The Armed Forces Institute of Pathology
Department of Veterinary Pathology

Conference Coordinator:
Todd M. Bell, DVM

WEDNESDAY SLIDE CONFERENCE 2008-2009

Conference 16

4 February 2009

Conference Moderator:

Dr. Tabitha Viner, DVM, DACVP

CASE I – 08-0165-WSC-2 (AFIP 3115836)

Signalment: 10-year-old, male white faced ibis (*Plegadis chihi*)

History: Animal presented to the Department of Animal Health for digital abnormalities. During amputation of P2 and P3 of right D2, bird arrested under anesthesia. Resuscitative efforts (IM and IV epinephrine, atropine, dopram, and fluids; cardiac massage; positive pressure ventilation with 100% oxygen) were unsuccessful.

Gross Pathology: The white-faced ibis is in excellent postmortem condition. There is sufficient musculature overlying the keel and moderate stores of coelomic and subcutaneous adipose tissue. The small intestine contains minimal amounts of tan, mucoid digesta, and the colon contains small amounts of urates. There is marked curvature of the spine between T4 and T7. There is a healed fracture at P2 of digit 3 on the left. Digit 2 on the right is absent distal to P1.

Laboratory Results: Hypophosphatemia (phosphorus 0.7 mg/dl) was observed in a blood sample collected near the time of death.

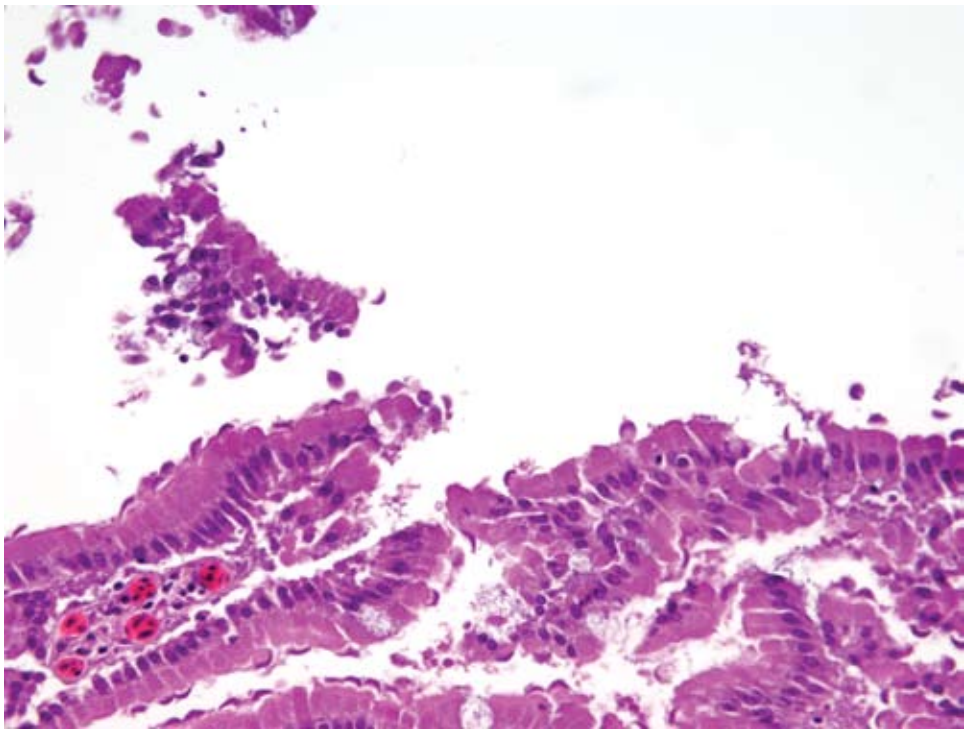
Histopathologic Description: Small Intestine: There are a few heterophils scattered throughout the lamina propria. Multifocally throughout the sections there are many, 5 x 10 micron, crescent-shaped to oval trophozoites overlying the mucosa (*Giardia*) **(Fig. 1-1)**.

Contributor's Morphologic Diagnosis: Intestine, small: Giardiasis, moderate, with mild, heterophilic enteritis

Contributor's Comment: *Giardia* sp. are flagellated protozoa that can be found in the upper intestine of many species of mammals, birds, reptiles, and amphibians. Though the taxonomy is poorly understood and subject to debate, it is generally accepted that *Giardia duodenalis* (a.k.a. *Giardia lamblia*) is classified into approximately eight separate assemblages.[5] Giardia appear to be host specific [5] with assemblages A and B being most common in humans; dogs are associated with assemblage C; and organisms from assemblage E have been isolated from livestock. *G. psittaci* has been isolated from budgerigars [6], *G. ardeae* from herons, and a genetically similar strain from straw-necked ibis.[2] Many animals harbor the organism in their intestinal tracts without evidence of clinical illness, indicating that there is a natural carrier state.[1]

Giardia colonize the upper small intestine, usually the duodenum, in the trophozoite form.[1] The trophozoites

*Sponsored by the American Veterinary Medical Association, the American College of Veterinary Pathologists, and the C. L. Davis Foundation.



1-1. Small intestine, ibis. Within the intestinal lumen, and adherent to crypt and villar enterocytes are numerous, 10 x 6 micron, flagellated, binucleate protozoal trophozoites. (HE 400X)

are piriform, with four paired flagella, paired nuclei and a ventral disk on the concave surface which attaches to the host enterocyte. Reproduction by binary fission occurs in the gut lumen. This form may be passed in the feces, but more commonly, oval cysts are seen in direct smears. Transmission of the environmentally-stable cyst is by the fecal-oral route.[1] Diagnosis is best made by seeing the organism in a direct fecal preparation from a clinically ill animal, but ELISA and PCR tests have been developed.

Though giardiaisis is generally an asymptomatic condition, the presence of the organism has been associated with vitamin E/selenium deficiencies in cockatiels.[6] The proposed mechanism involves malabsorption of these nutrients due to the presence of the organisms on the intestinal mucosal surface. Though speculative, hypophosphatemia in this white-faced ibis may also have resulted from a focally extensive and heavy parasite load. Phosphorus is absorbed from the diet in the proximal small intestine where the heaviest load of parasitism was found in this bird. The bird of this report had adequate fat and muscling, but antemortem bloodwork revealed a PO4 of 0.7mg/dl (ref. 3.1-6.6mg/dl). Additionally, marked thoracolumbar scoliosis was present in addition to the fractured and dislocated digits. Thus, while the protist in this particular animal did not appear to cause a direct effect on intestinal health, it may have acted indirectly on bone health by blocking absorption of some nutrients needed for maintenance of healthy, structural bone.

AFIP Diagnosis: Small intestine: Enteritis, heterophilic, diffuse, mild with numerous surface-associated trophozoites, etiology consistent with *Giardia* sp.

Conference Comment: Dr. Viner, the conference moderater, submitted this case and did an excellent job elucidating the importance of this organism in domestic species. The preservation of morphologic detail of the organism in the sections was excellent. This particular slide did not have extensive associated tissue reaction, thus driving home the point that Giardia species often cause minimal gross and histologic lesions and also often cause subclinical infections.

Giardia is an ubiquitous organism with worldwide distribution and is the most common flagellate of mammals and birds.[3] Giardiasis is also a zoonotic disease. The life cycle of Giardia species is direct. Trophozoites and cysts are present in feces and passed from infected hosts into the environment. Once outside the host the trophozoites die, but the cysts are resistant. They are protected from the environment by a membranous layer with an inner and outer cyst membrane and filamentous layer forming a dense mat of interlacing branches.[2] Cysts are ingested via fecal-oral transmission. Enzymes within the stomach and small intestine cause excystation releasing two trophozoites. The organisms attach and feed in the upper small intestine and multiply by binary fission. Once the organisms reach the colon, they encyst in preparation for

the external environment.

