The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 18

27 February 2008

Moderator:

Dr. James Raymond, DVM, MS, DACVP

CASE I – 47678 (AFIP 3066297).

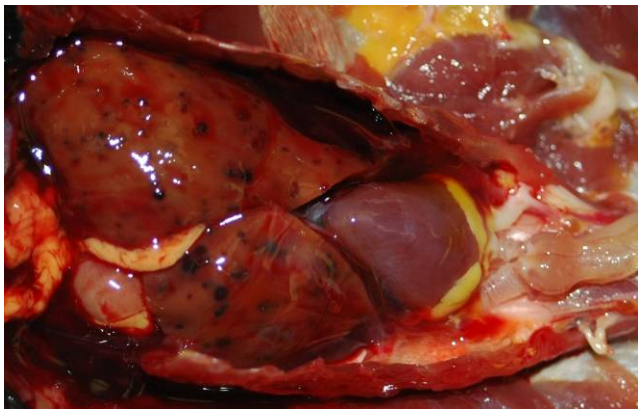
Signalment: 10-year-old, male, Magnificent Bird of Paradise (*Diphyllodes magnificus hunsteini)*

History: This Magnificent Bird of Paradise was housed in a bird breeding facility, where it was introduced to a female once daily. The bird had no history of medical problems, and was found on the ground, "puffed up, disoriented, and unable to fly", with blood on the beak. The bird was subsequently transported to the hospital where it died soon thereafter (less than 6 hours later).

Gross Pathology: The caudal coelom contained a moderate amount of unclotted blood. The liver was diffusely mottled green to brown with approximately two to three dozen, 1 to 3 mm diameter black foci scattered randomly throughout the parenchyma (hemorrhage) **(Fig. 1-1).** The intestines were diffusely pale. Body condition was assessed as fair.

Laboratory Results: Polymerase chain reaction (PCR) of fresh frozen liver was performed, using consensus primers for an avian malarial mitochondrial cytochrome B gene segment, and was positive in 9 of 10 cases. Sequencing of the gene product revealed that the protozoa were consistent with *Haemoproteus* spp., and not consistent with *Leukocytozoon* or *Plasmodium*. *In situ* hybridization was done in two animals with a mitochondrial cytochrome B probe and was positive only in megaloschizonts. Phylogenetic analyses showed that the protozoan was related to *Haemoproteus* spp. reported from asymptomatic native North American passerine birds.

Histopathologic Description: The liver parenchyma is multifocally disrupted by variably sized poorly demarcated regions of hemorrhage and necrosis ranging from 0.5 to 2 mm in diameter, typically with intralesional protozoal organisms (megaloschizonts) **(Figs. 1-2).** Protozoal megaloschizonts range from 200 to 500 µm in diameter and consist of a thin, 1-2 µm wide basophilic wall bordering a 30-60 µm thick peripheral basophilic rim. This rim delineates the perimeter of the schizont, and is comprised of numerous round to oval often poorly preserved, coalescing basophilic 1-5 µm diameter zoites and larger cytomeres. Cytomeres range from 6 to 15 µm in diameter, within which are often clear acicular clefts. Central cystic regions of the megaloschizonts contain amorphous wispy amphophilic to eosinophilic material. Rare megaloschizonts do not have a central cystic region, and are entirely comprised of well demarcated cytomeres **(Fig. 1-3).** In few locations, amorphous basophilic to amphophilic material resembling the protozoal zoites conforms to and expands sinusoids. Surrounding the megaloschizonts and intermixed within the regions of hemorrhage, there are small to moderate numbers of in-



1-1. Liver, Bird of Paradise. Scattered foci of hemorrhage. Photograph courtesy of Zoological Society of San Diego, San Diego, CA, USA

