



WEDNESDAY SLIDE CONFERENCE 2012-2013

Conference 14

6 February 2013

CASE I: RP18165 (JPC 4018046).

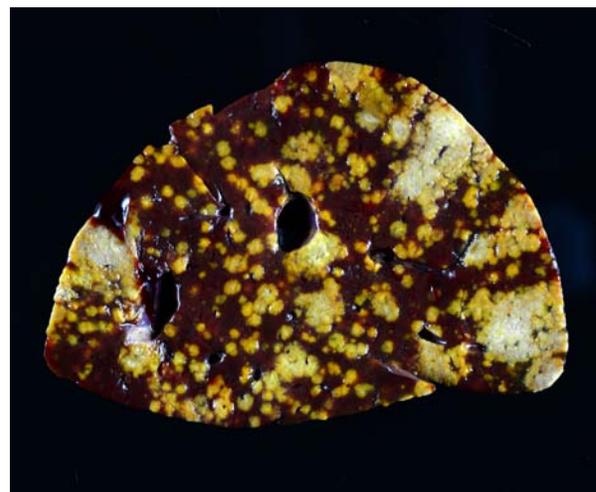
Signalment: Young adult female American white pelican, (*Pelecanus erythrorhynchos*).

History: This native bird was found dead on the grounds of a zoological institution.

Gross Pathology: The liver and spleen were enlarged and dark red with approximately 60-75% of the capsular surface and parenchyma replaced by

multifocal to coalescing, 1-3 mm, irregular, slightly bulging, yellow, dry, soft to firm foci. Myriad endoparasites were present: pouch lice in the oral cavity, trematodes in the trachea, and nematodes in the esophagus and proventriculus.

Laboratory Results: PCR (fresh frozen liver): PCR targeting a portion of the eukaryote 18S rRNA and subsequent sequencing identified a 126 bp fragment of DNA that was 96% identical with *Tetratrichomonas gallinarum* partial 18S rRNA gene



1-1, 1-2. Liver, pelican: There are multifocal to coalescing well-delineated areas of coagulative necrosis throughout all lobes of the liver. (Photo courtesy of: Wildlife Disease Laboratories, San Diego Zoo Institute for Conservation Research, San Diego Zoo Global, PO Box 120551, San Diego, CA 92112-0551, <http://www.sandiegozoo.org/conservation/>)

when compared to known sequences using the Basic Local Alignment Tool (BLAST).

Electron microscopy (formalin-fixed liver): Flagellated protozoal organisms consistent with a trichomonad were seen. On transmission EM within areas of necrosis there were numerous 10.3 X 6.3 µm single-celled microorganisms with 4 anterior flagella, one recurrent flagellum, basal bodies, pelta, axostyle, costa, hydrogenosomes, and glycogen granules. With scanning EM, round to oval spherules were mixed with cell debris. Occasionally these organisms had four anterior flagella and a single recurrent flagellum, but most lacked flagella and an undulating membrane.

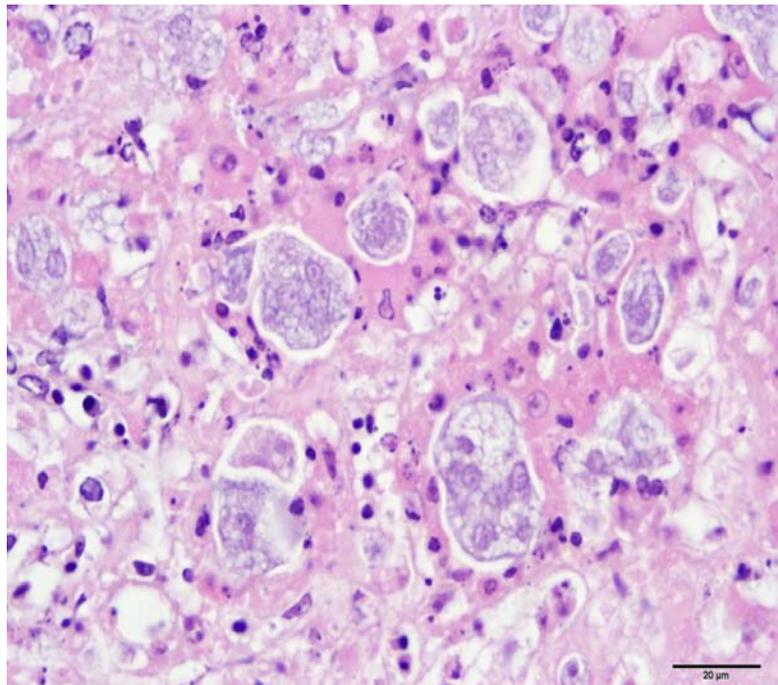
Histopathologic Description: Liver: The parenchyma is severely disrupted by multifocal to coalescing, areas of necrosis characterized by loss of cellular and architectural detail and accumulation of hypereosinophilic cellular debris and hyaline eosinophilic material (fibrin). Within these necrotic foci are myriad round to pleomorphic organisms that are amphophilic to basophilic, approximately 5-20 µm wide, and have one to multiple round nuclei. The adjacent hepatic parenchyma is multifocally infiltrated by variable numbers of lymphocytes and plasma cells, and diffusely there are perivascular infiltrates of hematopoietic cells (extramedullary hematopoiesis).

Contributor's Morphologic Diagnosis: 1. Liver: severe, acute to subacute, multifocal to coalescing, necrotizing hepatitis with intralesional protozoal trophozoites (*Tetratrichomonas gallinarum*).
2. Liver: moderate extramedullary hematopoiesis.