Contributing Institution: http://nationalzoo.si.edu/

References:
1. Brown CC, DC Baker, IK Barker: Alimentary system. *In:* Pathology of Domestic Animals, ed. MG Maxie, 5th ed., pp. 277-279. Elsevier, Philadelphia, PA, 2007
2. Cheville NF: Ultrastructural Pathology, pp. 722-723. Iowa State University Press, Ames, Iowa, 1994
3. Gardiner CH, Poynton SL: An Atlas of Metazoan Parasites in Animal Tissues, 2nd ed., pp. 6-7. Armed Forces Institute of Pathology, Washington, DC 1984
4. McRoberts KM, BP Meloni, UM Morgan, R Marano, N Binz, SL Erlandsen, SA Halse, RCA Thompson: Morphological and molecular characterization of *Giardia* isolated from the straw-necked ibis. J Parasitol **82**(5):711-718, 1996
5. Monis PT, RCA Thompson: *Cryptosporidium* and *Giardia*-zoonoses: fact or fiction? Infection, Genetics and Evolution **3**:233-244, 2003
6. Ritchie B, GJ Harrison, LR Harrison: Avian Medicine: Principles and Application. pp. 732, 1014-5. Wingers Publishing, Lake Worth, FL, 1994

CASE II – 07-18567 (AFIP 3103629)

Signalment: Adult, intact female, great blue heron (*Ardea herodias*)

History: The heron was presented to the wildlife clinic with a poor body condition score (1.5/5), as well as being dehydrated and weak. The heron was initially treated with metacam, claforan, and lactated Ringer's solution. Over the next 24 hours the heron developed subcutaneous edema ventral to the bill and was started on colloid therapy (Hetastarch). The heron failed to improve and was found dead 36 hours after presentation.

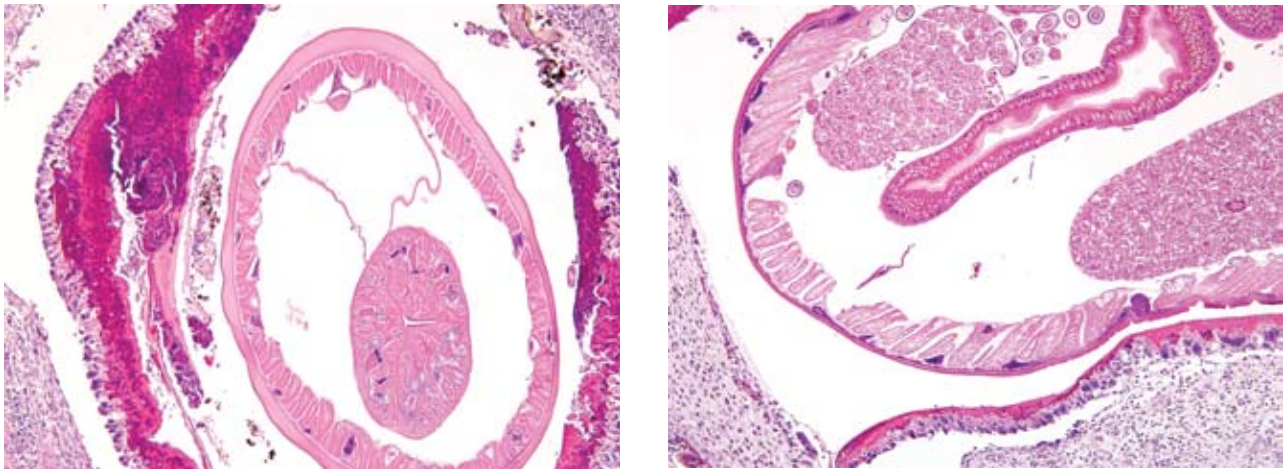
Gross Pathology: The pectoral muscles were moderately atrophied and the keel was prominent on palpation. The subcutaneous tissues ventral to the bill down to the thoracic inlet were moderately edematous. The ventral serosal surface of the proventriculus and adjacent ventriculus was focally overlain by a mass of multiple, tortuous intertwining tubules and a few ovoid cysts. Tubule diameters were up to 2 mm and cysts measured up to 1 cm long X 0.5 cm in diameter. The tubules and cysts were filled with dark red to brown, crumbly, soft debris and multiple pale nematodes 0.5 mm in diameter and up to 3 cm long. The ventriculus contained scant digested blood.

Laboratory Results:
Initial laboratory results:
Blood glucose = 297
PCV = 45
Total protein = 3.8
Fecal floatation detected ascarids

Histopathologic Description: The wall of the proventriculus, including the mucosa, muscularis, and serosa and the adjacent mesentery contain multiple variably sized granulomas. The core of each granuloma consists of a layer of eosinophilic, granular and necrotic debris mixed with degenerate eosinophils and heterophils surrounding longitudinal and cross sections of well to poorly preserved nematodes. The layer of eosinophilic debris is, in turn, surrounded by a prominent layer of macrophages and multinucleated giant cells. The outermost layer is a thick but sparsely collagenous, reticular capsule composed of both immature and mature fibrocytes. Scattered throughout the capsule are eosinophils and macrophages, fewer lymphocytes, plasma cells, and heterophils, and small foci of hemorrhage. The capsule is multifocally intersected by variably sized vessels lined by plump endothelial cells. Structural characteristics of the nematodes include one or more of the following: a thick, eosinophilic, smooth cuticle; polymyarian-coelomyarian musculature; a pseudocoelom; a ventral nerve cord; a glandular esophagus with associated pseudomembranes; many thick shelled, operculated, oval eggs 70 microns X 40 microns; an intestinal tract lined by tall columnar cells; and male or female reproductive tracts **(Figs. 2-1, 2-2)**. Occasionally, the profile of nematodes is obscured and replaced by eosinophilic necrotic debris, degenerate granulocytes, and/or colonies of bacilli along with free floating nematode eggs. The proventriculus contains multiple aggregates of eosinophils, macrophages, and lymphocytes and is mildly edematous. Glands of the proventriculus often contain sloughed epithelial cells, granular eosinophilic debris, and few leukocytes. The mesentery is mildly edematous and adipose tissue is atrophied.

Contributor's Morphologic Diagnosis: Proventriculus: Proventriculitis, chronic-active, granulomatous **(Fig. 2-3)**, eosinophilic, and heterophilic, multifocal, moderate, with multifocal mesenteric granulomas, serous atrophy of fat and intralesional adult nematodes consistent with *Eustrongylides* sp.

Contributor's Comment: *Eustrongylides* sp. infections have been reported from birds throughout the