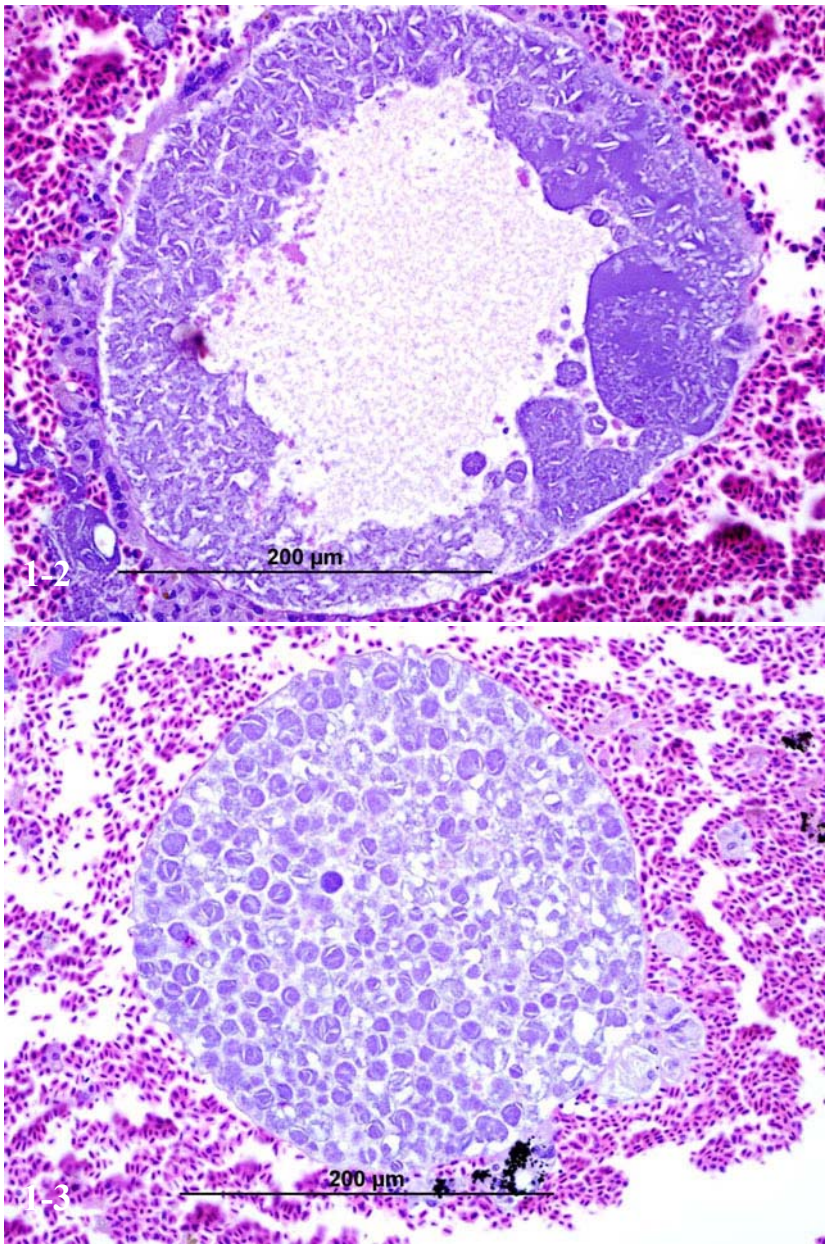
flammatory cells, including lymphocytes, plasma cells, macrophages and multinucleate giant cells. Multifocally, hepatocytes surrounding the parenchymal hemorrhage exhibit degenerative and necrotic features characterized by hypereosinophilia, anisocytosis, anisokaryosis, pyknosis, karyolysis and karyorrhexis. Golden brown granular anisotropic pigment is extracellular and intracellular, found within hepatocytes, erythrocytes and Kupffer cells, typically at foci of hemorrhage (acid hematin or malaria pigment, Fig. 4). Inflammatory cell populations including lymphocytes, plasma cells, macrophages and fewer heterophils are randomly disseminated or present at portal regions. Capsular mesothelial cells are multifocally hypertrophied. Diffusely, hepatocytes contain abundant golden brown granular pigment (hemosiderin).

Contributor's Morphologic Diagnosis: 1. Liver: Severe, acute multifocal hemorrhage and necrosis with intralesional protozoal megaloschizonts (*Haemoproteus*-like spp.) and moderate multifocal lymphoplasmacytic histiocytic hepatitis
2. Liver: Moderate diffuse hemosiderosis

Contributor's Comment: Slides vary with respect to number and morphology of protozoal megaloschizonts. Ten cases of protozoal infection associated with multifocal hepatic hemorrhage, hemocoelom and intralesional protozoal schizonts have been observed in passerine birds at this zoological park. The gross lesions were particularly striking, as multifocal hemorrhages were disseminated throughout the hepatic parenchyma, and the coelom contained unclotted blood. Severe blood loss into the abdominal cavity was the proposed mechanism of sudden death. Other lesions consistently present included non-suppurative endocarditis, epicarditis, myocarditis, and occasional endarteritis. Also common were coelomitis and airsacculitis, possibly secondary to widespread migration of the hemoparasites, or a reaction to the coelomic hemorrhage. Splenomegaly was a common finding, with reticuloendothelial hyperplasia or lymphoid hyperplasia diagnosed in the majority of cases. Although Isosporoid coccidia (formerly called *Atoxoplasma*)[14] were found in lung impression smears in the bird presented in this case and two others, no other hemoprotozoal parasites were found in lung impression smears of any bird. In this case and all but one of the ten cases, hepatic protozoa were identified as *Haemoproteus* via polymerase chain reaction (PCR) and sequencing of the gene product. *In situ* hybridization was done in three birds with a mitochondrial cytochrome B probe and was positive only in megaloschizonts.

Hemoparasitism in birds typically consists of 3 genera of Apicomplexan parasites in the family Plasmodiidae: *Leukocytozoon*, *Haemoproteus* and *Plasmodium*.[4] These protozoa can cause severe clinical disease, or hosts may be asymptomatic. *Haemoproteus* and *Plasmodium* are distributed almost worldwide,[16] with high species diversity. Over 120 species of *Haemoproteus* have been reported in birds.[4] *Haemoproteus* spp. are characterized by schizogony (merogony) within visceral endothelial cells (typically the lung, liver or spleen), with gametocyte development in circulating erythrocytes.[4,5] Biting flies, characteristically louse flies (Hippoboscidae) and biting midges (Ceratopogonidae) transmit the protozoa. In our cases, Ceratopogonidae are considered to be the more likely vector. Sexual development of *Haemoproteus* occurs in the intermediate host (insects), with asexual development in the bird.[5] Clinical disease is typically associated with anemia due to erythrocytic parasitism, frequently in a compromised or immunocompromised host. Few species of *Haemoproteus* are reported to induce clinical disease, including *H. meleagridis* in turkeys, *H. mettionis* in ducks and geese, and *H. columbae* in pigeons and doves.[13]

