



## WEDNESDAY SLIDE CONFERENCE 2018-2019

### Conference 18

6 February 2019

**Conference Moderator:**

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**CASE I:** 13-161654 (JPC 4048673).

**Signalment:** 25 year-old, female intact Congo African Grey Parrot (*Psittacus erithacus erithacus*)

**History:** This parrot was a permanent resident of a pet shop collection and was fed a diet of pellets, seeds, apples, and peanuts. The previous medical history included a prolapsed oviduct in May of 2013 with egg retention, but the bird was otherwise healthy. The bird was presented to the referring veterinarian in November of 2013 for a recent onset of lethargy, anorexia, and feather picking. On presentation, blood work abnormalities included increased bile acids and hyperproteinemia (absolute values were not provided).

**Gross Pathology:** Both brachiocephalic arteries are prominent, diffusely yellow, hard and do not collapse. On section, the luminal surface is expanded by a locally extensive 1mm thick, rough, yellow plaque (atherosclerosis).



*Presentation, parrot: The arteries are thickened and yellow and there is marked hepatomegaly. (Photo courtesy of Cornell University – Animal Health and Diagnostic Center, 240 Farrier Road, Ithaca, NY 14850)*

The coelomic cavity contains multiple aggregates of dark red gelatinous material that cover the viscera. The spleen is uniformly enlarged measuring 4.7 x4.5 cm and diffusely mottled white to light tan to red. The liver is enlarged with rounded margins and the parenchyma is diffusely mottled green to black to tan with dozens of random 0.1 cm round white foci (necrosis, presumptive).

**Laboratory results:** University of Miami – Avian and Wildlife Laboratory

- Increased beta and gamma globulins on electrophoresis consistent with acute inflammation and active humoral immune response
- High titer (1:25) for *Chlamydophila psittaci*

**Microscopic Description:**

**HEART** (not submitted): Randomly scattered throughout the parenchyma are small foci of cardiomyocyte loss with replacement by foamy macrophages and lymphocytes.

**VESSEL, NOS:** Arteries: Diffusely the tunica intima and media is disorganized with the tunica media expanded by abundant foam cells creating an irregular luminal surface. Occasionally the tunica media contains multifocal clear acicular clefts (cholesterol) that are variably surrounded by foamy macrophages and includes locally extensive regions of cellular loss with replacement by large clear spaces (fatty infiltrate) and several foci of chondroid metaplasia. The tunica



*Heart and great arteries, parrot: The arteries are thickened, do not collapse under their own weight and yellow and there is marked hepatomegaly. (Photo courtesy of Cornell University – Animal Health and Diagnostic Center, 240 Farrier Road, Ithaca, NY 14850)*

intima and media contain numerous small aggregates of granular amorphous deeply basophilic material (mineral). In cross section the arteriolar lumen is occluded up to 60% by a confluent mass composed of a deeper region of large foam cells, acicular clear clefts (cholesterol), large clear spaces (fatty infiltration), multifocal aggregates of mineral, and islands of chondroid matrix covered by a thin variably present endothelial lining (atheroma).

**LIVER** (not submitted): Randomly scattered throughout the hepatic parenchyma large coalescing areas of hepatocyte loss with the remaining hepatocytes being pale eosinophilic with loss of nuclear detail (necrosis). Admixed within these foci are small numbers of macrophages with fewer plasma cells and lymphocytes and rare heterophils. Surrounding hepatocytes contain fine granular golden brown cytoplasmic pigment (lipofuscin). The remaining hepatocytes show moderate anisocytosis and anisokaryosis, with occasional binucleate cells.

**SPLEEN** (not submitted): Diffusely expanding the red pulp are large coalescing aggregates of foamy macrophages admixed with few degenerate and non-degenerate heterophils.

**Contributor's Morphologic Diagnoses:**

Arteries: Severe, diffuse, chronic atherosclerosis

Heart: Mild, multifocal, lymphohistocytic myocarditis

Liver: Severe, multifocal to coalescing, chronic, hepatocellular necrosis with granulomatous, lymphoplasmacytic hepatitis

Spleen: Severe, multifocal, chronic, granulomatous splenitis



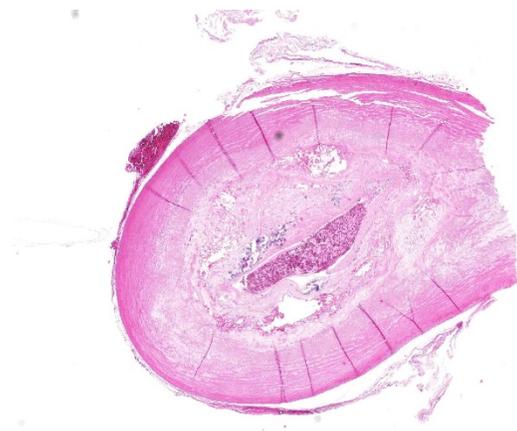
*Liver: The liver is enlarged, with a cobblestoned mottled parenchyma. (Photo courtesy of Cornell University – Animal Health and Diagnostic Center, 240 Farrier Road, Ithaca, NY 14850)*

**Contributor’s Comment:** The histologic features seen here are consistent with atherosclerosis of a grade IV out of VII,<sup>5</sup> although grade varies between submitted sections. The changes in the liver and spleen are seen with *Chlamydophila psittaci* infection, but other systemic bacterial infections (e.g. *Escherichia coli*, *Salmonella* sp, *Mycobacterium* sp, *Staphylococcus* sp, and *Streptococcus* sp) can create similar changes. Antemortem testing for *Chlamydophila psittaci* were consistent with an active infection. In this case the cause for coelomic hemorrhage remained unclear, as thrombotic events and vessel rupture are uncommon complications of atherosclerosis in the avian species.

Atherosclerosis is a chronic inflammatory fibroproliferative disorder, which commonly causes spontaneous disease in psittacines, as well as numerous other avian species. Most notably the Japanese Quail (*Coturnix coturnix japonica*) and pigeon (*Columa livia domestica*) have been used as inducible experimental models for the human disease.<sup>2,3,11</sup> The pathogenesis of disease has been largely explained by the response-to-

injury hypothesis. Here the principle inciting cause is endothelial damage, secondary to endothelial dysfunction and oxidative stress that creates a local atherogenic environment<sup>2,5</sup>. Endothelial dysfunction is characterized by a chronic state of endothelial cell activation leading to increased production of procoagulative and proinflammatory factors.<sup>2</sup> In a recent study endothelial cell changes were appreciated in mild pre-atheroma lesions; giving further support to the hypothesis that endothelial damage precedes lipid deposition.<sup>5</sup> A recent experimental model showed that type VIII collagen is up-regulated in arteriosclerotic lesions and allows for smooth muscle cell proliferation and migration, which is important for fibrous cap formation.<sup>7</sup>

Currently the association between histologic severity and clinical disease course is unknown. As atherosclerosis in psittacines represents a similar spontaneous disease progression to that seen in humans, a similar grading scheme based on the American Heart Association’s human grading scheme has been proposed for better characterization and future prognostication of disease. This scheme divides atherosclerotic lesions into seven grades based on the light microscopic,