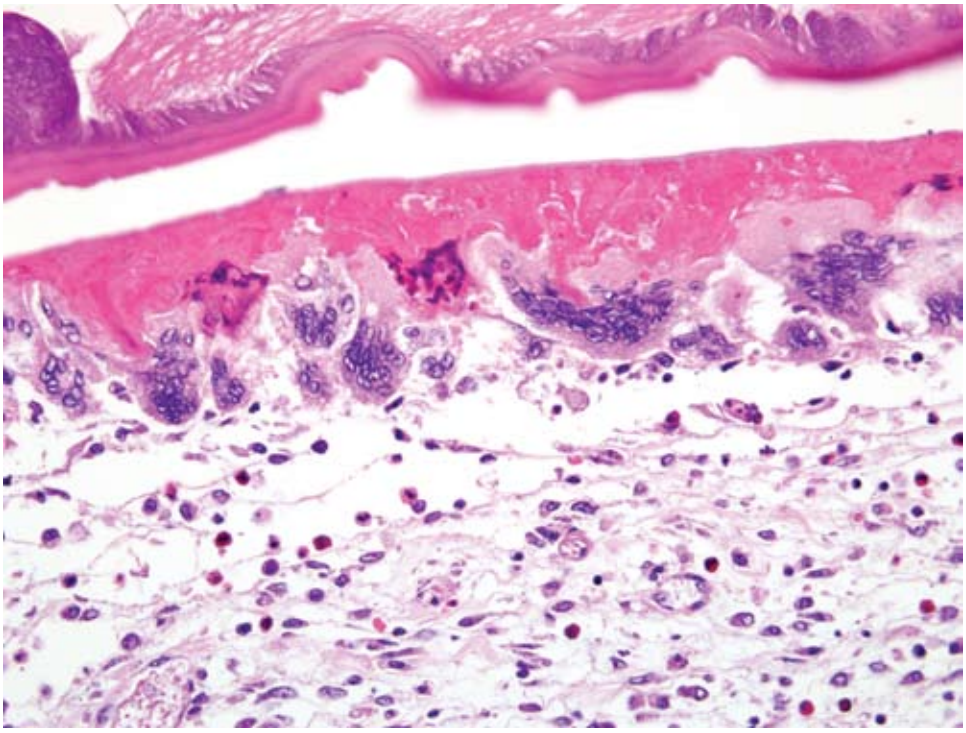


2-1, 2-2. Proventriculus, heron. Nematodes are characterized by a 7 – 10 micron thick cuticle, a pseudocoelom, coelomyarian-polymyarian musculature, bacillary bands, a glandular esophagus attached to the body wall with pseudomembranes, an intestine lined by uninucleate cells with a prominent brush border, male and female gonads, and a ventral nerve chord. (HE 100X)

world and have been implicated as a significant cause of morbidity and mortality in wading birds.[1,4,5,7,9,11] *Eustrongylides* undergo four developmental stages and require two intermediate hosts. Ciconiiformes (wading birds) are a definitive host for *Eustrongylides* [11] and shed eggs in the feces into the environment. The first larval stage then develops within the eggs and is consumed by freshwater oligochaetes (worms). The eggs then hatch and develop into second and third stage larvae. Small fish feed on the oligochaetes and the third stage larvae become encysted within the fish and develop into the infective fourth stage larvae. Wading birds then consume the fish and become infected. Alternatively, transport hosts, including amphibians, reptiles, and other fish, consume the smaller fish with the fourth stage larvae and then are consumed by wading birds. Larvae penetrate the wall of the proventriculus and ventriculus within 3 to 5 hours following ingestion of infected fish [7] and become adults in 2 to 8 days.[7] The shedding of eggs has been reported 10-17 days [1] and 14-23 days [7] post infection. *Eustrongylides* have no oral structures to allow attachment to the mucosal surface of the gastrointestinal tract, which may explain the rapid penetration of the proventriculus and ventriculus as well as clinical signs of regurgitation and vomiting as a way to remove the parasite.[7] Additional clinical signs reported include lethargy, depression, and emaciation.[11]

Significant lesions are frequently observed in birds infected with *Eustrongylides*.[4] Gross lesions reported following acute infections include minimal inflammation and hemorrhage, perforations of the proventriculus, ventriculus, and other organs, hematoma formations, severe fibrinous coelomitis involving multiple visceral organs, necrosis, and/or the presence of nematodes in the coelom.[2,4,5,7,8,11] Histologic evaluation of tissues show a severe granulomatous response including heterophils, macrophages, and multinucleated giant cells surrounding the organisms, hemorrhage or hematomas, fibrinous coelomitis with or without bacteria, and necrosis.[1,4,7,11] Lesions associated with more chronic infections include firm granulomas, air sacculitis, large, intertwining, firm, pale tan tubules crossing over the serosal surfaces of multiple organs often containing intact or decomposing organisms and/or necrotic debris.[1,4,7,11] Tubules are usually patent and open into the ventriculus.[7] Histologic lesions include the presence of intact and degenerate nematodes surrounded by a zone of cellular debris, bacteria, fragments of cuticle, and eggs which, in turn, is surrounded by eosinophilic debris, multinucleated giant cells, and fibrous connective tissue.[7] Active inflammation may also be present on the surface of the tubules.[6]

Eustrongylidosis in ciconiiformes is caused by three species of *Eustrongylides*: *E. tubifex, E. ignotus*, and *E. excisus*, and the prevalence of *Eustrongylides* sp. varies worldwide. A survey of ciconiiformes in Brazil revealed a 31% infection rate.[4] In the United States, eustrongylidosis has been implicated as a cause or contributor to losses of colonies of wading birds in Indiana [9], Delaware [8, 11], Louisiana [5], Florida [6], Texas [1], and Virginia.[1] In one survey from Florida, 13% of nestling wading birds and 24% of adult wading birds were infected.[6] The most commonly infected birds in that survey were adult Great blue herons (51%) followed by adult and nestling Great egrets (34% and 21%, respectively). 35% of nestlings in Texas, 6% in California, and 4% in Rhode Island were infected in a separate survey.[2] High mortality due to *Eustrongylides ignotus* has been observed in Delaware,



2-3. Proventriculus, heron. Nematodes (top) are surrounded by granulomas that consist of eosinophilic cellular and proteinaceous debris bounded by numerous multinucleated and epithelioid macrophages, further bounded by granulocytic and mononuclear inflammatory cells and fibroblasts. (HE 400X)

where the mortality rate reached 84% in snowy egret nestlings.[8] Multiple surveys have shown that nestlings and hatchlings are particularly sensitive and have high rates of mortality.[1,6,8,11] Infected adult birds, on the other hand, have lower rate of mortality, which serves as a source of continued shedding of eggs into the environment.[6] This can result in the reinfection of ciconiiformes, significantly impacting the overall health and reproductive success of wading bird colonies. Furthermore, the report of a human infection by *Eustrongylides* sp. resulting from the consumption of sushi [10] highlights the zoonotic potential of this parasitic nematode.

AFIP Diagnosis: Proventriculus: Proventriculitis, granulomatous, multifocal, moderate with nematodes consistent with *Eustrongylides* sp.

Conference Comment: *Eustrongylides* species are nematodes belonging to the subclass Aphasmidia, which have very characteristic morphologic features. These parasites have stichosomes, which are basophilic structures that surround the esophagus. The stichosome is formed from a row of cells called stichocytes, which are esophageal gland cells.[3] Aphasmids also have a bacillary band, which is a boomerang shaped, densely basophilic band of nuclei present in the hypodermis. Adult female aphasmids have only one genital tract in contrast to two tracts in phasmid nematodes. Eggs in most aphasmid species have bipolar plugs. Commonly encountered aphasmid parasites include *Trichuris* sp., *Eustrongylides* sp., *Dioctyophyma* sp., and *Capillaria* sp.

