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Conference Moderator:
Gary D. Coleman, DVM, PhD, Diplomate ACVP, Diplomate ACVPM

CASE I: 09A731 (AFIP 3165087).

Signalment: 5.65-year-old, female, Indian macaque (*Macaca mulatta*).

History: Found dead in an outside breeding corral without history of previous illness.

Gross Pathology: The animal was dehydrated with evidence of fecal soiling around the anus. Small intestine and colon were distended with fluid and gas. Contents were blood-tinged and foul smelling. Mesenteric lymph nodes were severely enlarged.

Laboratory Results: Colonic swab cultures contained *Yersinia pseudotuberculosis* and *Campylobacter coli.*

Histopathologic Description: Small intestine: Small intestinal mucosa contains multiple to confluent areas of necrosis and hemorrhage that in some areas extend to the muscularis mucosa. Necrotic foci are filled with neutrophils forming microabscesses with dense microcolonies of extracellular bacteria. Scattered hyalinized eosinophilic deposits around crypts and vessels in the lamina propria demonstrate apple green birefringence when stained with Congo red and observed with polarized light.

Contributor's Morphologic Diagnosis: 1. Enteritis, necrohemorrhagic, suppurative, multifocal, severe, with intralesion bacterial colonies. Etiology: *Yersinia pseudotuberculosis*
2. Amyloidosis, secondary, small intestine and mesenteric lymph node (not submitted)

Contributor's Comment: *Yersinia pseudotuberculosis* is a disease of rodents and birds but has been reported in rabbits, deer, dogs, cats, swine, sheep, goats, chinchillas, horses, non-human primates, man and exotic mammals. In cases where multiple pathogens are isolated, it may be difficult to associate lesions with a specific pathogen. However, necrotizing lesions in the small intestine, obvious bacterial colonization, and severe neutrophilic response are hallmarks of Yersiniosis.[1]

Three species of *Yersinia* are pathogenic for humans and non-human primates: *Y. pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis*. *Yersinia pestis* is transmitted by flea bite, while *Y. enterocolitica* and *Y. pseudotuberculosis* are typically self-limiting enteric infections transmitted by ingestion of contaminated food and water. All three organisms contain a virulence plasmid P (approximately 70 kb) that codes for a set of proteins called YOPS (for Yersinia outer membrane proteins) that are actually secreted virulence factors necessary for replication in host cells.[2] Additional plasmids pMT1 and pPCP1 in *Y. pestis* impart increased virulence.

Yersinia enterocolitica and *Y. pseudotuberculosis* pass through intestinal M cells, replicate in Peyers patches, and, in rodents, frequently disseminate to mesenteric lymph node, spleen, liver, and lungs. Septicemia is rare in humans and non-human primates. Some literature suggests *Yersinia* resist phagocytosis by neutrophils and induce apoptosis of macrophages.[4] Recent work suggests that *Yersinia* can alter antibacterial functions, survive within macrophages, and replicate.[4] Two genes in *Y. enterocolitica*, ompR and gsrA, controlling response to altered osmolarity and heat shock, are associated with survival in macrophages.[5] *Yersinia pseudotuberculosis* and *Y. pestis* may inhibit phagosome acidification,[5] can infect naïve macrophages, and continue to replicate when exposed to interferon gamma. The latter ability may be conferred by a Pgm (102 kb) segment of the bacterial genome that reduces production of nitric oxide (NO) in infected macrophages.[5]

AFIP Diagnosis: Small intestine: Enteritis, necrotizing and suppurative, acute, diffuse, marked, with multifocal hemorrhage, microabscesses, amyloidosis, and colonies of coccobacilli.

Conference Comment: Some participants noted the presence of fibrin thrombi within the submucosal and mesenteric vasculature; this histologic finding is not present on all slides due to section variability.

Each of the three species of *Yersinia* mentioned by the contributor (*Y. pestis, Y. pseudotuberculosis,* and *Y. enterocolitica*) produces a distinct syndrome in various species of animals. *Yersinia pestis* may infect cats in the southwestern United States following consumption of infected rodents. The disease syndrome in cats, like humans, can be bubonic, septicemic and pneumonic; the bubonic form is associated with the lowest mortality rate. In cats the bubonic form occurs most frequently, and affected animals are febrile, dehydrated, have lymphadenomegaly and hyperesthesia. Suppuration of lymph nodes may result in draining fistulous tracts. Delay or failure to treat infected animals increases the risk of developing the septicemic form via lymphatic or hematogenous spread. Histologically, there is marked suppurative and necrotizing lymphadenitis with hemorrhage.[3] The septicemic form results in involvement of nearly all organs and clinical signs of septicemic shock and dissemination intravascular coagulation (DIC); death typically occurs in 1-2 days. The pneumonic form occurs as a primary infection through direct inhalation, or can occur secondary to either the bubonic or septicemic forms. Of the three forms, the pneumonic form carries the worst prognosis, with nearly a 100% fatality rate.[3]

Yersinia pseudotuberculosis produces caseonecrotic foci in mesenteric lymph nodes, spleen and liver of susceptible animals, of which there are many. There is marked necrosis, parenchymal collapse and vasculitis in the liver in addition to multiple pyogenic granulomas visible grossly as variably sized white foci. The spleen and lymph nodes have similar foci, and these organs are enlarged by lymphoid and histiocytic hyperplasia.[7] The organism is also linked to abortions in cattle, sheep and goats; the bacteria localize in the caruncle with passage to the chorioallantois and fetus. The lesions are typically that of placentitis of the cotyledons observed histologically as villar necrosis with granulocytic and histiocytic infiltration of the chorioallantois, and fibrinoid necrosis of the media in placental vessels with mononuclear and neutrophilic infiltrates. Septal vessels in the caruncles are thrombosed, resulting in necrosis and hemorrhage with marked neutrophilic and mononuclear infiltrates. The fetus is often delivered in a good state of preservation, with thoracic and abdominal effusion and foci of hepatic necrosis admixed with granulocytic and mononuclear infiltrates. The myocardium, lymph nodes and conjunctiva may have similar inflammatory infiltrates.[6]