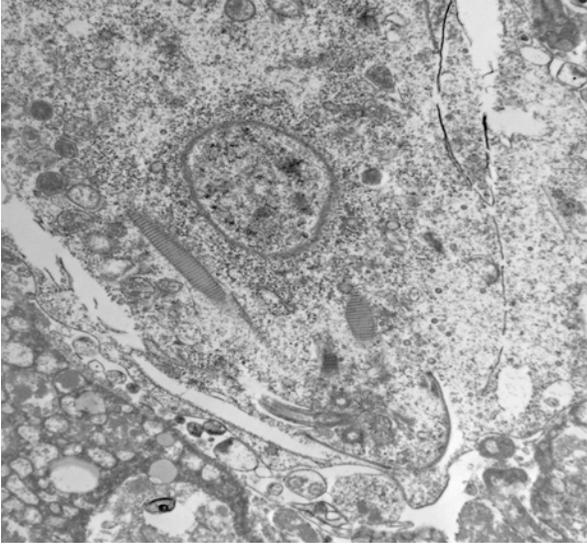
Contributor's Comment: The organisms in the hepatic lesions in this case were identified as trichomonad flagellates by electron microscopy and were a close match (96%) to *Tetratrichomonas gallinarum* by PCR of the 16s rRNA gene. This organism is most commonly isolated from the intestinal tract (cecum) of galliform and anseriform birds, where its role as a pathogen remains unclear. In turkeys and some species of ducks, it has been described as a cause of typhlohepatitis

similar to histomoniasis.⁹ However, other experimental infections in turkeys and have shown no clinical significance and only mild to absent changes in the cecum.^{1,7} Disease in chickens infected with *T. gallinarum* also appears to be rare.^{1,4,6} Recent comparison of *T. gallinarum* isolates from multiple species of galliform and anseriform birds by PCR detected differences in nucleotide sequences of up to 8.9%, which exceeded differences between some species of trichomonads.³ This marked heterogeneity among *T. gallinarum* isolates could account for some of the differences in pathogenicity between studies.

The appearance of the hepatic lesions on gross and histopathologic examination was similar to those of histomoniasis caused by the trichomonad *Histomonas meleagridis*. The pleomorphic size and shape of the organisms was comparable to that of *H. meleagridis* in its amoeboid phase, the form that occurs in tissues.⁶ Therefore, a similar change in morphology between organisms in the intestinal lumen or culture and those in tissue might occur with *T. gallinarum*. The presence of basal bodies and the variable presence of flagella of the organisms in the liver lesions were seen with electron microscopy and consistent with the PCR results. The presence of apparently multinucleated



1-3. Liver, pelican: Areas of coagulative necrosis contain numerous 25 µm amoebic trophozoites. (Photo courtesy of: Wildlife Disease Laboratories, San Diego Zoo Institute for Conservation Research, San Diego Zoo Global, PO Box 120551, San Diego, CA 92112-0551, <http://www.sandiegozoo.org/conservation/>)



1-4. Liver; pelican: Amoebic trophozoites have 4 anterior flagella, one flagellum, basal bodies, pelta, axostyle, costa, hydrogenosomes, and glycogen granules. (Photo courtesy of: Wildlife Disease Laboratories, San Diego Zoo Institute for Conservation Research, San Diego Zoo Global, PO Box 120551, San Diego, CA 92112-0551, <http://www.sandiegozoo.org/conservation/>)

forms on routine histopathology in this case was unusual and was not appreciated on EM. Replication by binary fission and dense crowding of the organisms could possibly account for this appearance.

To our knowledge, there are no reports of *Tetratrichomonas* sp. infections in pelicans, and the reason for disease in this bird is unknown. The liver and spleen were most severely affected with necrotizing lesions effacing large areas of the parenchyma, but smaller foci of necrosis with intralumenal organisms were also occasionally seen in the lung, heart, skeletal muscle, bone marrow, proventriculus and intestine, consistent with hematogenous dissemination of the infection. The likely origin of these trichomonads was the intestinal tract, although no areas of colonization of the lumen or crypts were seen. However, in addition to the variety of endoparasites noted grossly, myriad small trematodes invaded and multifocally disrupted the small intestinal, colonic and cecal mucosa. Secondary bacterial infections were also present in the ceca, and it is possible that this damage to the intestinal mucosa allowed invasion and dissemination of the flagellates. Poor immune function could also have been a contributing factor, as this pelican was young, heavily parasitized and in poor nutritional condition. In a previous report of this infection in ducks, all affected ducks were juveniles or subadults.⁹

Finally, as previously noted, isolates of *T. gallinarum* have a high degree of molecular polymorphism which could indicate the presence of multiple species or subspecies within this group, many of which could be host-adapted.³ Infection of a non-galliform or anseriform bird (such as a pelican) or cross-infections between galliforms and anseriforms, might be more likely to result in disease. A previous report of encephalitis due to *T. gallinarum* was in a mockingbird, a passerine bird, and in the previous outbreak in ducks, a turkey was a possible source of the parasite.^{8,9} The pelican in this report could have been exposed to a variety of native and exotic anseriform and possibly galliform birds at our zoological institution, none of which to date have been found to have a disseminated form of this infection.

JPC Diagnosis: Liver: Hepatitis, necrotizing, multifocal to coalescing, random, severe, with numerous trichomonads.

Conference Comment: See the contributor's comment for an excellent review of infection by the trichomonad *Tetratrichomonas gallinarum*. Trichomonads are protozoa in the phylum *Parabasalia*; they are flagellated, pear-shaped organisms with a single nucleus and an axostyle that protrudes from the posterior end. Trichomonads include the pathogens *Tritrichomonas foetus*, *Trichomonas gallinae*, and *Histomonas meleagridis*, as well as several nonpathogenic (commensal) species.

As the contributor notes, the pathogenicity of *Tetratrichomonas gallinarum* is unclear, as it is often identified in the cecum and colon of domestic species without causing disease.² However, recently a case of fatal hepatic tetratrichomoniasis was reported in a captive-raised juvenile Waldrapp ibis (*Geronticus eremita*).⁵ Similar to the pelican in this case, the Waldrapp ibis had necrotizing hepatitis associated with numerous protozoa. The protozoa showed positive immunoreactivity to antibodies against *Tritrichomonas foetus*. RNA sequence analysis (of the first ribosomal internal transcribed spacer region (ITS1), 5.8S ribosomal RNA, and ITS2 regions) revealed 96-97% genetic similarity to members of the *Tetratrichomonas gallinarum* complex. Cross-species transmission is suspected as the source of infection in this case as well. Further studies are needed to fully elucidate the pathogenicity of members of the *T. gallinarum*

complex and their role in disease in wild and captive birds.