Contributing Institution: Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign (http://www.cvm.uiuc.edu/path/)

References:

1. Cole RA: Eustrongylidosis. *In:* Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds, Biological Resources Division, ed. Ciganovich EA, pp. 223-228: United States Geological Survey, United States Department of the Interior, Washington, DC, 1999
2. Franson JC, Custer TW: Prevalence of eustrongylidosis in wading birds from colonies in California, Texas, and Rhode Island, USA. Colonial Waterbirds **17**:168-172, 1994
3. Gardiner CH, Poynton SL: *In:* An Atlas of Metazoan Parasites in Animal Tissues, 2nd ed., pp. 40. Armed Forces Institute of Pathology, Washington, DC, 1998
4. Pinto RM, Barros LA, Tortelly L, Teixeira RF, Gomes DC: Prevalence and pathology of helminths of ciconiiform birds from the Brazilian swamplands. Journal of Helminthology **78**:259-64, 2004
5. Roffe TJ: *Eustrongylides* sp. epizootic in young common egrets (*Casmerodius albus*). Avian Diseases **32**:143-7, 1988
6. Spalding MG, Bancroft GT, Forrester DJ: The

epizootiology of eustrongylidosis in wading birds (ciconiiformes) in Florida. Journal of Wildlife Diseases **29**:237-49, 1993
7. Spalding MG, Forrester DJ. Pathogenesis of *eustrongylides ignotus* (nematoda: Dioctophymatoidea) in ciconiiformes. Journal of Wildlife Diseases **29**:250-60, 1993
8. Wiese JH, Davidson WR, Nettles VF: Large scale mortality of nestling ardeids caused by nematode infection. Journal of Wildlife Diseases **13**:376-382, 1977
9. Winterfield RW, Kazacos KR: Morbidity and mortality of great blue herons in Indiana caused by *eustrongylides ignotus*. Avian Diseases **2**:448-51, 1977
10. Wittner M, Turner JW, Jacquette G, Ash LR, Salgo MP, Tanowitz HB: Eustrongylidiasis-a parasitic infection acquired by eating sushi. The New England Journal of Medicine **320**:1124-1126, 1989
11. Ziegler AF, Welte SC, Miller EA, Nolan TJ: Eustrongylidiasis in eastern great blue herons (*Ardea herodias*). Avian Diseases *44*:443-8, 2000

CASE III – 08N0017 (AFIP 3102498)

Signalment: 7-year-old male Fulvous whistling duck (*Dendrocygna bicolor*)

History: Marked caudal coelomic distension with a very firm mass was noted. Otherwise, the duck showed no clinical signs. A CBC revealed a heterophilic (13,710/ul) leukocytosis (21,240/ul), and on serum chemistry analysis there was marked hypoalbuminemia (0.2 g/dl) and mildly increased uric acid (10.4 mg/dl). Radiographs confirmed a large soft tissue density extending from the mid- to caudal coelomic cavity and displacing the intestines caudally. The duck was euthanized.

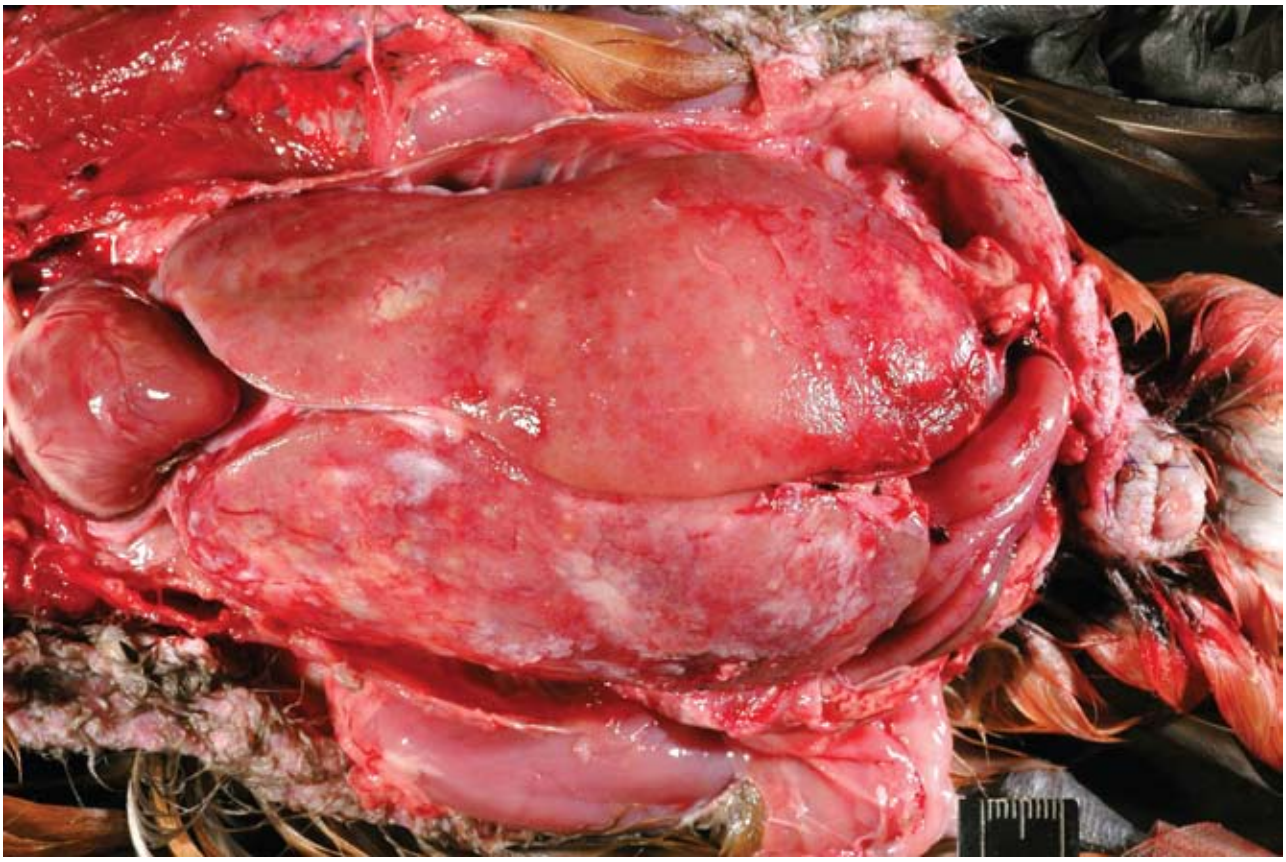
Gross Pathology: A 7-year-old male fulvous whistling duck was presented in fair nutritional condition with marked firm distension of the caudal coelomic cavity due to a massively and diffusely enlarged liver (21% of body weight). The capsular surface of the liver was rough, slightly irregular, and tan with several indistinct irregular white foci, predominantly on the right lobe **(Fig. 3-1)**. On cut section, these foci corresponded to firm, coalescing 0.2 to 1 cm diameter nodules with thick white capsules around brown caseous centers which replaced approximately 95% of the parenchyma of the right lobe and were scattered in small numbers throughout the left lobe **(Fig. 3-2)**. The remaining hepatic parenchyma was pale tan, firm and waxy. Additional very firm, multinodular, pink and tan mottled masses composed of encapsulated caseous material were present in the fascia of cervical area, dorsal to the syrinx, and attached to the serosa of several loops of intestine. The spleen and kidneys were also markedly enlarged.

Laboratory Results: With Congo red staining, the suspected amyloid was diffusely dull brick red and had a variable apple green birefringence under polarized light. This staining was lost when tissues were pretreated with potassium permanganate ($KMnO_4$). Electron microscopy confirmed that the deposits were extracellular and composed of long, haphazardly-arranged, non-branching fibrils that were on average 10 nm in diameter.

Both Ziehl-Neelsen and Fite's stains showed very large numbers of thin, variable length, acid-fast rods admixed with the debris at the center of the granulomas and occasionally within the surrounding multinucleated giant cells. These bacteria were gram positive with a Brown & Brenn stain. Immunohistochemical staining for Bacillus Calmette-Guerin (BCG) showed abundant amorphous immunoreactive material within the granulomas. The organism was identified as *Mycobacterium avium* complex by HPLC at the National Jewish Medical Center.

Histopathologic Description: In two sections of liver examined, the parenchyma is moderately to severely disrupted and replaced by abundant amorphous pale eosinophilic extracellular hyaline material (consistent with amyloid) and multifocal to coalescing granulomas **(Fig. 3-3)**. Deposition of the hyaline material variably expands the space of Disse and vessel walls, separating and disrupting cords of shrunken (atrophic) hepatocytes and occluding sinusoids. In other areas, coalescing sheets of hyaline material replace large areas of the parenchyma, with only small islands of hepatocytes and hyperplastic bile ducts remaining **(Figs. 3-4, 3-5)**. Hyaline material is also frequently present within Kupffer cells and the numerous multinucleated giant cells. The granulomas consist of a dense core of amorphous and cellular hypereosinophilic debris mixed with large numbers of wispy, amphophilic bacterial colonies surrounded by a thick rim of multinucleated giant cells and numerous fibroblasts, lymphocytes and plasma cells with fewer heterophils.