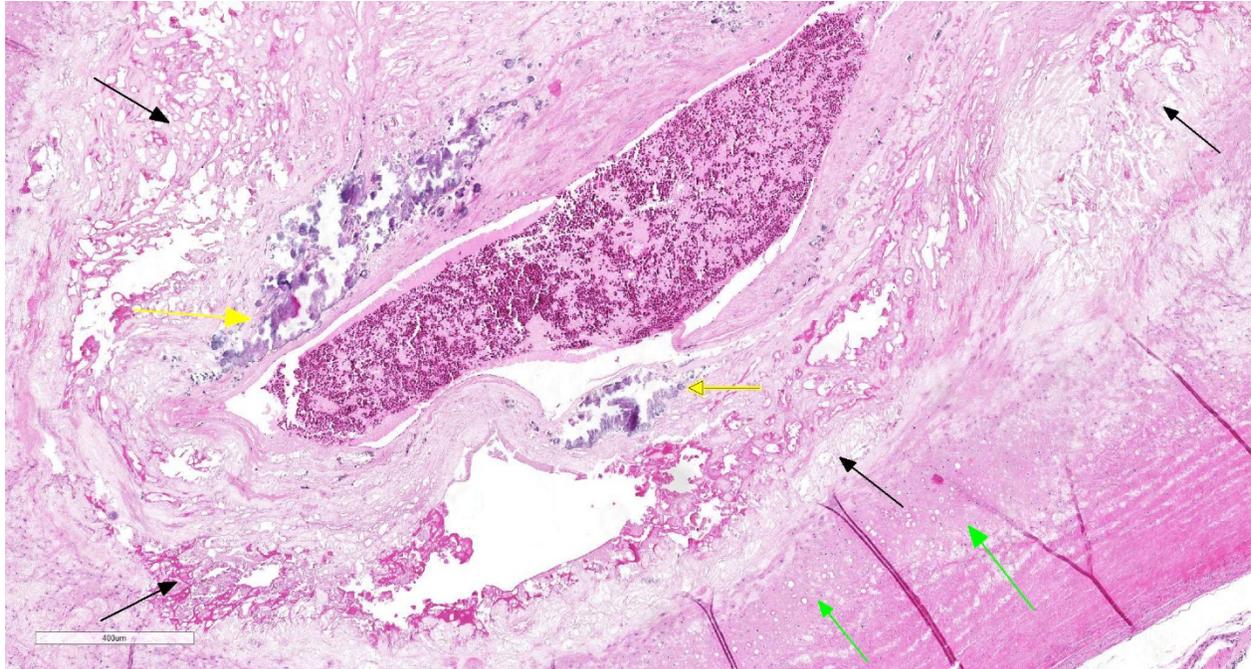


*Muscular artery, parrot. The tunica intima is effaced and the tunica media is markedly expanded by a complex atheroma. (HE 20X).*

histochemical, and electron microscopic features, with grades greater than IV of VII being considered clinically significant.<sup>5</sup>

Several risk factors have been proposed for the development of atherosclerosis in psittacines. Diet has been implicated, as many animal models for experimental disease are diet-inducible. In one study increasing the cholesterol within the feed of Quaker parrots (*Myiopsitta monachus*) by 1% induced clinically significant lesions within 4-6 months.<sup>6</sup> With hypercholesterolemia, lipoproteins themselves can allow for activation of endothelial cells and leukocyte adhesion. Oxidized lipoproteins act as strong chemoattractants for monocytes. Once they enter the vascular wall and transform, to macrophages, they express scavenger receptors that allow for uptake of additional low density lipoproteins (LDLs) and stimulation of smooth muscle cells to foam cells<sup>2</sup>. In addition increased age, female sex,

concurrent reproductive or hepatic disease, and the genera *Psittacus*, *Amazona*, and *Nymphicus* were found to increase the risk of development of severe atherosclerotic lesions.<sup>4</sup> The synthesis and transport of VLDLs and vitellogenin to the oocyte and future egg yolk in response to estrogen may be the underlying pathogenesis for this predisposition in female birds.<sup>2</sup> As VLDLs have been shown to be pro-inflammatory.<sup>2</sup> Infectious agents have been implicated as risk factors for lesion development. In one study there was a significant association between *Chlamydophila psittaci* antigen in the blood and development of atherosclerosis, however this correlation was not appreciated in one large retrospective study. However it is interesting to note that this patient had concurrent histologic findings and antemortem test findings consistent with active *Chlamydophila psittaci* infection. Marek's disease (herpes virus infection) has



**Muscular artery, parrot. Higher magnification of the atherosclerotic plaque reveals a thick, often artifactually cleaved layer of foam cells, cholesterol clefts and abundant collagen (black arrows), mineral (yellow arrows), and at the periphery, foci of cartilaginous metaplasia. (HE 66X).**

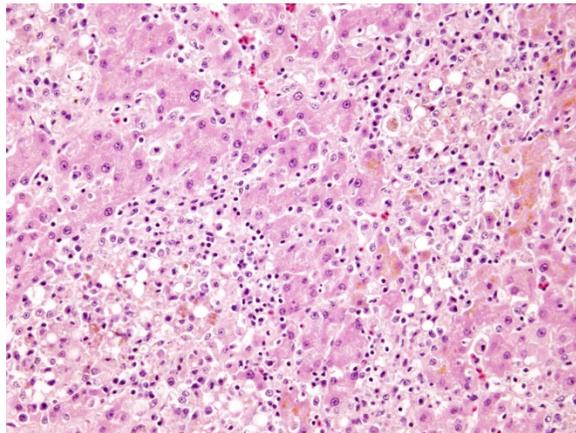
been shown in chickens to promote atherosclerotic lesion formation.<sup>2</sup>

Underlying genetic factors can predispose a patient to endothelial dysfunction. In one study in White Carneau Pigeons there was a divergence in the genes responsible for cytoskeleton remodeling, proteasome activity, cellular respiration, and immune response. These genes were postulated to predisposition to the development of atherosclerotic lesions.<sup>1</sup> The importance of gene divergence in other avian species has not been investigated.

**Contributing Institution:**

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**JPC Diagnosis:** Elastic artery: Atherosclerosis, circumferential, diffuse, severe, with marked mural fibrosis, cartilaginous metaplasia and mineralization.



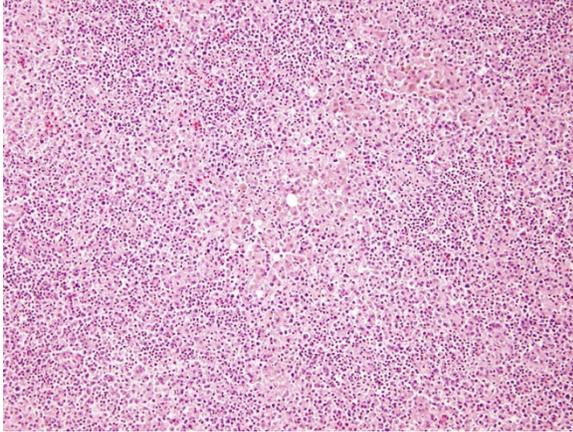
*Liver: There are multifocal to coalescing foci of granulomatous inflammation containing large numbers of macrophages with fewer often peripheral lymphocytes and plasma cells. Remaining hepatocytes often contain abundant lipofuscin. (HE 200X) (Photo courtesy of Cornell University – Animal Health and Diagnostic Center, 240 Farrier Road, Ithaca, NY 14850)*

2. Elastic artery, adventitial adipose tissue: Atrophy, diffuse, severe.

**JPC Comment:** The contributor has provided an excellent discussion of atherosclerosis in psittacines and other avian species, highlighting a number of abnormalities which are involved in this complex condition. Species such as humans, non-human primates, pigs, rabbits and hamsters are considered susceptible to atherosclerosis and its predisposing factor, hypercholesterolemia. Other laboratory animals such as mice, rats, dogs and shrews exhibit resistance to these conditions.<sup>9</sup>

The recent literature abounds with studies on various areas of research into animal models of atherosclerosis, in a number of areas of current interest in the pathogenesis of human atherosclerosis – genetic abnormalities, the contribution of inflammation and inflammatory mediators on the development of atheromatous plaques, endothelial dysfunction, and diet/dyslipidemia. Genetic abnormalities, such as the deficiency of low-density lipoprotein receptors (LDLR), are widespread in humans (with over 1700 separate genetic mutations to date)<sup>9</sup> and have been a subject of investigation since the use of the Watanabe rabbit in the 1970's. Today, genetic manipulation of knockout mice and rabbits involved in the pathway of production and degradation of the LDLR continues to further research in this area.<sup>9</sup>

Diet-induced atherosclerosis (or enhancement of disease in atherosclerosis – resistant species) is a time-honored research avenue which is still being applied today to new animal models. The Gottingen minipig has been a model for atherosclerosis for the last decade. Diets high in fat/cholesterol and cholate have the potential for inducing advanced coronary and atherosclerotic



*Liver: There are also foci of granulomatous inflammation within the spleen as well. (Photo courtesy of Cornell University – Animal Health and Diagnostic Center, 240 Farrier Road, Ithaca, NY 14850)*

lesions in the species<sup>10</sup>, as has been demonstrated in a number of other species over the years, to include laboratory rodents as well as non-human primates.

