Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2025-2026

Conference #13 — 26 November 2025

CASE I:

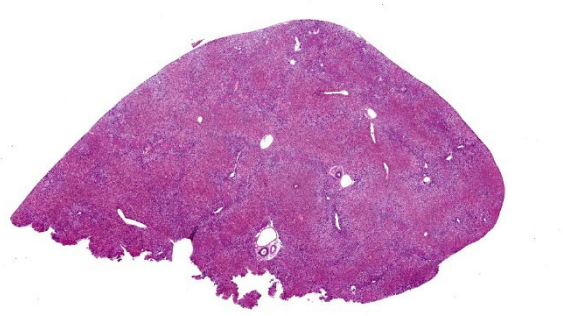
Signalment: 1.5-year-old male New Zealand white rabbit (*Oryctolagus cuniculus*)

History: This 1.5-year-old rabbit (R-857) was found dead in his cage on 4/25/2023. He was on a behavioral study with a high cholesterol diet. An autopsy was performed, and formalin fixed liver, heart, and kidney were submitted to the Michigan State University Veterinary Diagnostic Laboratory.

Gross Pathology: Veterinarian who performed the autopsy noted the liver was diffusely dark green and stomach contained food but was not distended.

Laboratory Results: Not applicable.

Microscopic Description: Approximately 60% of the hepatocytes in mostly periportal and centrilobular regions are markedly expanded by high numbers of microvesicular vacuolations (steatosis/lipid) and more rarely macrovesicular vacuolations or a mixture of the two. Sinusoids contain increased myofibroblasts (activated hepatic stellate cells) causing multifocal collapse of hepatocellular cord architecture. The myofibroblasts extend near portal areas, but distinct biliary hyperplasia is not appreciated. Occasionally hepatocytes are dissociated and composed of hypereosinophilic cytoplasm and lack a nucleus (necrosis). There are occasional binucleated hepatocytes. Canaliculi are multifocally, mildly expanded by tan

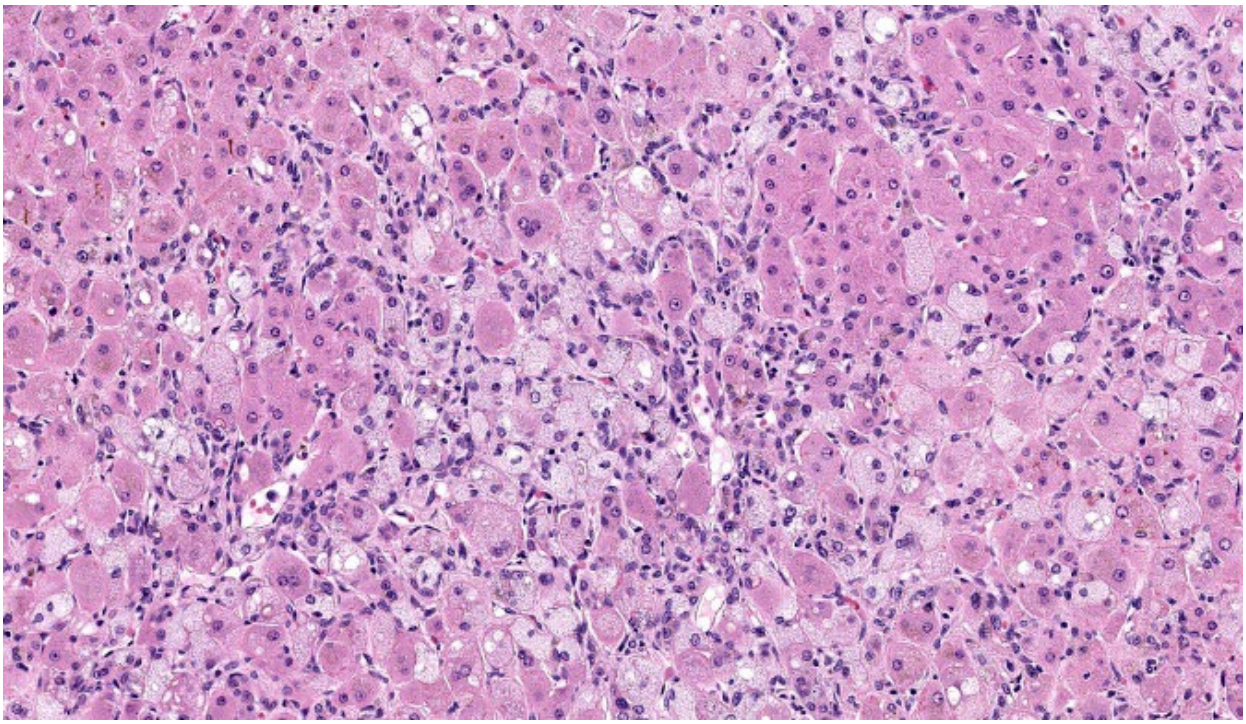


Liver, rabbit: One section of liver is submitted for examination. At subgross magnification, there are retiform patterns of pallor within the section. (HE, 21X)

to brown material (bile). Very rare lymphocytes are within sinusoids and in portal areas.

Contributor's Morphologic Diagnoses: Severe, chronic, multifocal to coalescing microvesicular and macrovesicular steatosis with sinusoidal fibrosis and cholestasis

Contributor's Comment: Hepatic steatosis or lipidosis is a common sequela to high cholesterol diets in rabbits, including New Zealand white rabbits.[6] Rabbits are highly susceptible to hypercholesterolemia and subsequent atherosclerosis and hepatic steatosis.[1,6] Hypercholesterolemia and hyperlipidemia also occur in rabbits affected by inflammatory processes and disease, without a high cholesterol diet.[9] In rabbits, hypercholesterolemia is accompanied by increased circulating very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL).[1] The VLDL

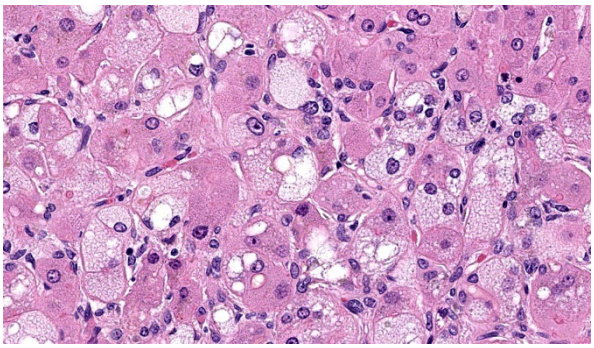


Liver, rabbit: There is diffuse loss of sinusoidal architecture. Within centrilobular and periportal areas of the lobule, hepatocytes are markedly swollen due to accumulation of vary ing sizes of lipid vacuoles within their cytoplasm. Hepatocytes are separated and surrounded by proliferating stellate cells and small amounts of collagen. (HE, 318X)

(specifically β- VLDL) lead to the lesions of atherosclerosis, and both VLDL and LDL are transported to and from hepatocytes via LDL receptors.[1,11] In rabbits and humans, hepatic steatosis can occur if cholesterol and triglycerides, in the form of VLDL and LDL, overload hepatocytes. In humans and animals with metabolic syndrome, ruminants, and cats, adipose tissue is broken down, releasing triglycerides or free fatty acids which go to the liver and similarly overload the hepatocytes.[3,11] Synthesis of VLDL in hepatocytes occurs in the endoplasmic reticulum (ER) and there is some mitochondrial oxidation normally. When overloaded, there can be ER stress and mitochondrial dysfunction associated with lipotoxic metabolites of fatty acids, thus leading to more steatosis.[5] Therefore, hepatic steatosis pathogenesis includes both the triglycerides themselves, as well as lipotoxic metabolites.