Contributor's Morphologic Diagnosis:
Liver: Severe multifocal to coalescing granulomatous hepatitis with intralesional acid-fast rod bacteria **(Fig. 3-6)** (*Mycobacterium avium* complex)
Liver: Severe amyloidosis

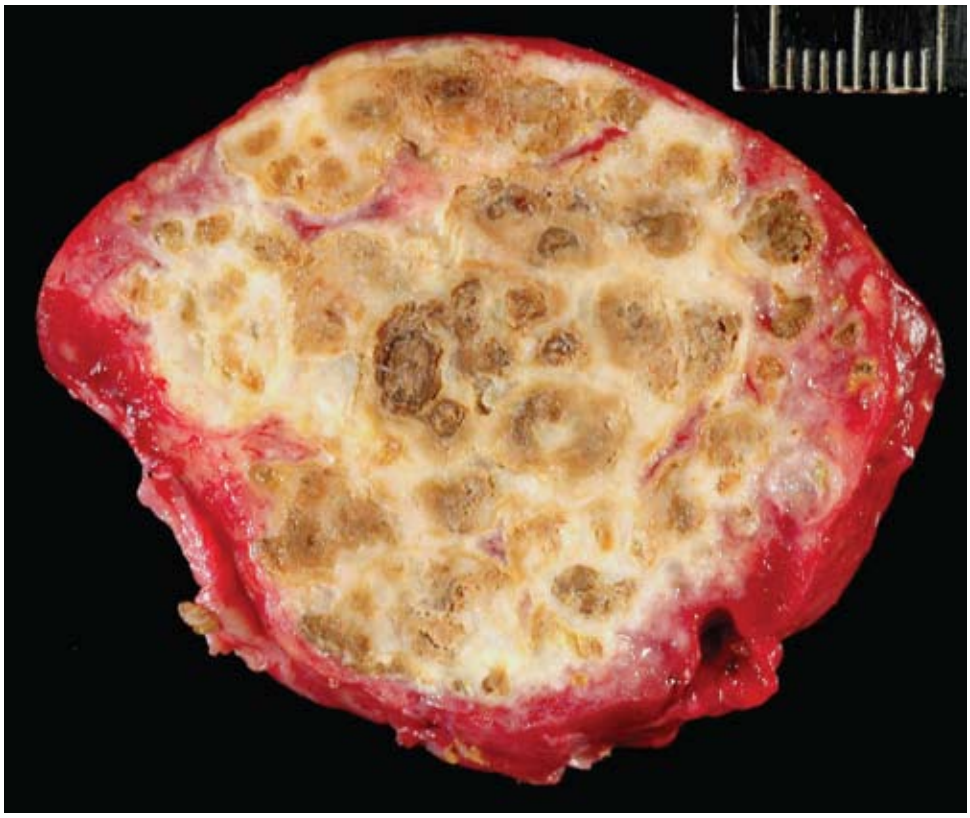


3-1. Liver, duck. Diffuse hepatomegaly, with tan, waxy discoloration and irregular capsular surface. Photograph courtesy of Rebecca M. Gruffey, Anatomic Pathology Service Manager, University of California – Davis, Veterinary Teaching Hospital, Anatomic Pathology, Davis California.

Contributor's Comment: This duck had systemic amyloidosis secondary to a chronic disseminated mycobacterial infection. In both birds and mammals with this type of amyloidosis, the liver, spleen, and kidney are most consistently involved, with variable involvement of other organs.[1,5] In addition to the liver in this case, the spleen, kidneys (interstitium and glomeruli), and adrenal glands were severely affected, but amyloid was also present in the vessel walls/interstitium of the thyroid glands, parathyroid glands, testes, bone marrow, lungs, and heart. As was demonstrated in this case, the deposits begin in the space of Disse (liver), within vessel walls, and along basement membranes, eventually leading to disease through compression of adjacent tissue and/or restriction of blood flow.[1,5]

The term amyloid encompasses a group of biochemically distinct proteins with a similar beta-pleated sheet conformation arranged in variable length, 7.5-10 nm wide, nonbranching filaments (visible by electron microscopy) which give amyloid its characteristic Congo red staining and green birefringence, as well as its fluorescence with thioflavin-T or S.[1,4,5] At least 17 amyloid proteins have been characterized in humans and animals, with AA (derived from serum amyloid A), AL (derived from immunoglobulin light chains), and Aβ (β-amyloid protein found in cerebral Alzheimer disease lesions) being the most common in humans.(1) Of these, except for a single report of β-amyloid in cerebral vessels of an aged woodpecker, [7] only AA-amyloidosis has been reported in birds.[5] The precursor protein for AA, serum amyloid A (SAA), is a soluble acute phase response protein synthesized in the liver in response to inflammation via cytokines Il-1, Il-6, and TNF.[1,4,5] The mechanisms by which insoluble derivatives of SAA are deposited and accumulate are poorly understood, but because of its association with persistently elevated SAA concentrations, as occurs in chronic inflammatory conditions, this type of amyloidosis is known as secondary, or reactive, amyloidosis.

Among birds, amyloidosis has been reported in most orders, but is particularly common in captive Anseriformes where



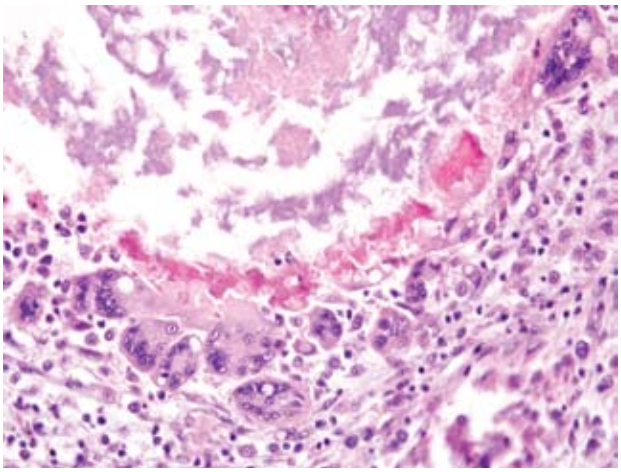
3-2. Liver, cross section, duck. Multifocal to coalescing 0.2 – 1 cm diameter granulomas with necrotic centers. Photograph courtesy of Rebecca M. Gruffey, Anatomic Pathology Service Manager, University of California – Davis, Veterinary Teaching Hospital, Anatomic Pathology, Davis California.

incidences may be almost 80% in ducks and 50% in geese and swans examined at necropsy.[5] In approximately 60-70% of amyloidosis cases in Anseriformes, an associated chronic inflammatory or infectious disease, such as mycobacteriosis, fungal disease, or enteric parasites, can be identified.[2,5,10] It has also been associated with bumblefoot in Pekin ducks and can be induced experimentally in ducks and chickens with injections of a variety of bacterial and adjuvant components.[5] The cases in which systemic amyloidosis is present without inflammation may be considered idiopathic or a result of nonspecific stresses associated with environmental conditions. For example, a study in white Pekin ducks free of chronic disease and parasites showed that increased crowding corresponded to increased rate and incidence of development of amyloidosis.[3] Amyloidosis has been seen in approximately 20% of avian mycobacteriosis cases overall, [6] and in up to 50-60% of the cases in Anseriformes.[2,8]