An excellent review from a species perspective on atherosclerosis was part of a recent review of aging changes in great apes.<sup>8</sup> In contrast to early literature stating that chimpanzees were suitable animal models for coronary atherosclerosis (especially if subjected to atherogenic diets), the actual incidence of coronary atherosclerosis is quite low in apes in well-managed colonies and collection; however more data needs to be collected on aortic atherosclerosis in these species.<sup>8</sup>

We are unable to confirm the diagnosis of chlamydia in this case, as liver and splenic tissue were not part of the submission. The connection between chronic inflammation and atherosclerosis is well-documented in the literature; however, the connection between the two lesions in this animal and its significance would be very difficult to assess.

Early on in the discussion, the moderator pointed out the difficulty in even establishing that whether this section represented an

elastic or a muscular artery. Without the use of special stains, the mural changes make it difficult to visually quantitate the amount of elastin within the wall. Muscular arteries, which deliver blood to specific organs, tend to have walls composed primarily of smooth muscle, while elastic arteries have a higher concentration of elastic fibers. Both the moderators from this week and last week conference agreed that the differentiation of elastic versus muscular arteries is extremely difficult in very diseased vessels.

The moderator reviewed atherosensitive species including rabbits, guinea pigs, birds, and pigs, as well as atheroresistant species - dogs, cats, cattle, goats, and unmanipulated mice/rats. In psittacines, the disease is common in Amazons, African greys, cockatiels and Quaker parrotss. The participants also discussed the response to injury (endothelial damage and the generation of a procoagulative and pro-inflammatory state) versus the response to retention theory (binding of LDL's by arterial extracellular matrix and promotion of further endothelial damage and recruitment of additional lipids and leukocytes) in atherogenesis. The moderator also reviewed the currently published grading system for atherosclerosis in psittacines.<sup>6</sup>

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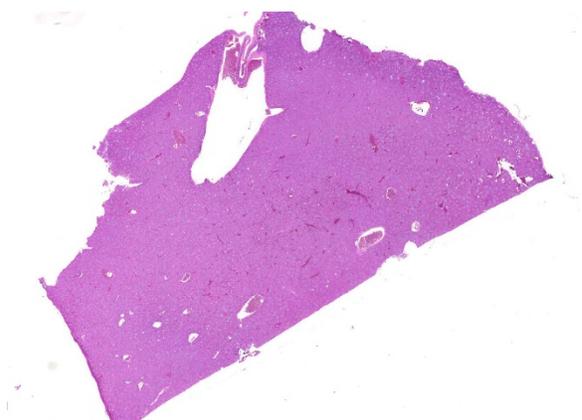
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**CASE II: B or blank (JPC 4066242).**

**Signalment:** 40 year old male Orange-winged Amazon parrot, *Amazona amazonica*

**History:** A 40 year old male Orange-winged Amazon parrot (*Amazona amazonica*) presented to the Ontario Veterinary College, Health Sciences Center, Avian and Exotics service for a one day history of severe



*Liver, amazon parrot. Subgross examination reveals numerous small areas of pallor scattered throughout the section. (HE, 7X)*

weakness, falling from his perch and inability to right himself or stand. The bird was initially purchased as a hatchling, and lived with the current owners for the last 40 years. There were no other birds in the house. The bird had a previous clinical history of feather plucking and hypovitaminosis A.

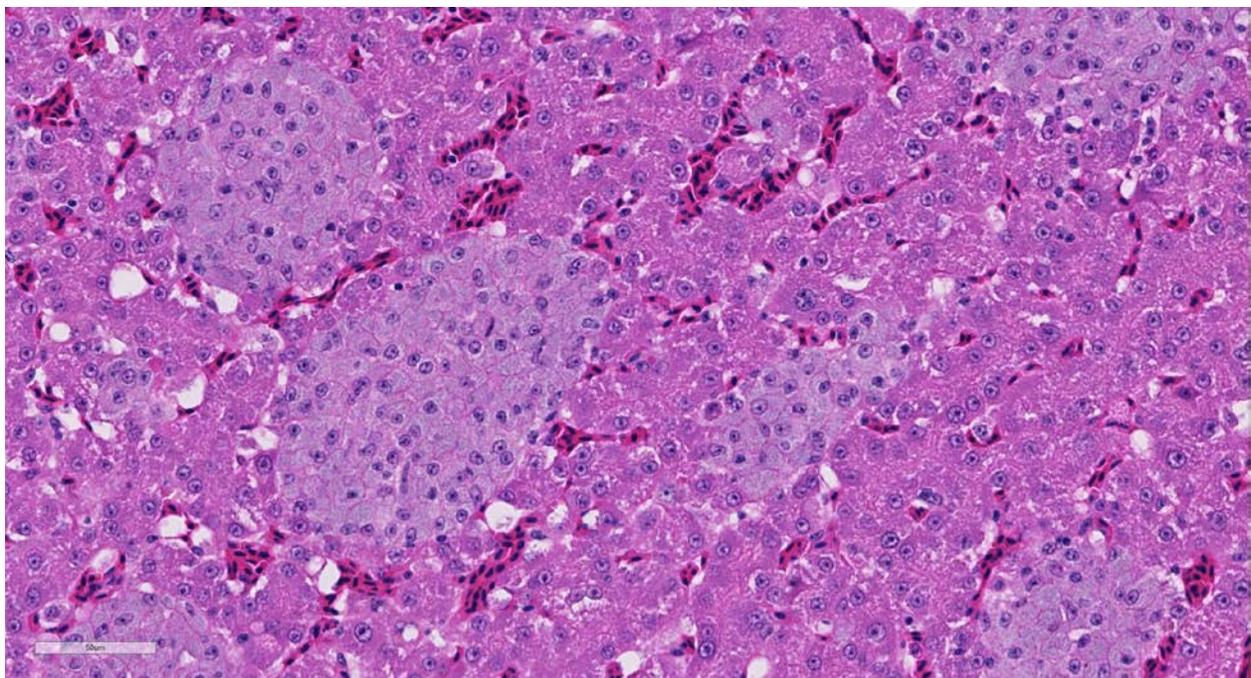
On physical examination, the bird was dull and lethargic. There was mild overgrowth of the rhamphotheca and nails. Complete blood count and serum biochemistry revealed leukocytosis (white blood cells  $28.2 \times 10^9/L$  [reference  $1.2-10.1 \times 10^9/L$ ], heterophils  $21.96 \times 10^9/L$  [78 %; reference 21.9-40.7 %]<sup>5</sup>) and increased concentration of liver enzymes (AST 1012 U/L [reference range 150-344 U/L]<sup>2</sup>). Due to the age of the patient and severity of clinical signs, the owners declined further diagnostics and the bird was euthanized.

**Gross Pathology:** The bird was in thin body condition, with decreased subcutaneous fat stores and mildly depleted pectoral muscle mass. The majority of the contour feathers were missing from the ventrum, but down feathers were still present. The gnathotheca was overgrown, with flaking of keratin at the lateral edges. On internal examination, the liver was enlarged and mottled tan-red. The liver weighed 9 grams (2.8 % of body weight). The gastrointestinal tract was empty, with the exception of a small amount of gravel in the ventriculus.

**Laboratory Results:**

Sections of liver and brain were stained with Ziehl Neelsen acid-fast stain, revealing myriad acid-fast bright red intracytoplasmic bacilli, morphologically compatible with mycobacteria.

Samples of liver and spleen were sent to an external laboratory (Public Health Laboratory, Toronto) for culture and



*Liver, amazon parrot. High magnification demonstrates the presence of numerous foci of non-caseating granulomatous inflammation throughout the section. Macrophages contain numerous intracytoplasmic bacilli which impart an amphophilic color to the cytoplasm. (HE, 400X)*

mycobacteria species identification. On tissue smears of both organs, 4+ acid-fast bacilli were seen. GenoType® Mycobacterium molecular genetic test systems (Hain Lifescience) identified the bacilli as *Mycobacterium genavense*. No mycobacteria were isolated after 7 weeks of mycobacterial culture.