Yersinia enterocolitica is known to cause disease in sheep, cattle, goats, deer and pigs in addition to humans and non-human primates. Gross lesions vary with the severity of infection, with fluid intestinal contents and congestion in mild cases, and edema to hemorrhage, ulceration and fibrin exudation in more severe cases. Histologic lesions include large colonies of coccobacilli in the lamina propria and villi in the distal small intestine and marked neutrophilic infiltration with microabscessation, ulceration, hemorrhage, and occasional pyogranulomas. In addition to enteritis, *Y. enterocolitica* is also associated with caseous mesenteric lymphadenitis.[1]

Contributor: Department of Comparative Pathology, Tulane National Primate Research Center, 18703 Three Rivers Rd, Covington, LA 70433
www.tpc.tulane.edu

References:

1. Brown CC, Barker DC, Barker IK The alimentary system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 5th ed., vol. 2. Philadelphia, PA: Elsevier Ltd; 2007:204-206.
2. Cornelis GR, Boland A, Boyd AP, et al. The virulence plasmid of *Yersinia*, an antihost genome. *Microbiol Molec Bio Rev*. 1998;62(4):1315-1352.
3. Macy D. Plague. In Greene CE, ed. *Infectious Diseases of the Dog and Cat*. Saint Louis, MO: Saunders Elsevier; 2006:439-445.
4. Monack DM, Mecsas J, Bouley D, Falkow S. *Yersinia* induced macrophage apoptosis in vivo aids in the establishment of systemic infection in mice. *J Exp Med*. 1998;188(11):2127-2137.
5. Pujol C, Bliska JB. Turning *Yersinia* pathogenesis models inside out: subversion of macrophage function by intracellular yersiniae. *Clin Immunol*. 2005;114:216-226.
6. Schlafer DH, Miller RB. Female genital system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 5th ed., vol. 3. Philadelphia, PA: Elsevier Ltd; 2007:500.
7. Valli VEO: Hematopoietic system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 5th ed., vol. 3. Philadelphia, PA: Elsevier Ltd; 2007:298-299.

CASE II: WCS20097531 (AFIP 3167495).

Signalment: Juvenile, male, black howler monkey (*Alouatta caraya*).

History: This animal was part of a group of wild howler monkeys from two species, brown (*Alouatta guariba clamitans*) and black (*Aloutatta caraya*), found dead between November 2007- February 2009 in northeastern Argentina. In total, 65 animals were found dead. This particular animal was found dead by people living in the area and was left at a local human health office.

Gross Pathology: The only necropsy finding reported in the animals that were suitable for postmortem examination was mild to moderate yellow discoloration of the mucous membranes, skin and sclera (icterus).

Laboratory Results: Serum, liver and splenic samples from multiple animals examined during the outbreak were positive for Yellow Fever virus by virus isolation, direct immunofluorescence, RT-PCR and genome sequencing.

Histopathologic Description: Liver: Slides contain one sample of liver. Throughout the section there is disruption of the normal liver architecture with diffuse dissociation of the hepatic cords. There is necrosis of most midzonal to centrilobular hepatocytes characterized by rounding of cellular margins, hypereosinophilia of the hepatocyte cytoplasm and deep basophilic staining of the nucleus and loss of cellular detail. Throughout the zones of necrosis there is marked hepatocellular loss, collapse of hepatic cords and the remaining space is filled with erythrocytes. All remaining hepatocytes are swollen with numerous, well demarcated, variably sized, round, clear cytoplasmic vacuoles (microvesicular fatty change). Many hepatocytes contain variably sized hypereosinophilic foci within the cytoplasm (eosinophilic degeneration). Sinusoids adjacent to zones of necrosis contain many shrunken, rounded hypereosinophilic bodies occasionally with an associated small, basophilic, condensed nucleus (Councilman bodies). Some Kupffer cells contain cytoplasmic erythrocytes (erythrophagocytosis) and a small amount of cytoplasmic golden brown, granular pigment (hemosiderin). Occasional portal areas and central veins contain small numbers of lymphocytes, plasma cells and fewer neutrophils. In the background of many sections there are scattered black to brown amorphous microcrystalline granules consistent with acid hematin. These granules are an artifact of tissue fixation that we see in samples collected in the field in unbuffered formalin or paraformaldehyde.

Contributor's Morphologic Diagnosis: Necrosis, acute, midzonal to centrilobular, severe, with parenchymal collapse, hepatic cord dissociation, eosinophilic degeneration and Councilman body formation and diffuse microvesicular hepatocellular fatty change.

Contributor's Comment: This animal is one of a large group of wild howler monkeys of two species that died during a yellow fever outbreak in northeastern Argentina. Serum, liver and splenic samples from numerous animals were positive for yellow fever virus by virus isolation and direct immunofluorescence, followed by RT-PCR for generic flaviviruses and genome sequencing. Limited tissues were available for histologic examination but in all cases the liver demonstrated the classic features of yellow fever virus infection including:

1. Midzonal acute hepatocellular necrosis
2. Eosinophilic degeneration of hepatocytes/Kupffer cells and Councilman body formation
3. Hepatocellular microvesicular fatty change

Additional findings in other organs examined from these cases, but not provided as part of this submission, included acute renal tubular necrosis with evidence of regeneration and hemoglobin casts and marked lymphocytolysis throughout the splenic white pulp and within multiple lymph nodes.

