

**The Armed Forces Institute of Pathology
Department of Veterinary Pathology**

Conference Coordinator
Matthew Wegner, DVM



WEDNESDAY SLIDE CONFERENCE 2010-2011

C o n f e r e n c e 8

6 October 2010

Conference Moderator:
Mike Garner, DVM, PhD, Diplomate ACVP

CASE I: S981/08 (AFIP 3163067).

Signalment: 1-year-old, male, Snowy owl (*Nyctea scandiaca*).

History: The owl lived in a farm with several other birds, including owls and different birds of prey. The animal had no history of previous illnesses and developed clinical signs one day before death in autumn 2008. The vaccination status of the owl is unknown. Predominant clinical signs were apathy and a severe cyanosis, thus the private veterinarian started a therapy against shock. Because the clinical symptoms did not improve, the owl was euthanized due to poor prognosis.

Gross Pathology: The carcass was in good body condition with plenty of adipose tissue. Both liver and spleen were dark red to reddish purple and were mild to moderately enlarged (hepato- and splenomegaly). During cut sections, a small amount of blood flowed from the organs (mild hyperaemia). Throughout the whole parenchyma of liver and spleen, numerous yellow-white partly coalescing caseous foci (approximately 1.0 to 2.0 mm size in diameter) were found. These were interpreted to be necrosis. The serous membranes of both organs were normal. Further findings during necropsy were moderate numbers of granulomas diffusely distributed among the aerosaccula, and mild to moderate uric acid scaling within the kidneys. Additionally, some bird lice

(*Menacantus sp.*) were found on the feathers. All other organs, including the bone marrow, appeared normal.

Laboratory Results: Microbiological and virological examination was performed from samples of the liver, spleen and the lungs. The results are listed below.

Lungs: *Aspergillus* spp.; coagulase-negative *Streptococcus* spp.; and *Escherichia coli*.

Liver and spleen: no bacterial growth observed.

Extracts from liver and spleen were cultured on chicken-embryo-hepatocytes and chicken-embryo-fibroblasts in cell culture and the supernatant was analyzed on an electron microscope. These examinations revealed *Herpesviridae* in both organ samples.

Histopathologic Description: Liver: The parenchyma is multifocally replaced by areas of degenerated hepatocytes and acute central(ly) coagulating necrosis. Several affected areas are coalescing. Adjacent to these areas, the hepatocytes show variable stages of degeneration with pyknosis and karyorrhexis and peripheral nuclear hyperchromasia. Numerous affected hepatocytes, primarily at the edges of necrotic regions, show eosinophilic intranuclear inclusion bodies (Cowdry A-type). There is no inflammatory infiltration or demarcation of the necrotic regions. Occasionally, intravascular coagulopathy is seen in some arterial and venous vessels of the portal fields and central veins sometimes contain fibrin thrombi.

Some portal vessels and central veins frequently show intravascular leucocytostasis. The capsule of the organ, as well as portal fields, bile ducts and hepatocytes of unaffected areas are normal.

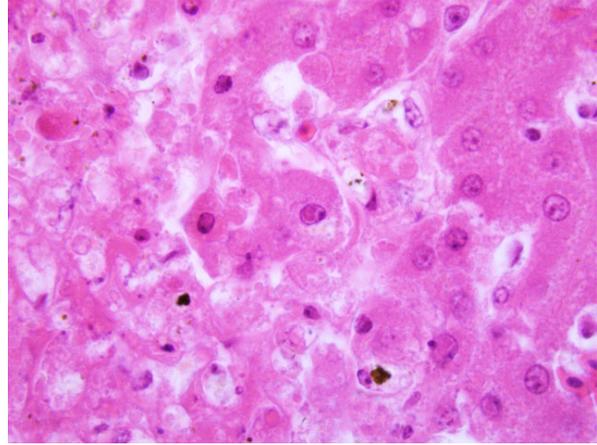
Spleen: The parenchyma is replaced by multifocal to coalescing areas of acute necrosis, partly associated with acute haemorrhages resulting in complete loss of the typical architecture of the organ. Several reticuloendothelial cells of the white pulp closely adjacent to these regions contain eosinophilic intranuclear inclusion bodies (Cowdry A-type). A beginning demarcation of necrotic regions by granulocytes is seen in some areas of the spleen. The unaffected white pulp is highly depleted. Furthermore, numerous histiocytes are filled with a brown, chunky pigment (haemosiderocytes). Frequently, areas with an accumulation of dark brown pigment that appears green at the borders can be seen (formalin-pigment). The capsule of the organ is normal.

Contributor's Morphologic Diagnosis: 1. Liver: Moderate, acute, multifocal sometimes coalescing necrosis, with multifocal eosinophilic intranuclear inclusion bodies, *Herpesviridae*.

2. Spleen: Severe, acute, multifocal to coalescing necrosis, with multifocal eosinophilic intranuclear inclusion bodies, *Herpesviridae*.

Contributor's Comment: The clinical and pathological findings in this case represent the typical syndrome of *hepatosplenitis infectiosa strigum* (HSIS) in a snowy owl. This infectious disease of wildlife and captive birds usually runs fatal within a few days and is caused by a herpesvirus.

The family of avian herpesviruses comprises three subfamilies, including alpha-, beta- and gamma-Herpesviruses. Each name for the genera derives from the predominant clinical and pathological findings.¹⁰ *Hepatosplenitis infectiosa strigum* (HSIS) is caused by the *strigid herpesvirus-1* (SHV-1) and is dedicated to the betaherpesviruses (synonym "hepatosplenitis viruses"). This disease occurred in 1915 in Austria for the first time (retrospective examination of fixed tissue samples of affected birds),³ but was first described in the United States in 1936.⁸ It is seen frequently in Germany since 1969.^{4,12} The SHV-1 is a strict host-specific pathogen that reveals close similarities to the *falconid herpesvirus-1* (FHV-1) and the *columbid herpesvirus-1* (CHV-1). Due to the large genomic homology, a PCR styled to detect the CHV-1 can also be used to detect SHV-1 and FHV-1.¹ Reported susceptible species of owls for HSIS are: the Eagle Owl (*Bubo bubo*), the Great Horned Owl (*Bubo virginianus*), the Striped Owl (*Asio clamator*), the Long-eared Owl (*Asio otus*), the Snowy Owl (*Nyctea scandiaca*), the Little Owl (*Athene noctua*), and the Boreal Owl (*Aegolius funereus*), whereas the Eurasian