In these cases, disease manifestation is presumed to occur with the pre-erythrocytic stages, rather than circulating intra-erythrocytic gametocytes, as no gametocytes were identified with lung impression smears (a representation of peripheral blood). Pre-erythrocytic schizont stages of *Haemoproteus* spp. have been reported to occur in many organs including the lung, liver, spleen, heart, kidney and cecum.[2] Schizont development within capillary endothelial cells and myofibroblasts suggests that the parasite can use a variety of host cells.[2] The reason for the apparent tropism to the liver in our cases is unknown. Because of the location of the schizont formation and sequelae to



1-2. Liver, Bird of Paradise. Megaloschizont with central lumen containing proteinaceous fluid and a peripheral basophilic rim of protoplasm containing variable numbers of merozoites.
1-3. Liver, Bird of Paradise. Megaloschizont containing numerous merozoites.

Photomicrographs courtesy of Zoological Society of San Diego, San Diego, CA, USA. (H&E 400X).

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hepatic parenchymal disruption (hemorrhage and hemocoelom), it is plausible that there is not enough time for erythrocytic forms to be identified. Experimental *Haemoproteus* infection in turkey poults, a naturally infected wild turkey, and naturally infected bleeding heart doves caused severe myositis with intralesional megaloschizonts, muscle necrosis and lameness.[2] Significant myositis was not found in any of our ten cases.

Descriptions of lesions virtually identical to those found in our cases have been reported in psittacine and nonpsittacine birds,[12] with the designation of "Haemosporozoa of undetermined taxonomic status". As described by Gardiner, this group of protozoal organisms is characterized by protozoal cysts within viscera and skeletal muscle without identification of gamonts or gametes in peripheral blood cytology.[8] A recent report describing similar hepatic lesions in passerine and nonpasserine birds used PCR to confirm *Haemoproteus* as the etiologic agent.[7] Interestingly, DNA sequence analysis of a conserved area of the cytochrome B gene reveals that the *Haemoproteus* sp. in the aforementioned cases is identical to the *Haemoproteus* in our cases. The presence of the same species of *Haemoproteus* in different orders of birds is a surprising finding, which challenges the previous designation of high host specificity of this protozoal disease.

Host sharing involving both *Haemoproteus* and *Plasmodium* was reported to occur between populations of migrating European birds and resident African birds,[16] suggesting that these hemoparasites may be less host specific than previously believed. Host switching of protozoal hemoparasites into naïve populations has been linked with changes in virulence and increased morbidity.[1,3,14] These findings imply that host switching is a conceivable mechanism for *Haemoproteus* infection of birds from different localities assimilated into a novel environment, as is typical in a zoological setting. Although avian species from similar regions of the world are typically grouped together in a zoo, insect vectors can move between groups, and may play a role in transmission of infectious organisms. Interestingly, the majority of the birds affected (9 of 10) at our institution are from South America, excluding the bird from this case, the magnificent bird of paradise. In general, hemoprotozoa of neotropical birds are rare.[15] Therefore, birds from this

region of the world may have an increased susceptibility to hemoprotozoal infections. Sequence analysis of PCR products from our cases revealed that the sequences were identical and most consistent with *Haemoproteus* spp. from North American passerine birds. Thus, it is possible that infection with North American *Haemoproteus* in South American birds (aberrant hosts) may result in an aberrant parasitic form or a more prominent or virulent pre-erythrocytic form of the parasite. A similar example may be the introduction of new *Plasmodium* species to Hawaii, which resulted in high mortality rates and limited range habitation of native passerine species[1], illustrating the impact engendered by introduction of a novel parasite to a naive population.

Iron stains confirmed the presence of hemosiderin within hepatocytes. Hemosiderosis is a common finding in many avian species, particularly in frugivorous or insectivorous birds. Birds of paradise are listed as a family within the order Passeriformes (Paradisaeidae) in which hepatic or multisystemic hemosiderosis is often found.[10] Other commonly affected families include Ramphastidae and Sturnidae. Iron storage disease in these families may be primary or due to vulnerability to dietary overload, as opposed to secondary causes, including infection or inflammation.[10] One must be careful to differentiate between hemosiderosis, meaning that there is excessive stainable iron in parenchymal or phagocytic cells, and hemochromatosis, meaning that the excess iron damages the cell, tissue or organ. In this case, although hemosiderin is widespread throughout the liver, no cellular damage or reactive changes attributable to the hemosiderin are apparent, which warrants a diagnosis of hemosiderosis.

AFIP Diagnosis: 1. Liver: Hemorrhage and necrosis, multifocal with megaloschizonts, Magnificent Bird of Paradise (*Diphyllodes magnificus hunsteini*), avian.
2. Liver: Hepatitis, portal, lymphoplasmacytic, multifocal, moderate.
3. Liver, hepatocytes: Hemosiderosis, diffuse, moderate.