### **Microscopic Description:**

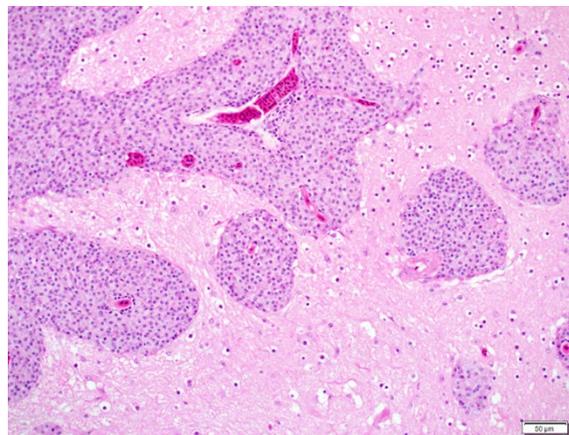
**Liver:** There are numerous, multiple, well-demarcated, expansile to coalescing, variably-sized (up to 250 µm in diameter) nodular infiltrates scattered randomly throughout the hepatic parenchyma, comprising up to 40% of the tissue. The infiltrates are composed of relatively uniform, approximately 15 µm diameter polygonal round cells morphologically compatible with epithelioid macrophages, with distinct cell margins and abundant faintly basophilic cytoplasm containing large numbers of faintly discernable, 1-2 µm long organisms. Nuclei are round and eccentric, with finely stippled chromatin and prominent basophilic nucleoli. Rare larger nodules contain central aggregates of pyknotic cellular debris and amorphous eosinophilic fibrillar material (necrosis).

**Brain (tissue not included on slides submitted):** Similar infiltrates of macrophages are present beneath the leptomeninges and within Virchow-Robins spaces throughout the sections of brain examined.

### **Contributor's Morphologic Diagnoses:**

**Liver:** Hepatitis, multifocal nodular, histiocytic with intracytoplasmic bacilli (*Mycobacterium genavense*).

**Contributor's Comment:** A presumptive diagnosis of mycobacterial hepatitis (avian mycobacteriosis) was initially made based on the presence of intracytoplasmic acid-fast



*Brain, amazon parrot. Infiltrates of similar bacilli-laden macrophages expand Virchow-Robins spaces. (HE, 100X)*

bacilli. As there was potential zoonotic risk in this case,<sup>1</sup> samples of liver and spleen were submitted for mycobacterial culture and species identification at a local public health laboratory (Public Health Laboratory, Toronto). The lab identified acid-fast bacilli on direct smears of the tissues and speciated the sample using GenoType Mycobacterium test, which can differentiate between 16 different mycobacterial species (including *Mycobacterium genavense*).<sup>6</sup> Despite the large numbers of bacilli noted histopathologically and on tissue smear, the laboratory was unable to isolate the mycobacteria after 7 weeks of culture.

Avian mycobacteriosis is one of the most common diseases of birds world-wide, affecting pet birds, domestic poultry, zoo collections and wild avian species.<sup>8</sup> The disease is often insidious, presenting as a chronic wasting disease with relatively non-specific clinical signs or sudden death with no premonitory signs at all.<sup>1,3,8,10</sup> Antemortem diagnosis is challenging, and diagnosis is often made on characteristic post-mortem and histopathological findings, with or without confirmatory culture and speciation.<sup>7, 8</sup> Lesions may exist as multifocal, multi-organ granuloma

formation, intestinal paratuberculosis-like histiocytic infiltration, or grossly apparent organomegaly.<sup>8</sup> The gross and histological appearance may be impacted by the species of bird affected, or the immune status of the affected bird.<sup>8</sup>

*Mycobacterium genavense* has been reported to be the most common mycobacterial species isolated from pet birds,<sup>4,8</sup> but *Mycobacterium avium* subsp. *avium* and *Mycobacterium intracellulare* are other commonly identified species. Other mycobacterial species (of which there are at least ten identified in various species of birds) are uncommon.<sup>7</sup> Mycobacteria are often ubiquitous in the environment, and can be isolated from acidic or wet soil or contaminated water sources.<sup>8</sup> Environmental contamination is the presumed source of the organism, and infection is contracted by ingestion of the bacteria. Disease susceptibility is variable depending on the species of bird, with a species predilection (amongst pet birds) for amazons, parakeets, pionus parrots and budgerigars.<sup>8</sup> The propensity for a bird to develop disease likely depends on several compounding factors, including nutritional status, stress and overall immune status. Once infected, mycobacteria are phagocytosed by macrophages, often within the intestinal tract, and stimulate a cell-mediated immune response. The exact mechanism of survival of mycobacteria in avian macrophages is not completely understood, but is thought to be the result of impaired enzyme function and phagosome-lysosome fusion.<sup>8</sup>

**Contributing Institution:**

Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada

<http://ahl.uoguelph.ca>

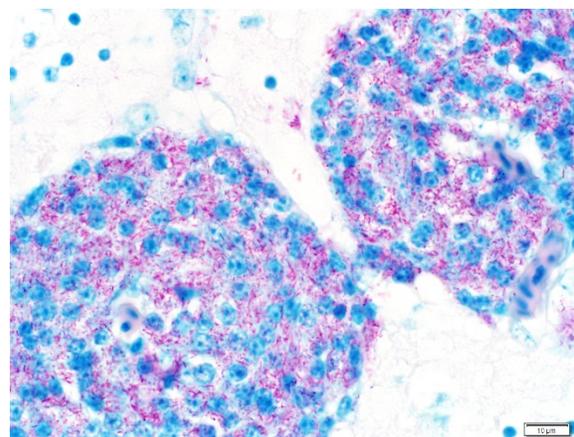
**JPC Diagnosis:** Liver: Hepatitis, granulomatous (histiocytic), multifocal to

coalescing, marked with numerous intracytoplasmic bacilli.

**JPC Comment:** The contributor has provided an excellent and concise review of mycobacteriosis (often incorrectly referred to as “avian tuberculosis”) in birds. Mycobacteriosis is the result of infection by non-tuberculous mycobacteria – over 160 species of ubiquitous environmental bacteria, a small percentage of which have been documented to cause disease in humans and other species. In humans, pulmonary disease is the most common manifestation, and there is well-documented variation in the most common clinical isolates based on location, even within individual countries.<sup>9</sup>

Once a problem in commercial poultry, today mycobacteriosis is a rare finding due not only to improved husbandry conditions, but most importantly, the rapid turnover of birds from hatching to market – which essentially does not leave enough time for the development of this chronic disease.

A very interesting study performed at the Zoological Society of San Diego (ZSSD) investigated the characteristics and risk



*Brain, amazon parrot. An acid fast stain reveals numerous acid-fast-positive bacilli in the cytoplasm of macrophages within Virchow-Robin's spaces. (ZN, 400X)*

factors associated with avian mycobacteriosis in often rare and expensive species.<sup>10</sup> The true incidence in many zoo populations is likely underestimated as a result of the chronic nature and long latency period. While previously considered largely an intestinal disease in zoo birds, retrospective study at the ZSSD identified respiratory lesions in 76% of cases, and 27% of cases with only respiratory signs (contrasting with 58% of cases which had intestinal lesions and only 8% with intestinal lesions only) citing a need for screening for respiratory disease as well. The study showed that animals imported into the ZSSD had higher odds of being infected than those born on the property – previous exposure to infected birds prior to import, as well as immunosuppression related to shipping stress (and other forms of stress including conspecific aggression and concurrent disease were considered as potential reasons for this finding. Additional factors included birds that were moved more often between enclosures, species of infected birds, and likely species of infecting mycobacteria.<sup>10</sup>

The moderator noted the presence of occasional karyomegalic hepatocytes in this section, musing that these may be polyploid and may reflect the age of the bird. The moderator's preference in the morphologic diagnosis of this lesion is histiocytic which is absolutely acceptable, understandable, and has launched heated discussions around the world for many years. Conference participants showed amazing restraint and tolerance in this instance. The moderator concluded the discussion with a review of mycobacterial disease in a number of species, to include fish, amphibia, reptiles, ruminants, and elephants.