Steatosis occurs in a macrovesicular or microvesicular form. Macrovesicular steatosis is more often associated with hepatic lipidosis as seen in cats, ruminants, and animals with metabolic syndrome. Both macrovesicular and microvesicular steatosis are due to triglyceride accumulation. Microvesicular steatosis is also associated with mitochondrial dysfunction[4] and is most common in toxic hepatopathies, like Reye's syndrome in humans[3,4]. The mitochondrial dysfunction and lipotoxicity are likely why microvesicular steatosis has a poorer prognosis.

Hepatic steatosis in humans is referred to as non-alcoholic or metabolic dysfunction-associated fatty liver disease (NAFLD and MAFLD) and can lead to steatohepatitis (NASH or MASH)[7]. Metabolic dysfunction-associated steatohepatitis (MASH) is associated with steatosis, inflammation, hepatocellular degeneration, and fibrosis,



Liver, rabbit: Higher magnification of lipid-laden hepatocytes with small lipid vacuoles (microsteatosis) and larger lipid vacuoles (macrosteatosis). Hepatocytes often contain lipofuscin granules in their cytoplasm as well. Stellate cells proliferate along the sinusoids. (HE, 881X)

almost all clearly displayed in this rabbit and in a rabbit model[2]. This rabbit and humans with MASH often also have concurrent hypercholesterolemia and atherosclerosis[2].

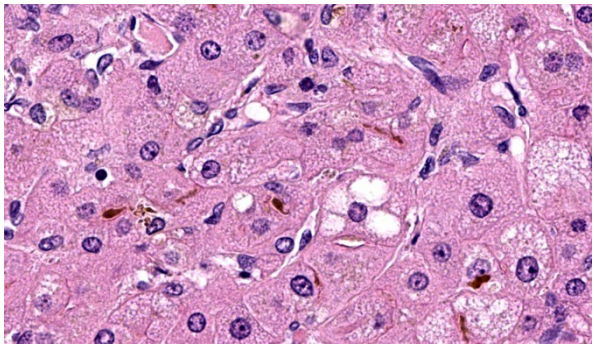
The liver histopathology in this case also provided opportunity to review the classic lesions of liver repair. One group of progenitor cells in the liver (also termed oval cells in rodents) is located within the canals of Hering. When the liver is damaged, these cells are activated and lead to histopathologic "biliary duct hyperplasia", "ductular reaction", or "oval cell activation"[8]. In addition to this population of progenitor cells are hepatic stellate cells. Hepatic stellate cells reside in the space of Disse along the sinusoids and are activated from relatively quiescent cells into myofibroblasts when there is liver damage[3,8]. This case highlights early fibrosis within the sinusoids or space of Disse due to activation of hepatic stellate cells.

Contributing Institution:
Michigan State University Veterinary Diagnostic Laboratory
4125 Beaumont Road
Lansing, MI 48910-8104
https://cvm.msu.edu/vdl

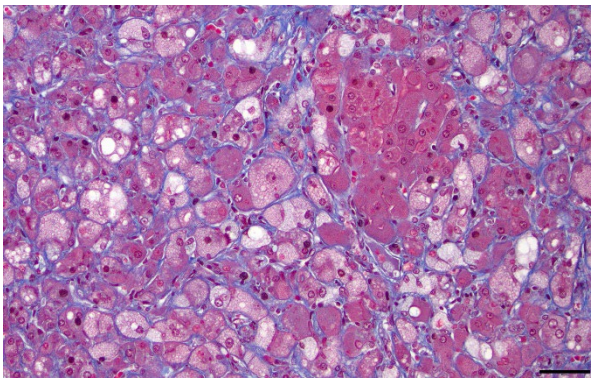
JPC Diagnoses: Liver: Hepatocellular micro- and macrovesicular lipidosis, chronic, multifocal to coalescing, severe, with marked stellate cell hyperplasia and cholestasis.

JPC Comment: Conference 13 was moderated by the Dr. Louis Huzella, a previous graduate of this program during the AFIP years. As his conference was held the day before the Thanksgiving holiday here in the U.S., he selected cases that he felt were loosely in keeping with that theme. This made for an enjoyable and light-hearted conference to with which to wrap up the 2025 conferences.

The contributor in this case provided a well-thought and informative writeup of hepatic lipidosis and hyperlipidemia, and much of what was discussed in conference is covered in their comment. Conference participants were, for the most part, readily able to achieve the reach the correct morphologic diagnosis in this case, but there was some speculation on the origin of the hyperplastic spindle cell population on the H&E. As mentioned by the contributor, there are two main possibilities: oval cells or stellate cells. Oval cells are bipotential progenitors of both hepatocytes and biliary ductular epithelial cells. Hepatic stellate cells, also called Ito



Liver, rabbit: Bile canaliculi are often expanded with bile. (HE, 921X)



Liver, rabbit: A Masson's trichrome demonstrates the fibrous connective tissue lining sinusoids and surrounding occasional hepatocytes. (Masson's trichrome, 200X). (Photo courtesy of: Michigan State University Veterinary Diagnostic Laboratory, https:/cvm.msu.edu/vdl)

cells, live primarily in the space of Disse, store Vitamin A, and can be activated into myofibroblasts following injury that will produce connective tissue components.[3,8] Participants wondered if there was both oval cell and stellate cell hyperplasia in this case due to the presence of mild ductular reaction, cholestasis, and fibrosis, but unanimously agreed that there was at least stellate cell activation due to the fibrosis highlighted by a Masson's trichrome. This is a difficult distinction to make on H&E alone.

Of laboratory animal species, rabbits are more similar to humans in lipoprotein metabolism, atherosclerosis, and myocardial function than are rats and mice.[10] The Watanabe heritable hyperlipidemic (WHHL) rabbit is a specific strain of New Zealand White rabbit that was developed in Japan and is used as an animal model of familial hypercholesterolemia. Within the WHHL strain, there are two additional advanced strains used for specific hyperlipidemia-related diseases processes and include the coronary atherosclerosis-prone WHHL rabbit and the myocardial infarction-prone WHHL rabbit.[10] Hypercholesterolemia in the WHHL rabbit family is due to reduced LDL uptake by the liver.[10] Watanabe rabbits have played an important role in advancing these research areas and enabling a better understanding of these diseases in humans and animals alike. Additionally, studies in Watanabe rabbits have led to the development of a number of statins, which are inhibitors of cholesterol biosynthesis, to help treat hypercholesterolemia in humans.[10]

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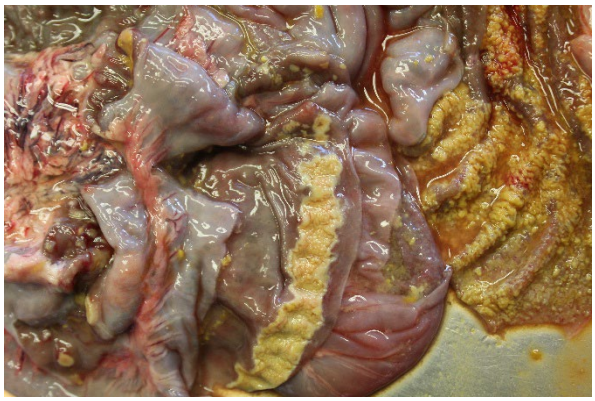
CASE II:

Signalment: Necropsy was performed on two male castrated pre-fattening pigs each of about 25 kg body weight (*Sus scrofa domesticus*).

History: The owner reported an increased mortality rate in a herd of pre-fattening pigs. Clinically affected animals showed yellowish to greyish, watery diarrhea with a sudden worsening of their general condition before they died spontaneously.