1-1. Liver; owl. Multifocally, degenerate hepatocytes at the periphery of areas of necrosis occasionally contain eosinophilic intranuclear inclusion bodies that marginate the chromatin. (HE 1000X)

Tawny Owl (*Strix aluco*) and the Barn Owl (*Tyto alba*) seem to be naturally resistant.⁴ However, other authors have shown that American Kestrels, Budgerigars and Ring-Necked Doves are susceptible for experimental infections, as well.¹²

Additionally, evidence for latent infections was found, given the fact that antibody-positive but healthy animals could be observed.⁹ As reported by Burtcher and Sibalin,⁴ only owls with a yellow or orange coloured iris (of the aforementioned species) have proven to be susceptible, whereas species with dark irises (e.g. Tawny Owls and Barn Owls) were resistant, even to massive experimental infections. However, the authors considered this finding not to be significant, but remarked further investigations were needed to clarify.

The virus has a tropism for mesenchymal cells and to a lesser extent epithelial cells.^{2,4} An oropharyngeal route of infection is assumed. Virus-shedding takes place through the pharynx and urine. After an incubation period of 7 to 10 days, affected animals show apathy, anorexia and ruffled feathers. At later stages, they support themselves with their wings standing on the soil and fall into the prone position in which they are perishing in the following period.^{2,8,11}

As in the present case, findings during necropsy are: good nutritional condition, swelling of the liver (hepatomegaly) and pale white foci of caseous necrosis in liver, spleen and bone marrow (not recorded in this case). Microscopic examinations reflect the necrotic regions to be mostly without any inflammatory reaction presumably due to the fulminant clinical progress of the disease. Viable and degenerating hepatocytes, particularly at the edges of the necrotic foci, show intranuclear eosinophilic inclusion bodies (Cowdry A-type).^{2,8,11}

It was not possible to establish the source of the infection in this case. Even intense anamnestic survey could not identify potential reservoirs (no acquisition of animals, no contact to wildlife animals and no changes in the acquisition of prey animals). Regardless, no further infections were recorded in this aviary. This might be due to the strict hygienic prevention (including the prey animals) that was performed acting upon the advice of the private veterinarian and the Institute of Pathology of the University of Leipzig.

AFIP Diagnosis: 1. Liver: Hepatitis, random, necrotizing, acute, multifocal, moderate, with hepatocellular intranuclear eosinophilic inclusion bodies.
2. Spleen: Splenitis, necrotizing, acute, multifocal to coalescing, marked with intranuclear eosinophilic inclusion bodies.

Conference Comment: Most participants readily identified the intranuclear hepatocellular inclusions, which all diagnosed as consistent with herpesviral infection. A brief discussion of other causes of intranuclear inclusion bodies followed, which in addition to viral etiologies, include heavy metals, such as lead or bismuth; cytoplasmic nuclear invaginations that result in pseudoinclusions; and occasionally, neoplastic cells accumulate tubules and filaments resulting in histologically visible intranuclear inclusions.^{5,6} In addition to infection by other herpesviruses, other avian viruses producing intranuclear inclusions include avian adenoviruses, psittacine circovirus, avian paramyxovirus 1 (Newcastle disease) and avian polyoma virus. Avian adenoviruses and herpesviruses are the primary agents causing intranuclear inclusions in the hepatocytes of owls.

Some discussion focused on the epidemiology of HSIS in wild birds of prey. The moderator mentioned that this disease is only seen in wild, and not captive, falcons and owls. Recent research demonstrated that the herpesviruses isolated from owls, falcons, hawks and pigeons were all identical. The authors concluded that owls, falcons and hawks are infected with the same virus, Columbid herpesvirus-1, and therefore captive birds of prey should not be fed pigeons.⁷

The moderator commented that a few avian herpesviruses are associated with neoplasia. One example, Psittacid herpesvirus-1, the etiology of Pacheco's disease, is known to cause psittacine papillomatosis; gross findings are papillomas in the choana and cloaca.¹⁴ The herpesviral induced proliferative epithelial lesions can undergo malignant transformation to squamous cell carcinoma or adenocarcinoma. More recently, DNA from this virus

was detected in a pancreatic duct carcinoma in a macaw.¹³

Contributor: Institut für Veterinär-Pathologie, An den Tierkliniken 33, 04103 Leipzig, Germany
<http://pathologie.vetmed.uni-leipzig.de/>

References:

1. Aini I, Shih LM, Castro AE, Zee YC. Comparison of herpesvirus isolates from falcons, pigeons and psittacines by restriction endonuclease analysis. *J Wildl Dis.* 1993;29:196-202.
2. Burki F, Burtscher H, Sibalin M. Herpesvirus strigis: a new avian herpesvirus. I. Biological properties. *Arch Gesamte Virusforsch.* 1973;43:14-24.
3. Burtscher H. Die virusbedingte Hepatosplenitis infectiosa strigum. 1. Mitteilung: Morphologische Untersuchungen. *Pathol Vet.* 1965;2:227-255.
4. Burtscher H, Sibalin M. Herpesvirus strigis: host spectrum and distribution in infected owls. *J Wildl Dis.* 1975;11:164-169.
5. Cheville NF. Response to cellular injury. In: *Ultrastructural Pathology: The Comparative Basis of Cellular Disease.* 2nd ed. Ames, IA: Wiley-Blackwell; 2009:13.
6. Cheville NF. The neoplastic cell. In: *Ultrastructural Pathology: The Comparative Basis of Cellular Disease.* 2nd ed. Ames, IA: Wiley-Blackwell; 2009:7665
7. Gailbreath KL, Oaks JL. Herpesviral inclusion body disease in owls and falcons is caused by the pigeon herpesvirus (Columbid herpesvirus 1). *J Wildl Dis.* 2008; 44(2):427-433.
8. Green RG, Shillinger JE. A virus disease of owls. *Am J Pathol.* 1936;12:405-410.
9. Kaleta EF, Drüner K. Hepatosplenitis infectiosa strigum und andere Krankheiten der Greifvögel und Eulen. *Zentralblatt Vet Med.* 1976;25(Suppl):173-180.
10. Kaleta EF. Herpesviruses of birds: a review. *Avian Pathol.* 1990;19:193-211.
11. Lee LF, Armstrong RL, Nazerian K. Comparative studies of six avian herpesviruses. *Avian Dis.* 1972;16:799-808.
12. Mare CJ. Herpesviruses of birds of prey. *J Zoo Animal Med.* 1975;6:6-11.
13. Mundhenk L, Müller K, Lierz M, et al. Psittacid herpesvirus DNA in a pancreatic duct carcinoma in a macaw. *Vet Rec.* 2009;164:306-308.
14. Styles DK, Tomaszewski EK, Jaeger LA, Phalen DN. Psittacid herpesviruses associated with mucosal papillomas in neotropical parrots. *Virology.* 2004;325(1):24-35.