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References:

1. Amin A, Liebhart D, Weissenbock H, Hess M. Experimental infection of turkeys and chickens with a clonal strain of *Tetratrichomonas gallinarum* induces a latent infection in the absence of clinical signs and lesions. *J Comp Path.* 2011;144:55-62.
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CASE II: N2012-0372 (JPC 4017930).

Signalment: 5.5-year-old, male Muscovy duck (*Cairina moschata*).

History: This duck was found dead with no premonitory signs.

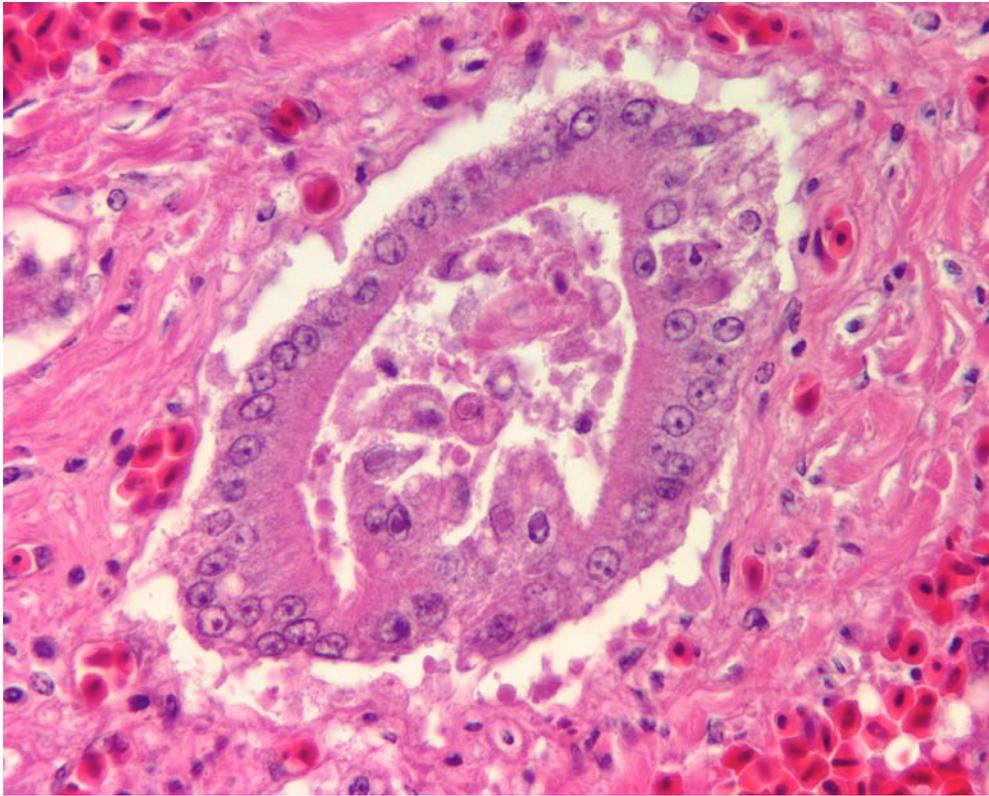
Gross Pathology: There was moderate, diffuse enlargement of the liver with multifocal to coalescing tan foci. These foci were not raised, extended into the parenchyma and bulged minimally on section. Red-tinged, dark brown fluid filled the duodenum, the distal small intestine, ceca and colon. There were multiple raised tan foci on the ileal and cecal mucosa. Pinpoint to 1.0 mm diameter red to dark brown foci were present on the esophageal mucosa. Red streaking and mottling was present on the epicardial surface and the endocardium of the left ventricle, and pinpoint red foci were identified in the pancreas and coelomic adipose tissue.

Laboratory Results: PCR was performed using primers targeting the herpesvirus DNA polymerase gene, and an appropriately-sized PCR product of

180 base pairs was amplified. The product was sequenced and found to be 100% identical to Anatid herpesvirus 1 (Genbank accession No. JQ647509).

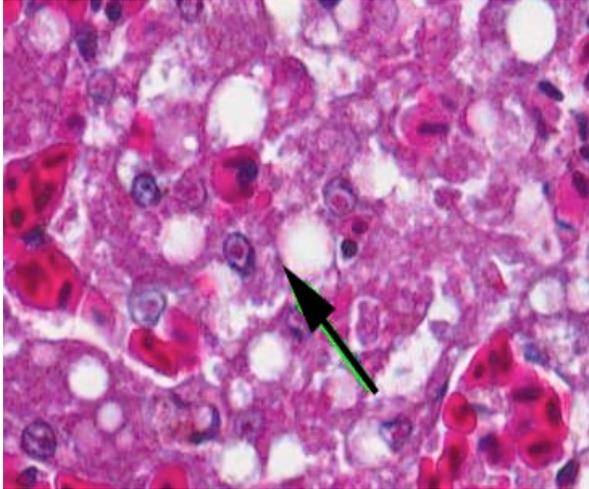
A Gram stain performed on the liver identified intravascular and intrasinusoidal Gram negative bacilli.

Histopathologic Description: Liver: There is extensive disruption of hepatic architecture with moderate to large numbers of free erythrocytes (hemorrhage) interrupting hepatic cords. Individualized hepatocytes with eosinophilic and shrunken cytoplasm, and karyorrhectic or karyolytic nuclei are scattered throughout the section (individual cell necrosis). Additionally, there are multifocal, random areas of necrosis characterized by diffuse eosinophilia and variable retention of architecture. Hepatocytes in less affected portions of the liver frequently contain large, clear cytoplasmic vacuoles or have a lacy cytoplasmic appearance. Throughout the liver, in areas of necrosis, hemorrhage and architectural disruption, as well as in less affected areas, moderate numbers of hepatocytes contain eosinophilic intranuclear inclusion bodies.

