other reported species-specific features of the clinical progression of rabies include the following: cattle commonly exhibit excessive salivation and vocalization, swine are often found dead with no preceding clinical signs, sheep display passive behavior and dogs appear agitated or anxious. There are typically no gross lesions, although in horses infection may be associated with spinal cord hemorrhage. Historically, intracytoplasmic viral inclusions (i.e., Negri bodies) occur in the Purkinje cells of the cerebellum in herbivores and in neurons of the hippocampus in carnivores. In most mammalian species, Negri bodies are identified in 70% to 85% of affected animals; however, in horses this figure falls to 30% to 50%.[3,4] As an example of equine rabies with lesions limited to the spinal cord, without demonstrable Negri bodies, this case illustrates the potential variability of gross and histopathologic findings associated with rabies virus infection.

Contributing Institution: Kord Animal Health Diagnostic Lab
Ellington Agricultural Center
Porter Building
436 Hogan Road
Nashville, TN 37220
http://www.tn.gov/agriculture/regulatory/kord.shtml

References:

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2. Green S, Smith L, Vernau W, et al. Rabies in horses: 21 cases (1970-1990). *J Am Vet Med Assoc*. 1992;200:1133-1137.
3. Maxie MG, Youssef S. Nervous system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol 1. 5th ed. Philadelphia, PA: Elsevier; 2007:413-416.
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CASE III: 12-258-13 (JPC 4032444).

Signalment: 36-year-old male chimpanzee, (*Pan troglodytes*).

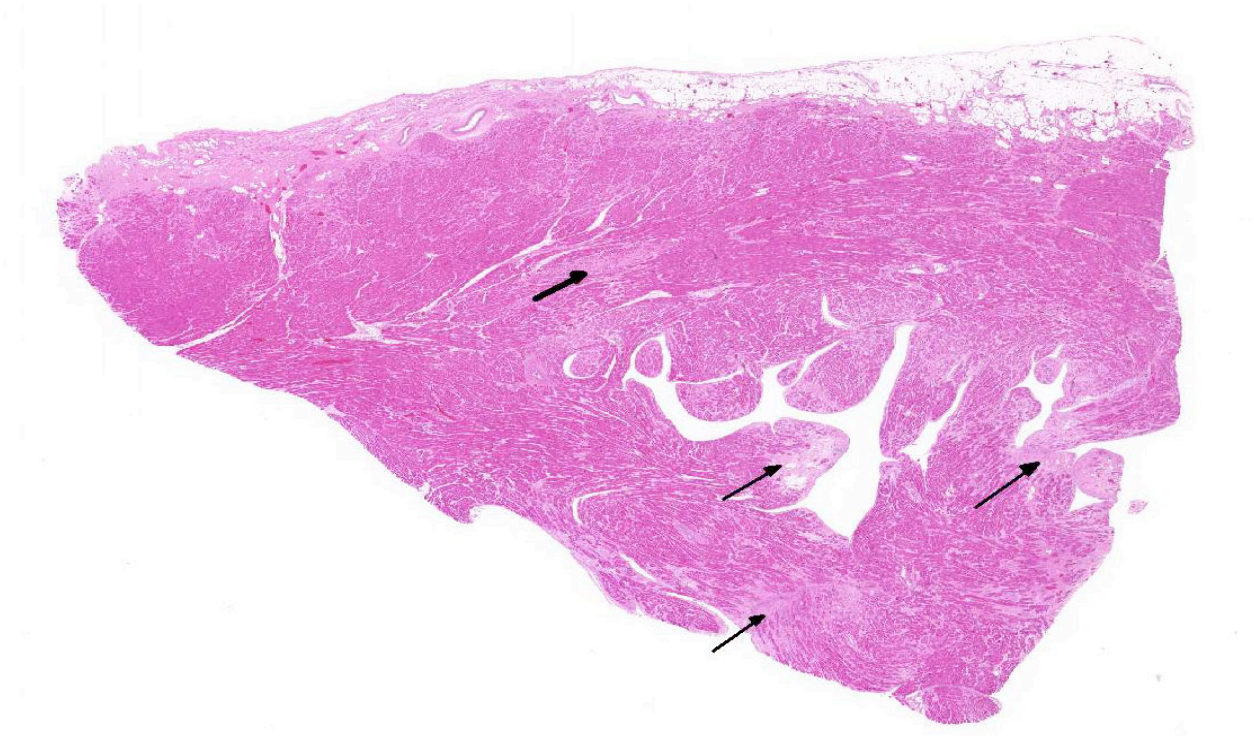
History: The chimpanzee was found dead. There was no history of prior illness, other than a single episode of vomiting several days prior to death.