Conference Comment: The contributor gives an excellent overview of *Haemoproteus* infections. The typical life cycle of *Haemoproteus* consists of gametocytes within the host erythrocyte cytoplasm that are taken up by blood-sucking vectors (hippoboscid flies or midges of the genus *Culicoides*).[6] The parasite undergoes several stages of development within the insect host to become sporozoites within the insect's salivary gland. These sporozoites are injected into a new susceptible host when the insect feeds.[6] The sporozoites enter the bird's endothelial cells and tissues (lung, liver, bone marrow, and spleen), undergo schizogony, and form large round cysts containing numerous multinucleated bodies (cytomeres) that in turn produce numerous merozoites.[6] The merozoites escape into the bloodstream and enter erythrocytes to become gamatocytes (macrogametes and microgametes).[9] Occasionally extraerythrocytic macrogametes and microgametes are found within the peripheral blood.[6]

Haemoproteus, *Plasmodium*, and *Leucocytozoon* gametocytes can all be found within the peripheral blood, although several differences exist among the three groups. In *Leucocytozoon* sp., gametocytes may also be found in leukocytes and will severely distort host cells.[9] Mature *Haemoproteus* gametocytes within the erythrocytes of birds are located within the cytoplasm and partially encircle the nucleus without causing nuclear displacement (halter shape).[6] Megaloschizonts are frequently present in tissues in cases of leucocytozoonosis and with infection of some species of *Haemoproteus*, but are not characteristic of *Plasmodium* sp.

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http://cres.sandiegozoo.org/

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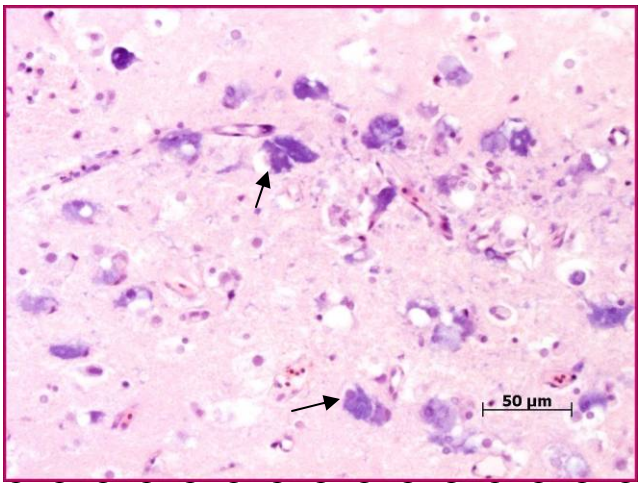

2-1. Brain, Atlantic cod. Neuronal vacuolation (arrows). (H&E 400X).
Photomicrograph courtesy of Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE, C1A 4P3, Canada. www.upei.ca

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Laboratory Results:
Viral isolation – Nodavirus recovered
Immunohistochemistry – Positive for nodavirus

Histopathologic Description: Each slide contains a tangential section through the cranium of 4 fish. All slides have between 1 and 4 visible sections of brain and associated optic structures.

Telecephalon: The most prominent changes are present in the olfactory lobe of the telencephalon. There are neurons present with clear cytoplasmic vacuoles, often with scalloped edges **(Fig. 2-1).** The affected neurons are typically markedly basophilic, and in more advanced cases there is more prominent neuronal degeneration with mild gliosis. Some cases have similar changes in more caudal aspects of the brain, often extending into the cranial spinal cord.

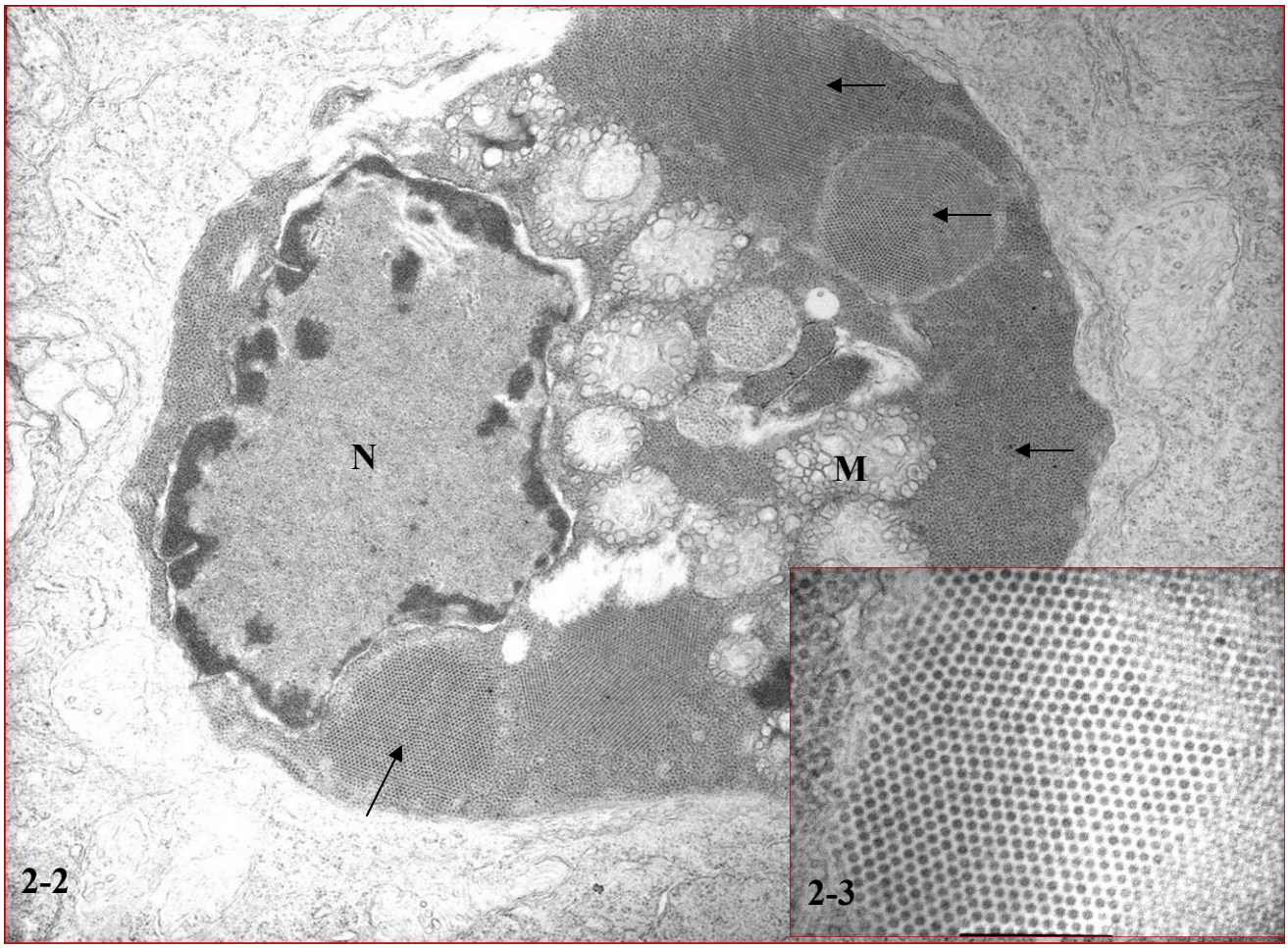
Eye: The posterior chamber commonly contains a lightly eosinophilic fluid admixed with loose histiocytic cells. There are commonly adherent to the surface of the lens, and occasionally to the retina. Within the retina, sparsely dispersed vacuolated neurons are present predominantly in the inner nuclear layer and nerve fiber layer.