Yellow fever virus is an arbovirus of the genus Flavivirus, family Flaviviridae and is the cause of severe hemorrhagic disease in both humans and non-human primates.[2] The flaviviruses are RNA-viruses and include yellow fever virus, dengue virus, West Nile virus, Japanese encephalitis virus and tick-borne encephalitis virus.[2] Mosquitoes are the primary vector of yellow fever virus and include *Aedes aegypti* in urban areas, *Aedes albopictus* in suburban areas and tree-hole-breeding mosquitoes (*Haemagogus spp*.) in forests.[3] *Aedes aegypti* are found throughout most tropical to subtropical regions of the world including much of the United States. *Haemagogus* spp. are found primarily in the forests of central and South America.[2,8] Currently, yellow fever virus distribution is limited to sub-Saharan Africa and Central and South America. Maintenance of the yellow fever virus can occur in a

sylvatic cycle between non-human primates and forest mosquitoes or in an urban cycle between humans and *Aedes aegypti* or *albopictus*. Humans and non-human primates in these situations serve as temporary amplifiers while mosquitoes remain infected for life and serve as the reservoir of infection.[2,8]

While all primates are susceptible to yellow fever virus infection, new world monkeys are most susceptible, likely due to their evolution in the absence of the virus.[3] In particular spider, wooley and howler monkeys are extremely sensitive and infection is almost always fatal. In yellow fever endemic areas, mortality in highly susceptible howler monkeys can act as an early warning signal for the presence of the virus.[3] In this particular mortality event, after the death of the first four howler monkeys, the National Health Authority of Argentina was alerted and a massive human vaccination campaign against yellow fever was carried out in the surrounding areas.

The characteristic midzonal hepatocellular necrosis seen in yellow fever is reflected clinically in humans and non-human primates as severe jaundice.[2,8] It has been suggested that the zonal nature of this lesion is due to hypoxia secondary to terminal shock; however, yellow fever antigen and RNA have been demonstrated in midzonal hepatocytes.[6] Macrophages are the principle site of initial viral replication and support the rapid spread of infection. The innate immune response is thought to contribute significantly to the pathogenesis of yellow fever infection with the release of cytokines from infected macrophages and dendritic cells disrupting normal vascular function and promoting coagulation.[7] Hepatic damage is proposed to be one of the mechanisms of hemorrhage in yellow fever infection due to a reduction in synthesis of coagulation factors in combination with consumptive coagulopathy.[2]

Eosinophilic degeneration and Councilman body formation are consistent histological features of yellow fever virus infection in both humans and non-human primates. They do not represent viral inclusions, but instead are the result of cell injury.[2] Councilman bodies begin as areas of eosinophilic degeneration within hepatocytes or Kupffer cells due to injury to the endoplasmic reticulum. These foci of condensed cytoplasm then detach from the wall of the sinusoid (if they are of Kupffer cell origin) or from the hepatocellular cord (if they are of hepatocyte origin). In some cases the condensed cytoplasmic material carries along a nuclear remnant. Within the sinusoid, the Councilman body either lies free or is phagocytosed by a Kupffer cell.[2] The cytoplasmic eosinophilic degeneration and condensed nuclear chromatin of these Councilman bodies is most consistent with cellular apoptosis. Virally-induced apoptosis is thought to contribute to hepatocellular death.[6] While this mechanism has not been thoroughly investigated for yellow fever virus, West Nile virus has been shown to trigger apoptosis *in vitro* via the intrinsic pathway.[1] Hepatic lesions in yellow fever have a paucity of inflammation and heal in surviving patients with minimal fibrosis, a finding that is argued to support apoptosis over necrosis as a primary mechanism for cell death. Microvesicular lipidosis was consistently seen in remaining hepatocytes in the livers of these monkeys. This is also described as a constant finding in human cases.[2]

In addition to the severe hepatic damage, yellow fever virus can also result in acute renal failure. Acute renal tubular necrosis is reported in severe human cases of yellow fever[2] and was seen in several of the black howler monkeys examined as part of this outbreak. Again, the tubular necrosis is thought to be the result of hypoxic damage compounded by hemoglobinuria; however, viral antigen has also been demonstrated in renal tubular epithelial cells.[5] These renal lesions are also associated with a paucity of inflammation and an apoptotic mechanism has been suggested as a primary mechanism but to date has not been proven.

From a conservation perspective, yellow fever outbreaks can have devastating effects on howler monkey populations. The initial outbreak in this case affected four groups of howler monkeys that had been under study since 2005, all of which are believed to have died. Brown howler monkeys are endangered in Argentina due to habitat loss, fragmentation and hunting pressures. Currently yellow fever virus is also feared to put these populations at additional risk.[3] An unfortunate added risk to howler monkeys in yellow fever outbreaks is the misconception that they are the cause of human infection, rather than mosquitoes, which has led to campaigns of poisoning during human outbreaks of the disease.

AFIP Diagnosis: Liver: Hepatocellular degeneration and necrosis, centrilobular to midzonal, diffuse, severe, with hepatocellular disassociation, lipid type microvacuolar change, and Councilman bodies.