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**CASE III: 1013/15 (JPC 4085103).**

**Signalment:** 18-year-old, female, bird, blue-eyed cockatoo (*Cacatua sanguinea*)

**History:** Bird suddenly presented respiratory distress and apathy, culminating in death few hours later. The bird was born in a house where it lived for seven years, and then moved to a small zoo where it lived for 11 years. This blue-eyed cockatoo shared premises with other bird species and, eventually, was transferred to a visiting area inside another aviary. The daily diet was a commercial ration (MegaZoo, Vale Verde, Minas Gerais, Brazil) and varied tropical fruits. Febendazole (4%) diluted in water or oral administration of ivermectin and albendazol was given once a year.

**Gross Pathology:** Grossly, lungs were markedly hyperemic and edematous. Visceral pleura was whitish and mildly thickened. The pericardial space and coelomic cavity contained small amount of a transparent fluid. In addition, there were moderate splenomegaly and multifocal black foci in the ovary.

**Laboratory Results:** None.

**Microscopic Description:**

In the lungs, there was marked hyperemia and air spaces contained homogenous to fibrillar eosinophilic material. In some sections there were many erythrocytes within parabronchi. Cellularity was increased throughout the lungs, resulting in mild

thickening of air capillary walls. In several perivascular areas there was mild to moderate infiltration of lymphocytes, histiocytes and few plasma cells. There was multifocal *micro-thrombosis* within capillaries. In some sections, lymphocytes, histiocytes and fewer plasma cells were also seen in the interstitium. Associate to these lesions, there were many sinuous schizonts lining the periphery of the capillaries. These schizonts were elongated, and ranged from 15 to 20  $\mu\text{m}$  in length and from about 7 to 10  $\mu\text{m}$  in cross-sectional diameter. There were some basophilic mature free merozoites within capillary lumen, which were most evident on the cross-sections.

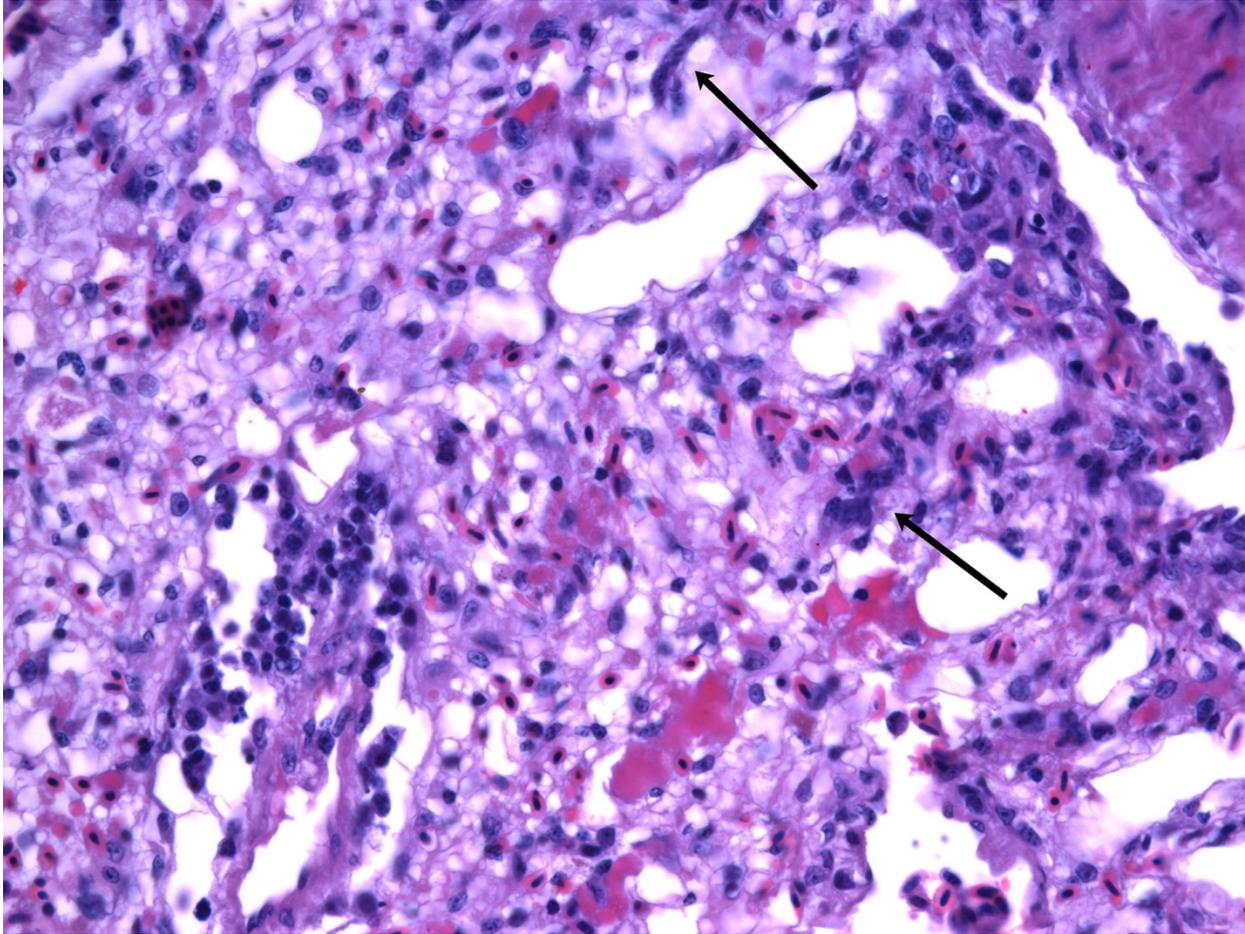
Other organs (not submitted): in the heart there was mild to moderate, multifocal perivascular infiltration of lymphocytes and plasma cells. The liver had moderate to marked increase in the cellularity due to accumulation of histiocytes, lymphocytes and plasma cells in the sinusoids. In the kidneys, there was multifocal, mild interstitial and perivascular infiltration of lymphocytes and plasma cells. In the spleen, there was mild increased in the differentiation of lymphocytes to plasma cells. In the ovary, there was multifocal melanosis.

**Contributor's Morphologic Diagnoses:**

Lungs: pneumonia interstitial lymphoplasmacytic acute, diffuse, moderate, with protozoa morphologically compatible with *Sarcocystis falcatula*.

**Contributor's Comment:** Clinical history, gross and histologic lesions associated with sinuous schizonts in the lungs, suggests that this bird had fatal pulmonary sarcocystosis, presumed to be due to *Sarcocystis falcatula*.

*Sarcocystis falcatula* utilizes two-host life cycle. The only known definitive host in North America is the opossum (*Didelphis*



**Lung, cockatoo:** There is diffuse mild edema, multifocal microthrombosis and perivascular infiltration of lymphocytes, plasma cells and histiocytes. There are multifocal immature *Sarcocystis* schizonts (arrows). (HE, 400X) (Photo courtesy of: Universidade Federal de Minas Gerais School of Veterinary Medicine – [www.vet.ufmg.br](http://www.vet.ufmg.br))