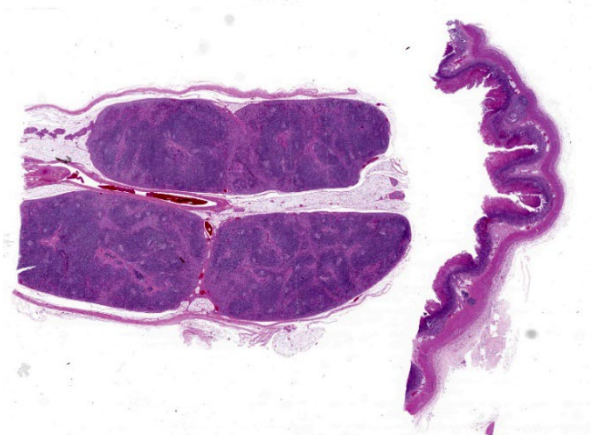
Gross Pathology: Both animals were in a moderate nutritional condition and showed severely sunk bulbi (dehydration). Multifocal to coalescing, yellowish, not removable plaques (fibrin) were observed on the mucosa of the stomach. The large intestine contained brown, fluid material and the mucosa of cecum and colon ascendens was multifocally to coalescing covered with yellowish, not removable plaques (fibrin/necrosis). The fibrin strands and necrotic areas were sometimes associated with the lymphatic tissue and band-like or sometimes appeared button-shaped. The rectum was empty.



Colon: Fibrinonecrotic plaques, both linear and button-shaped, are scattered throughout the colon. (Photo courtesy of: Institute of Veterinary Pathology, Vetsuisse-Faculty (University of Zurich), www.vetpathology.uzh.ch)

Laboratory Results: The bacteriological examination (culture) revealed the following result: *Salmonella enterica subsp. enterica serovar Typhimurium*

Microscopic Description: Large intestine (Colon ascendens): Multifocal to coalescing, a thick layer of fibrin, sometimes intermingled with degenerated neutrophils, and abundant coccoid to rod-shaped bacteria are attached to the mucosa. In these areas, the mucosa is diffusely destroyed (necrosis) and replaced by fibrin, cell debris and fewer coccoid to rod-shaped bacteria. The lamina propria and the submucosa show a moderate infiltration with neutrophils as well as fewer lymphocytes and histiocytes. The few remaining crypts are dilated and some of them contain neutrophils and cell debris. Some capillaries and venules contain fibrin thrombi or are dilated and filled with neutrophils.



Colon and colonic lymph nodes: At subgross magnification, there is pallor of the colonic mucosa. Within the colonic lymph nodes, there are no apparent germinal centers as well as a diminished paracortex. (HE, 17X)

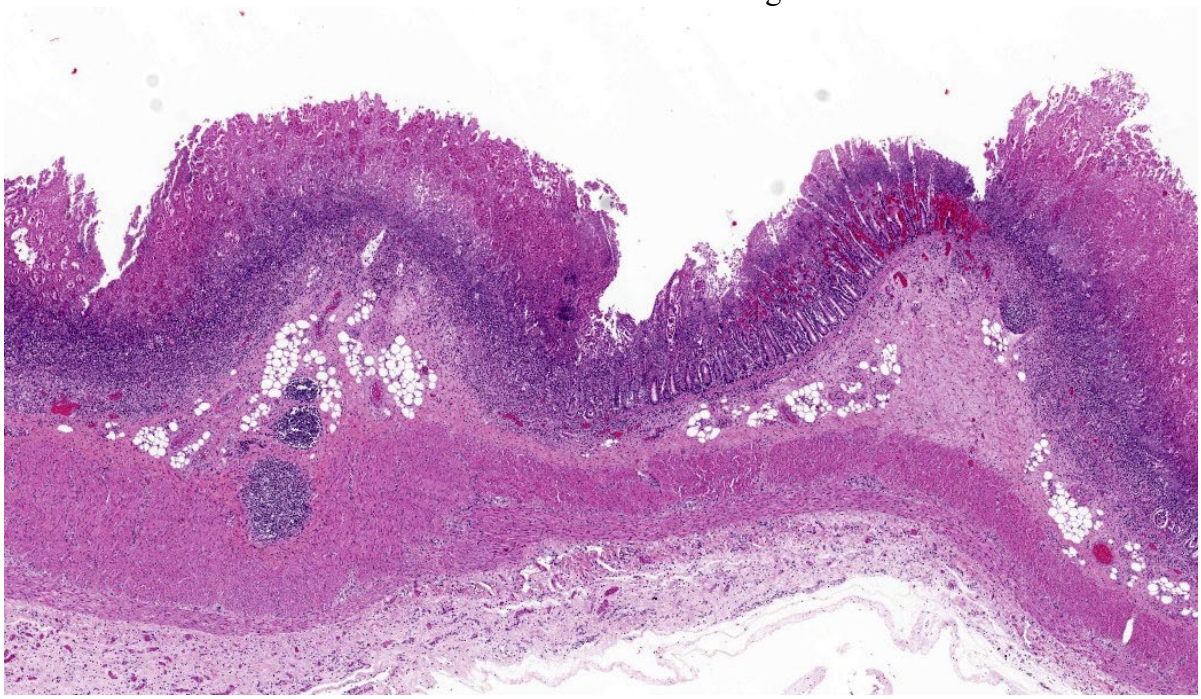
Mandibular lymph node: Lymphoid follicles are indistinct as a result of a severe lymphocyte depletion and replacement by aggregates of epithelioid macrophages with abundant eosinophilic cytoplasm.

Immunohistochemistry examination: Immunohistochemistry using an antibody directed against PCV-2 antigen revealed positive labeling in the cytoplasm of the epithelioid macrophages.

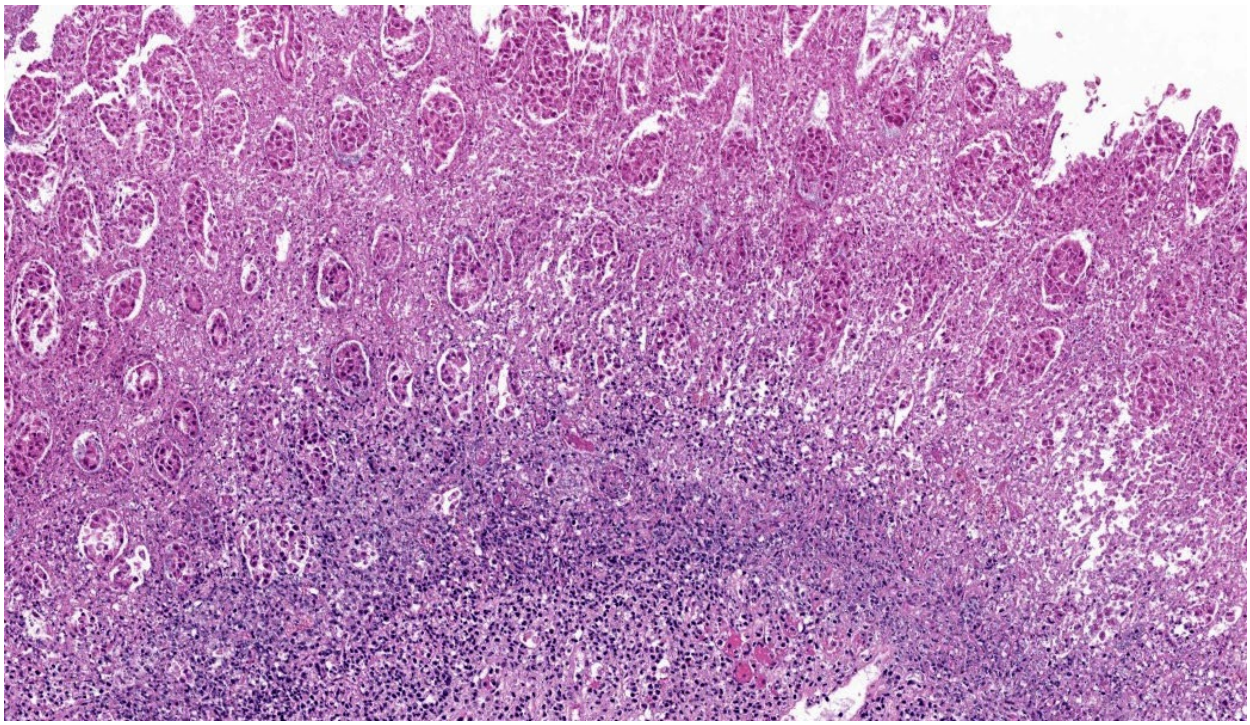
Contributor's Morphologic Diagnoses:
Large intestine: fibrinous to necrotizing enteritis, severe, chronic, multifocal to coalescing