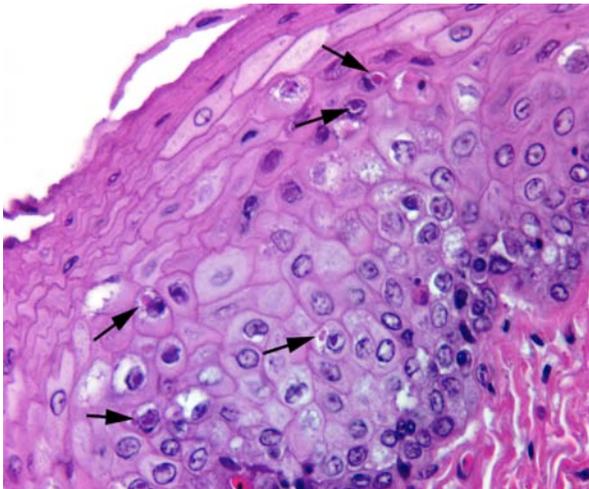


2-1. Liver; Muscovy duck: Multiple biliary epithelial cells contain eosinophilic intranuclear viral inclusions consistent with herpesvirus infection. There is sloughing of most of these cells. (HE 600X) (Photo courtesy of: The Wildlife Conservation Society, Zoological Health, 2300 Southern Blvd., Bronx, NY 10460, <http://www.wcs.org>)

These are often centrally located with peripheral chromatin clearing. They occasionally fill the nucleus, leaving only a thin clear halo within the nuclear envelope. Similar intranuclear inclusion bodies are rarely found within biliary epithelial cells and affected cells are often sloughed into the ductular lumen. Necrotizing vasculitis, predominantly affecting central veins, is uncommon. There are multifocal, small, dense



2-2. Liver, Muscovy duck: Intranuclear inclusions are also seen in hepatocytes. (HE 400X)



2-3. Esophagus, Muscovy duck: Scattered squamous epithelial cells contain variably sized, eosinophilic, intracytoplasmic inclusion bodies. Intranuclear inclusion bodies are also present, and some cells contain both intracytoplasmic and intranuclear inclusions. (HE 600X) (Photo courtesy of: The Wildlife Conservation Society, Zoological Health, 2300 Southern Blvd., Bronx, NY 10460, <http://www.wcs.org>)

accumulations of bacteria (predominantly short bacilli) in sinusoidal and intravascular spaces and, occasionally, in foci of hepatocellular necrosis.

Esophagus: The mucosal epithelium contains multifocal ulcers and erosions and exhibits scattered necrosis of epithelial cells. There is disruption of normal layering, accumulation of karyorrhectic debris, few transmigrating leukocytes and variable intracellular edema (ballooning degeneration) in affected areas. Epithelial cells in these areas also occasionally contain intranuclear inclusion bodies similar to those described in the liver. Rare, variably sized, eosinophilic intracytoplasmic inclusion bodies

are also present. Rafts of sloughed epithelial cells (with occasional intranuclear inclusion bodies) admixed with keratin, necrotic cell debris and mixed bacteria often cover the affected mucosa. Underlying some epithelial lesions is accumulation of karyorrhectic debris in the submucosa, accompanied by mild basophilia and disruption of the fibrillar collagen architecture. Submucosal glands are frequently obliterated; these glands contain intraluminal sloughed epithelia (with occasional intranuclear inclusion bodies), necrotic cellular debris and mucinous material. Intranuclear inclusion bodies are also present in remaining, viable glandular epithelium. Scant mononuclear infiltrates are scattered throughout the submucosa and are predominantly perivascular.

- Contributor's Morphologic Diagnosis:**
1. Liver: Necrosis, acute, random, multifocal and individual cell, marked, with intranuclear inclusion bodies, necrotizing vasculitis, hemorrhage and hepatocellular vacuolar change.
 2. Liver: Bacteremia, acute, moderate.
 3. Esophagus: Necrosis, acute, multifocal, moderate, with intranuclear and intracytoplasmic inclusion bodies, multifocal erosions and ulcers, and submucosal gland necrosis.

Contributor's Comment: The submitted liver and esophagus exhibited necrosis with intranuclear inclusion bodies, lesions typical of herpesvirus infections across species. In addition to the tissues examined here, there was severe fibrinonecrotic ileitis and segmental necrosis in the remainder of the small intestines. These sites were heavily infiltrated by bacteria and loss of mucosal epithelium precluded identification of discrete inclusion bodies (sloughed epithelial cells occasionally harbored presumptive intranuclear inclusions). Intestinal lesions were potentially secondarily invaded by opportunistic bacteria following initial damage incurred from viral infection. As noted in the submitted tissue, bacteremia and septicemia followed.

Based on the presence of intranuclear inclusion bodies, tissue necrosis and the species affected, anatid herpesvirus 1 (AnHV-1) was suspected and molecular diagnostics were pursued. PCR performed on a sample of frozen liver amplified an appropriately sized PCR product which was sequenced and found to be identical to AnHV-1 (DNA polymerase gene).

Anatid herpesvirus 1 causes predominantly a gastrointestinal disease in waterfowl which is referred to as “duck plague” or “duck virus/viral enteritis” (DVE).^{1,3,4} Disease severity depends on the strain of the virus and species infected.² Muscovy ducks, like the one in this case, are considered to be one of the more sensitive species, while mallard ducks (*Anas platyrhynchos*) are more resistant.³ Many anseriform species, including geese and swan, are susceptible, and recently a crested and a common coot (*Fulica cristata* and *F. atria*, respectively) reportedly died as a result of DVE.⁴ As with other herpes viruses, ducks surviving initial infection become latently infected and can intermittently shed the virus.^{3,4}

Typical presenting signs for DVE include lethargy, polydipsia, emesis, bloody or watery diarrhea and prolapse of the phallus.^{3,4} In many cases, especially in the more sensitive species, there may be no clinical signs prior to the animal being found dead.^{3,4} Infection typically occurs via oral ingestion of the virus, which is shed in the feces and orally from infected ducks; ingestion of contaminated water is thought to be the major route of transmission.^{3,4} Typical gross lesions include petechiae in multiple organs, annular band necrosis and hemorrhage in the intestinal tract, ulcers and erosions in the esophagus (especially at the esophageal/proventriculus junction) and on the ventral surface of the tongue, and multifocal necrosis in the liver.^{3,4} In addition to these findings, necrosis of lymphoid tissue and necrotizing vasculitis can be identified histologically.^{1,3,4} Intranuclear inclusion bodies would be expected in areas of necrosis, including lymphocytes. Barr, et al¹ reported the presence of intracytoplasmic inclusion bodies in the esophagus and cloacal epithelium of DVE-affected Muscovy ducks.¹ Ultrastructurally, these inclusions were membrane-bound and contained enveloped virions.¹ Rare (and not present in all slides), variably sized eosinophilic intracytoplasmic inclusion bodies similar to those described in the above report were identified in the esophagus of this patient. Although other causes of intracytoplasmic inclusion bodies were not completely ruled out, they were considered less likely given the nature of the lesions, the findings in other organs and PCR identification of AnHV-1.