Conference Comment: As noted by the contributor, hemorrhage and massive hepatic damage often result in consumptive coagulopathy, also referred to as disseminated intravascular coagulation (DIC). Clotting is initiated by release of tissue factor (i.e. tissue thromboplastin or factor VII) beginning the extrinsic coagulation pathway; or activation of factor XII after contact with collagen, which commences the intrinsic pathway. Both pathways enter the common pathway, converting prothrombin to thrombin which converts fibrinogen to fibrin. As thrombin is

carried away from the site of clotting, it binds to endothelial cell thrombomodulin and becomes an anticoagulant. The thrombin-thrombomodulin complex activates protein C, which in turn inhibits procoagulant factors V and VII. Activated coagulation factors are removed from circulation by the liver.[4]

Disseminated intravascular coagulation can be triggered by the release of tissue factor or thromboplastic substances into the circulation and/or by marked endothelial damage. Damaged endothelial cells release tumor necrosis factor (TNF), which promotes coagulation via increased tissue factor expression and decreased thrombomodulin expression on endothelial cells and up-regulation of adhesion molecules for leukocytes, which themselves can damage the endothelium. Widespread activation of the coagulation cascade results in fibrin thrombi lodging in the microvasculature, leading to ischemia and erythrocyte fragmentation referred to as microangiopathic hemolytic anemia. Additionally, there is massive consumption of platelets, clotting factors and plasminogen resulting in hemorrhagic diathesis.[4]

Several tests are available and widely used for clinical assessment of coagulation including activated clotting time (ACT), activated partial thromboplastin time (APTT), and prothrombin time (PT). The chart below summarizes the key points of each assay:[9]

	ACT	**APTT**	**PT**
What is measured	Time for fibrin clot to form in fresh whole blood after addition of contact activator	Time for fibrin clot formation in citrated plasma after addition of an activator of the intrinsic pathway	Time for fibrin clot formation in citrated plasma after addition of thromboplastin (factor III) and calcium
Factors/pathways assessed	Intrinsic (PK, HMWK, factors XII, XI, IX, VII) and common pathways (factors X, V, II, fibrinogen)	Intrinsic (factors XII, XI, IX, VII) and common pathways (factors X, V, II, fibrinogen)	Extrinsic (factor VII) and common pathway (factors X, V, II, fibrinogen)
Conditions associated with prolonged times	Thrombocytopenia, factor deficiency <5% normal activity, anticoagulants, coagulation inhibitors (FDPs)	Hemophilia (factor VIII or IX), hereditary factor XII deficiency, hereditary PK deficiency, DIC, vitamin K factor deficiency secondary to rodenticide intoxication, bile stasis and liver failure	Inherited factor VII deficiency, DIC, acquired vitamin K factor deficiency

PK: prekallikrein; HMWK: high molecular weight kininogen; FDP: fibrin degradation products

In DIC, ACT, APTT and PT are all prolonged; additionally, d-dimers are routinely used to diagnose DIC.[9]

Contributor: Wildlife Conservation Society, Global Health Program – Pathology, 2300 Southern Boulevard, Bronx, NY 10460
http://www.wcs.org

References:

1. Chu JJH, Ng ML. The mechanism of cell death during West Nile virus infection is dependent on initial infectious dose. *J Gen Virol*. 2003;84: 3305-3314.
2. del Rio C, Meier FA. Yellow fever. In: Nelson AM, Horsburgh CR eds. *Pathology of Emerging Infections*. Washington DC: American Society for Microbiology; 1998:13-41.
3. Holzmann I, Agostini I, Areta JI, Ferreyra H, Beldomenico P, diBitetti MD. Impact of yellow fever outbreaks on two howler monkey species (*Alouatta guariba clamitans* and *A. caraya*) in Misiones, Argentina. *Am J Primatol*. 2010;72:475-480.
4. Kumar V, Abbas AK, Fausto N, Aster JC. Red blood cell and bleeding disorders. In: Kumar V, Abbas AK, Fausto N, Aster JC, eds. *Robbins and Cotran Pathologic Basis of Disease*. 8th ed. Philadelphia, PA: Elsevier Saunders; 2009:673-674.
5. Lima EQ, Nogueira ML. Viral hemorrhagic fever-induced acute kidney injury. *Sem Nephrol*. 2008;28(4) 409-415.
6. Monath TP. Yellow fever: an update. *Lancet Infect Dis*. 2001;1:11-20.
7. Pastorino B, Nougairede A, Wurtz N, Gould E, de Lamballerie, X. Role of host cell factors in flavivirus infection: Implications for pathogenesis and development of antiviral drugs. *Antiviral Res*. 2010;doi:10.1016/j.antiviral. 2010.04.014.

8. Sallis ESV, Souza de Barros VLR, Garmatz SL, Fighera RA, Graca DL. A case of yellow fever in a brown howler (*Alouatta fusca*) in Southern Brazil. *J Vet Diagn Invest*. 2003;15:574-576.
9. Topper MJ, Welles EG. Hemostasis. In: Latimer KS, Mahaffey EA, Prasse KW, eds. *Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology*. 4th ed. Ames, IA: Blackwell Publishing; 2003:126-133.

CASE III: 10-5392-2 (AFIP 3167230).

Signalment: 10-year-old, neutered, male, domestic short hair, cat (*Felis catus*).

History: A 10-year-old previously healthy male neutered domestic short hair cat from Oregon was examined after it had shown labored breathing of one day's duration. The indoor/outdoor cat had an unknown vaccination status and no previous health problems. Radiographs showed pneumonia with air bronchograms in caudal lung lobes and mixed interstitial alveolar pattern. The cat's breathing difficulty worsened and it repeatedly received oxygen therapy. A second radiograph two days later showed consolidation of the ventral aspect of the cranial lung lobes. The cat died on the subsequent day.