Mandibular lymph node: granulomatous lymphadenitis, mild, multifocal, with lymphoid depletion

Contributor's Comment: *Salmonella* spp. are gram-negative, aerobic or facultative anaerobic and motile bacteria. The route of bacterial entry is mostly fecal-oral. The bacteria are mainly detected in contaminated food, water and aerosols but flies and fomites can also act as transmission sources. Furthermore, transplacental infection is also possible. Salmonellosis is an important zoonotic disease and all known species are pathogenic. It is a significant cause of acute and chronic



Colon, pig: There is partial to full-thickness necrosis of the colonic mucosa, multifocal hemorrhage, and depleted Peyer's patches. (HE, 47X)

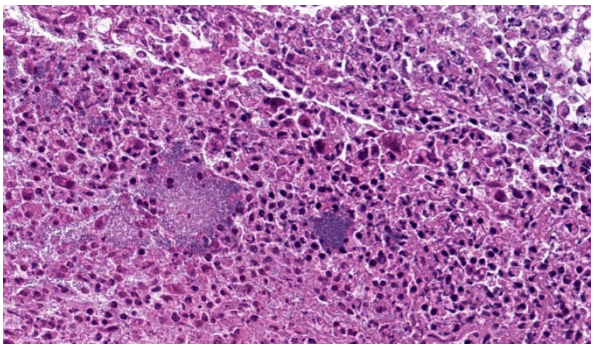


Colon, pig: There is coagulative necrosis of the superficial mucosa and lytic necrosis of the deep mucosa which effaces the muscularis mucosa and extends into the underlying submucosa (HE, 227X).

diarrhea and might even result in death of animals or humans. The most commonly isolated serovars in animals are *Salmonella Typhimurium, Salmonella Enterica, Salmonella Dublin, Salmonella Choleraesuis and Salmonella Typhosa*. Three forms of salmonellosis are reported: septicemic, acute enteric and chronic enteric. Which form occurs is depending on many factors such as infectious dose, previous exposure to the bacterium and stress (overcrowding, transport, cold temperatures, feed changes, pregnancy, parturition, surgery, anesthesia, antibiotic administration). It is possible that some of the recovered animals become carriers shedding the organism in their feces for at least 3-5 months.

Salmonella septicemia: The peracute Salmonella septicemia occurs in calves, foals and pigs and is mostly fatal. Younger animals get more frequently infected. This form of disease is usually caused by *Salmonella Choleraesuis*. Gross lesions are minimal or can present as fibrinous polyserositis and fibrinoid necrosis of blood vessels with microvascular thrombosis, which results in extensive petechiation and a blue discoloration of the extremities, ventrum, ears and tail in pigs due to endotoxemia. Furthermore, petechial hemorrhages can develop in many organs and tissues.

Acute enteric salmonellosis: The acute enteric form occurs in cattle, pigs, horses and rarely carnivores and is most frequently caused by *Salmonella Typhimurium*. A catarrhal enteritis with focal to diffuse fibrinonecrotic ileotyphlocolitis is the characteristic sign; the intestine is filled with mucus, fibrin, and occasionally blood. Moreover, multifocal hepatocellular necrosis, mesenteric lymphadenopathy and fibrinous cholecystitis are pathognomonic for the acute enteric salmonellosis.



Colon, pig: There are scattered colonies of coccobacilli within the necrotic mucosa. (HE, 960X).

Chronic enteric salmonellosis: The chronic form occurs in pigs, cattle and horses. Multiple necrotizing and ulcerated foci are commonly seen in the cecum, colon and rectum ("button ulcers") of pigs. Rectal strictures can develop due to vascular thrombosis caused by the infection.

The co-infection with PCV-2 in this case is interpreted as an underlying disease leading to an immunosuppression and rendering the pigs more susceptible for secondary bacterial infection, in this case *Salmonella enterica* subsp. *enterica* Serovar t*yphimurium.*

Contributing Institution:

Institute of Veterinary Pathology
Vetsuisse-Faculty (University of Zurich)
Winterthurerstrasse 268, CH-8057 Zurich
www.vetpathology.uzh.ch

JPC Morphologic Diagnosis:

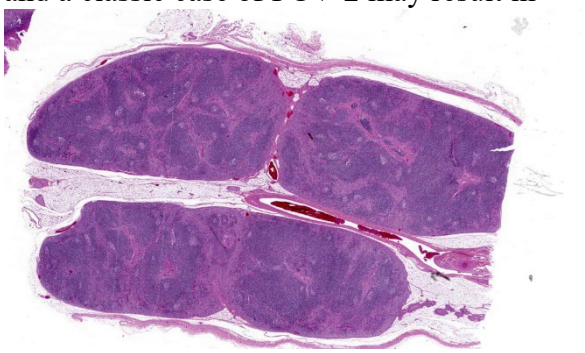
1. Colon: Colitis, necrotizing, subacute, diffuse, severe, with Peyer's patch depletion and numerous coccobacilli.
2. Lymph nodes: Lymphoid depletion, diffuse, severe.

JPC Comment:

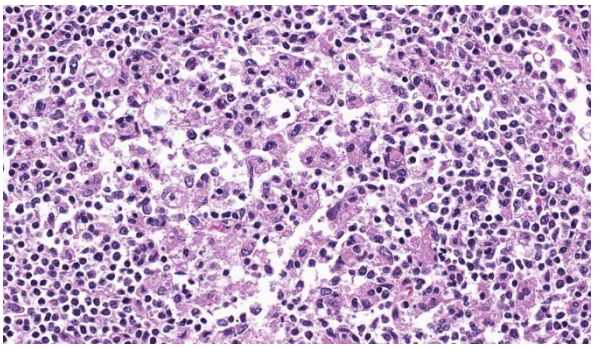
What a fantastic case this is! It highlights some of the classic GI lesions in a severe case of salmonellosis, and the degree of lymphoid depletion present in both the GI-associated lymphoid tissue and the mandibular lymph nodes were excellent clues for the pathologist to suspect a primary cause of immune suppression as well. Many thanks to the contributor for providing this highly educational case to the WSC!

Due to the degree of necrosis and inflammationin the submitted section of colon. some participants had difficulty deciding in which segment of the GI tract this sample originated. The presence of occasional intact crypts and multiple lymphoid follicles were helpful in establishing an anatomic location of "intestine" (remember, there are no lymphoid follicles in the stomach except in severe cases of *Helicobacter spp* infection in some species), but it can be challenging to get further than "intestine" when a section is as inflamed and necrotic as the tissue is in this case.

There was discussion on the difference between lymphoid necrosis and lymphoid depletion, the former of which implies active targeting of lymphocytes while the latter can be an aftermath of lymphoid necrosis or can be a separate process secondary to any number of pathogeneses (i.e. SCID). The target cell for porcine circovirus-2 is macrophages, and a classic case of PCV-2 may result in



Colonic lymph nodes, pig: Colonic lymph nodes are markedly depleted, with no germinal centers and a sparse paracortex. (HE, 13X)



Colonic lymph nodes, pig: There are small aggregates of macrophages scattered throughout the nodes. (HE, 720X)

granulomatous lymphadenitis, an unusual result of most viral infections. Lymphocytes are not directly targeted in the pathogenesis of PCV-2 but are rather secondary victims to the inflammation from immune response to the virus, making this case a great example of lymphoid depletion. This is further exacerbated in cases of secondary bacterial infection when the offending bacteria colonize Peyer's patches, which is an important step in the pathogenesis of enteric salmonellosis.