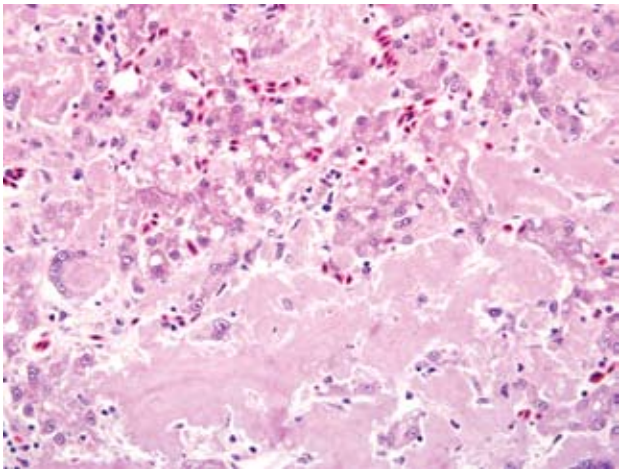
Mycobacteriosis in birds is predominantly caused by *Mycobacterium avium-intracellulare* (MAI) complex organisms or *Mycobacterium genavense* and is spread by fecal-oral transmission (rarely aerogenous) from environmental contamination.[9] They have low zoonotic potential, except to immunocompromised individuals, who are nevertheless more likely to acquire the infection from a common environmental source.[9] Some studies have found disproportionate susceptibility of waterfowl, especially perching ducks, to *Mycobacterium* sp. in zoologic collections.[9] The infection in this duck was systemic with severe involvement of the liver and scattered granulomas present in the spleen, on the serosal surface of the intestinal tract, and in the fascia of the cervical and syringeal regions. It was identified as a *Mycobacterium avium* complex species.

AFIP Diagnosis: 1. Liver: Granulomas, multiple, with acid fast bacilli, etiology consistent with *Mycobacterium* sp.
2. Liver: Amyloidosis, diffuse, severe, with moderate hepatocellular atrophy, loss, degeneration, and necrosis and multifocal, moderate granulomatous hepatitis

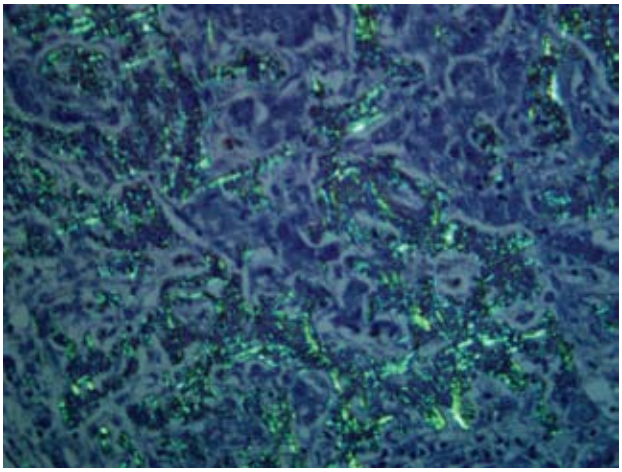
Conference Comment: The conference discussion centered predominately on the different types of amyloid and the cytokines involved in inflammation and amyloidosis. Scattered granulomatous inflammation in some areas did not appear to be associated with the acid-fast bacteria. The contributor did an outstanding job of describing this entity.



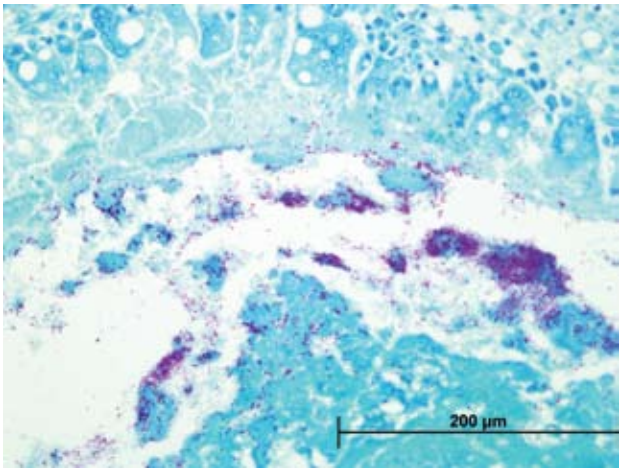
3-3. Liver, duck. Granulomas are composed of a central area of dropout and necrosis admixed with poorly staining 0.5 x 2 micron bacilli. This is bounded by numerous multinucleated giant cells, epithelioid macrophage, mononuclear inflammatory cells, and fibroblasts. (HE 400X)



3-4. Liver, duck. Hepatic cords and sinusoids are effaced by large amounts of waxy homogenous eosinophilic material (amyloid), which is occasionally found within multinucleated giant cells. Remaining hepatocytes are degenerative, characterized by cytoplasmic vacuoles and faded, swollen nuclei. (HE 400X)



3-5. Liver, duck. Congophilic amyloid material effacing hepatic cords and sinusoids displays apple green birefringence under polarized light. (CongoRed 400X) Photomicrograph courtesy of Rebecca M. Gruffey, Anatomic Pathology Service Manager, University of California – Davis, Veterinary Teaching Hospital, Anatomic Pathology, Davis California.



3-6. Liver, duck. There are numerous acid fast bacilli within granulomas. (Fite-Foraco 400 X) Photomicrograph courtesy of Rebecca M. Gruffey, Anatomic Pathology Service Manager, University of California – Davis, Veterinary Teaching Hospital, Anatomic Pathology, Davis California.

Contributing Institution: University of California Veterinary Medical Teaching Hospital, VM3-A, Anatomic Pathology, One Shields Avenue, Davis, CA 95616

References:

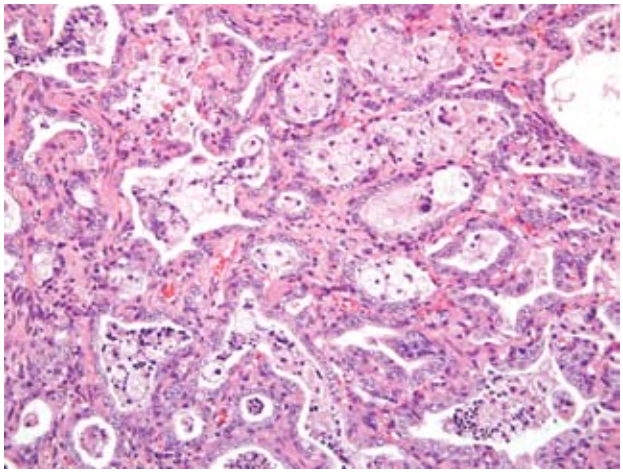
1. Abbas AK: Diseases of immunity: amyloidosis. *In*: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 258-264. Elsiever, Inc., Philadelphia, PA, 2005

2. Brassard A: Amyloidosis in captive Anseriformes. Can J Comp Med Vet Sci **29**:253-258, 1965

3. Cowan DF, Johnson WC: Amyloidosis in the white Pekin duck. Lab Invest **23**:551-555, 1970

4. King NW, Alroy J: Intarcellular and extracellular depositions, degenerations: Amyloidosis. *In:* Veterinary Pathology eds., Jones TC, Hunt RD, King NW, 6th ed., pp. 50-54. Lippincott Williams & Wilkins, Baltimore, 1997