Gross Pathology: Gross examination of the thoracic cavity by the practitioner revealed pneumonia affecting approximately three fourths of the lung field. No other lesions were noted in the thoracic cavity. Nasal secretions and formalin-fixed lung tissue collected from the cat were submitted to the Veterinary Diagnostic Laboratory (VDL) at Oregon State University. All lung samples received at the VDL were diffusely firm, dark and poorly collapsed. One piece had, on cut section, an enhanced peribronchiolar pattern.

Laboratory results: Nasal secretions tested positive for the influenza A matrix gene and neuraminidase 1 gene of pandemic (H1N1) 2009 influenza virus by rRT-PCR at the VDL at Oregon State University. The results were confirmed by the USDA's National Veterinary Services Laboratory in Ames, Iowa. Common feline respiratory viruses were not isolated from the nasal swab. Fluorescent antibody (FA) of smears generated from scraped cell cultures for feline herpesviral and feline caliciviral antigen was negative.

Histopathologic Description: Lung: Lesions may vary slightly among sections. In most bronchioles, there is complete or segmental epithelial necrosis; multinucleated cells are occasionally observed. Areas lacking epithelium are covered with fibrin. Bronchiolar lumens contain a few macrophages and sloughed epithelial cells and small amounts of cellular debris. Where present, bronchiolar epithelium is attenuated or appears normal on light microscopy. Alveolar lumens are flooded with wispy to dense protein-rich material (occasionally in the form of hyaline membranes) and is admixed with small to moderate numbers of macrophages and desquamated type II pneumocytes, scattered neutrophils, and rare multinucleated giant cells. In approximately half of the sections some peribronchiolar alveoli have cuboidal epithelial cells (type II pneumocyte hyperplasia) and/or scattered multinucleated cells. There is also multifocal epithelial necrosis or loss, and lumens are occasionally occluded by dense fibrin accumulations. Lesions not present in sections included multifocal, mild, lymphoplasmacytic to histiocytic bronchitis and minimal epithelial degeneration of bronchial mucosal glands; multifocal, perivascular, mild to moderate edema; and mild, multifocal, pleural, mesothelial hypertrophy and hyperplasia.

Immunostaining for influenza virus antigen was performed. A few of the bronchioles with segmental necrosis had individual or small groups of positive, intact, non-ciliated or necrotic epithelial cells adjacent to negative epithelium with normal morphology. Intense staining traced the surface of some non-ciliated bronchiolar epithelial cells. Denuded bronchiolar surfaces showed irregular, linear to clumped staining. In some bronchiolar and alveolar lumens, cellular debris had intensely staining globules. Individual to small groups of positive-reactive cells were observed in peribronchiolar alveoli with type II pneumocyte hyperplasia. In alveoli with acute damage there was intense staining of some pneumocytes and/or luminal macrophages or sloughed pneumocytes.

Contributor's Morphologic Diagnosis: Severe, diffuse, acute to subacute, bronchointerstitial, fibrino-necrotizing pneumonia; some sections also had multifocal, severe type II pneumocyte hyperplasia of peribronchiolar alveoli.

Contributor's Comment: In the past, cats were considered relatively resistant to influenza virus infections.[3] In recent years this notion was changed by reports of respiratory and systemic disease in domestic cats after experimental and natural infections with highly pathogenic avian influenza virus (HPAIV) H5N1.[7,8,15,16] Infection of cats with HPAIV H5N1 can result in bronchointerstitial pneumonia with epithelial necrosis and fibrin exudation in bronchioles and alveoli. The pneumonia in cynomolgus monkeys, BALB/c mice, miniature pigs, and ferrets after

experimental and natural infections with pandemic (H1N1) 2009 influenza virus, and in human patients with lethal swine origin influenza virus (SOIV) infections presents similarly,[6,10,12,14] as did the pneumonia in the cat in this report.[9] The main infectious differential etiologies for acute bronchointerstitial pneumonia in cats are feline herpesvirus and calicivirus,[2] which were not isolated from the nasal swab of this cat.[9]

When viral infections and lesions extend to involve alveoli, there is alveolitis with sero-fibrinous to neutrophilic exudate. It has been observed in domestic cats naturally infected with HPAIV H5N1, ferrets, and mice infected with pandemic (H1N1) 2009 influenza virus, and human patients with lethal pneumonia due to pandemic (H1N1) 2009 influenza virus, [6-10,12,14-16] and was also present in this cat.[9]

It is notable that neither necrosis nor viral antigen was observed in bronchial epithelium in the cat, which can be seen in human patients with lethal pandemic (H1N1) 2009 influenza virus-associated pneumonia, and in ferrets experimentally infected with pandemic (H1N1) 2009 influenza virus.[6,10-12,14] The type II pneumocyte hyperplasia in this cat may have been due to damage to the respiratory epithelium caused by the acute viral infection, rather than a preexisting condition, as time course was prolonged enough.[2] Co-infections with bacteria are surprisingly uncommon in lethal pandemic (H1N1) 2009 influenza virus infections in humans.[1] Unfixed lung tissue was not available for bacterial culture, but a deep bronchial swab taken from this cat during postmortem examination did not yield bacterial growth.[9]

Transmission of pandemic 2009 (H1N1) influenza virus from a family member to the cat is highly likely in the case presented here. The owner of the cat and single household member had severe influenza-like illness (ILI). Pandemic 2009 (H1N1) influenza was confirmed by PCR testing at a hospital. The cat in this report had no known contact with other animals with respiratory disease due to pandemic (H1N1) 2009 influenza virus. Transmission from an owner was recently implicated in pandemic 2009 (H1N1) infection in a cat,[17] and transmission of Hong Kong pandemic influenza (H3N2) virus from a human patient to two domestic cats has been documented in the literature.[13]

AFIP Diagnosis: Lung: Pneumonia, bronchointerstitial, fibrinonecrotizing, acute to subacute, diffuse, severe, with alveolar edema.