Approximately one week following the death of this duck, another Muscovy duck at the same facility was acutely lethargic one evening and found dead the following morning. Gross and histopathological lesions were similar to those reported here. This was the second DVE-related mortality event affecting ducks at our institutions in the past decade. The first was responsible for the death of four Muscovy ducks and at least two wild mallards. It is presumed that wild, latently infected ducks sharing waterways and housing with the Muscovies were responsible for the infection, but the possibility of latently infected permanent residents intermittently shedding the virus has not been fully investigated.

JPC Diagnosis: 1. Liver: Hepatitis, necrotizing, multifocal, moderate, with intranuclear viral inclusion bodies and necrotizing vasculitis.
2. Esophagus: Esophagitis, necrotizing, multifocal, moderate, with intranuclear and intracytoplasmic viral inclusion bodies and submucosal gland necrosis.

Conference Comment: The contributor provides a thorough summary of anatid herpesvirus 1 infection in anseriforms. Conference participants were intrigued by the presence of both intranuclear and intracytoplasmic viral inclusions in esophageal epithelium, a phenomenon more commonly associated with paramyxoviruses. Herpesviruses, which are DNA viruses that replicate in the host cell nucleus, generally produce intranuclear inclusions.² However, intracytoplasmic viral inclusions are observed with certain herpesvirus infections, as illustrated by this case. In addition to Anatid herpesvirus-1, cytomegaloviruses (in the subfamily *Betaherpesvirinae*) and Gallid herpesvirus-2 (Marek’s Disease virus) can also be associated with intracytoplasmic viral inclusions in addition to the classic intranuclear inclusions.¹ Awareness of the possibility of observing intracytoplasmic inclusions in these herpesviral infections can help avoid misdiagnoses.¹

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References:

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CASE III: 11-1482 (JPC 4003259).

Signalment: Yearling, male, Harbor seal (*Phoca vitulina*).

History: This animal was found dead on a beach in the Pacific Northwest.

Gross Pathology: The animal was emaciated and featured corneal abrasions and numerous nasal mites.

Laboratory Results: PCR of pooled tissues proved negative for canine distemper virus and influenza virus and positive for *Apicomplexa*. No bacteria were recovered from the lung, lymph node, brain or spleen and there was heavy growth of *Escherichia coli* from the small intestine. Trace mineral and vitamin A analysis results of the liver proved within in house reference limits.

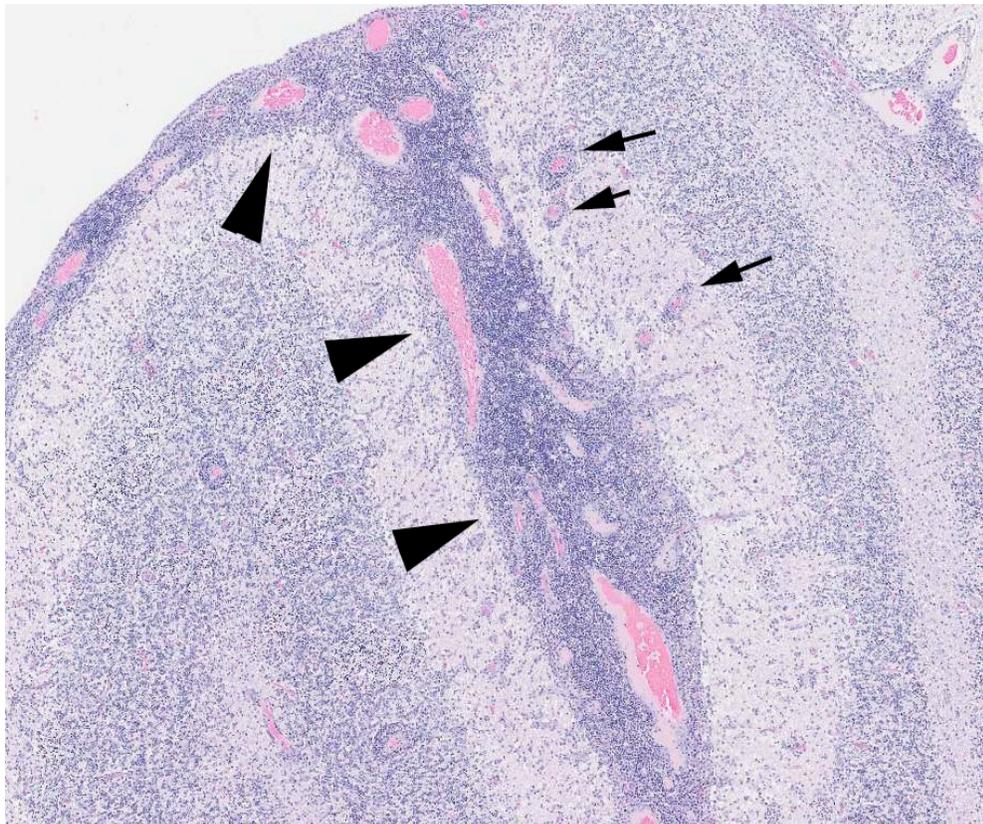
Histopathologic Description: Throughout the cerebellum, expanding the meninges, extending focally to segmentally throughout the molecular layer and expanding and disrupting the molecular

and granular layers of numerous folia, there is multifocal to coalescing accumulations of lymphocytes and histiocytes with fewer plasma cells. In more severely affected areas, there is multifocal edema fluid with neovascularization, reactive endothelia, lymphoplasmacytic perivascular cuffing and edema. Randomly interspersed within the inflammatory infiltrate are numerous protozoa with multifocal karyorrhectic and pyknotic debris.