Contributor's Morphologic Diagnosis: 1. Multifocal neuronal vacuolation and degeneration, with focal gliosis – telencephalon.
2. Multifocal neuronal vacuolation and degeneration,

CASE II – U24048-04A-A1, C; U24883 A1-B2 (AFIP 2988631).

Signalment: Atlantic cod (*Gadus morhua*)

History: The samples are from approximately 12-month-old fingerlings, 5 cm-in-length, taken from a population of fish in grow out tanks held indoors at 15 degrees Celsius. Over a 2 week period, there was an increase in mortalities. Grossly the skin was darkened.



2-2. Retina, Atlantic cod. Large numbers of intracytoplasmic viral particles (arrows) and several degenerating mitochondria (M) within retinal cell; nucleus (N). Transmission electron micrograph.
2-3 (inset). Higher magnification of virions.
Electron micrographs courtesy of Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE, C1A 4P3, Canada.
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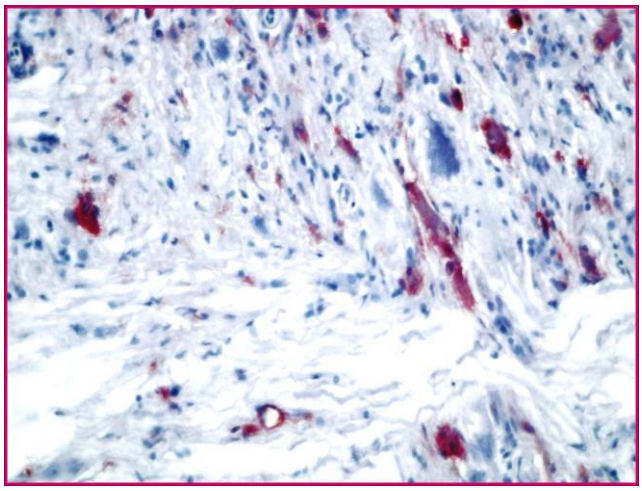
retina (variable)
3. Diffuse histiocytic uveitis, mild to moderate, (variable)

Contributor's Comment: The changes in the telencephalon and retina are considered highly compatible with a disease condition referred to as viral encephalopathy and retinopathy, caused by an aquatic Nodavirus.[1]

Nodaviruses are species specific non-enveloped, icosahedral agents, 25-30nm in diameter.[1,5] A large number of marine species are affected by this family of viruses which cause significant mortality in juveniles. The viruses have been a major impediment to the commercialization of numerous fish species. Consistently, the virus affects the central nervous system (with some exceptions noted – spinal ganglia of Japanese parrotfish).[5] Characteristically the lesions are in the anterior section of the brain (telencephalon – in most fish, the olfactory lobe comprises the largest portion) and consist of the presence of vacuoles in the grey matter which appear to be cytoplasmic. Other lesions included pyknosis and basophilia of affected cells. Similar lesions are noted in the neural component of the retina.[1,4,5] The relative lack of gliosis is likely due to the acute nature of the infection.