Conference Comment: This case was studied in consultation with the AFIP Department of Pulmonary and Mediastinal Pathology, whose pathologists indicated that most of the pathology in the lung of this cat is attributed to viral infection. They also commented that, given the diffuse involvement of alveoli, diffuse alveolar damage (DAD) is an appropriate histologic interpretation in this case, despite the paucity of characteristic hyaline membranes, and indicated that oxygen therapy may have been contributory. During conference, several participants also observed histologic features suspicious for acute alveolar damage, including alveolar septal necrosis, fibrin, and edema, although none observed organized hyaline membranes lining alveolar walls. Upon disclosure of the clinical history, conference participants agreed that oxygen therapy may have exacerbated the alveolar wall lesion induced by viral infection, and concluded this case may represent an example of acute lung injury (ALI), which is a complication of diverse or multiple contributory predisposing conditions manifested histologically as DAD.

Oxygen therapy/toxicity is one of a number of primary or contributory causes of ALI, although the molecular basis by which oxygen causes the pulmonary lesion is not completely understood; the current favored hypothesis is capillary endothelial and type I pneumocyte damage due to reactive oxygen species.[2] Early in the course of ALI, there is up-regulation of IL-8 by pulmonary macrophages; IL-8 then attracts neutrophils to the lung, and also serves as a potent neutrophil activator. Together with IL-1 and TNF, IL-8 activates endothelial cells and neutrophils, and causes neutrophil sequestration in the pulmonary microvasculature.[5] Release of cytotoxins, chemokines and cytokines from neutrophils contributes to and enhances the inflammatory damage to alveoli. As the attack on the endothelium and alveolar epithelium continues, there is increased vascular permeability and loss of surfactant, both of which contribute to the loss of the ability of the alveoli to expand. The exudation of fibrin and edema, admixed with necrotic epithelial and inflammatory cells, contributes to the formation of hyaline membranes.[5]

The histologic appearance of DAD progresses through three phases: acute exudative phase, subacute proliferative phase, and chronic fibrosing phase. Congestion and edema of alveolar septa,with infiltration of neutrophils and macrophages, characterize the acute exudative phase, although hyaline membrane formation is typically considered the hallmark histologic finding. With loss of type I pneumocytes, the type II pneumocytes spread out to cover the denuded areas, and by 2-3 days post-injury type II pneumocyte proliferation is evident; it peaks at six days. The final phase, interstitial fibrosis, occurs via two pathways. First, fibroblasts invade affected alveoli and organize into fibrous tissue akin to granulation tissue, which is then incorporated into the alveolar septa and covered by type II

pneumocytes. Second, continued damage to endothelial and epithelial cells and inflammation within alveolar septa can recruit fibroblasts via TGF-β, resulting in direct interstitial fibrosis which is often well-organized by day 14.[2]

As noted by the contributor, the immunohistochemical profile included staining not only of bronchiolar epithelium but also of type II pneumocytes and sloughed, necrotic alveolar epithelial cells. Interestingly, Herfst *et al* produced strikingly similar lesions and immunohistochemical staining profiles (i.e. type I and II pneumocytes) in experimentally-infected cynomolgus macaques.[3]

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Contributor: Veterinary Diagnostic Laboratory, Department of Biomedical Sciences, Oregon State University, Magruder 144, PO Box 0429, Corvallis, OR 97331-0429, USA
http://oregonstate.edu/vetmed/diagnostic

References:

1. Centers for Disease Control and Prevention (CDC). Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) - United States, May-August 2009. MMWR Morb Mortal Wkly Rep 58(38):1071-1074, 2009.
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; 2007:523-653.
3. Herfst S, van den Brand JMA, Schrauwen EJA et al. Pandemic 2009 H1N1 influenza virus causes diffuse alveolar damage in cynomolgus macaques. *Vet Pathol*. 2010;47(6):1040-1047.
4. Hinshaw VS, Webster RG, Easterday BC, Bean WJ Jr. Replication of avian influenza A viruses in mammals. *Infect Immun*. 1981;34:354-361.
5. 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; 2009:680-682.
6. Itoh Y, Shinya K, Kiso M, et al. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. *Nature*. 2009;460:1021-1025.
7. Klopfleisch R, Wolf PU, Uhl W, et al. Distribution of lesions and antigen of highly pathogenic avian influenza virus A/Swan/Germany/R65/06 (H5N1) in domestic cats after presumptive infection by wild birds. *Vet Pathol*. 2007;44:261-268.
8. Kuiken T, Rimmelzwaan G, van Riel D, et al. Avian H5N1 influenza in cats. *Science*. 2004;306:241.
9. Löhr CV, DeBess EE, Baker RJ, et al. Pathology and viral antigen distribution of lethal pneumonia in domestic cats due to pandemic (H1N1) 2009 influenza A virus. *Vet Pathol*. 2010;47:378-386.
10. Mauad T, Hajjar LA, Callegari GD et al. Lung pathology in fatal novel human influenza A (H1N1) infection. *Am J Respir Crit Care Med*. 2010;181:72-79.
11. Memoli MJ, Tumpey TM, Jagger BW, et al. An early 'classical' swine H1N1 influenza virus shows similar pathogenicity to the 1918 pandemic virus in ferrets and mice. *Virology*. 2009;393:338-345.
12. Munster VJ, de Wit E, van den Brand JM, et al. Pathogenesis and transmission of swine-origin 2009 A (H1N1) influenza virus in ferrets. *Science*. 2009;325:481-483.
13. Paniker CKJ, Nair CMG. Infection with A2 Hong Kong influenza virus in domestic cats. *Bull World Health Org*. 1970;43:859-862.
14. Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, et al. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. *N Engl J Med*. 2009;361:680-689.
15. Rimmelzwaan GF, van Riel D, Baars M, et al. Influenza A virus (H5N1) infection in cats causes systemic disease with potential novel routes of virus spread within and between hosts. *Am J Pathol*. 2006;168:176-183.
16. Songserm T, Amonsin A, Jam-on R, et al. Avian influenza H5N1 in naturally infected domestic cat. *Emerg Infect Dis*. 2006;12:681-683.
17. Sponseller BA, Strait E, Jergens A, et al. Influenza A pandemic (H1N1) 2009 virus infection in domestic cat. *Emerg Infect Dis*. 2010;16:534-537.