5. Landman WJM, Gruys E, Gielkens ALJ: Avian amyloidosis. Avian Pathol **27**:437-449, 1998
6. Montali RJ, Bush M, Thoen CO, Smith E: Tuberculosis in captive exotic birds. JAVMA **169**:920-927, 1976
7. Nakayama H, Katayama KI, Ikawa A, Miyawaki K, Shinozuka J, Uetsuka K, Nakamura SI, Kimura N, Yoshikawa Y, Doi K: Cerebral amyloid angiopathy in an aged great spotted woodpecker (*Picoides major*). Neurobiol Aging **20**:53-56, 1999
8. Saggese MD, Riggs G, Tizard I, Bratton G, Taylor R, Phalen DN: Gross and microscopic findings and investigation of the aetiopathogenesis of mycobacteriosis in a captive population of white-winged ducks (*Cairina scutulata*). Avian Pathol **36**:415-422, 2007
9. Tell LA, Woods L, Cromie RL: Mycobacteriosis in birds. Rev Sci Tech Off Int Epiz **20**:180-203, 2001
10. Zschiesche W, Linke RP: Immunohistochemical characterization of spontaneous amyloidosis in captive birds as AA-type, using monoclonal and polyclonal anti-AA antibodies against mammalian amyloid. Acta Histochem **86**:45-50, 1989



4-1. Lung, cat. Alveolar septa are markedly expanded by large amounts of fibrosis, smooth muscle proliferation, and an inflammatory infiltrate composed of histiocytes and lymphocytes. Alveolar are flooded by either a cellular exudates composed of viable and degenerate neutrophils and fewer macrophages, or by numerous large foamy alveolar macrophages which contain many small indistinct vacuoles. Diffusely, alveoli are lined by type II pneumocyte hyperplasia. (HE 200X)

CASE IV – BA 652/07 (AFIP 3102244)

Signalment: 9-year-old, female neutered, Persian cross-bred, feline (*Felis domesticus*)

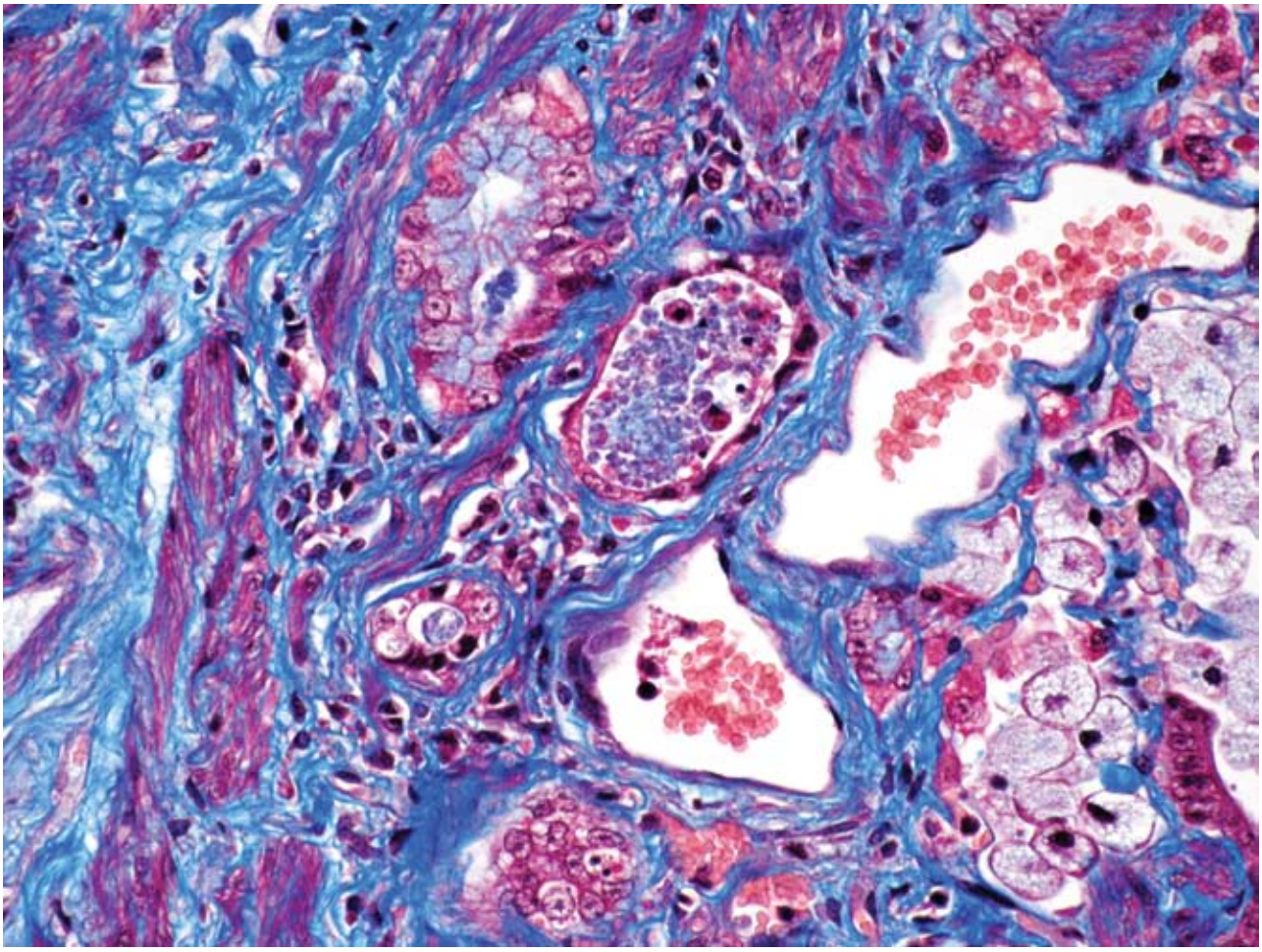
History: The cat was referred to the Royal (Dick) School of Veterinary Studies with a 4 month history of intermittent coughing and retching, which was unresponsive to furosemide (diuretic) and Synulox (antibiotic). Broncho-alveolar lavage fluid prior to referral contained very large numbers of monomorphic epithelial cells but was otherwise non-diagnostic. The cat presented with a severe tachypnoea (100-160 bpm). Thoracic radiographs were performed and revealed a mixed alveolar, interstitial, nodular and bronchial pattern with bullous change in the caudo-dorsal lung lobes. PCR for *Mycoplasma* sp. was negative. A clinical diagnosis of Idiopathic Pulmonary Fibrosis was made. The cat was prescribed steroids, Sildenafil (Viagra), Colchicine and Salbutamol (Ventolin). Some response to the steroid treatment was observed but this was limited. Over the following 9 months the cat deteriorated with several episodes of hospitalization.

Gross Pathology: The cat was in obese body condition (4.2kg). The lungs were diffusely mottled dark and light pink with numerous multifocal to coalescing firm, yellow-brown to yellow-green, depressed areas over the surface. These ranged from 2-12 mm diameter and extended into the parenchyma. This was more pronounced in the caudal right lung lobe. The airways were clear. Mild, multifocal bilateral chronic renal cortical infarcts were also noted, characterized by firm, depressed, tan foci.