Porcine circovirus-2 is a bit of a catch-all virus in that it causes a wide variety of clinical signs in affected pigs. It should *always* be on the differential list for sick pigs due to the many ways it manifests. PCV-2 is widely distributed in domestic pigs and wild boars, and has also been isolated from cattle, dogs, deer, chamois, mice, and various arthropods (i.e., ticks and mosquitoes). PCV-2 infection in swine can be either subclinical or cause numerous forms of illness that are collectively referred to as "PCV-associated diseases" (PCVAD). These includes post-weaning multisystemic wasting syndrome (PMWS), porcine respiratory disease complex, porcine dermatitis and nephropathy syndrome, and both reproductive and enteric diseases.[1] The immunosuppression associated with PCV-2 is characterized by lymphopenia, lymphoid cell (both B and T cell) depletion, and altered cytokine production.[1] Because of its immunosuppressive effects, PCV-2 is frequently diagnosed concurrently with various other pathogens, and these combinations often provoke more severe disease together than they would individually, as seen in this case with concurrent salmonellosis.

During the initial phase of enteric *Salmonella* infection, the bacteria invades intestinal epithelial cells by employing an SPI-1 Type III secretion system (T3SS). This induces alterations in intestinal folds and in actin structures of the intestinal epithelial cells, facilitating the uptake of *Salmonella* by these non-phagocytic cells.[4] Alternatively, *Salmonella* can invade M cells that cover Peyer's patches, enabling entry into lymphoid follicles.[4] From here, the bacteria are taken up by phagocytes such as dendritic cells and macrophages. Upon internalization by these cells, Salmonella forms its own specialized membrane-bound blebs known as "*Salmonella*-containing vacuoles" (SCVs). Once within these vacuoles, the expression of SPI-1 T3SS is down-regulated and the SPI-2 T3SS is up-regulated.[4] Where the SPI-1 system facilitates entry into host cells, the SPI-2 system promotes survivability and replication of the bacteria within host cells. Within these vacuoles, the bacteria can also deliver various effector proteins into the cytoplasm of phagocytes, triggering caspase-1 activation and subsequent apoptosis.[4] The lysis of these cells releases additional *Salmonella* bacteria into the host, further perpetuating infection.

Lastly, there was brief discussion during conference on host-adapted vs non-host-adapted *Salmonella* spp. and the histologic clues that may key the pathologist in to suspecting one versus the other - of course, morphology is not definitive, and culture and PCR are required for confirmation. That being said, the

targeting of blood vessels is a feature seen most often with host-adapted *Salmonella* infections (*S. cholerasuis* and *S. typhisuis* in swine). This feature is not generally seen in non-host-adapted *Salmonella* infections (*S. typhimurium,* as in this case, does not target vessels, and causes a necrotizing enterocolitis, as it does in many other mammalian species). All *Salmonella* spp. in swine will target lymphoid tissues as part of their pathogenesis. Wrapping up this case discussion was a quick reminder of the orientation of porcine lymph nodes…remember, they are "inside-out"; the medulla is on the outside!

References:

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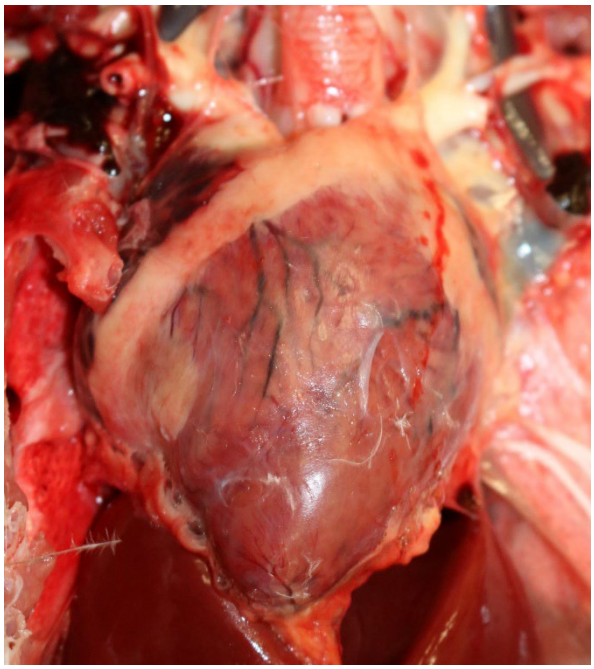
CASE III:

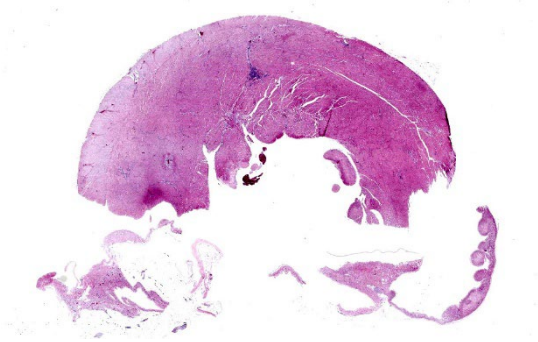
Signalment: A juvenile (<1 year old), male bald eagle (*Haliaeetus leucocephalus*)



Heart, bald eagle: There are multifocal areas of pallor within the myocardium and a small amount of fibrin on the epicardium. (Photo courtesy of: University of Connecticut, Connecticut Veterinary Medical Diagnostic Laboratory, https://cvmdl.uconn.edu/)

History: The eagle was admitted to a local raptor rehabilitation center in Connecticut two days before death, The bird was reported to be 10% dehydrated, showing neurologic signs including seizures and ventral flexion of the neck. The lead level was 4 ug/dL. The eagle received subcutaneous fluid therapy of unreported volume every six hours. The eagle was fed with quail once with assistance but regurgitated all the stomach contents afterward. The eagle was found deceased in the cage on the day of the necropsy submission.

Gross Pathology: This eagle was in adequate nutritional condition, with intra-coelomic adipose stores. A small amount (2-3 mL) of mildly greenish seromucous nasal fluid was discharged from the nostrils, along with two bright red threadlike, 1 cm-long nematodes. The myocardium had multifocal pale areas.



Heart, bald eagle. Three sections of heart, to include both ventricles and the interventricular septum. At subgross magnification, several foci of inflammation are visible in the myocardium. (HE, 15X)

Laboratory Results: Frozen brain tissue was positive for West Nile virus via real-time quantitative polymerase chain reaction (qPCR) performed by the Connecticut Veterinary Medical Diagnostic Laboratory. A cloacal swab sample was submitted for avian influenza virus (IAV) testing via PCR, and the result was not detected.

Microscopic Description: Heart: Multifocally infiltrating the myocardial interstitium are small to moderate numbers of lymphocytes, histiocytes, and fewer heterophils admixed with scattered cell debris. Adjacent cardiomyocytes are fragmented with loss of cross striation (myocardial necrosis). Occasionally, the myocardium contains protozoal cysts measuring up to 70 x 20 µm, containing numerous bradyzoites.