CASE IV: BA600/09 (AFIP 3165076).

Signalment: 5-year-old, female, Thoroughbred cross, equine (*Equus caballus*)

History: On the morning of presentation to the Royal (Dick) School of Veterinary Studies this mare had been found reluctant to move her limbs. Upon arrival at the school's large animal hospital she was recumbent with dilated pupils, reduced pupillary light reflex, decreased tongue tone and increased respiratory rate.

Gross Pathology: There was multifocal ecchymotic haemorrhage on the entire surface of the diaphragm, extending into the internal diaphragmatic musculature on cut section. The pelvic musculature also contained multifocal areas of ecchymosis which were particularly obvious in the muscle of the tail head and the gluteal muscles. The pelvic muscles were also slightly paler than normal. The urine was dark brown.

Laboratory Results: AST and CK levels were dramatically elevated and serum required a 1:200 dilution to obtain measurable levels (off the scale in undiluted serum). AST was 16,690U/L and CK was 506,680 U/L (reference ranges 258-554 and 150-385, respectively). The mare was humanely killed due to poor prognosis.

Histopathologic Description: Skeletal muscle, hind limb: One longitudinal section is examined. There is marked multifocal and segmental myofiber necrosis, characterized by myofiber swelling, hypereosinophilia and loss of cross striations. The sarcoplasm is often glassy, consistent with Zenker's-type degeneration, and there are myofibers with contraction bands. Many have fragmented, smudged, granular, flocculent, "moth-eaten" or shredded, ribbon-like sarcoplasm. Very occasionally, small numbers of neutrophils are interspersed within necrotic myofibers.
Kidney: Multiple tubules contain intraluminal deeply eosinophilic, granular material (myoglobin). There are small numbers of lymphocytes and plasma cells in the interstitium. There is moderate autolysis.

Contributor's Morphologic Diagnosis: 1. Moderate to severe multifocal acute myofiber necrosis with mild neutrophilic inflammation - skeletal muscle, hind limb – equine
2. Moderate multifocal intratubular myoglobin pigment (myoglobinuric nephrosis) – kidney - equine

Contributor's Comment: The gross and histological findings, together with the signalment and clinical history, were considered to be consistent with equine atypical myopathy with secondary myoglobinuric nephrosis.

Equine atypical myopathy (AM) is an acute, frequently fatal rhabdomyolysis that is not associated with exertion or abnormal polysaccharide storage, and is characterixed clinically by weakness, stiffness, and recumbency. Atypical myopathy (previously known as atypical myoglobinuria) has been recognized since the mid-twentieth century; however, recently the number of reported cases has increased and it is now recognized as an emerging disease.[1,4,12] Cases have been reported in many European countries and reports of a seasonal pasture myopathy in the USA bear many similarities to AM.[3,12]

Cases of AM most commonly occur during autumn, with fewer cases in spring. Although the disease is not contagious, several horses on the same pasture may be affected, indicating the tendency for pasture-related factors to predispose to disease. Affected horses are usually young (<3 years old), in normal to poor body condition, and grazing poor quality or bare pasture.[1,12,13] Management practices and pasture characteristics associated with increased risk of disease include permanent pasture, manure spreading, high humidity, sloping pastures, accumulation of dead leaves, and the presence of a waterway.[12] Some epidemiological factors of AM are shared by equine dysautonomia (equine grass sickness) and cases of horses being affected by both diseases concurrently have been reported.[10]

Clinical signs of AM are largely due to degeneration of postural and respiratory muscles, and include stiffness, muscular weakness, recumbency and dyspnea. The onset of clinical signs is rapid, and animals are frequently found recumbent or dead.[12,15] The most consistent laboratory finding is markedly increased levels of creatine kinase (CK), which may reach >100,000 iu/L.[11] Hypocalcemia and hyperglycemia are commonly present, although other electrolyte imbalances are not usually evident, in contrast to exercise-induced rhabdomyolysis.[12] Myoglobinuria is frequently present, except in those animals which have died early in the course of disease. Affected animals often have a distended bladder and are unable to urinate normally; however, blood urea nitrogen and creatinine values are frequently within normal limits, indicating normal renal function.[4,5,11,13]