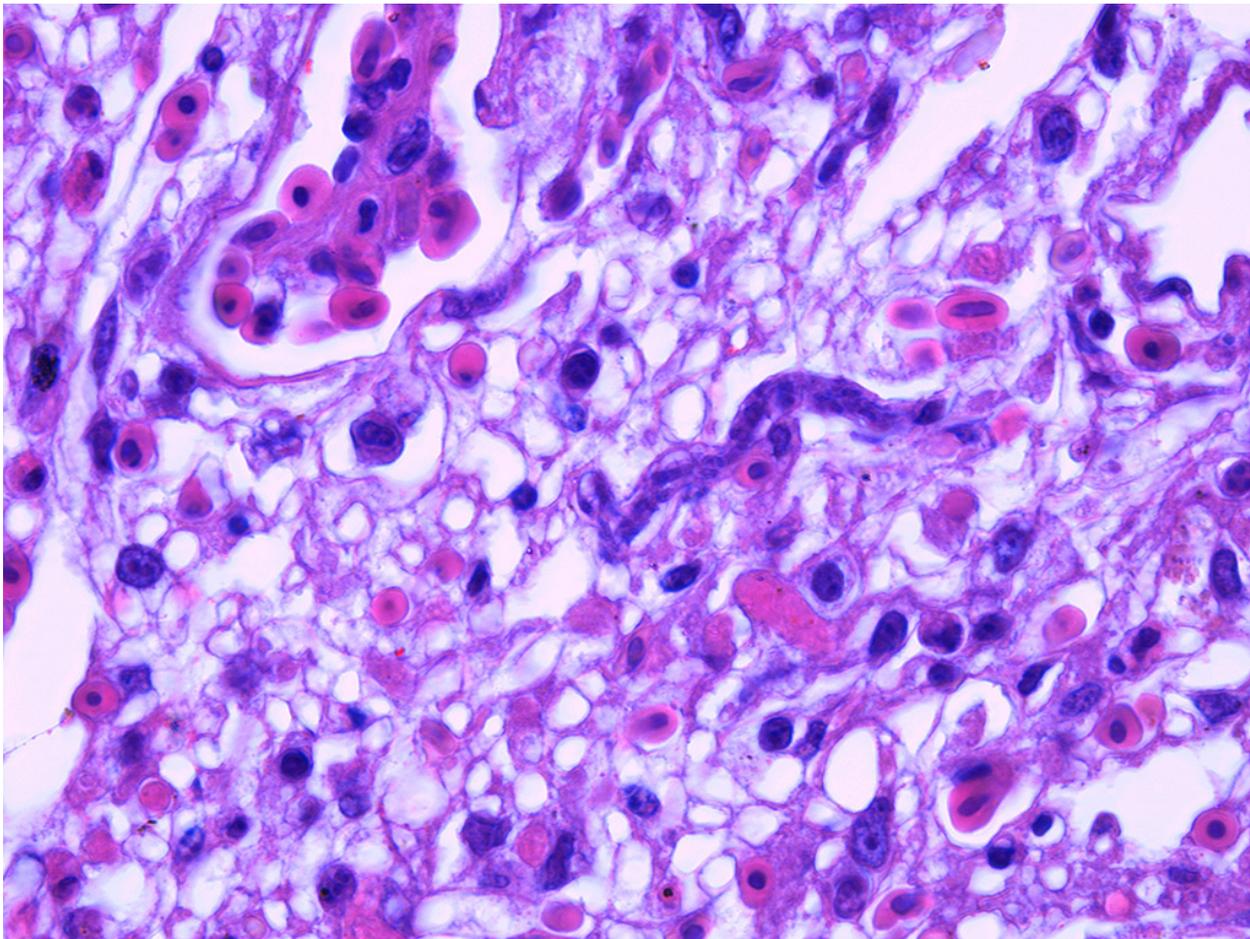
*virginiana*).<sup>12</sup> South American opossums (*D. marsupialis* and *D. albiventris*) can be the definitive hosts for *S. falcatula*, and *S. falcatula*-like protozoans.<sup>2,3</sup> The aviaries where this bird was kept were enclosed by wire mesh on all sides, including the roof and the contact with feces of wild animals probably occurred. The wild opossum (*Didelphis albiventris*) inhabits the forest surrounding the area of the small zoo, and is frequently seen at night in the area near the enclosures.

Asexual reproduction occurs in the intermediate host and is characterized by schizogony (merogony) and formation of sarcocysts in skeletal muscle. *Sarcocystis*

*falcatula* can use a large variety of bird species as intermediate hosts, including Passeriformes,<sup>9</sup> Psittaciformes,<sup>1,8</sup> and Columbiformes.<sup>5,16</sup> *S. falcatula* is highly pathogenic to intermediate hosts, especially to psittacines, because of its prolonged schizogony (5 months or more) and its fatal pulmonary presentation with many immature schizonts.<sup>1,3,7</sup> The merogony phase in the intermediate hosts takes place in arteries, capillaries, veins, and venules of lungs, liver, kidney and brain.<sup>12,13</sup> Ultimately, the merozoites give rise to sarcocysts in striated muscle. Then, mature sarcocysts can be found in cardiac and skeletal muscles.<sup>13</sup>

Clinical signs of acute fatal pulmonary sarcocystosis include severe dyspnea, anorexia, lethargy and loss of weight prior to death.<sup>12,16</sup> Pulmonary sarcocystosis was described in different species of birds such as pigeons<sup>16</sup>, cockatiels<sup>1</sup>, ring-necked parakeets and African grey parrots<sup>5</sup>. Like this blue-eyed cockatoo, similar clinical presentations and lesions are usually found in other captive birds of Psittacidae family (Psittacinae and Arinae subfamily). Mature sarcocysts in the pectoral muscles were found in a free ranged macaw (*Anodorhynchus hyacinthinus*) and in a dusky parrot (*Pionus fuscus*) necropsied in our lab.

Pulmonary lesions were the most prominent finding in this bird and, according to Smith et al.<sup>12,13</sup> are directly related to the pathogenesis of *Sarcocystis* infection, because endothelial cell invasion by schizonts occurs during asexual multiplication. The schizogony of this protozoan parasite in all bird species usually begins in the endothelium of capillaries and venules in the lamina propria of the small intestine.<sup>7</sup> In parrots and parakeets, pulmonary schizogony is more intense than in other organs. In contrast, schizogony in pigeons is more intense in the liver.<sup>14,16</sup> There are two hypotheses for these differences: the host cellular immune



**Lung, cockatoo: Higher magnification demonstrating mild perivascular infiltration of lymphocytes and histiocytes, fibrin and an immature *Sarcocystis* schizont. (HE, 1000X) (Photo courtesy of: Universidade Federal de Minas Gerais School of Veterinary Medicine – [www.vet.ufmg.br](http://www.vet.ufmg.br))**

response for controlling *Sarcocystis* infection differs among bird species<sup>15</sup>, the type of host cell infected influencing the growth and persistence of *S. falcatula* merozoites<sup>6</sup> and amount of sporocysts ingested by a bird<sup>7</sup>. Pulmonary hyperemia and edema result from stenosis of the capillaries and venules caused by the protozoan parasite, in addition to microthrombi formation induced by endothelial lesions during schizogony.<sup>12</sup>

The species of *Sarcocystis* that infected the bird in the study could not be determined and awaits further study. Sarcocystosis should be considered in the differential diagnosis of sudden death and pneumonia in captive birds. The usual occurrence of sarcocystosis is probably associated with the frequency of the definitive host and the close contact between birds and these animals.

**Contributing Institution:**

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**JPC Diagnosis:** Lung: Pneumonia, interstitial, histiocytic, diffuse, moderate with mild multifocal necrosis, and occasional intraendothelial apicomplexan meronts.