Contributor's Morphologic Diagnosis:

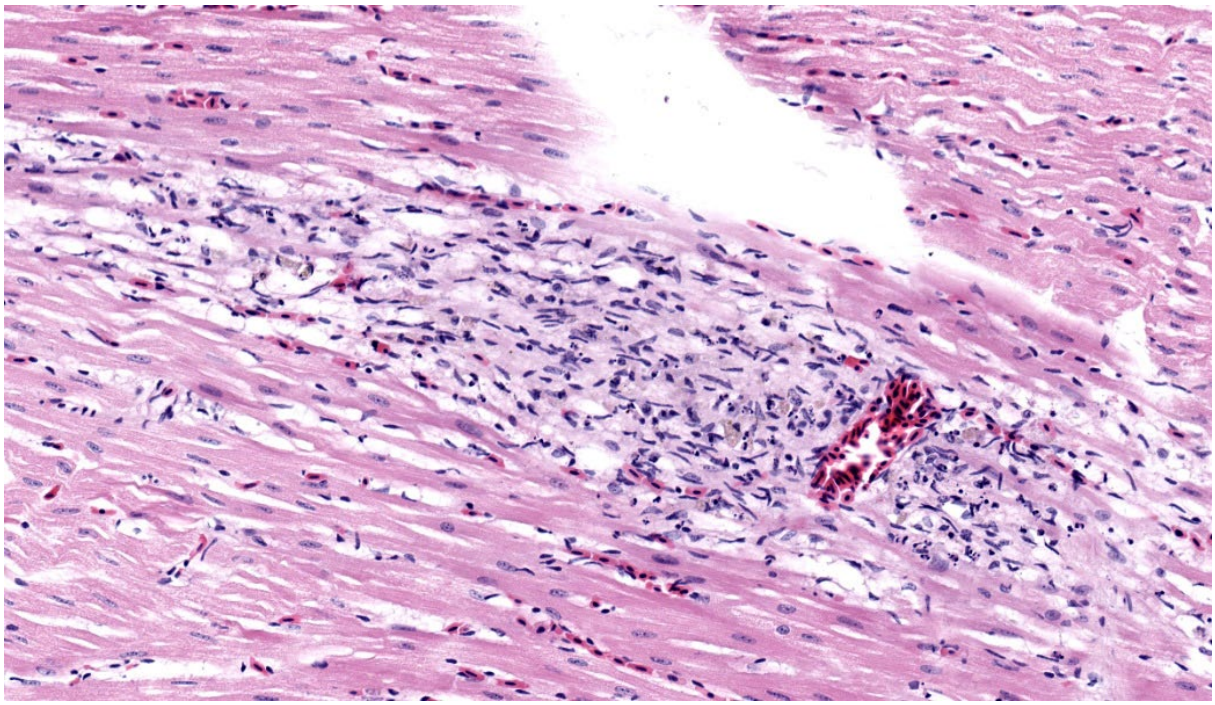
Lymphohistiocytic myocarditis, multifocal, moderate, with myocardial necrosis and protozoal cysts, heart.

Contributor's Comment: Additional histologic findings in this case included lymphohistiocytic encephalitis with perivascular cuffing. The histopathological findings in the heart and the brain are consistent with West Nile virus (WNV) infection, confirmed by the molecular testing of frozen brain tissue via qPCR. West Nile virus is an arthropod-borne, enveloped RNA virus of the family Flaviviridae, genus Flavivirus. The virus is primarily transmitted by mosquitoes, but direct contact with infected animals and via oral uptake of virus-infected tissues have also been reported.[1,3] WNV causes fatal diseases in humans, horses, and a wide variety of birds, including bald eagles.[7] Gross lesions caused by WNV in bald eagles included bilaterally symmetrical cerebral pan-necrosis with hydrocephalus ex vacuo, retinal scarring, myocardial pallor, and rounded heart apex.[7] Histologic lesions included lymphoplasmacytic encephalitis, myocarditis, pectenitis, and choroiditis.[7] Diagnosis of WNV requires a combination of detection of viral antigen and/or RNA or virus-specific antibodies in the cerebrospinal fluid.

Differential diagnoses for the protozoa observed in the cardiomyocytes included *Sarcocystis* and *Toxoplasma*. Protozoal encephalitis seemed to be unlikely based on the absence of protozoal organisms in the H&E-stained sections, although the co-existence of protozoal encephalitis cannot be ruled out entirely in this case.

The histological characteristics of the nematode in the trachea are consistent with tracheal worm (*Syngamus trachea*), and this finding is considered incidental in this case.

Contributing Institution:
University of Connecticut Veterinary Diagnostic Laboratory
61 N Eagleville Rd (yes, Eagleville), Storrs, CT 06269



Heart, bald eagle. There are areas of myocardial necrosis and loss with infiltration of low to moderate numbers of macrophages and heterophils admixed with few siderophages edema and cellular debris. Cardiomyocytes are lost in this region – remnant cardiomyocytes are markedly shrunken and occasionally demonstrate karyorrhexis. There is hypertrophy of interstitial cell and fibroblast nuclei. (HE, 459X)

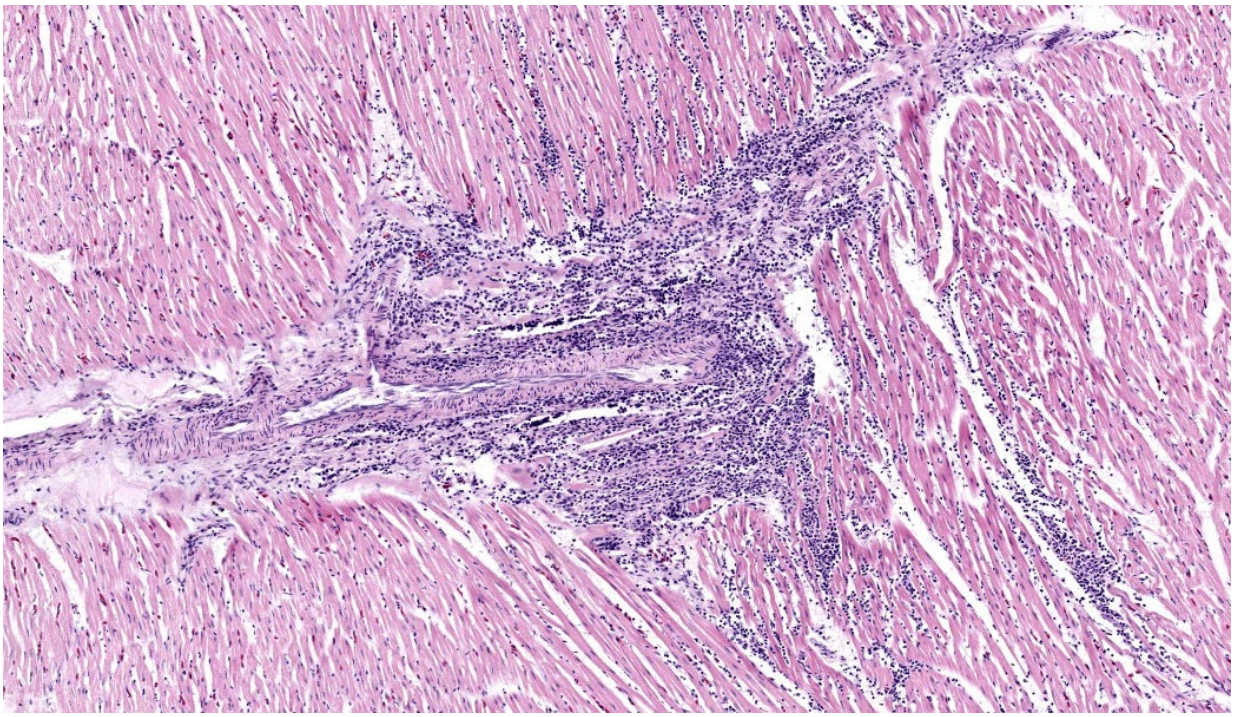
JPC Morphologic Diagnosis:

1. Heart: Myocarditis, lymphoplasmacytic and histiocytic, chronic, multifocal, moderate, with cardiomyocyte necrosis.
2. Heart, cardiomyocytes: Intrasarcoplasmic apicomplexan schizonts, multiple.