CASE II: A09-35528 (AFIP 3138059).

Signalment: Group of less than 1-year-old, mixed sex, African cichlid fish, Family Cichlidae, Genus and species unknown.

History: The fish were part of a large shipment of pond raised African cichlid fish that had been held in quarantine approximately two weeks prior to the onset of mortalities. Losses had reached 25% at the time of submission. Reported gross necropsy findings included abdominal distension and cloudy, gelatinous intracoelomic fluid.

Gross Pathology: None provided.

Histopathologic Description: Tissue: Changes vary slightly between sections from individual fish. The normal architecture of the gastric wall has been transmurally effaced and replaced by broad sheets of predominantly epithelioid macrophages, interspersed with variable mixed numbers of lymphocytes, proliferating fibroblasts, and occasional discrete granulomas. However, more extensive, multifocal to coalescing granuloma formation, with central necrosis and wide mantles of epithelioid cells are more frequently encountered in some sections. Only isolated remnants of gastric mucosa and smooth muscle remain. Present throughout are small vacuolated spaces containing one to several, elongated pyriform, approximately 5 x 10 µm organisms with a distinct nucleus and dense basophilic kinetoplast located peripherally against one long body axis. Large numbers of mixed bacteria are widely distributed in most sections. The degree to which the inflammatory process extends beyond the gastric serosa also varies between individual fish. In some sections, isolated foci of granulomatous inflammation and granulomas are scattered throughout the mesenteric fat, while in others extensive sheets of inflammatory cells and fibroplasia encroach upon and infiltrate the hepatic capsule. In these sections, multiple foci of necrosis, granuloma formation, and organisms as previously described can be found in the liver and head kidney.

Contributor's Morphologic Diagnosis: 1. Stomach: Gastritis, necrotizing and granulomatous, transmural, diffuse, severe, with multifocal granulomas, adjacent granulomatous peritonitis and intralesional protozoa.
2. Liver and Kidney (variable between sections): Multifocal necrosis, granulomatous inflammation and epithelioid granulomas with intralesional protozoa.

Contributor's Comment: Consistent with the referring veterinarian's findings, follow-up necropsies performed on euthanized moribund fish revealed abdominal distension and cloudy ascites. Stomachs were extremely friable when manipulated and large

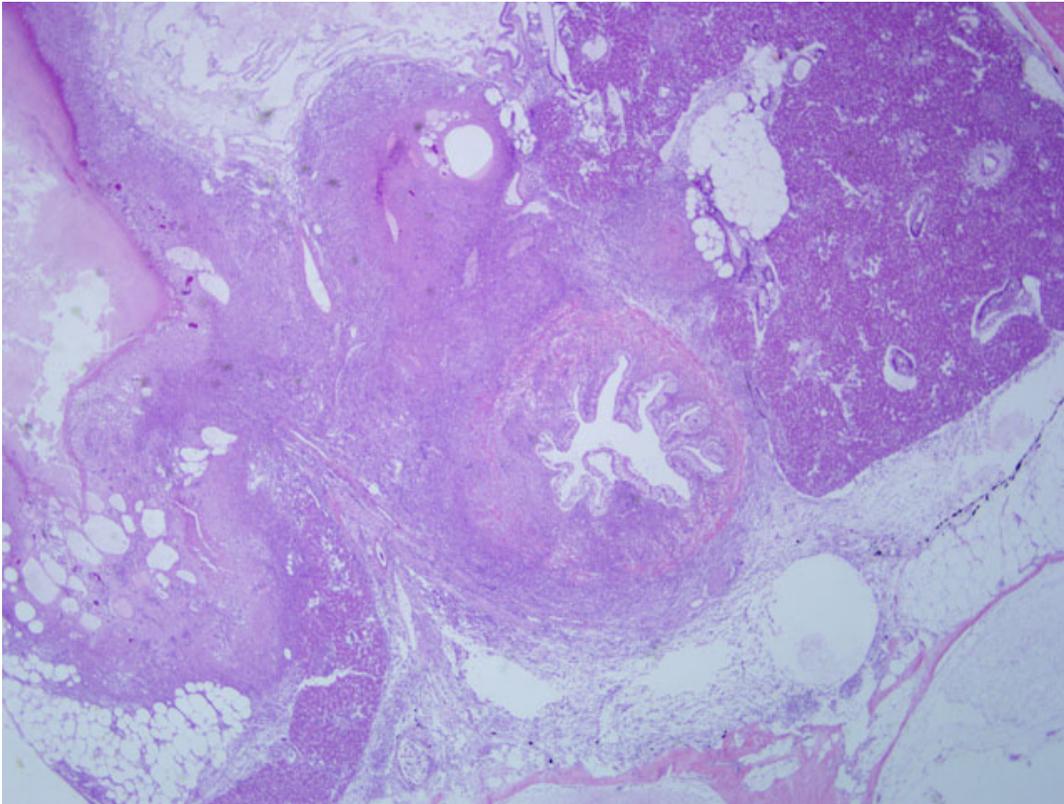
numbers of bi-flagellated protozoans, with undulating motility, were observed in gastric mucosal scrapings.