Contributor's Morphologic Diagnosis: Brain: Meningoencephalitis, severe, multifocally extensive, necrotizing, lymphohistiocytic, with numerous intralésional protozoa.

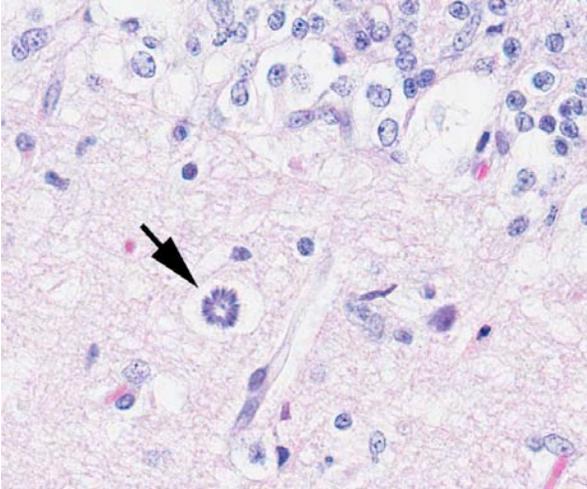
Contributor's Comment: Since 2000, increasing numbers of marine mammals have stranded within the Pacific Northwest with protozoal encephalitis. In the initial stages of this phenomenon, *Toxoplasma gondii* was the primary pathogen, however, more recently, *Sarcocystis neurona* has emerged as the dominant etiologic agent. Based on review of brain pathology, dual infections appear to have potentiated the pathogenicity of both parasites. Malnutrition, contaminant loads and other environmental stressors

may have also potentiated the pathology of infections. The increase in *S. neurona* infections is attributed to the extension of opossums north, into Washington State and British Columbia.

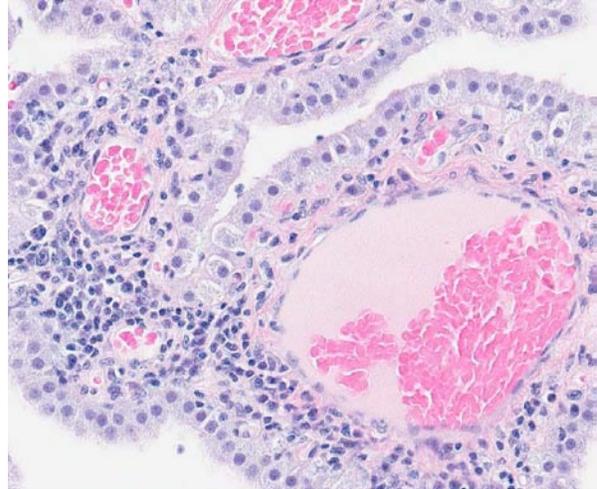


3-1. Cerebellum, harbor seal: The meninges (arrowheads) and Virchow-Robins spaces (arrows) are expanded by a profound inflammatory infiltrate. (HE 40X)

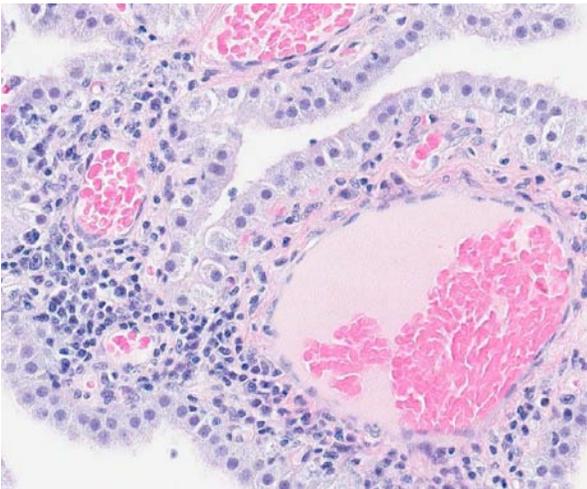
JPC Diagnosis: Cerebellum and brain stem: Encephalitis, necrotizing, multifocal to coalescing, severe, with diffuse lymphohistiocytic and neutrophilic meningitis, choroid plexitis, and numerous intracellular schizonts.



3-1. Cerebellum, harbor seal: The meninges (arrowheads) and Virchow-Robins spaces (arrows) are expanded by a profound inflammatory infiltrate. (HE 40X)



3-2. Cerebellum, harbor seal: Areas of inflammation contain numerous 20 µm apicomplexan schizonts in which zoitae reproduce by endopolygony (arrow). (HE 400X)



3-3. Choroid plexus, harbor seal: There is a moderate lymphoplasmacytic choroid plexitis (a common finding in animals with inflammatory encephalitis). (HE 360X)

Conference Comment: Conference participants discussed the differential diagnosis for this case, and favored *Sarcocystis neurona*; however, since mature schizonts and merozoites of *Sarcocystis* are difficult to distinguish from *Toxoplasma*, we consulted with Dr. J.P. Dubey. Immunohistochemical staining with antibodies against *Sarcocystis neurona* performed by Dr. Dubey resulted in strong positive immunoreactivity in numerous schizonts. Furthermore, the diagnosis was confirmed by Dr. Dubey based on the morphology of the immature schizonts, as *S. neurona* immature schizonts are morphologically unique in that their nucleus becomes multilobed, eventually giving rise to many merozoites, and often leaving a residual body. (J.P.

Dubey, personal communication).

S. neurona is an apicomplexan, best known for causing equine protozoal myeloencephalitis (EPM), a severe neurologic disease in horses.¹ Opossums spread the disease by shedding sporocysts into the environment.¹ In addition to horses and sea mammals (sea otters, Pacific harbor seals), other animals including cats, striped skunks, and nine-banded armadillos can be infected with *S. neurona*; however, in these species, the sarcocysts tend to develop in muscle rather than the central nervous system.^{1,2} Raccoons have also been reported to have myocarditis and encephalitis due to infection with *S. neurona*.¹

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References:

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2. Dubey JP, et al. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, and *Sarcocystis canis*-like infections in marine mammals. *Vet Parasitol*. 2003;116(4):275-96.

CASE IV: HN2633 (JPC 4003689).

Signalment: Juvenile, Whooper swan (*Cygnus cygnus*).