JPC Comment: This case was challenging due to the subtlety of the myocardial necrosis, but conference participants astutely managed to come up with a reasonable list of differentials, all of which must be considered in cases of myocardial necrosis in Bald eagles: lead toxicosis, highly pathogenic avian influenza (HPAI), and West Nile virus (WNV). All participants basically waved "Hello", to the protozoal bradyzoites in the myocardium and drove on, realizing that, although fun to look at, they were likely not the cause of this bird's lesions and are a common finding in wild avians.

WNV was first isolated in Uganda in 1937 but did not rise to notoriety until its relatively recent spread throughout Europe and the Americans.[2] Within the US, WNV broke onto the scene in the summer of 1999 in New York City as an unknown cause of high mortality in bird populations and an encephalitis of unknown origin in horses.[5] These cases in animals preceded the disease in humans, which ultimately resulted in 62 deaths in this initial outbreak. From the US outbreak, WNV spread to Canada and Mexico, where it was identified in both countries in 2002. WNV continues to cause occasional outbreaks with severe neurological disease in both humans and horses, and is maintained in the environment predominantly within wild bird and mosquito populations.[2,5]

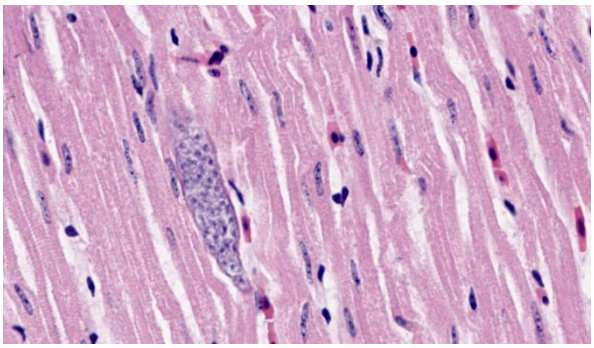
Birds are the main vertebrate hosts of WNV.[2,5] They are usually asymptomatic, but



Heart, bald eagle. There is profound periarterial inflammation in one area of the left ventricle, with macrophages, lymphocytes and plasma cells extending into the adjacent myocardium. In areas in which the inflammation extends, there is degeneration and necrosis of cardimyocytes. (HE, 172X)

if clinical signs do manifest, they most commonly include ruffled feathers, lethargy, difficulty moving, and loss of appetite that can be severe enough to cause emaciation. Affected birds may also have profuse oronasal secretions, dehydration, head tremors, and/or seizures.[5] Death of infected birds typically occurs within 24hrs of the onset of clinical signs. Of bird species, corvids are exquisitely susceptible to WNV and the only sign of infection may be that they drop dead out of the sky. Histologically, the most common lesion of WNV in birds is necrosis +/- hemorrhages and the most frequently affected organs are the brain, liver, heart, kidney, and spleen.[5] There may also be ocular lesions (i.e., neuritis, retinal inflammation, iris degeneration) that can result in clinical blindness.[5]

Circling back to the two other primary differentials to consider in this case, the histologic lesions in this case are less consistent with lead toxicosis, but blood lead levels should be considered in any case of this nature. Histologic lesions of lead toxicity in bald eagles are those of a primary vascular injury and are classically characterized by fibrinoid necrosis of small to medium-caliber arteries in the heart, brain, and eyes.[4] Blood lead concentrations greater than 4ppm are associated with a higher likelihood of cardiac lesions in bald eagles.[4] In cases of HPAI in bald eagles, gross and histologic lesions are similar to those seen in WNV and PCR is required to achieve definitive diagnosis. Histologic lesions of HPAI in bald eagles include a leukocytoclastic and fibrinoid vasculitis, as well as necrotizing and hemorrhagic encephalitis, myocarditis, pancreatitis, adrenalitis, histiocytic splenitis, and anterior uveitis.[6]



Heart, bald eagle. Multifocally, cardiomyocytes contain numerous round 2um zoites (HE, 1800X)

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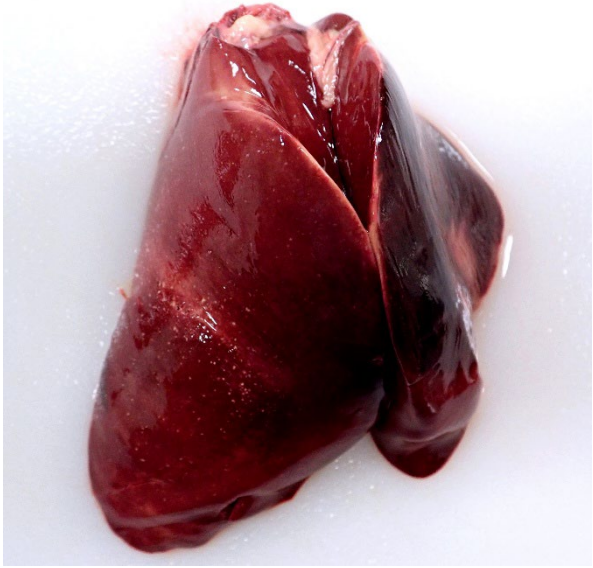
CASE IV:

Signalment: Adult, female, *Antigone canadensis*, greater sandhill crane

History: Two lesser snow geese and two greater sandhill cranes were submitted for postmortem examination to investigate the



Liver, sandhill crane. The liver was enlarged, congested, and contained numerous random pinpoint tan foci. (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services, https://nmdeptag.nmsu.edu/labs/veterinary-diagnostic-services.html

Spleen, sandhill crane. The spleen was enlarged and contained numerous coalescing pinpoint tan foci. (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services, https://nmdeptag.nmsu.edu/labs/veterinary-diagnostic-services.html

death of at least 30 birds that included a variety of duck species, lesser snow geese and greater sandhill cranes at a waterfowl management area.

Gross Pathology: The crane was in good body condition and with minimal postmortem decomposition. The liver was enlarged, congested, and contained numerous random pinpoint tan foci. The spleen was enlarged and contained numerous coalescing pinpoint tan foci.

Laboratory Results

Pasteurella multocida was isolated from the liver and spleen.

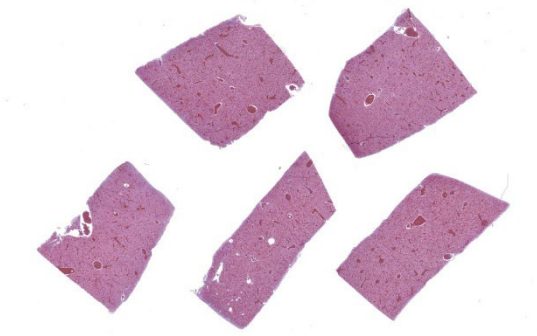
PCR testing was negative for avian influenza virus and avian paramyxovirus.

Microscopic Description: The liver has multiple random foci of necrosis that are filled with necrotic debris, fibrin, variable numbers of intact and degenerate heterophils and macrophages, and variable numbers of Gram-negative coccobacilli. There are multiple sinusoids that contain coccobacilli with small numbers of sinusoids that contain fibrin thrombi. The hepatocytes adjacent to the sinusoids with fibrin thrombi are often necrotic. There are small numbers of perivascular aggregates of lymphocytes.

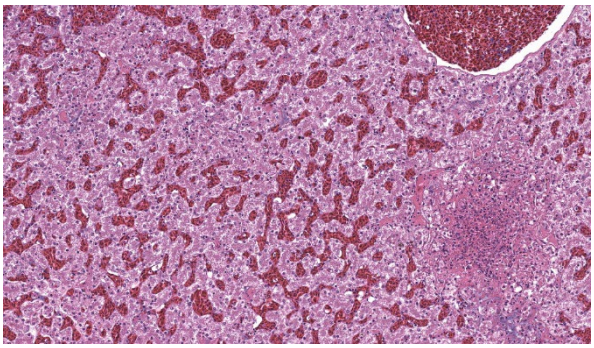
Contributor's Morphologic Diagnosis:

Liver – Hepatitis, necrotizing, heterophilic and histiocytic, random, multifocal with intralesional and intravascular Gram-negative coccobacilli and sinusoidal fibrin thrombi; etiology *Pasteurella multocida*.