Microscopic findings of severe granulomatous gastritis are consistent with descriptions of *Cryptobia iubilans* (order Kinetoplastida, family Bodonidae) infection in other cichlid fishes.^{1,4,6} The Kinetoplastidea are flagellates with long, tubulous mitochondria curved inside the cell, which contain a kinetoplast, an organized DNA "nucleoid." Easily detected by Giemsa and Feulgen stains, it is usually single and located close to the kinetosome. One or two flagella may be present and most parasitic forms are transmitted by a vector.

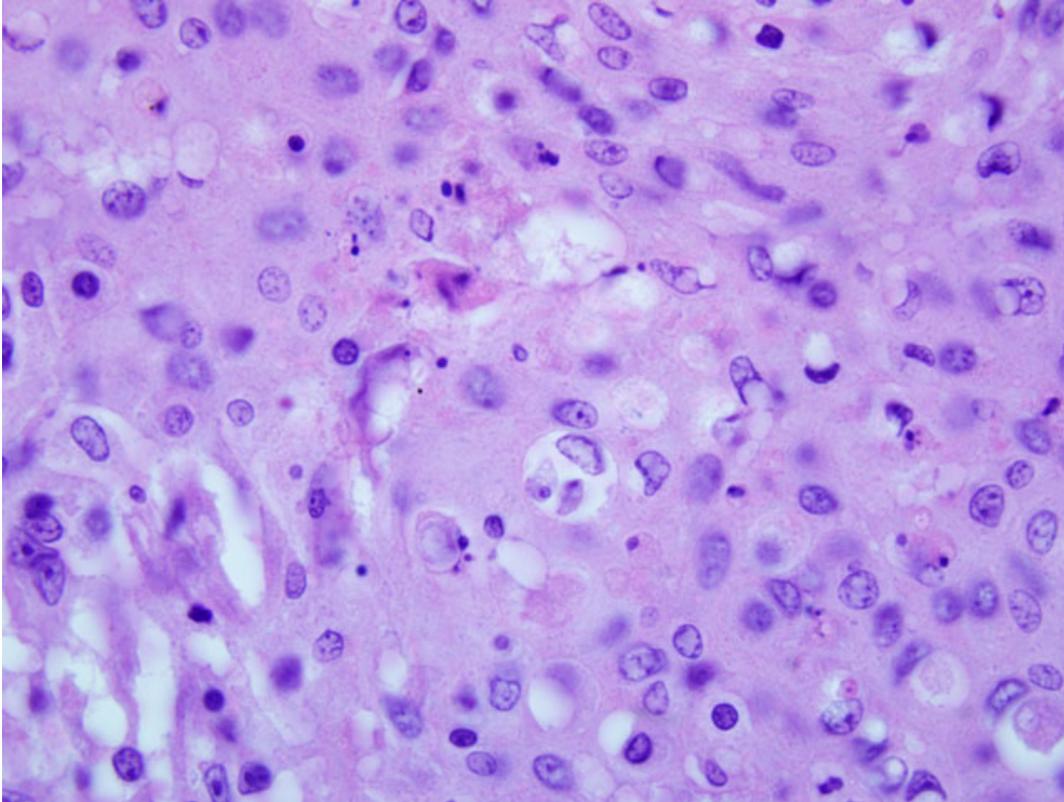
Although 52 *Cryptobia* spp. have been tentatively identified in fish, most are leech transmitted hemoparasites and it has been proposed they be assigned to the Trypanoplasma.^{2,3} The only fish pathogenic intestinal species, *C. iubilans*, has a direct life cycle and is common in cichlid fishes. The parasite is ovoid to elongate and averages 19 x 5 µm. The anterior flagellum is 1.5 - 2 times the body length and the recurrent flagellum, attached along the ventral axis, extends 11-19 µm beyond the body. Posterior to the flagellar pocket is a slender triangular kinetoplast. A spherical nucleus lies in the anterior half of the body and the cytoplasm contains large vacuoles filled with glycogen reserves.³ Additional electron microscopic features are available in the literature.⁶

Histopathologic lesions in light infections are confined primarily to the stomach and may range from isolated granulomas to diffuse granulomatous gastritis. In severe cases there can be multi-organ involvement associated with a similar necrotizing and granulomatous response, as seen in the livers and head kidney of some sections. The parasite may occur extracellularly or intracellularly within macrophages, where it is confined to a large parasitophorous vacuole.^{2,6}

It has been suggested that *C. iubilans* may be a part of the normal gastrointestinal fauna, becoming pathogenic only under certain stressful conditions.⁶ There are no effective treatments and losses can be acute and severe. Differentials for *C. iubilans* infection include other flagellates, primarily *Spironucleus vortens*. This organism is seen typically in the intestinal lumen and while it does not evoke as intense an inflammatory response, systemic spread can occur as well. On wet mounts this diplomonad is differentiated from *C. iubilans* by its linear movement and 8 flagella.⁶ Although mycobacteriosis was considered, acid-fast stains and mycobacterial cultures performed on multiple fish were negative.



2-1, 2-2. Stomach, African cichlid fish. Transmurally, the stomach is infiltrated and effaced by many epithelioid macrophages, fewer lymphocytes, and fibroblasts. Admixed with inflammatory cells are clear vacuoles that contain one to several $5 \times 10 \mu\text{m}$ elongated pyriform organisms. (HE 20X, 1000X)



AFIP Diagnosis: 1. Stomach and intestines: Gastroenteritis, transmural, granulomatous and necrotizing, diffuse, severe with protozoal trophozoites and many bacilli.

2. Liver; pancreas; mesentery; and kidney: Granulomas, with coelomitis.

Conference Comment: The moderator pointed out that most cases of *Cryptobia* spp. infection in fish are not as visually appealing as in the slides of this case in which there is excellent tissue preservation and minimal autolysis. The moderator commented on the difficulty in detecting this organism during routine histologic examination within necrotic tissues, because the dead organisms appear remarkably similar to degenerate inflammatory cells. Finally, the moderator noted that cryptobiasis is the most important infectious disease of cichlids. Another species in the genus, *Cryptobia salmositica*, infects Pacific salmonids in the western United States.