Gross Pathology: The intact heart is moderately, diffusely enlarged and weighs 497.3 grams. The heart:BW ratio is 0.68 (normal 0.54 - 0.60). There are multifocal streaks of pale, firm fibrosis, predominantly within the left ventricular free wall, interventricular septum, and apex of the heart. The right ventricle appears mildly dilated. Additionally, there is a focal discrete, white, firm 2 cm diameter multicystic nodule within the superficial to mid cortex of the right kidney.

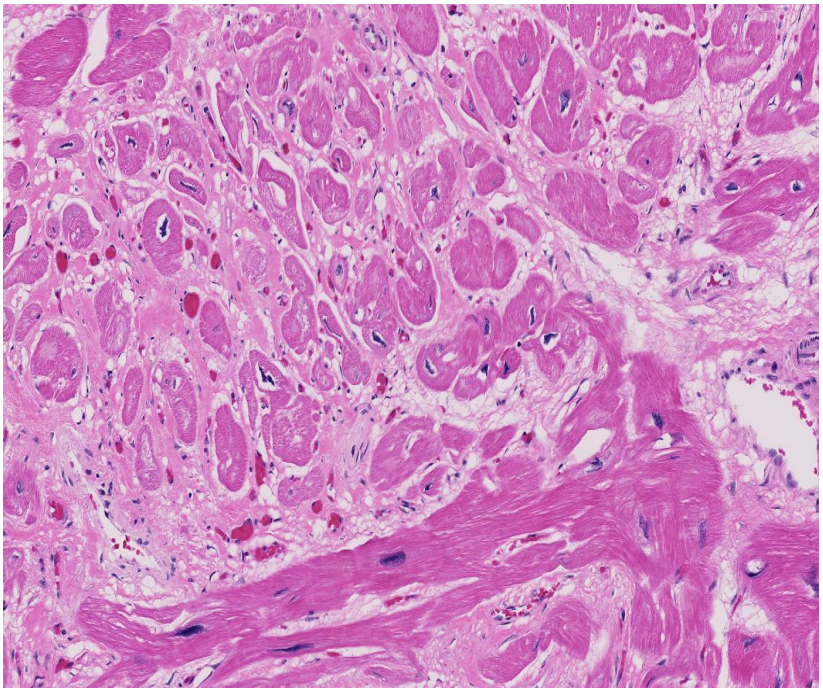
Histopathologic Description: There is moderate to severe, multifocal to coalescing myocardial fiber degeneration, characterized by myocardiocyte pallor and loss of cross striations. There is multifocal, moderate cardiac myofiber disorganization and separation and replacement of myofibers by pale pink, predominantly mature fibrous connective tissue (fibrosis) and adipose tissue. There is significant fibrosis at the base of the mitral valve. The cardiac myocardial fiber nuclei are frequently moderately enlarged, basophilic, and bizarre. Minimal, light brown intracytoplasmic granular pigment (lipofuscin) is present in scattered myocytes. Rare, minimal to mild atherosclerotic changes are seen in random coronary arterioles, characterized by few mural macrophages, foamy cells and eosinophilic matrix material. There is multifocal mild lymphocytic and lesser histiocytic inflammation within the areas of fibrosis or surrounding degenerate myofibers and within the epicardial connective tissue.