Contributor's Comment: Fowl cholera is a disease of birds caused by the Gram-negative rod-shaped bacterium *Pasteurella multocida*.[3,4,6,7,8] The bacterium is known to infect numerous species of birds including but not limited to domestic poultry, wild waterfowl, birds of prey, and birds in zoological collections. Fowl cholera typically occurs as an acute fatal respiratory and septicemic disease with death occurring within 24-48 hours. Chronic and even benign infections with *P. multocida* can occur. Of the domestic poultry, turkeys are more susceptible to infection with *P. multocida* than chickens.[4,7] Adult chickens are most susceptible to infection with *P. multocida* that young chickens.[4,7] Among wild birds, outbreaks of fowl cholera typically occur in North American waterfowl in wetlands or nesting areas.[3,6,8]



Liver, sandhill crane. Multiple sections of liver are submitted for examination. (HE, 10X)



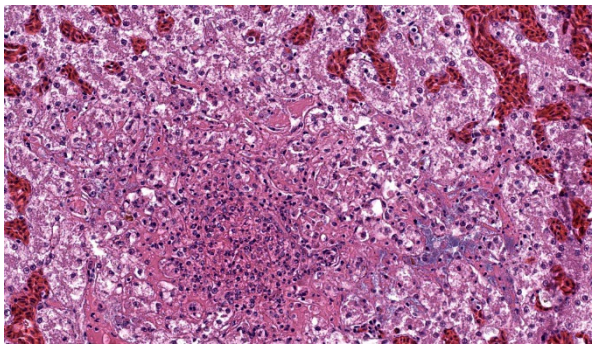
Liver, sandhill crane. Numerous foci of hepatocellular necrosis are scattered throughout the section. A small more recent area is at left, with a larger, more advanced lesion at right. (HE, 290X)

P. multocida is likely transmitted to susceptible birds from the contaminated environment or bird-to-bird contact.[3,6,8] Large numbers of *P. multocida* are shed in nasal, oral and ocular secretions of live sick birds, and large numbers of *P. multocida* contaminate the environment from the carcasses of dead birds.[3,4,6,7,8] Immediate removal of carcasses from the outbreak area is important for the management of avian cholera in wild birds as the bird carcasses will continually contaminant the environment with *P. multocida* if left to naturally decompose or be consumed by scavengers.[3,6,8] How *P. multocida* is transmitted between outbreak areas is not known, but it is believed that some lesser snow geese and Ross's geese can be carriers of *P. multocida*.[3,8,9] In some wetlands, an increase in the population of lesser snow geese has been associated with an increase in the incidence and mortality numbers of avian cholera.[2,3,8,9] The ability of *P. multocida* to persist in the environment has not been fully clarified.[3,6,8] However, there are some studies that show *P. multocida* cannot be isolated from the wetland environment after 7 weeks of an outbreak.[1,8] Thus, long term persistence of the bacterium in the environment is not likely the source of multiple outbreaks in the same area that occur years apart.

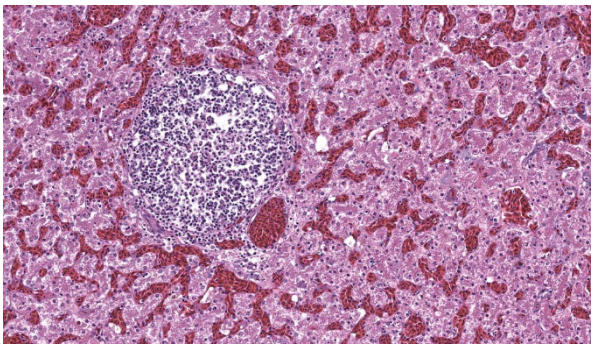
In acute fowl cholera, the most likely sign of disease first noticed is the death of large numbers of birds.[4,6,7,8] Clinical signs are only evident for a short period prior to death and include anorexia, ruffled feathers, depression, oral and nasal discharge, increased respiratory rate, diarrhea, and cyanosis of nonfeathered skin. Birds that die of acute fowl cholera are in good body condition and often will still have food in the esophagus and crop. These birds may have congestion of their organs with hemorrhages on serosal surfaces and in the heart, lungs, fat, and other organs. Birds dying acutely with fowl cholera can have multifocal random tan foci in an enlarged liver. The spleen can be enlarged and have multifocal random tan foci. Microscopically, the liver, spleen and lung can have random foci of necrosis filled with heterophils and bacteria. Lesions in the lung are most common in turkeys. There are often bacteria within numerous blood vessels.

Chronic *P. multocida* infections in birds are often localized infections in comparison to the fatal septicemia of acute fowl cholera.[4,7]

Localized infections of *P. multocida* most commonly occur in the respiratory tract. Any part of the respiratory tract can be involved



Liver, sandhill crane. High magnification of an area of hepatocellular lytic necrosis. Colonies of coccobacilli are entrapped in fibrin at right; there are small colonies within the sinusoids at the periphery of the necrotic focus. (HE, 619X)



Liver, sandhill crane. Foci of extramedullary hematopoiesis abut portal areas. (HE, 318X).

including the lungs, sinuses, and pneumatic bones. A common chronic respiratory tract lesion in turkeys with *P. multocida* is pneumonia. Other chronic infections with P. multocida in birds include conjunctivitis, arthritis, pododermatitis, salpingitis, and otitis media. The microscopic lesions of chronic P. multocida infections in birds tend to be heterophilic inflammation mixed with macrophages and multinucleated giant cells.

Contributing Institution:
New Mexico Department of Agriculture Veterinary Diagnostic Services

https://nmdeptag.nmsu.edu/labs/veterinary-diagnostic-services.html

JPC Morphologic Diagnosis:

Liver: Hepatitis, necrotizing, acute, random, marked, with fibrin thrombi and numerous colonies of coccobacilli.

JPC Comment: Fowl cholera was last seen in the WSC in Conference 22, Case 1 of the 2014-2015 WSC. Sandhill cranes are not usually a species that comes to mind in a classic diagnosis of fowl cholera. However, *Pasteurella multocida* happens to be one of the leading bacterial causes of death for sandhill cranes in the U.S. and is commonly isolated from wild bird populations. There is some evidence that migratory bird populations may contribute to outbreaks.[3,7] The contributor's comment in this case provides a thorough overview of *P. multocida* in common avian species and is worth the read. Much of the discussion held in this case revolved around topics in their write-up.

P. multocida is associated with a wide range of diseases in several species of animals. Some of the major diseases in domestic species include hemorrhagic septicemia in ungulates, atrophic rhinitis in swine, and fowl cholera in wild and domestic birds. *P. multocida* sports a handful of key virulence factors that include a capsule, lipopolysaccharide (LPS), iron-regulated proteins, and outer membrane proteins OmpA, OmpH, sialylation of outer membrane components, and the adhesion protein filamentous hemagglutinin B2 (FhaB2).[5] The FhaB proteins are both surface-associated and secreted by the bacteria, the latter of which is necessary to establish infection in turkeys upon mucosal exposure.

It is thought that FhaB2 in *P. multocida* functions as an adherence molecule involved in colonization and invasion of respiratory mucosal surfaces in turkeys.[5] Due to the key role FhaB2 plays in the pathogenesis of fowl cholera, there have been vaccine trials using recombinant FhaB2 peptides that demonstrated anti-FhaB2 antibodies effectively protected turkeys against challenge with both homologous and heterologous strains of *P. multocida*.[5] This wide cross-protection is thought to be due to the high degree of conservation of *P. multocida* FhaB2 protein sequences.

References:

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wetlands following avian cholera outbreaks. *J Wildl Dis*. 2006;41(1):33-39.

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