At necropsy, areas of pallor are present within the postural and respiratory musculature, in particular the intercostal muscles, diaphragm, neck and shoulder musculature, and to a lesser extent the muscles of the back and hind quarters. Cardiac muscle may also be affected; however, this is not a consistent finding.[12,15] Histologically, there is multifocal, monophasic myodegeneration, consistent with Zenker's degeneration/necrosis. Type I myofibers are predominantly affected.[2,7]

Staining with periodic-acid-Schiff has failed to indicate increased or abnormal cytoplasmic glycogen or polysaccharides within affected fibers, whereas staining for lipid has revealed abnormal accumulations of neutral fat within myofibers, and staining for nicotinamide adenine dinucleotide (NADH) reductase and succinate dehydrogenase (SDH) in myofibers has highlighted a weak oxidative potential in these cells.[2,7] Recently, a study identified multiple deficiencies of several mitochondrial dehydrogenases, including those involved in β-oxidation of fatty acids. It is speculated that such deficiencies may be possible etiological factors in AM.[14] A separate study has identified the lethal toxin of *Clostridium sordellii* as a possible trigger or lethal factor in this disease.[8]

AFIP Diagnosis: 1. Skeletal muscle: Myocyte degeneration and necrosis, acute, multifocal, moderate, with contraction bands.
2. Kidney: Cortical tubular degeneration and necrosis, acute, multifocal, moderate, with cortical and medullary intratubular eosinophilic granular casts (myoglobin).

Conference Comment: Skeletal muscle degeneration and necrosis often involve different portions of the myofiber dependent on the amount of injury involved, and classically there are four ways to classify skeletal muscle necrosis:[9]

- Focal monophasic: isolated, single mechanical injury
- Multifocal monophasic: single event with widespread involvement with all affected myofibers exhibiting the same change often due to toxins or metabolic defects
- Focal polyphasic: isolated lesion with affected myofibers in different stages of alteration due to repeated trauma
- Focal polyphasic: widespread involvement of myofibers over an extended period of time resulting in different stages of reaction, which can be due to genetic disease or nutritional deficiencies.

In this case, conference participants agreed with the contributor in classifying the lesion as multifocal monophasic degeneration and necrosis based on the extent and relative uniformity of the lesion.

In discussing the renal lesion, participants were impressed with the tubular changes. The changes are consistent with acute tubular necrosis, the cause of which appears to be shock and ischemic necrosis. As noted by the moderator, myoglobin is not directly toxic to the renal tubules; however, during periods of hypotension it contributes to necrosis.[6] Tubulorrhexis is an important finding that implies disruption of the basement membrane, which affects reparative capability as regenerating epithelial cells lack the necessary scaffolding.

Contributor: Veterinary Pathology Unit, Division of Veterinary Clinical Sciences, Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, EH25 9RG, UK

References:
1. Bowen JN, Craig JF. Myoglobinuria in horses. *Vet Rec*. 1942;35:354-355.
2. Cassart D, Baise E, Cherel Y, et al. Morphological alterations in oxidative muscles and mitochondrial structure associated with equine atypical myopathy. *Equine Vet J*. 2007;39:26-32.
3. Finno CJ, Valberg SJ, Wunschmann A, Murphy MJ. Seasonal pasture myopathy in horses in the midwestern United States: 14 cases (1998–2005). *J Am Vet Med Assoc*. 2006;229:1134-1141.
4. Harris P, Whitwell K. Atypical myoglobinuria alert. *Vet Rec*. 1990;127: 603.
5. Hosie BD, Gould PW, Hunter AR, Low JC, Munro R, Wilson HC. Acute myopathy in horses at grass in east and south east Scotland. *Vet Rec*. 1986;119:444-449.
6. Maxie MG, Newmann SJ. Urinary system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol. 2, 5th ed. Philadelphia, PA: Elsevier Ltd; 2007:466-467.
7. Palencia P, Rivero JLL. Atypical myopathy in two grazing horses in northern Spain. *Vet Rec*. 2007;161:346-348.
8. Unger-Torroledo L, Straub R, Lehmann AD, et al. Lethal toxin of *Clostridium sordellii* is associated with fatal equine atypical myopathy. *Vet Microbiol*. 2010;144:487-492.
9. Van Vleet JF, Valentine BA. Muscle and tendon. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol. 1, 5th ed. Philadelphia, PA: Elsevier Ltd; 2007:198-199.
10. Vercauteren G, van der Heyden S, Lefère L, Chiers K, Laevens H, Ducatelle R: Concurrent atypical myopathy and equine dysautonomia in two horses. *Equine Vet J*. 2007;39:463-465.
11. Votion DM, Linden A, Saegerman C, et al. History and clinical features of atypical myopathy in horses in Belgium (2000–2005). *J Vet Intern Med*. 2007;21:1380-1391.

12. Votion DM, Serteyn D. Equine atypical myopathy: a review. *Vet J*. 2008178:185-190.
13. Votion DM, Linden A, Delguste C, et al. Atypical myopathy in grazing horses: A first exploratory data analysis. *Vet J*. 2009;180:77-87.
14. Westermann CM, Dorland L, Votion DM,et al. Acquired multiple acyl-CoA dehydrogenase deficiency in 10 horses with atypical myopathy. *Neuromuscul Disord*. 2008;18:355-364.
15. Whitwell KE, Harris P, Farrington PG. Atypical myoglobinuria: an acute myopathy in grazing horses. *Equine Vet J*. 1988;20:357-363.