Contributor's Morphologic Diagnosis: Heart: Moderate to severe, multifocal to coalescing, chronic myocardial degeneration and fibrosis with mild multifocal chronic myocarditis.

Contributor's Comment: Cardiomyopathy was first reported in the chimpanzee in 1984, and the initial case report describes a 26-year-old male chimpanzee with history of heart murmur progressing to weight loss, heart failure, and coma



3-1. Heart, chimpanzee: Throughout the myocardium, there are numerous areas of myofiber loss and replacement by fibrous connective tissue (arrows). (HE 0.63X)



3-2. Heart, chimpanzee: Within areas of fibrosis, remaining myofibers are often atrophic and contain enlarged basophilic nuclei. (HE 116X)

over a 10 month period.[2] Gross lesions in this case included dilated cardiomyopathy, pericardial effusion, cerebral infarct, and hepatomegaly, and cardiac histopathology showed myocardial fibrosis and coronary atherosclerosis.[2] Subsequently, interstitial myocardial fibrosis has emerged as a major cause of heart disease and sudden death in chimpanzees, and in most cases there are no associated lesions of atherosclerosis.[3] Sudden death is thought to be secondary to conduction abnormalities and arrhythmias. A 2009 study of 87 adult chimpanzees found a 68% prevalence of heart disease and a 52% prevalence of idiopathic cardiomyopathy, with heart disease the primary cause of death.[5] In another study, interstitial myocardial fibrosis was identified as the most common cause of sudden death in chimpanzees, and was present in 92% cases of sudden cardiac death and 81% cases of all sudden death.[5] In humans, myocardial fibrosis occurs secondary to systemic hypertension, myocarditis, cardiomyopathy, or as response to myocardial injury, and can be a replacement (scarring) response to injury such as myocardial infarction or reactive and triggered by external stimuli such as pressure or volume overload.[5] In great apes, myocardial fibrosis with atrophy and hypertrophy of cardiac myocytes occurs with minimal or no inflammation and often no apparent cause or associated disease.[5] Potential biomarkers for myocardial fibrosis in chimpanzees have been investigated, and brain natriuretic protein and cardiac troponin I were elevated in cases of cardiovascular disease in one report.[1] In this study, neither a lipid panel including cholesterol, LDL, and triglycerides nor hsCRP, one of the best biomarkers for indicating ischemic disease in man, were useful in the diagnosis of heart disease in chimpanzees.[1]

JPC Diagnosis: Heart: Fibrosis, multifocal, moderate, with myofiber degeneration, atrophy and loss.

Conference Comment: The contributor provides an excellent overview of cardiomyopathy in great apes; the microscopic lesions in this case are consistent with this well-documented condition. Interestingly, myocyte degeneration and fibrosis appear most severe in the subendocardial and subepicardial tissue, while sparing the rest of the myocardium; the clinical significance of this finding is unknown. Additionally, moderate numbers of Anitschkow cells, also known as caterpillar cells due to their wavy nuclei, are present within affected areas of myocardium. These are thought to be macrophages or attempts at myofiber regeneration and are found in the myocardium in certain disease states.[4] Other striking features include widespread cardiomyocyte hypertrophy, characterized by fiber thickening and a change in nuclear morphology from spindle/cigar shaped to a “box car” like (rectangular) silhouette (see WSC 2013-2014, case conference 17, case 2), as well as frequently enlarged, bizarre cardiomyocyte nuclei. Conference participants speculated that these nuclear changes may represent abortive attempts at regeneration.

Contributing Institution: University of Washington
Department of Comparative Medicine (http://depts.washington.edu/compmed/)
Washington National Primate Research Center (*www.wanprc.org/)*

References:

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CASE IV: 46184-1 (JPC 4001561).

Signalment: 8-month-old female sheep, (*Ovis aries*).