2-4. Forebrain, Atlantic cod. Neurons exhibit positivity in immunohistochemical staining for nodavirus. Photomicrograph courtesy of Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE, C1A 4P3, Canada. www.upei.ca

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Participants are referred to the electron microscopic images **(Figs. 2-2 and 2-3 [inset])** which display large numbers of intracytoplasmic viral particles. Note the degenerating mitochondria (M) in figure 2-2. An immunohistochemistry image is also submitted demonstrating strongly positive neurons in the telencephalon **(Fig. 2-4).**

Nodavirus encephalitides are common in the Mediterranean and Indo-Pacific regions, however, recently (2002) a nodavirus of Atlantic cod was demonstrated along the Atlantic seaboard of Canada.[4]

From a comparative view point, the inflammatory response in teleosts differs somewhat from the higher vertebrates. Whilst teleosts possess oligodendrocytes and astrocytes, it has not been reported that they possess microglia. Additionally, in teleosts even mature ependymal cells retain the capacity for differentiation, implying that neuronal regeneration is possible.[3]

AFIP Diagnosis: Brain, telencephalon: Encephalitis, histiocytic, multifocal, moderate with necrosis, neuronal vacuolation, and spongiform change, Atlantic cod (*Gadus morhua*), piscine.

Conference Comment: Nodaviruses were originally isolated from insects (termed alpha-nodavirus), then from fish (termed beta-nodavirus).[7] Betanodavirus are the agents causing viral encephalopathy and retinopathy (VER), also referred to as viral nervous necrosis (VNN).[8] The brain, spinal cord and retina are the primary target organisms for infection, causing vacuolation and neuronal degeneration.[7] The virus has been described in over 40 species of fish, affecting primarily larval and juvenile fishes[2], and has been a major limiting factor of marine aquaculture development world wide.[7] Transmission is not fully understood, although it is believed to occur vertically from eggs or sperm, or horizontally from water or feed.[7]

Characteristic histologic features of vacuolation and degeneration occur most frequently in the anterior brain. Additional lesions include focal pyknosis and karyorrhexis of neural cells, granularity of neuropil, and mononuclear cell infiltrates.[6] There are conflicting reports in the literature on the extent of optic involvement among different species of fish.[6] Optical lesions, when described, include vacuolation of the rod and cone layer, as well as ophthalmitis of both the anterior and posterior chambers.[6] The cells that most often contain the virus, as identified through electron microscopy, are the neurons, astrocytes, oligodendrocytes, and microglia.[6]

There is multifocal, moderate histiocytic inflammation of the vitreous body or humor (hyalitis) which was not evident in all sections. Retinal and uveal lesions were not evident in the sections evaluated at AFIP.

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CASE III – 05-3426 (AFIP 3027074).

Signalment: 10-year-old, female Alpaca (*Lama pacos)*

History: The alpaca had be en healthy until last month, had a s uccessful pregna ncy the past year. Started with anorexia, and lethargy with rapid weight loss over the last 2 weeks.

Gross Pathology: Mass within small intestine (jejunum) and small circular white nodules in liver

Histopathologic D escription: Received 2 fragments of tissue, o ne a s ection o f l iver 5 x 5 cm and t he other a fragment o f small in testinal tract 16 cm lo ng with attached m esentery. Th e section of liv er has a m ultifocal infiltrate of neoplastic cells throughout the hepatic parenchyma (not i ncluded on t he slides). Th e in testinal sections reveal the orig in of the tumor within the mucosa on the majority of th e slides. The cells ex hibit marked anisokaryosis and have large oval open vesicular nuclei with a variable mitotic rate. This rate is highest in the sections of the mucosa that have the neoplastic process. Necrosis is common to the center of the masses present throughout the su pporting m esentery an d within t he muscle layers. There is a co nnective tissu e reaction within th e supporting m esentery an d c ells i n t his a rea vary from no dular masses to ind ividual cells within th e connectiv e tissu e stroma. A m ucinous m atrix is app arent main ly with in the supporting mesentery. The cells ex tend full thickness through t he subm ucosa, m uscle layers, serosal s urfaces and within the mesentery**.**

Contributor's Morpho logic Diag noses: Intestinal ad enocarcinoma poorly differentiated; jejunum.

Contributor's Comment: Tumors in llamas and alpacas are reported to be relativ ely rare.[5] The re are fe w reported cases i n t he literature and alth ough t his m ay reflect the popul ation of alpacas in North America, it m ay also re flect in herent di fferences within the im mune sy stem of Ne w World cam elids as com pared to ot her s pecies.

Small intestinal primary epithelial tumors are rare in most species, a nd in man are m ore likely to be benign rat her than malignant. Extensive researc h has b een do ne o n colonic neoplasia or c olorectal ne oplasia i n m an including identification of g enetic alteratio ns, famil ial tendencies and chromosomal abnormalities. The low number of small in testinal ad enocarcinomas has precluded th is type of ev aluation. In m an th e maj ority o f small intestinal adenocarcinomas can be surgically resected with substantial benefits in terms of 5-year survival.[1]

In domestic a nimals, sm all intestinal ad enocarcinomas are al so co nsidered t o be ra re, but cat s do t end t o have mid-jejunal a nd ileocecal origins for t his tum or at a higher rate t han the other domestic species. T umors are classified as a denocarcinoma, mucinous, undifferentiated or solid, and signet ring carcinomas.

In the cat, surgical excision of the tumors yields a reasonable prognosis.[2]

AFIP Dia gnosis: 1. Small intestine; mesentery: Carcinoma, anaplastic, alpaca (*Lama pacos*), camelid.
2. Small in testine: En teritis, necrotizing, acu te, diffuse, severe, with fibrin, h emorrhage, ed ema, v asculitis, an d fibrin thrombi.