The contributor provides an excellent review of this important disease of cichlids. Clinically, parasitemia results in anemia, exophthalmia, ascites, positive Coombs' test, microcytic hypochromic anemia, and anorexia. As a brief additional note, isolation of a cysteine protease and metalloprotease from the organism recently provided useful information regarding the pathogenesis of disease; current research is focused on the metalloproteinase that causes lysis of erythrocytes. Additionally, the protein degrades types I, IV and V collagen and laminin, aiding invasion and generation of the observed histologic lesions.⁵

Contributor: Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602

<http://www.vet.uga.edu/VPP/index.php>

References:

1. Dykova I, J Lom. Definition of Flagellates and Lesions Due to Various Flagellates. In: *Histopathology of Protistan and Myxozoan Infections in Fishes: An Atlas*. Praha, CR: Academia; 2007:79-87.
2. Francis-Floyd R, R Yanong. *Cryptobia iubilans* in Cichlids. University of Florida Institute of Food and Agricultural Sciences Extension Publication VM 104.
3. Gou FC and PTK Woo. Selected parasitosis in cultured and wild fish. *Vet Parasitol*. 2009;163: 207-216.
4. Lom J, I Dykova. Flagellates (Phylum Mastigophora Diesing, 1866). In: *Protozoan Parasites of Fishes*. Amsterdam, The Netherlands: Elsevier Science Publishers; 1992:48-53.
5. Woo PTK. Immunological and therapeutic strategies against salmonid cryptobiasis. *J Biomed Biotechnol*. 2010;10(Article ID 341783):9 pages.

6. Yanong RP, Curtis E, Russo R, et al. *Cryptobia iubilans* infection in juvenile discus. *J Am Vet Med Assoc*. 2004;224:1644-1650.

CASE III: G08-049679 (AFIP 3167328)

Signalment: Male, mature, captive bred, Timneh African Grey parrot (*Psittacus erithacus timneh*).

History: This bird was one member of a research colony that had been created to study fecal virus shedding in psittacine birds with proventricular dilatation disease (PDD). All birds in this colony had a history of exposure to other birds with PDD. This individually caged bird had not shown any signs of neurological or gastrointestinal disease over the course of the study.

Gross Pathology: No gross lesions seen at necropsy.

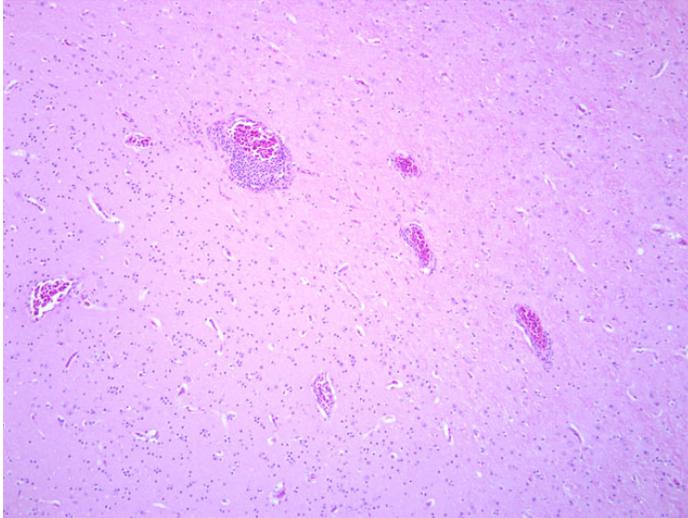
Laboratory Results: Histopathological lesions consistent with PDD were identified in cerebrum, cerebellum, brainstem, spinal cord, brachial nerve, vagus nerve, adrenal gland, crop, proventriculus, ventriculus and heart. Tissues positive for avian bornavirus (ABV) antigen by immunohistochemistry (IHC) include cerebellum, brainstem, spinal cord, adrenal gland and ventriculus. Tissues positive for ABV antigen by RT-PCR include cerebrum, cerebellum, brain stem, spinal cord and adrenal gland.

Histopathologic Description: Brain: Multiple cerebral and meningeal vessels are lined by mildly hypertrophied endothelial cells with partial to complete perivascular cuffs of variable thickness (1-8 cells thick) composed of predominantly lymphocytes and plasma cells with fewer macrophages.

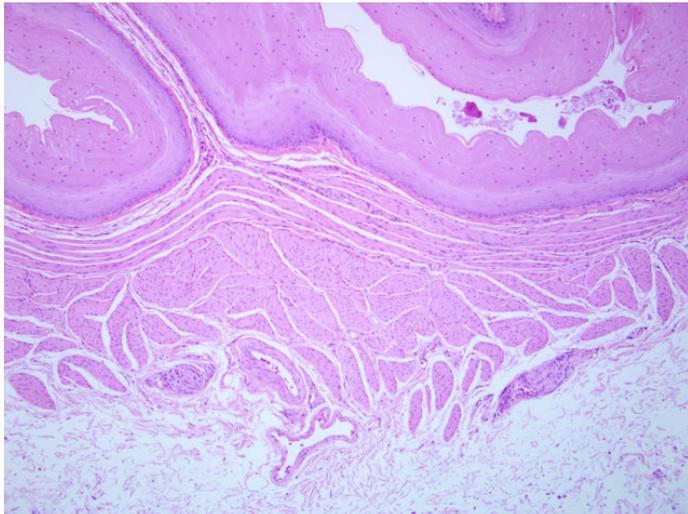
Crop: Variable numbers of plasma cells and lymphocytes infiltrate around and within blood vessels, nerves and ganglia in the tunica muscularis. Infrequently, a few plasma cells and lymphocytes have infiltrated between smooth muscle fibers of the tunica muscularis. Occasional moderate sized lymphoid aggregates are present in the lamina propria.

Contributor's Morphologic Diagnosis: 1. Mild to moderate cerebral and meningeal lymphoplasmacytic perivascular cuffing.
2. Mild to moderate multifocal lymphoplasmacytic ganglioneuritis and mild multifocal non-suppurative leiomyositis of the crop compatible with proventricular dilatation disease (PDD).