History: The lamb had been growing well until 4 weeks earlier when it was noticed to have edematous ears and was lying in the shade during the day. Over the following 4 weeks the lamb progressively lost weight and skin sloughed from around the eyes, the dorsal aspect of both ears, and on the face. The owner elected euthanasia on humane grounds because the lamb had not shown any signs of improvement and was constantly rubbing its face and ears.

Gross Pathology: On post-mortem examination, adipose tissue throughout the carcass was pale yellow. The liver was normal in shape, but slightly firm and had a bronze discoloration. No other gross abnormalities were apparent.

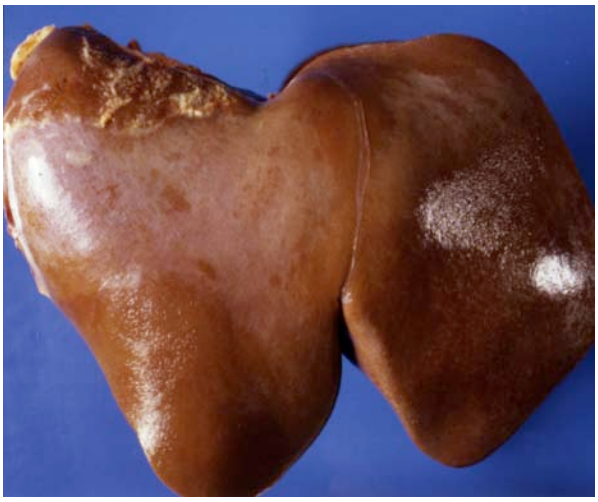
Histopathologic Description: Throughout the section, portal triads are variably expanded by increased numbers of bile ductules and loose fibrous connective tissue infiltrated with moderate numbers of lymphocytes and plasma cells. Many large and medium-sized bile ductules are lined by dysplastic epithelial cells and surrounded by an edematous adventitia containing capillaries and reactive fibroblasts. Some large ducts have been either completely replaced by edematous granulation tissue or are represented by a markedly attenuated lumen. Occasional large blood vessels in portal regions have segmental thickening of their wall adjacent to damaged bile ducts. Scattered aggregates of neutrophils and occasional foci of fibrosis are present in the parenchyma but hepatocytes are unaffected.

Contributor's Morphologic Diagnosis: Subacute lymphocytic/plasmacytic cholangitis with periductular edema, replacement fibrosis and recanalization.

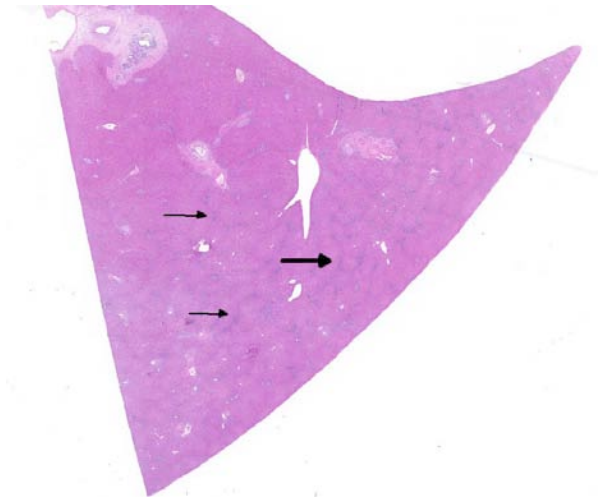
Contributor's Comment: These hepatic lesions are typical of those caused by exposure to sporidesmin, a toxin produced by the fungus *Pithomyces chartarum.* This fungus grows readily on dead plant material in ryegrass pastures and spores containing sporidesmin can reach high levels during periods of warm, moist weather, as often occurs during the fall. Sporidesmin toxicity is an important cause of production loss, ill thrift and sometimes death in ruminants and camelids in the North Island of New Zealand. The disease is also reported in southern Australia and South Africa. The disease is characterized by hepatogenous photosensitivity and is commonly known in New Zealand as facial eczema.