Conference Comment: According to the World Health Organization I nternational Histological C lassification o f Tumors of the Alimentary System of Domestic Animals[3], there are si x categ ories o f m alignant in testinal ep ithelial neoplasia in do mestic an imals: Acin ar ad enocarcinoma, papillary a denocarcinoma, m ucinous a denocarcinoma, signet ri ng ce ll carci noma, un differentiated ca rcinoma, and adenosquamous carcinoma (table 3-1).

A recent re view of neoplasia i n l lamas an d al paca c onducted by Val entine et. al.[6] indicated that although the overall prevalence of n eoplasia was h igher in llamas, the mean age of affected alpacas was sign ificantly lo wer. The most com mon malignant ne oplasm in cam elids was cutaneous an d m ucocutaneous s quamous c ell carci noma with lymphoma being the second most common.[6]

In intestinal adenocarcinoma cells of sheep, there is altered expression of b-catenin, E-cadherin, cycloxygenase-2, and p53 protein.[4] The rates of these altered expressions were lower than that of corresponding rates in human colonic neoplasms, but these findings suggest the use of sheep as potential animal models.[4] b-catenin is a component of the WNT signaling pathway, and increased concentrations of this protein promote genes that regulate the cell cycle.[4] Neoplasm dedifferentiation, invasion and metastasis are promoted by the loss of E-cadherin.[4] COX-2 is often found in increased levels in colonic neoplasm, although the influence it has on tumor behavior is currently under investigation.[4] p53 protein is one of the key regulators of cell cycle regulation and apoptosis.[4]

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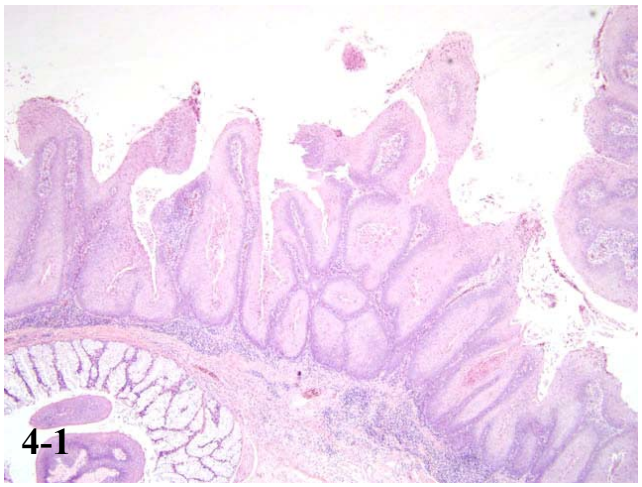
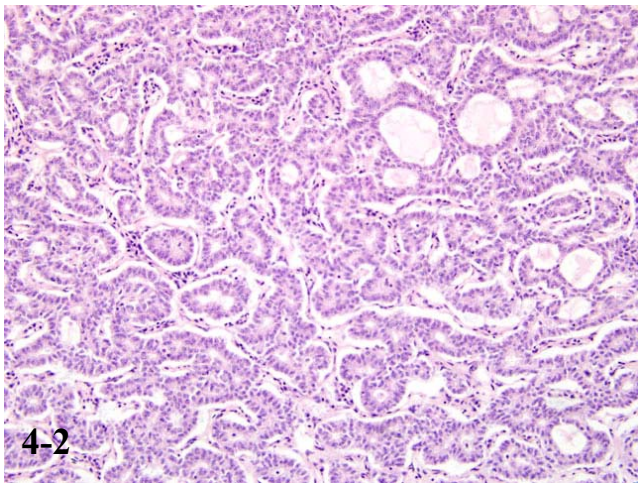
Table 3-1. Malignant intestinal epithelial neoplasms. Adapted from the WHO classification[3]

Type of Neoplasm	Characteristic Histologic Features
Acinar adenocarcinoma	Variably sized **acinar structures** replacing intestinal mucosa, arise from hypercellular crypts, infiltrate submucosa and musclular layers, tumor cells may contain occasional goblet cells. In colon, infiltrates Peyer's patches at the primary site
Papillary adenocarcinoma	**Papillary projections** lined by multiple layers of anaplastic columnar cells with little stroma, may have cribriform pattern, mostly intraluminal
Mucinous adenocarcinoma	Acinar or irregular crypts **filled or distended with mucin** replacing the mucosa, mostly goblet cells are seen, infiltrates intestinal wall and mesentery, mostly annular lesions
Signet ring cell carcinoma	**Signet-ring cells** characterized by mucin-filled cytoplasm and peripheralized crescent-shaped nuclei, replace mucosa, infiltrate bowel wall, severe desmoplasia, rare multinucleated cells, must differentiate from adenocarcinoid (which contains neuroendocrine and signet-ring cells)
Undifferentiated carcinoma	Solid sheets of large **anaplastic or pleomorphic cells** with little stroma, desmoplasia, may be rare mucin or signet-ring cells
Adenosquamous carcinoma	Glandular forming adenocarcinoma with **areas replaced by squamous cells** with varying degrees of keratinization





4-1. Cloaca, Scarlet Macaw. Multifocally extending from the mucosa are multiple epithelial lined fibrovascular papillary frondlike projections. Papilloma. (H&E 200X).