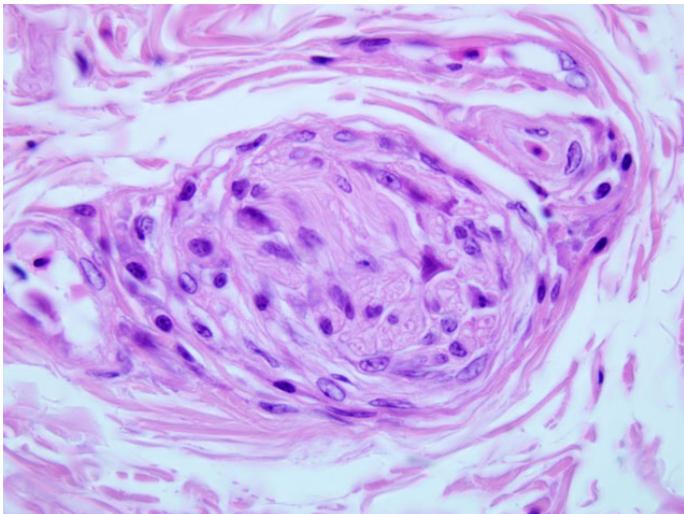
Contributor's Comment: Plasmacytic and lymphocytic infiltrates in the myenteric ganglia of the crop, proventriculus, ventriculus and duodenum are



3-1. Brain, African grey parrot. Multifocally, cerebral vessels are surrounded by low to moderate numbers of lymphocytes, macrophages, and plasma cells. (HE 100X)



3-2, 3-3. Crop, ganglia, African grey parrot. Multifocally, ganglia are infiltrated by low numbers of lymphocytes, macrophages, and plasma cells. (HE 100X, 1000X)



characteristic of proventricular dilatation disease (PDD). This disease has also been called macaw wasting disease, neuropathic gastric dilatation in psittaciformes⁷ and myenteric ganglioneuritis⁶ and has been reported in many species of psittacine birds.

Similar pathological findings have been identified in Canada geese² and recently in various Passeriformes.¹¹ Psittacine birds affected with PDD can exhibit gastrointestinal signs including anorexia, emaciation, weight loss, regurgitation, delayed crop emptying, diarrhea and the presence of undigested seeds in the feces and/ or neurological signs such as ataxia, tremors, seizures and motor or proprioceptive defects.³ Sudden death with the absence of clinical signs has also been reported. It is now known that the characteristic lymphoplasmacytic infiltrates can occur in many tissues of the central and peripheral nervous system and not all affected birds exhibit nervous signs clinically¹ as demonstrated by this bird. This bird did not show any signs of neurological disease for a year prior to euthanasia but histologically had widespread cerebral and meningeal perivascular lymphoplasmacytic cuffing.

The characteristic cellular infiltrates of PDD are variable in size and distribution as demonstrated by the lesions in the sections of brain and crop provided for this case. In some sections of crop, numerous plasma cells and lymphocytes are present within the nerves and ganglia, while in other sections, only rare plasma cells or lymphocytes can be identified. Historically, examination of multiple sections of biopsied tissues, such as crop, or multiple sections of gastrointestinal tract collected at necropsy was needed to improve diagnostic accuracy.

An avian bornavirus (ABV) has recently been proposed as the etiological agent of PDD, and evidence from bird inoculation studies is strongly supportive.^{4,5} Through collaborative efforts of researchers from the University of California, San Francisco, the Ontario Veterinary College and the Animal Health Laboratory, University of Guelph, an ABV specific RT-PCR screening test and immunohistochemistry (IHC) that detects the ABV nucleocapsid protein have been developed and tissues from psittacine birds with and without PDD were tested. Those results were compared with the histopathologic diagnoses and the sensitivity and specificity of IHC for detection of ABV antigens on a bird by bird basis were found to be 100% and 100%,

respectively. Many more tissues were positive for ABV RNA by RT-PCR than were positive histopathologically or for viral antigens by IHC. Brain tissues, but not crop tissues, from this bird were positive by RT-PCR for ABV antigen. Similar results were obtained with IHC testing. Overall, brain tissue appears to be the tissue that is most consistently positive with both PCR and IHC testing. It is suggested that IHC and or RT-PCR testing of biopsy specimens, in addition to histopathology, may help increase the sensitivity of diagnosis of PDD allowing for earlier identification of infected birds.¹¹

AFIP Diagnosis: 1. Brain: Meningoencephalitis, lymphohistiocytic and plasmacytic, perivascular, multifocal, mild.
2. Crop, ganglia: Ganglioneuritis, lymphohistiocytic and plasmacytic, multifocal, mild.

Conference Comment: Borna disease virus (BDV) is the sole member of the family *Bornaviridae*, order *Mononegavirales*. The virus is spherical and enveloped with negative-sense single-stranded RNA.⁸ Replication within infected cells occurs in the nucleus; viral inclusions typically are seen only in cell culture. BDV is neurotropic, resulting in persistent, non-cytolytic infection of the central nervous system.⁹ The virus is known to naturally infect a wide variety of animals, including horses, sheep, cattle and rabbits.⁸ The virus is implicated in human neuropsychoses, such as depression, schizophrenia, obsessive compulsive disorder and chronic fatigue syndrome.¹² The disease is best characterized in equids, where after a four week incubation period the infected horse exhibits non-specific signs of colic, fatigue, coughing and icterus followed by alternating periods of excitability and somnolence. Neurologic signs progress over the next 3-20 days, leading to the death of the animal.⁸

The pathogenesis and persistence of the virus within infected cells is complex. Once infection is established, the virus interferes with many intracellular signaling pathways involved in spread of the virus, maintenance of viral persistence, and modulation of neurotransmitter pathways.⁹ Neuronal death is due to effector CD8+ T cells which kill infected cells.¹² Studies in laboratory infected mice demonstrated a positive correlation between lesion severity and increased levels of IL-6, TNF- α , IL- α and inducible nitric oxide synthase mRNA.¹²

Contributor: Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada
<http://ahl.uoguelph.ca>

References:

- Berhane Y, Smith DA, Newman S, et al. Peripheral neuritis in psittacine birds with proventricular dilatation disease. *Avian Pathol.* 2001;30:563-570.
- Daoust PY, Julian R, Yason CV, Artsob H. Proventricular impaction associated with nonsuppurative encephalomyelitis and ganglioneuritis in two Canada geese. *J Wildlife Dis.* 1991; 27:513-517.
- Degernes LA, Flammer K, Fisher P. Proventricular dilatation syndrome in a Green-Winged Macaw. *Proceedings of the Annual Conference of the Association of Avian Veterinarians.* 1991;45-49.
- Gancz AY, Kistler AL, Greninger AL, et al. Experimental induction of proventricular dilatation disease in cockatiels (*Nymphicus hollandicus*) inoculated with brain homogenates containing avian bornavirus 4. *Virology.* 2009;6:100.
- Gray P, Hoppes S, Suchodolski P, et al. Use of avian bornavirus isolates to induce proventricular dilatation disease in conures. *Emerg Infect Dis.* 2010;16:473-479.
- Joyner KL, Kock N, Styles D. Encephalitis, proventricular and ventricular myositis, and myenteric ganglioneuritis in an Umbrella Cockatoo. *Avian Dis.* 1989;33:379-381.
- Mannl A, Gerlach H, Leipold R. Neuropathic gastric dilatation in Psittaciformes. *Avian Dis.* 1987;31:214-221.
- Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ. *Bornaviridae*. In: *Veterinary Virology*. 3rd ed. vol.1. San Diego, CA: Elsevier Academic Press; 1999:455-458.
- Planz O, Pleschka S, Wolff T. Borna disease virus: a unique pathogen and its interaction with intracellular signaling pathways. *Cell Microbiol.* 2009;11(6): 872-879.
- Perpiñán D, Fernández-Bellón H, López C, Ramis A. Lymphoplasmacytic myenteric, subepicardial and pulmonary ganglioneuritis in four non-psittacine birds. *J Avian Med Surg.* 2007;21:210-214.
- Raghav R, Taylor M, Delay J, Kistler A, Smith D. Avian bornavirus is present in many tissues of psittacine birds with histopathologic evidence of proventricular dilatation disease. *J Vet Diagn Invest.* 2010 Jul;22(4):495-508
- Thakur R, Sarma S, Sharma B. Role of Borna disease virus in neuropsychiatric illnesses: are we inching closer? *Indian J Med Microbiol.* 2009;27(3): 191-201.

CASE IV: SP-09-6422 (AFIP 31642241).

Signalment: Adult, female, red-footed tortoise (*Geochelone carbonaria*).

History: According to the provided history, an adult female red-footed tortoise (*Geochelone carbonaria*) from a privately-owned Midwestern zoological garden

presented with progressive lethargy. As the animal was non-responsive to treatment, necropsy was performed on site following euthanasia, and tissues were submitted to the Diagnostic Center for Population and Animal Health, Lansing, MI, for histopathologic examination.

Gross Pathology: No gross description was provided with the tissue submission.

Laboratory Results: After a diagnosis was made, water from the pond where this tortoise was kept was submitted for parasitological analysis but yielded no significant results.

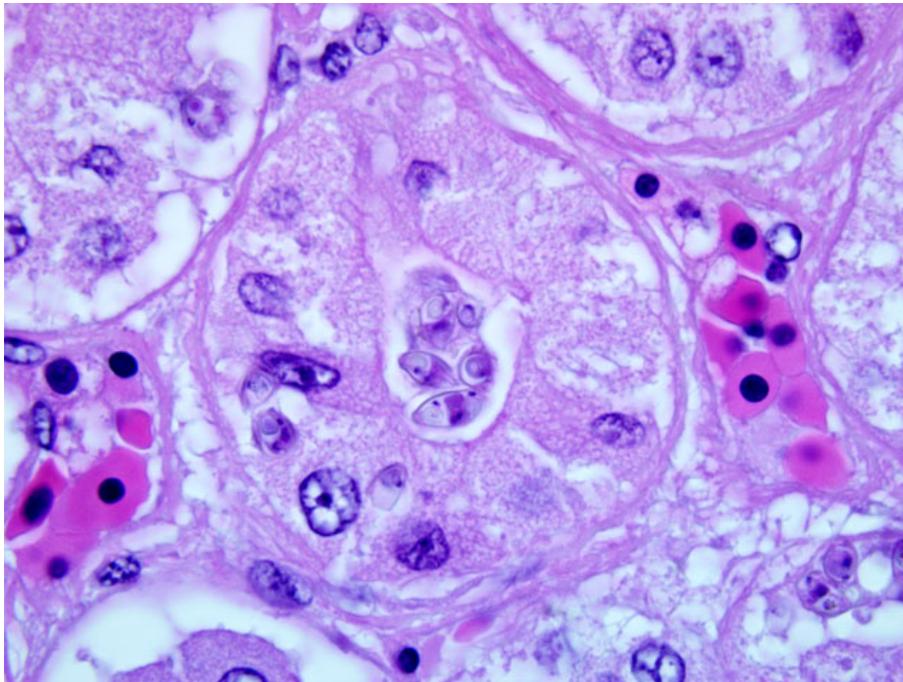
Histopathologic Description: Kidney: Diffusely in a section of kidney, the renal tubular epithelium is variably degenerate and necrotic. Within affected tubules, epithelial cells are often dissociated, variably swollen, with eosinophilic finely vacuolated to floccular cytoplasm, and pyknotic to karyolytic to lost nuclei (degeneration and necrosis). Few large, irregular, and mildly atypical cells with prominent vesicular nuclei are also present (regeneration). Multifocally, tubules are filled with small to moderate amounts of eosinophilic smooth to floccular material (proteinosis), sloughed-off epithelial cells, and few heterophils. Occasional tubular epithelial cells contain up to 10, 10-15 μm , intracytoplasmic, retractile, amphophilic ellipsoidal organisms obscuring the cytoplasm and nucleus. These organisms contain two apical polar capsules, approximately 2 μm in diameter, and basophilic nuclear material in between polar capsules (morphology suggestive of myxozoans).

Similar organisms are also present in tubular lumina, within sloughed-off epithelial cells or individually amidst intraluminal debris. Low numbers of lymphocytes and heterophils expand the renal interstitium, together with rare pigment-laden macrophages (hemosiderin). Diffusely within the section, glomerular tufts are segmentally to globally, mildly to moderately thickened. Multifocally, there is rare segmental synechiation and mild expansion of the urinary space, which is mostly devoid of contents (clear space). Rare intratubular basophilic casts (mineral) are also identified. Degenerate sloughed-off epithelial cells, heterophils, and myxozoan organisms are present in the renal pelvic lumen. Multifocally within the subepithelial stroma of the pelvis are occasional foci of coarse deeply basophilic salt deposition (dystrophic mineralization).