History: This wild swan was found weak at the northern lake of Japan in Jan, 2011. A local veterinarian euthanized the bird and detected influenza virus using rapid test kit. The carcass was transported to our university and dissected within the biosafety level 3 facility.

Gross Pathology: The bird was in relatively good nutritional condition. In pancreas, multiple hemorrhagic foci up to 8 mm were scattered.

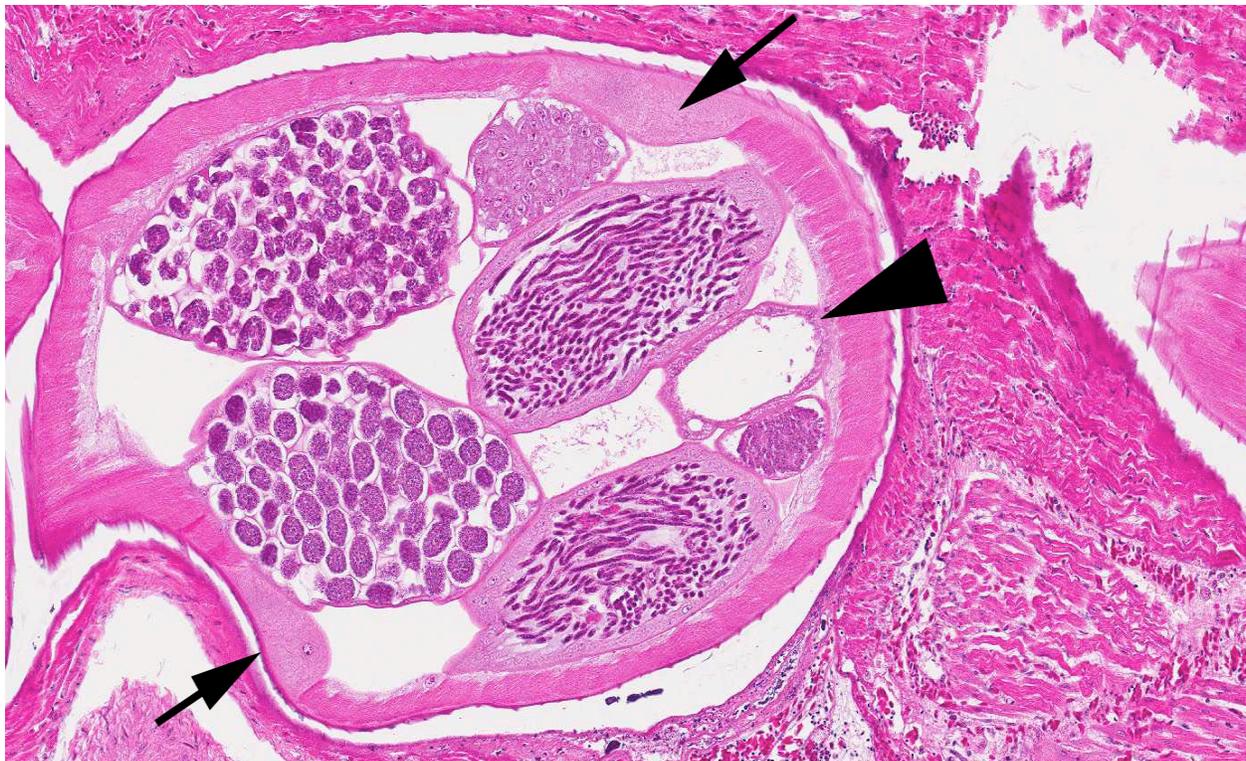
Laboratory Results: Highly pathogenic avian influenza virus of H5N1 subtype was isolated from the brain, trachea, lungs and colon.

Histopathologic Description: Heart: Multifocal to coalescing myocardial necrosis and infiltration of inflammatory cells were scattered throughout the heart. The lesions consisted of coagulative necrosis of myocardial fibers admixed with lymphocytes, macrophages and mild hemorrhage. By

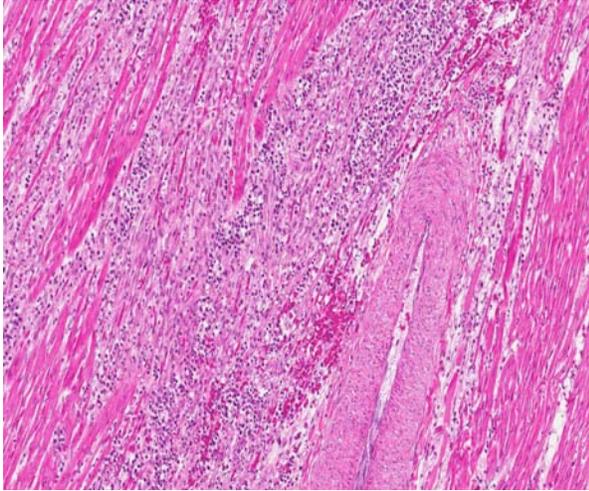
immunohistochemistry, strong positive signals of influenza virus antigens were confirmed in myocardiocytes around the inflammatory lesions. A few organisms enclosed in cuticula in the heart were consistent with parasitic nematodes. The nematodes contained microfilaria-like structures in the viscera. Inflammatory reaction to the parasites was indiscernible.

Contributor's Morphologic Diagnosis: Heart: Myocarditis, necrotic and nonsuppurative, multifocal to coalescing, severe, influenza virus infection and parasitic nematode infection.