Large and medium-sized intrahepatic and extrahepatic bile ducts are the primary target of the toxin and may become attenuated or completely occluded in severe cases. The changes in these ducts is virtually pathognomonic for sporidesmin toxicity,[3,4,6] the portal fibrosis and biliary ductular hyperplasia representing non-

4-1. Haired skin, sheep: This 8-month old female lamb progressively lost weight, and skin sloughed from around the eyes, the dorsal aspect of both ears and on the face. (Photo courtesy of: Institute of Veterinary, Animal, and Biomedical Sciences, Massey University, Tennant Drive, Palmerston North, New Zealand)



4-2. Liver, sheep: The liver was normal in shape, but was slightly firm and had a bronze discoloration. (Photo courtesy of: Institute of Veterinary, Animal, and Biomedical Sciences, Massey University, Tennant Drive, Palmerston North, New Zealand)



4-3. Liver, sheep: Within the section, the adventitia of bile ducts is edematous, and portal triads are prominent due to their hypercellularity (arrows). (HE 0.63X)

specific secondary changes following blockage of larger ducts.

Although lesions occur throughout the liver, the left (ventral) lobe is affected more severely than the right lobe. In chronic cases, especially those where animals are exposed to sublethal doses over more than one year, the left lobe may be markedly atrophic and exist only as a fibrous remnant, sometimes containing small remnants of hyperplastic hepatocytes. In such cases, the right lobe is typically hypertrophic and the liver is rounded in shape.

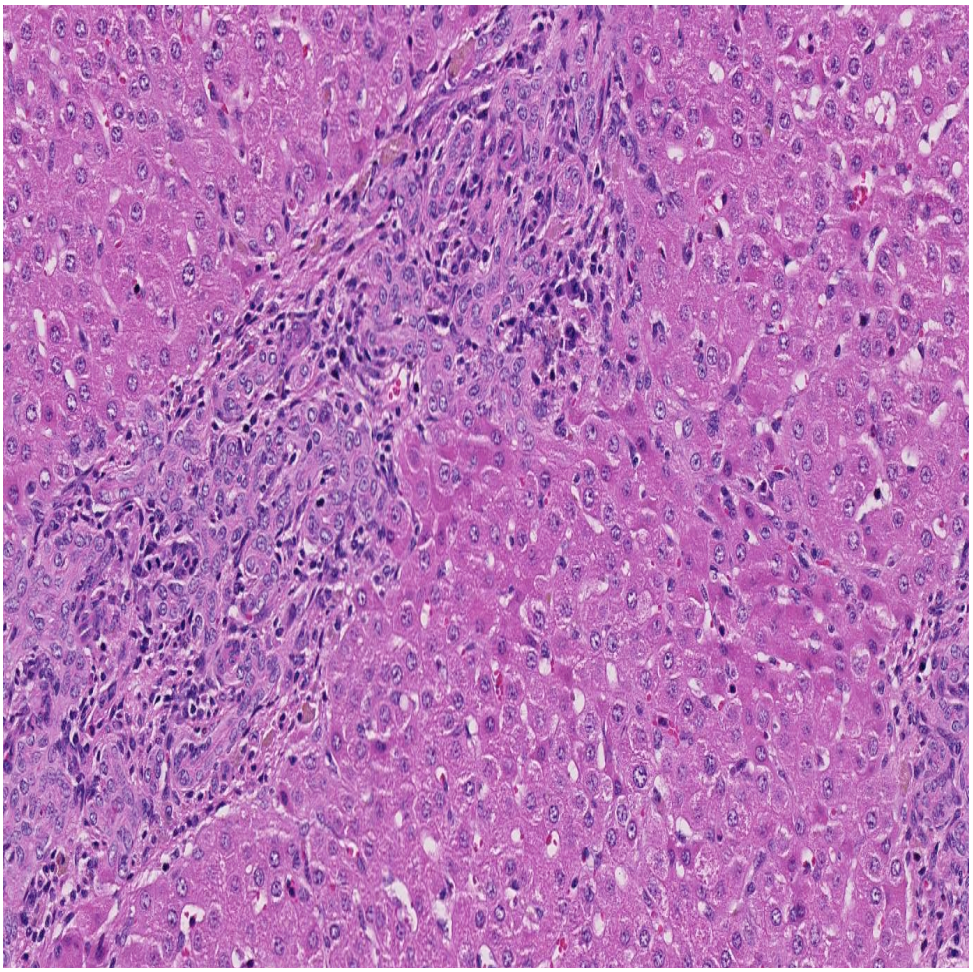
Subacute sporidesmin toxicity is characterized by a marked increase in the serum activity of GGT (often well above 1000 IU/L), which remains elevated for several months following exposure to the toxin.