**JPC Comment:** The contributor has provided an excellent review of the pathogenesis of *Sarcocystis falcatula* infection in the bird. The recent literature contains a number of articles on the isolation of this parasite from a number of species of its definitive host (opossums native to various regions of the world), as well as details of the various manifestations of infection in a wide range of avian species, such as the recent report of necrotizing meningoencephalitis in a bare-faced ibis.<sup>8</sup>

Historically, *S. falcatula* was named by Stiles in 1893 from a specimen of a rose-breasted grosbeak. The parasite's life cycle (similar to

other sarcocysts, utilizing herbivores as intermediate hosts and carnivores as definitive hosts) and potential for infecting a wide variety of avian species was determined by Box and Smith in 1982. They determined that *S. falcatula* utilized only avian species as intermediate hosts and were not infective for poultry. Until 1995, it was believed that *S. falcatula* was the only sarcocyst utilizing the Virginia opossum (*Didelphis virginianus*) as its definitive host, until the discovery in 1995 of *Sarcocystis neurona* by Dubey and Lindsey, another agent which causes potentially fatal disease in intermediate hosts.<sup>4</sup>

Recently Dubey et. Al. have posited that there may be more than one particular species of *Sarcocystis* that cycles between avian species and opossums, suggesting than many of the earlier reports of *S. falcatula* in which molecular or immunohistochemical identification of the parasite was performed may be in question.<sup>4</sup> In addition, this report discusses the potential immunohistochemical cross-reaction between the schizonts of *S. falcatula* and *S. neurona* based on the stage of the parasite used for immunization, method of preparation of zoites, stage of the parasite in test tissues, and individual variability among immunized rabbits.<sup>4</sup> According to Dubey, a god in this field (and for whom *Sarcocystis jaypeedubeyi* is phonetically named), the structure of the sarcocyst wall (best viewed by ultrastructure) is the most reliable way to differentiate between two species of *Sarcocystis*, especially within the same host species.<sup>4</sup>

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**CASE IV: BA214/11 B1 (JPC 4035109).**

**Signalment:** 2 year old, male entire, *Pogona vitticeps*, bearded dragon

**History:** An outbreak of fungal dermatitis occurred in a colony of bearded dragons.



*Presentation, bearded dragon. A round 1.25x1.5cm cutaneous plaque is present on the dorsal midline. (Photo courtesy of: Royal (Dick) School of Veterinary Studies, Easter Bush Campus, Roslin, Midlothian, EH25 9RG, UK., www.ed.ac.uk/vet)*

Seven responded successfully and six were euthanized with follow-up 3 months later apparently showing complete resolution of the disease in all animals. Several months later, this animal was presented with new skin lesions on the dorsum, left stifle and left hock which were treated with both topical preparations (F10, iodine and clotrimazole) and systemic antifungals (itraconazole). The animal's condition failed to respond to therapy and due to continued deterioration (including development of coelomic distension) it was euthanized in March 2011.

**Gross Pathology:** An adult (2 year old), male entire, bearded dragon, weighing 553g was presented for necropsy examination. On the midline of the dorsum, there was a large (1.27cm x 1.5cm), focal, well demarcated, raised, black, irregularly rounded midline plaque. There were similar smaller lesions, which were discoloured dark yellow to brown, located over the right elbow (1cm diameter), left elbow (8mm diameter) the lateral aspect of the right knee (5mm diameter), the plantar surface of the left foot (8mm), and the plantar surface of the right foot (1mm). The liver was mottled pink to yellow to red with three, well-demarcated,

firm, raised, pale yellow, irregularly rounded nodules ranging from 8-20mm in diameter. One nodule was incised and contained yellow to brown, granular material. There was a similar 5mm diameter lesion present within the omentum, near the spleen.

**Laboratory Results:** 3 months prior to euthanasia blood was evaluated to assess liver function and white cell count while on treatment. Total protein=74 g/l (Range=52-72); AST=103 U/l (Range=0-80); CK=5830 (Range=0-550); Leukocytes =0.4 cells x10<sup>9</sup>/L (Range=1.5-8.5). The owner was given a guarded prognosis due to the low leukocyte count. Cultures submitted previously from this colony from similar lesions were identified as *Chrysosporium anamorph* of *Nannizziaopsis vriesii* (CANV).

#### **Microscopic Description:**

Elbow skin (1 section) –There is full thickness loss of cellular and nuclear detail extending into the dermis (necrosis). In this area, multifocal remnants of the overlying stratum corneum are present and there are scattered subcorneal accumulations of palely basophilic 1µm round to oval organisms (yeasts-presumptive). Multifocally



*Scaled skin, bearded dragon. Closeup of the dorsal cutaneous plaque. (Photo courtesy of: Royal (Dick) School of Veterinary Studies, Easter Bush Campus, Roslin, Midlothian, EH25 9RG, UK., www.ed.ac.uk/vet)*

throughout the necrotic tissue there are negatively stained outlines of fungal hyphae (approx 2-4um diameter with occasional septae, branching and terminal buds) with parallel walls and occasional segmentation. At the edges of the necrosis there are large numbers of epithelioid macrophages with multinucleated giant cells, heterophils and scattered lymphocytes with mild to moderate vascular congestion. Scattered haemorrhage is present. Smaller granulomas which are centrally necrotic and contain fungal organisms (as described above) are present around the edges of the necrotic region. Intact skin is mildly to moderately hyperplastic with multifocal, mild heterophilic and mononuclear inflammation within the superficial dermis.

Liver- There is a focal, large centrally necrotic area composed within the hepatic parenchyma which contains fungal organisms as previously described. Histiocytic, lymphocytic and heterophilic inflammation is present around the periphery with moderate to large numbers of multinucleated giant cells with scattered admixed fibroblasts. Multifocal, small granulomas are present around the periphery

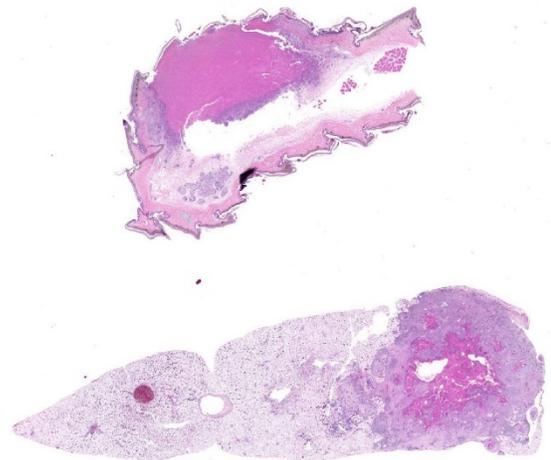


*Scaled skin and liver, bearded dragon. Coalescing granulomas with a brightly eosinophilic necrotic center are present within the skin and the liver. (HE, 5X)*

of the large necrotic lesion. The surrounding hepatocytes have marked, diffuse, swelling with large numbers of small to moderately sized, well demarcated, clear intracytoplasmic vacuoles (consistent with lipid) which often displace the nucleus to the periphery.

### **Contributor's Morphologic Diagnoses:**

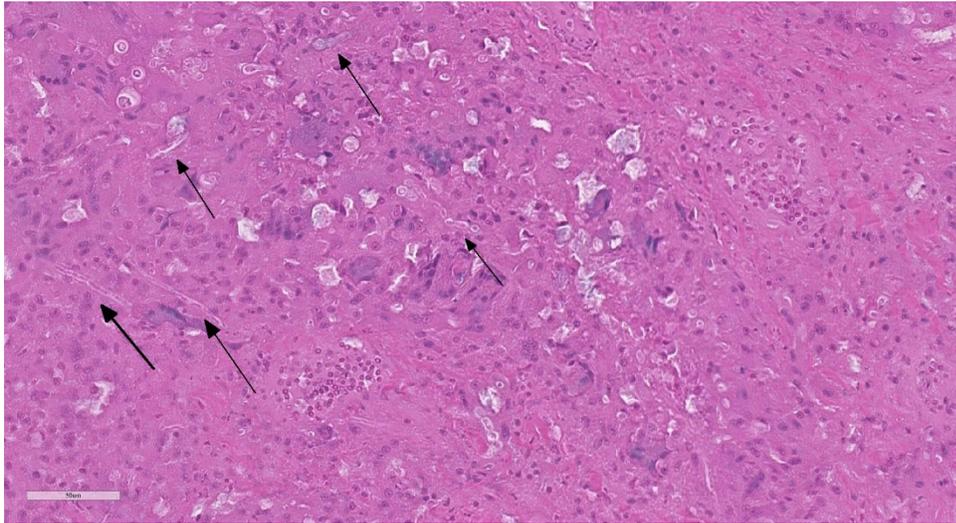
- 1) Severe, granulomatous and necrotising, ulcerative dermatomycosis and cellulitis with intra-lesional fungal organisms – elbow skin
- 2) Multifocal, severe, granulomatous hepatitis with intra-lesional fungal organisms – liver
- 3) Diffuse, moderate, hepatic lipodosis – liver



*Skin, bearded dragon: There are well-formed granulomas within the dermis, with large epithelioid macrophages and more brightly eosinophilic foreign body type macrophages. These granulomas will later coalesce. (HE, 106X)*

NOTE: Similar inflammation was present in sections from the dorsal skin lesion, lung and omental nodule (not included in the submission). Given the previous isolation of CANV in this colony/animal, the progression of the disease and the histological features of

the sections this was considered to be the most likely diagnosis.