4-2. Liver, Scarlet Macaw. Infiltrating and replacing normal hepatic architecture are numerous irregular, branching and anastomosing tubules of neoplastic biliary epithelium. Bile duct carcinoma. (H&E 200X).

CASE IV - H06-976B; 06-998 (AFIP 3063514).

Signalment: Adult, female, *Ara macao*, scarlet macaw

History: The caged scarlet macaw was presented to the consulting veterinarian with a history of weight loss and also straining to pass faeces and urates.

Gross Pathologic Findings: The bird was in poor body condition and there was evidence of wasting of the pectoral muscles. There were multiple small 2-4mm broad based papillomatous masses extending over the mucosa of the cloaca. A low number of papillomatous masses were seen in the choana. Within the left lobe of the liver was a firm white demarcated nodular mass (approximately 4-5mm across) extending to the capsular surface.

Laboratory Results: PCR analysis of emulsified cloacal tissue was positive for psittacid herpesvirus.

Histopathologic Description: 1. Cloaca: The normal epithelium is replaced by abundant papillary structures. The papillary structures are supported by a fibrovascular stroma and lined by markedly hyperplastic stratified squamous epithelium **(Fig. 4-1).** There is marked parakeratotic hyperkeratosis. There are an increased number of mitotitic figures among the basal epithelial cells (3 6 mitotic figures/10hpf). Between the folds of the papillary structures and extending along the surface is necrotic cellular debris, and aggregates of cocci bacteria. Within the fibrous stroma there is a mild diffuse mixed inflammatory infiltrate consisting of macrophages, plasma cells, heterophils and lymphocytes.

The second section of cloaca: There is mild erosion of the overlying mucosal epithelium.

2. Liver: There is a focally extensive mass with an invasive pattern of growth at the margins replacing the normal hepatic architecture. The mass consists of pleomorphic cells forming tubular structures supported by a fine fibrovascular stroma **(Fig. 4-2).** The cells vary from columnar to cuboidal, have a moderate to scant amount of eosinophilic cytoplasm, oval to round nucleus, reticular chromatin and prominent nucleolus. There is moderate anisokaryosis and increased nuclear to cytoplasmic ratio. Mitotic figures are rare. There are multiple small aggregates of lymphocytes within the mass.

Contributor's Morphologic Diagnosis:
1. Cloaca: Marked diffuse epidermal hyperplasia, cloacal papillomatosis
2. Liver: Bile duct carcinoma

Contributor's Comment: Internal papillomatosis of parrots is believed to be an infectious disease which results in papillomatous tumours of the cloaca and oral cavity.[5] Some macaws also show severe lesions within the oesophagus, ventriculus and proventriculus. Studies

of internal papillomatosis of parrots have shown a correlation between this disease and intercurrent carcinomas of the bile duct and pancreas.[5]

Recent studies have demonstrated an alphaherpesvirus, within cloacal, oral and cutaneous papillomatous lesions and normal cloacal tissue.[4,6] An alpha herpesvirus was identified by PCR analysis of cloacal tissue in the scarlet macaw presented in this case. Styles D and co-workers demonstrated the virus isolated from cutaneous & cloacal papillomas and the normal cloacal mucosa of African grey parrots (*Psittacus erithacus eritha cus*) was most closely related, phylogenetically, to the psittacid herpesvirus (which causes Pacheco's disease, psittacid herpesvirus 1), but demonstrated sufficient nucleotide and amino acid diversity to be considered a new alphaherpesvirus, psittacid herpesvirus 2.[6]

Pacheco's disease is a devastating disease with acute onset. Histopathological findings include marked hepatic necrosis, with intranuclear inclusion bodies. Splenic necrosis, enteritis, pancreatitis, tracheitis and air saccu litis are other lesions which are seen variably.[1]

AFIP Diagnosis: 1. Liver: Bile duct carcinoma, scarlet macaw (*Ara macao*), avian.
2. Cloaca: Papilloma.

Conference Comment: Internal papillomatosis of parrots (IPP) is characterized by the progressive development of tumors in the oral and clocal mucosa.[4] Cloacal lesions are most commonly found in the Amazon parrot. Oral papillomas are most common in the oral cavity with occasional extension into the esophagus, proventriculus and ventriculus.[4] Herpesvirus inclusion bodies, virions or PCR products identified as psittacid herpesvirus-2 have been recognized within cutaneous and mucosal papillomas or from healthy cloacal mucosa in African grey parrots, macaws, and a conure.[3,4,8] It is well documented in the literature that there is an association between the presence of papillomatous lesions in Amazon parrots with an increased incidence of pancreatic or bile duct carcinomas, although the exact relationship between these two has not been determined.[2,4]

Psittacid herpesvirus-2 DNA sequence differs from psittacid herpes virus-1, the cause of Pachec o's disease, by more than 20%.[8] All four psittacid herpesvirus-1 geneotypes have been shown to cause Pacheco's disease in Amazon parrots, but only genotypes 2, 3 and 4 result in disease in African grey parrots.[8]

Other causes of cloacal papillomas or papilloma-like lesions include: papillomavirus; chronic irritation with cell hypertrophy or hyperplasia; and malnutrition with vitamin A deficiency.[2] Papillomavirus infections in birds have been demonstrated in an African grey parrot, finch, and Cuban Amazon parrot.[3,6]

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