JPC Diagnosis: Liver: Biliary hyperplasia, diffuse, marked, with reactive dysplasia, moderate portal and bridging fibrosis and mild lymphoplasmacytic portal hepatitis.

Conference Comment: Although conference participants were not provided with gross necropsy findings or the clinical history of facial eczema, most suspected a toxic etiology based upon the presence of portal and bridging fibrosis. Moreover, the prominent biliary hyperplasia led many participants to consider toxins that target the biliary epithelium, specifically the mycotoxin sporidesmin; however, the differential diagnosis for these lesions must also include phomopsin, *Lantana*, aflatoxin and pyrrolizidine alkaloid hepatotoxicity, as well as the South African condition known as geeldikkop.

Phomopsin is a mycotoxin produced by the saprophytic fungus *Phomopsis leptostromiformis*, which commonly infects lupines; it is a potent microtubule inhibitor that results in mitotic arrest during metaphase. Thus, in addition to biliary hyperplasia and hepatic fibrosis, this condition is characterized microscopically by the presence of numerous bizarre mitoses. Phomopsin toxicosis is also associated with hepatogenous photosensitivity.[1,5] Toxic pentacyclic triterpenes (especially Lantadene A, B, and C) from the shrub *Lantana camara* induce hepatic cholestasis, icterus and hepatogenous photosensitization in ruminants, primarily cattle. *Lantana* hepatotoxicosis is distinguished histologically by megalocytosis, bile accumulation and bile duct proliferation.[5] Saponins of the South African plant *Tribulus terrestris* (alone or in combination with sporidesmin) are likely responsible for geeldikkop ("yellow bighead") in sheep, which is characterized by hepatocyte vacuolation and Kupffer cell hyperplasia in acute toxicosis, and the presence of crystalline material within bile ducts in chronic intoxication.[5]

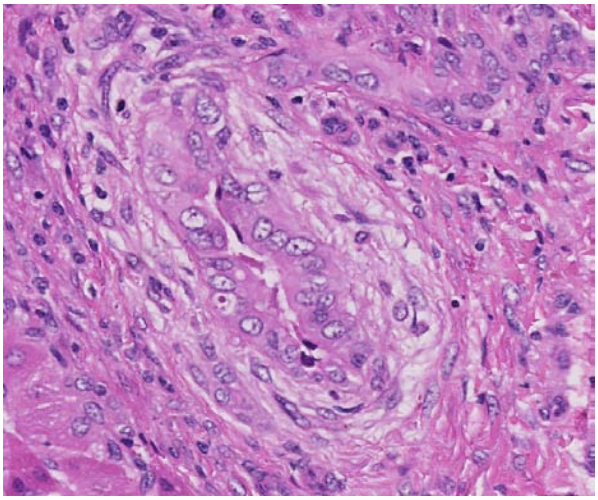
Aflatoxicosis and pyrrolizidine alkaloid toxicity are less commonly associated with photosensitivity in sheep and are considered less likely causes in this case. Of the numerous types