The above described organisms were not highlighted by PAS, acid-fast, or Giemsa histochemistries.

Additional findings in other organs (not included): multifocal myocardial mineralization and severe heterophilic hepatitis. No organisms were observed within any other examined tissues, including but not limited to liver, biliary ducts, and urinary bladder.

4-1. Kidney, red footed tortoise. Renal tubular epithelial cells are swollen and vacuolated (degeneration). Within the cytoplasm of epithelial cells and in tubular lumens there are refractile, amphophilic, ellipsoid myxozoans that measure up to 15 μm in diameter. Occasionally, organisms contain two apical polar capsules and basophilic nuclear material between the capsules. (HE 1000X)



Contributor's Morphologic Diagnosis: 1. Kidney: Moderate diffuse tubular degeneration, and necrosis, with intralesional, intraepithelial and intratubular myxozoa-type parasites (*Myxidium* spp.).
2. Kidney: Moderate diffuse membranous glomerulonephritis, with mild tubular proteinosis.
3. Kidney: Minimal multifocal dystrophic mineralization.

Contributor's Comment: Microorganisms of the phylum *Myxozoa* are spore-forming, metazoan parasites that infect the biliary, urinary, and gastrointestinal tracts of cold-blooded aquatic vertebrates, especially fishes but rarely also reptiles, amphibians, and birds, with alternate life cycle stages in invertebrates.^{2,4,5} Infection is believed to be of little clinical importance in most species, although in the recent years, life-threatening disease has been documented in a variety of species.² In the reported cases, the kidneys, biliary tract, and liver are the most commonly compromised organs. Affected kidneys are often grossly pale and swollen, while histologically the infection is characterized by degeneration and necrosis of the tubular epithelium infected with intralesional spores.⁵ All myxozoans known to infect reptiles are in the genus *Myxidium*, and all reports involve aquatic turtles.⁵

In an attempt to elucidate the species involved in this case, additional ancillary tests such as ultrastructural analysis, phase contrast microscopy, and molecular analysis of the samples were performed.

Ultrastructural analysis on infected kidney tissue revealed 60x20 µm spores, with a mean polar capsule dimension of 15x10 µm. Phase contrast microscopy and transmission electron microscopy demonstrated only mature spores, with two asymmetrical valve cells and a binucleated sporoplasm between two opposing polar capsules. Valve cells were longitudinally striated, with two overlapping sigmoidal capsule sutures. Polar capsules contained a single polar filament, coiled 5 to 7 times and surrounded by a double-layered wall. Based on spore morphology, these myxozoa were classified in the genus *Myxidium*.

Macerated formalin-fixed tissue yielded plentiful spores from which DNA extraction was attempted. At least two distinct extraction methods were used (using *Myxozoa* and *Myxidium* generic primers) with no success, likely as a result of formalin-fixation nucleic acid damage to the samples (additional fresh samples were not readily available).

To the best of the contributor's knowledge, renal myxozoonosis has not been documented in terrestrial chelonids.

AFIP Diagnosis: 1. Kidney: Tubular degeneration and proteinosis, multifocal, mild with rare tubular epithelial necrosis and intraepithelial and intratubular myxozoon parasites.

2. Adipose tissue, perirenal: Fat atrophy, diffuse moderate.

Conference Comment: Several participants included some form of glomerulonephritis in the histomorphologic diagnosis. The moderator pointed out that early studies and descriptions of the histomorphology of the chelonian kidney mistakenly identified aging changes as glomerulonephritis, and he assessed the glomeruli in this tortoise as essentially normal. Tortoises and other reptiles possess smaller glomeruli with reduced vascularity as compared to amphibians, birds and mammals.³ This morphologic feature acts to conserve water for these animals that often live in an arid, dehydrating environment. The reduced glomerular size and vascularity results in an ostensibly thicker mesangium with pronounced mesangial cells, and these normal histoanatomical features may be mistaken for glomerulonephritis by pathologists lacking extensive experience in evaluating reptile tissues. Additionally, the moderator believed there was little "corroborative evidence" to support a diagnosis of glomerulonephritis, e.g., lack of interstitial inflammatory infiltrates.

A subtle, but important, histologic finding in this case is the depletion of perirenal adipose tissue. The moderator indicated this was the most striking lesion, as it indicates negative energy balance. When conducting gross and histologic examination of reptiles and amphibians, assessment of adipose tissue provides valuable insight into the metabolic state of the animal.

Participants discussed species affected by myxozoon parasites. In addition to cold-blooded vertebrates, the moderator indicated that several ducks, both native wild and captive exotic species, have been infected with *Myxidium anatum* n. sp.¹ Myxozoon parasites which infect ducts preferentially infect biliary epithelium.

Contributor: Michigan State University, Diagnostic Center for Population and Animal Health, 4125 Beaumont Rd, Lansing, MI 48910
www.animalhealth.msu.edu

References:

1. Bartholomew JL, Atkinson SD, Hallett SL, et al. Myxozoon parasitism in waterfowl. *Int J Parasitol.* 2008;38(10):1199-1207.
2. Garner MM, Bartholomew JL, Whipps CM, Nordhausen RW, Raiti P. Renal myxozoonosis in crowned river turtles *Hardella thurjii*: description of the putative agent *Myxidium hardella* n. sp. by

- histopathology, electron microscopy, and DNA sequencing. *Vet Pathol.* 2005;42:589-595.
3. Jacobsen ER. Overview of reptile biology, anatomy, and histology. In: *Infectious Diseases and Pathology of Reptiles*. New York, NY: CRC; 2007:13-14.
 4. Roberts JF, Whipps CM, Bartholomew JL, Schneider L, Jacobson ER. Myxidium scripta n. sp. identified in urinary and biliary tract of Louisiana-farmed red-eared slider turtles Trachemys scripta elegans. *Dis Aquat Organ.* 2008;80:199-209.
 5. Zwart, P. Renal Pathology in Reptiles. *Vet Clin North Am Exot Anim Pract.* 2006;9:129-159.