*Skin, bearded dragon. Within the necrotic centers, fungal hyphae and arthroconidia may be seen in bas-relief.*

**Contributor’s Comment:** Saprophytic fungi are often implicated in dermatomycoses of snakes and lizards and infections are often associated with poor husbandry, poor nutrition or immunosuppression.<sup>8,11</sup> *Chrysosporium* species may be isolated from normal reptile skin but the most clinically important (and rarely isolated) is considered to be *Chrysosporium anamorph* of *Nannizziopsis vriesii* (CANV).<sup>8,11</sup> This is a keratinophilic ascomycetous fungus reported to be the primary pathogen of “yellow fungus disease”, an emerging disease in reptiles.<sup>1-12</sup> CANV is a non-pigmented fungus (hyalohyphomycosis). CANV has been reported in a number of chameleon species, geckos, bearded dragons, leopard geckos, a girdled lizard, several snake species and saltwater crocodiles, among others.<sup>1-16</sup> CANV is difficult to identify on culture and may be mistaken for any of the following: *Trichophyton*, *Geotrichum*, *Malbranchea* and *Trichosporon*.<sup>6,12</sup> Generally more than one animal is infected in an outbreak

(suggesting that it is contagious) especially in bearded dragons. In this species it causes

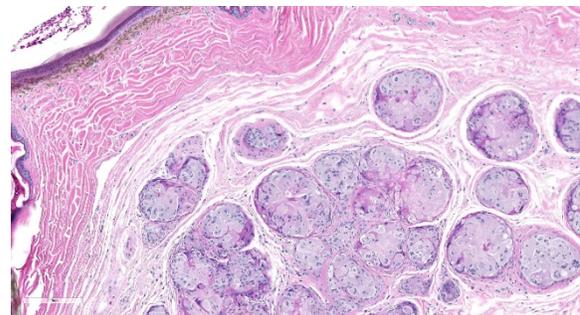
granulomatous dermatitis which often becomes systemic<sup>1,4,5,9</sup>. This presents as multifocal, yellow-brown discoloured lesions on the body with crusting and/or hyperkeratotic plaques. These lesions tend to develop necrosis, leading to sloughing of the skin and ulceration. This is followed by invasion of the

musculature and bones and may progress to lethal systemic infection.

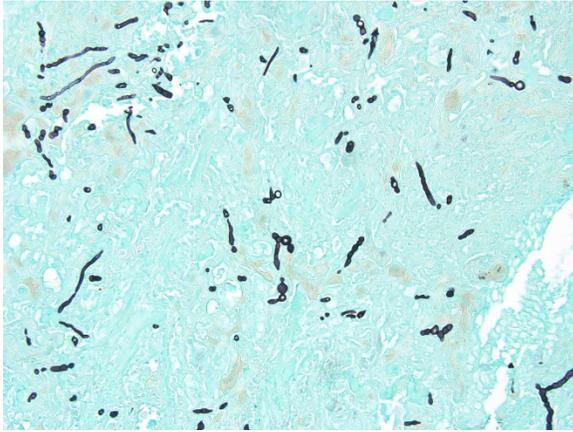
Conference participants discussed the orthokeratotic hyperkeratosis and elevation of the scale overlying the granulomas as potentially a localized form of dysecdysis.

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*Skin, bearded dragon. Within the necrotic centers, fungal hyphae and arthroconidia may be seen in bas-relief.*



**Skin, bearded dragon:** Fungal hyphae and arthroconidia (arrows) are easily demonstrated with a silver stain. (Grocott's methenamine silver, 400X)

**JPC Diagnosis:** 1. Scaled skin: Dermatitis, granulomatous and necrotizing multifocal to coalescing, severe, with numerous intra- and extracellular fungal hyphae and epidermal arthroconidia.

2. Liver: Hepatitis, granulomatous, focally extensive, severe, with numerous intra- and extracellular fungal hyphae.
3. Liver, hepatocytes: Lipidosis, diffuse, severe.

**JPC Comment:** Since the submission of this case in 2013, much has been written about the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) and related pathogens (referred to as *Chrysosporium* anamorph of *Nannizziopsis vriesii* complex (CANVC) which cause disease in reptiles. Anamorphs, most often seen in the *Ascomycota*, are asexual reproductive states (i.e. these fungi do not produce a fruiting body).

CANV is a keratophilic species that lives primarily on fragments of feathers and hair in the soil. While most cases of CANV have been identified in reptiles, related species of *Chrysosporium* infection have been documented in other species – *C. pannicola* (dog, horse, human), *C. tropicum* (chickens),

**Table 1.** Proposed species causing infection in reptiles

<i>Chrysosporium</i> -related fungi	Reptile species
<b><i>Nannizziopsis</i> Currah (1985)</b>	
<i>N. arthrosporioides</i> Stchigel et al. (2013)	Water dragon ( <i>Physignathus</i> sp.)
<i>N. barbata</i> Sigler et al. (2013)	Coastal bearded dragon ( <i>Pogona barbata</i> )
<i>N. chlamydospora</i> Stchigel et al. (2013)	Inland bearded dragon ( <i>Pogona vitticeps</i> )
<i>N. crocodili</i> Sigler et al. (2013)	Saltwater crocodile ( <i>Crocodylus porosus</i> )
<i>N. dermatitidis</i> Sigler et al. (2013)	Chameleons, geckos
<i>N. draconii</i> Cabañes et al. (2013)	Inland bearded dragon
<i>N. guarroi</i> Cabañes et al. (2013)	Green iguana ( <i>Iguana iguana</i> ), inland bearded dragon, lizard ( <i>Agama agama</i> ), snake
<i>N. pluriseptata</i> Stchigel et al. (2013)	Skink lizard ( <i>Eumeces inexpectatus</i> )
<i>N. vriesii</i> Currah (1985)	Lizard ( <i>Ameiva</i> sp.)
<b><i>Paranannizziopsis</i> Sigler et al. (2013)</b>	
<i>P. australiensis</i> Sigler et al. (2013)	Northern tuatara ( <i>Sphenodon punctatus punctatus</i> ), coastal bearded dragon, aquatic file snake ( <i>Acrochordus</i> sp.)
<i>P. californiensis</i> Sigler et al. (2013)	Tentacled snake ( <i>Erpeton tentaculatum</i> )
<i>P. crustacea</i> Sigler et al. (2013)	Tentacled snake
<i>P. longispora</i> Sigler et al. (2013)	Tentacled snake
<b><i>Ophidiomyces</i> Sigler et al. (2013)</b>	
<i>O. ophiodiicola</i> Sigler et al. (2013)	Snakes

**Proposed CAMVC species and infected reptile species.14,15**

and *C. keratinophilum* and *C. zonatum* (human).<sup>5</sup>

In 2013, Stchigel et al.<sup>14</sup> and Sigler et al.<sup>15</sup> published a taxonomic revision of *Chrysosporium*-related fungi and their likely host specificity (Table 1).<sup>5</sup> In most hosts, the lesion begins in the skin, with dermal and subcutaneous heterophilic granulomas which eventually extend into underlying bone, muscle, and disseminate to viscera, as seen in this case.<sup>16</sup> It is difficult, if not impossible, to differentiate members of the CANV in tissue section.<sup>5</sup>

A related fungus, *Ophidomyces ophidiicola*, which causes similar lesions in snakes was reassigned from the CANVC in 2013 to its own genus.<sup>15</sup> A case of this was published in the WSC earlier this year (Conference 8, Case 4).

Conference participants discussed the uniqueness of the epidermal lesion in this case. While granuloma formation is the rule in this infection, some participants interpreted with large area of necrosis within the dermis extending to the overlying epidermis as an infarct, and one participant saw hyphae involving a cutaneous vessel. The moderator commented that this was a unique presentation in his experience. Conference participants also discussed the orthokeratotic hyperkeratosis and elevation of the scale overlying the granulomas as potentially a localized form of dysecdysis.

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