4-4. Liver, sheep: Portal triads are expanded by marked biliary reduplication, and mild fibrosis which often breaches the limiting plate and bridges to adjacent portal areas. (HE 118X)

of aflatoxin reported, the most common is aflatoxin B1, which is typically produced by *Aspergillus* sp. Following metabolism by hepatic cytochrome p450 enzymes, in species that lack adequate glutathione-s-transferase, toxic metabolites cause multiple carcinogenic, toxic and teratogenic effects. In the liver, histological features include hepatocellular necrosis in acute cases, and hepatic fibrosis, hepatocellular megalocytosis and biliary hyperplasia in more chronic cases. Pyrrolizidine alkaloids from *Senecio, Crotalaria* and *Heliotropium* sp. are metabolized via hepatic cytochrome p450 enzymes into dehydropyrrolizidine (DHP) derivatives, which cause similar hepatic lesions to those described for aflatoxicosis. Sheep are thought to be relatively resistant to both aflatoxin and pyrrolizidine alkaloid toxicity; cattle, horses, farmed deer, and pigs are most susceptible.[1,5]

Photosensitization is generally classified into three broad categories: types 1, 2 and 3 (see included table). Type 1, or primary photosensitization occurs following ingestion of preformed photodynamic toxins, such as hypericin in St. John's Wort, fagopyrin in buckwheat, and certain drugs, including phenothiazine and tetracycline. Type 2 photosensitization is due to congenital enzyme deficiencies resulting in endogenous pigment accumulation. Bovine congenital hematopoietic porphyria results from deficient levels of uroporphyrinogen III cosynthetase, a key enzyme in heme biosynthesis. Porphyrins subsequently accumulate in dentin and bone, causing the teeth and bone to appear pink and fluoresce upon exposure to ultraviolet radiation. Porphyrins also accumulate in the skin, where they cause necrosis, likely via induction of reactive oxygen species or xanthine oxidase. Affected cattle are anemic, and the accumulated pigments are excreted in the urine, which appears brown. Bovine erythropoietic protoporphyria, on the other hand, is an autosomal recessive condition in Limousin cattle caused by a defect in ferrochelatase, which allows accumulation of protoporphyrin IX in the blood and tissue. This disease is characterized solely by the presence of photodermatitis. There is no anemia, and the teeth, bones and urine are not discolored. Facial eczema, as demonstrated in this case, is associated with type 3, or hepatogenous, photosensitization. This is the most common form. It occurs in conjunction with primary hepatocellular damage



4-5. Liver, sheep: Bile ducts are lined by dysplastic epithelium, which exhibits markedly enlarged anisokaryotic nuclei and multinucleated cells. (HE 288X)

(or, less commonly, bile duct obstruction) and is due to impaired hepatic excretion of the potent photodynamic agent, phylloerythrin. Phylloerythrin is a breakdown product of chlorophyll, formed by microbes in the gastrointestinal tract and transported via portal circulation; hepatocytes normally take up phylloerythrin and excrete it into bile. In animals on a chlorophyll-rich diet and generalized hepatic damage, phylloerythrin builds up in various tissues, including the skin. The distribution of the photodermatitis is similar in all types of photosensitization; it is generally confined to sparsely-haired, lightly pigmented, sunlight exposed areas of the skin.[2]

Table: Categories of photosensitization.[2]

Type	Causes
Type 1: Primary	Ingestion of preformed **photodynamic toxins** in plants and drugs: • St. John's Wort (hypericin) • Buckwheat (fagopyrin) • Phenothiazine
Type 2	Congenital **enzyme deficiencies** resulting in endogenous pigment accumulation: • Uroporphyrinogen III cosynthetase deficiency (bovine congenital hematopoietic porphyria) • Ferrochelatase deficiency (bovine erythropoietic protoporphyria)
Type 3: Hepatogenous	Build-up of **phylloerythrin** due to generalized hepatocellular damage or bile duct obstruction

Contributing Institution: Institute of Veterinary, Animal and Biomedical Sciences
Massey University
Tennant Drive, Palmerston North, New Zealand

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