Laboratory Results: None

Histopathologic Description: Lung – Right caudal lung lobe – 1 section

Approximately 70-80% of the lung parenchyma is remodeled by multifocal to coalescing areas of increased cellularity, predominantly located subpleurally, but also extending into the central parenchyma. In these areas, the pulmonary interstitium is markedly expanded by fusiform and plump mesenchymal cells (fibrocytes and fibroblasts), and thick bundles of plump spindloid cells with moderate amounts of eosinophilic cytoplasm, and a medium sized oval to elongated nucleus with a reticular chromatin pattern (myofibroblasts) **(Fig. 4-1)**. Where present, alveolar spaces are lined by a single to occasionally multiple layers of low columnar to cuboidal epithelial cells with indistinct cell borders, a moderate amount of pale eosinophilic, finely granular cytoplasm and a basally located, round to



4-2. Lung, cat. Masson's trichrome stain demonstrates alveolar interstitial fibrosis and smooth muscle proliferation. Photomicrograph courtesy of Veterinary Pathology Unit, University of Edinburgh, Royal (Dick) School of Veterinary Studies, Easter Bush Veterinary Centre, Easter Bush, Midlothian, Scotland. (MAS 400X)

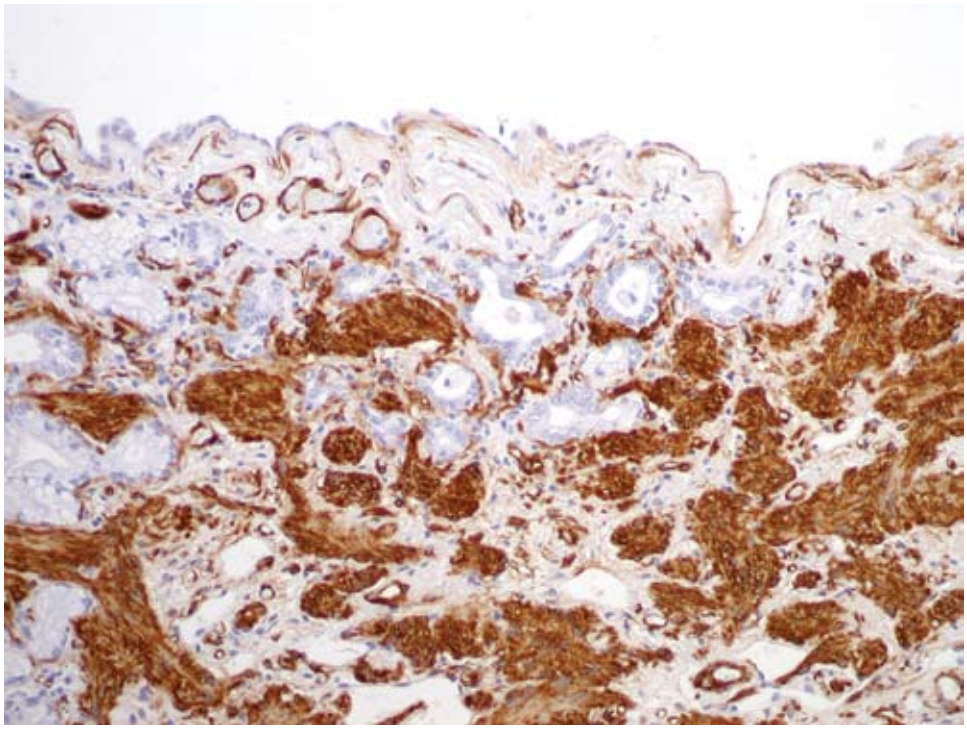
oval nucleus with a finely stippled chromatin pattern, and one to two medium sized nucleoli consistent with type II pneumocytes ("honeycomb lung").

Within the alveolar lumen are numerous, plump, polyhedral to round cells with abundant, highly vacuolated, pale eosinophilic cytoplasm and a medium sized oval nucleus with a finely stippled chromatin pattern and a single, medium sized nucleolus (alveolar macrophages). These cells are occasionally binucleated and multinucleated. Small aggregates of lymphocytes and plasma cells are scattered throughout the remaining pulmonary parenchyma.

Small to medium sized bronchi contain numerous alveolar macrophages interspersed with abundant pale amphophilic to basophilic, lightly fibrillar, acellular material (mucus), small areas of deeply basophilic, cracked acellular material (mineral) and acicular (cholesterol) clefts. At the periphery of the large bronchi are numerous, small peribronchiolar glands. In less severely affected areas, the alveolar lumens are multifocally enlarged by non-staining spaces (emphysema).

Special stains/Immunohistochemistry: The cuboidal cells lining the alveolae are strongly positive for cytokeratin, confirming their epithelial nature. The interstitial mesenchymal proliferation stained variably blue and pink (collagen and smooth muscle, respectively) **(Fig. 4-2)** and large areas stained strongly with smooth muscle actin confirming the presence of smooth muscle **(Fig. 4-3).**

Contributor's Morphologic Diagnosis: Lung – Right cranial lung lobe - Severe, multifocal to coalescing interstitial fibrosis with fibroblast and myofibroblast foci,



4-3. Lung, cat. Smooth muscle actin immunohistochemical stain demonstrating positive cytoplasmic immunoreactivity within areas of alveolar interstitial smooth muscle proliferation. Photomicrograph courtesy of Veterinary Pathology Unit, University of Edinburgh, Royal (Dick) School of Veterinary Studies, Easter Bush Veterinary Centre, Easter Bush, Midlothian, Scotland. (SMA 200X)

type II pneumocyte hyperplasia (honeycomb lung) and alveolar interstitial smooth muscle proliferation

Contributor's Comment: The severe, multifocal to coalescing interstitial fibrosis, interstitial smooth muscle proliferation, alveolar macrophages and excess mucus production are consistent with the clinical diagnosis of feline idiopathic pulmonary fibrosis (FIPF). The lesions were similar in nature throughout the lung but were more severe in the right caudal lung lobe than the left cranial lung lobe. There was also squamous metaplasia in sections of the cranial lung lobe, which is also a feature of this condition.

FIPF is an uncommon condition, first described in 2000 by Rhind and Gunn-Moore. [2] The average age of onset is approximately 8.7 years and the average time between onset clinical signs to death is 5.5 months.[1] This case has many features characteristic of this condition: sub-plural and caudo-dorsal distribution, persistent progressive fibrosis with temporal heterogeneity of lesions, alveolar type II pneumocyte hyperplasia and small foci of alveolar epithelial squamous metaplasia in some sections. This condition has been associated with the development of bronchoalveolar carcinoma, which was not a feature of this case. The aetiology is not currently known. Toxic, hypersensitivity, inflammatory and genetic origins have been investigated.

Parallels have been drawn between FIPF and Usual Interstitial Pneumonia (UIP) in man.[3] Salient features of UIP in man include interstitial fibrosis with fibroblast/myofibroblast proliferation, temporal heterogeneity of lung remodeling, subpleural orientation, "honeycomb" change, interstitial smooth muscle proliferation and scant inflammation.

A type II pneumocyte defect has been described in a familial form of human interstitial pulmonary fibrosis (IPF), with abnormal cytoplasmic lamellar body-like inclusions seen on electron microscopy.[3] Similar ultrastructural changes were observed in type II pneumocytes of affected cats by Williams et al. The authors suggested that FIPF may be due to a defect in the type II pneumocyte and that cats potentially represent a spontaneous model of IPF in humans.

AFIP Diagnosis: Lung: Interstitial fibrosis, multifocally extensive, severe with fibroblast and smooth muscle proliferation, type II pneumocyte hyperplasia, and alveolar histiocytosis

Conference Comment: Idiopathic pulmonary fibrosis is a respiratory disease of humans that affects 11 male and 8 female patients per 100,000 individuals per year. Consequently, a representative animal model to study this malady is highly coveted and bleomycin-treated rodents have not proved to be adequate subjects to date.[3]

The contributor did an excellent job of outlining what is currently known about this disease in cats.

Contributing Institution: Veterinary Pathology Unit, University of Edinburgh, Royal (Dick) School of Veterinary Studies, Easter Bush Veterinary Centre, Easter Bush, Midlothian, EH25 9RG, Scotland, UK.
www.vet.ed.ac.uk

References:

1. Cohn L, Norris E, Hawkins E, Dye J, Johnson C and Williams K: Identification and characterization of an Idiopathic Pulmonary Fibrosis-like condition in cats. J Vet Intern Med **18**:623-641, 2004
2. Rhind S and Gunn-Moore D: Desquamative form of cryptogenic fibrosing alveolitis in a cat. J Comp Path **123**:226-229, 2000
3. Williams K, Malarkey D, Cohn L, Patrick D, Dye J and Toews G: Identification of spontaneous feline idiopathic pulmonary fibrosis. Chest **125**:2278-2288, 2004