Contributor's Comment: The threat of highly pathogenic avian influenza virus (HPAI) of H5N1 subtype to humans as well as domestic and wild birds is of great concern to human public health and fowl industry. The first reported serious case of H5N1 influenza virus in Asia was in Hong Kong in 1997, and emerged in east and southeast Asian countries in 2003.⁵ Since then, Japan has also suffered small outbreaks of H5N1 infection in domestic fowls. HPAI surveillance activity in poultry farm and wild migrating birds is important to control and predict the epidemics of HPAI. The



4-1. Heart, swan: The myocardium contains numerous cross sections of an adult nematode measuring up to 100 μ in diameter, with a ridged cuticle, large lateral cords, a digestive tract with few multinucleated cells (arrowhead), ovaries with morulated eggs and a uterus with microfilariae (arrows). (HE 4X)



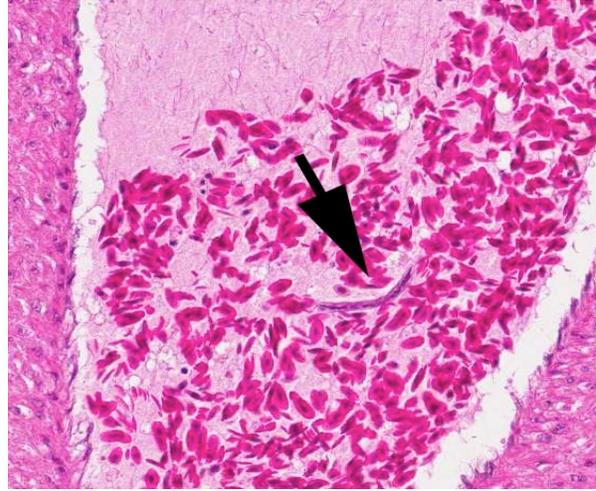
4-2. Heart, swan: There are multifocal to coalescing areas of myofiber necrosis and loss with infiltration by large numbers of histiocytes, lymphocytes, and plasma cells, as well as scattered hemorrhage. In this particular case, necrosis may have resulted from either migration of parasites within the myocardium, or the concurrent infection with highly pathogenic avian influenza (or both, concurrently). (HE 160X)

present case was one of the birds likely on migration from southern Asian countries to Siberia.

Histopathological examination of the systemic organs from this bird revealed necrotic changes and nonsuppurative inflammation in the heart, pancreas and brain that were consistent with HPAI infection lesion.⁶ Immunohistochemical analysis using anti-H5 subtype influenza virus polyclonal antibody also revealed the distribution of viral antigens in myocardium, necrotic foci in pancreas and in astrocytes and neurons in the brain, suggesting systemic infection of influenza virus.

In domestic fowls, an experimental study found HPAI infection causes rapid viremia and produces vascular endothelial cell injury.^{2,4} Necrotic and apoptotic changes of parenchymal cells of organs follow the endothelial damages. The most severely affected tissues are brain, heart, lungs and pancreas as well as primary and secondary lymphoid organs.^{2,6} But the lesions vary from mild to severe, although the affected birds usually die shortly after systemic infection. The histopathological lesions in the present case were relatively severe.

Adult heartworms containing microfilariae in their uterus were found in the heart of the present case. The morphology of the parasites presumably identified as *Sarconema eurycerca*, a filarial nematode of the superfamily *Filarioidea*. Several studies of *S. eurycerca* infection in wild swans and geese were reported.^{1,7} Although mortality cases in



4-3. Heart, swan: Coronary arterioles rarely contain microfilariae. (HE r00X)

wild birds are reported, the pathological significance of this worm is still not well understood. Grossly, an enlargement of the heart with dark red and white streaks due to the migration of adult worms in myocardium is described in previous reports. The heart failure is induced by myocardial necrosis when numbers of parasites infected. Hemorrhage, lymphocytic and histiocytic inflammation was multifocally observed. Migration of microfilaria into myocardium, which is accompanied with inflammation, is sometimes observed, but was not observed in the present case. The infection of heartworms may partly contribute to develop severe myocardial lesions, but the cause of death in this swan might be concluded as systemic influenza virus infection.

JPC Diagnosis: 1. Heart: Myocarditis, heterophilic and granulomatous, chronic, multifocal, moderate, with myofiber necrosis, atrophy and loss, with adult filarid nematodes and microfilaria.
2. Heart: Vasculitis, heterophilic and necrotizing, multifocal, with thrombosis and myofiber degeneration and necrosis.

Conference Comment: The contributor provides an informative summary of both HPAI and *Sarconema eurycerca* infection in this very interesting case. Conference participants discussed the two disease processes occurring simultaneously and speculated on the two distinct patterns of necrosis in the heart: The chronic lesions are attributed to adult filarid migration tracts while the

granulomatous inflammation observed in some sections is associated with microfilaria. Although the relationship between the chronic myocardial lesions and the more acute vasculitis, thrombosis and coagulative necrosis are unclear, participants attributed the latter to infection with HPAI.

HPAI is in the family *Orthomyxoviridae*, genus *Influenzavirus A*. Influenza A viruses commonly infect horses, swine, and domestic poultry, as well as humans.³ Wild birds, particularly ducks, shorebirds and gulls, are reservoir hosts for low-pathogenicity influenza A viruses; however, mink, seals, whales and dogs can also become infected. Influenza A viruses are currently classified into 16 hemagglutinin (H) and 9 neuraminidase (N) types. Gene reassortment results in numerous combinations of H and N subtypes; however, only a few combinations are important in naturally occurring infections in animals. Enzootic H7N7 and H3N8 viruses (previously called equine influenza viruses 1 and 2) cause respiratory disease in horses. Enzootic H1N1, H2N2, and H3N2 viruses affect swine. H3N8 and H3N2 viruses cause respiratory disease in dogs. Sporadic H10N4 viruses cause respiratory disease in mink. Sporadic H7N7 and H4N5 viruses cause disease in seals. In humans, H1N1, H2N2, H3N2, H5N1, H7N3, H7N7 and H9N2 viruses can all cause respiratory disease and virtually all of these types can be found in wild aquatic birds and domestic poultry. Of these, the H5 and H7 viruses can be associated with the high-pathogenicity phenotype. High pathogenicity is associated with changes in an amino acid sequence at the cleavage site of the hemagglutinin protein. These changes, which result in altered virulence, include the elimination of the glycosylation site, pH changes, and insertions or deletions in the amino acid sequence. While low pathogenicity subtypes are restricted to the intestinal and respiratory system in birds, highly pathogenic strains cause systemic disease characterized by viremia, endothelial injury and subsequent necrosis in multiple organs, as described by the contributor.³

Conference participants also discussed differential etiologic diagnoses for mononuclear myocarditis in birds including West Nile Virus, Newcastle Disease Virus, and protozoal myocarditis. As pointed out by the conference moderator, the presence of myocardial, pancreatic and/or hepatic necrosis in waterfowl should immediately raise the suspicion of HPAI infection.

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