Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2024-2025

Conference #22 — 29 January 2025

CASE I:

Signalment:
Young adult female wild pigeon (rock dove, *Columba livia*).

History:
The pigeon was found dead with no known history. Necropsy was performed as part of a wildlife disease screening program.

Gross Pathology:
Gross examination identified moderately thin body condition and mild pallor of the spleen.

Laboratory Results:
Immunohistochemistry for *Toxoplasma gondii* and *Sarcocystis* sp. showed strong positive immunolabeling of abundant intralesional zoites for *T. gondii* in multiple tissues (e.g., liver, lung, cloaca, brain) and no immunolabeling for *Sarcocystis* sp.

A pan-Apicomplexan PCR (18s rDNA) and sequencing of the amplicon confirmed a sequence with 100% identity to several isolates of *Toxoplasma gondii* in Genbank.

Microscopic Description:
The sections of cloaca include variable proportions of cloacal mucosa, oviduct, pericloacal skeletal muscle, subcutis, skin, and adjacent Bursa of Fabricius. There is marked, diffuse, necrotizing cloacitis and pericloacal cellulitis (panniculitis, myositis, dermatitis). Moderate numbers of inflammatory cells,

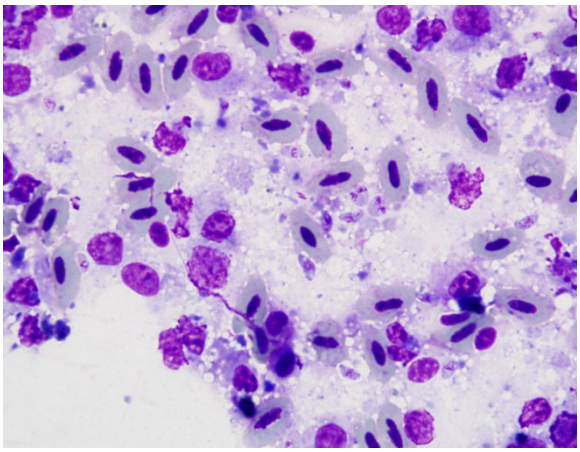

Figure 1-1. Impression smear, site unspecified, pigeon. The impression smear contains intact red blood cells, degenerate heterophils, macrophages, and free apicomplexan zoites. (*Photo courtesy of*: Wildlife Conservation Society, www.wcs.org)

abundant admixed pyknotic and karyorrhectic cell debris (necrosis), amorphous eosinophilic material (edema), and myriad protozoal zoites are present transmurally in the cloaca and throughout the pericloacal subcutis, skeletal muscle, and dermis. Inflammatory cells consist primarily of histiocytes with fewer heterophils, lymphocytes, and plasma cells. Zoites (tachyzoites) are approximately 2-3 um diameter and variably oval to crescentic (banana-shaped) with a pinpoint central nucleus and pale basophilic cytoplasm; many are individualized and extracellular, and moderate numbers are intracellular and range from individual zoites to haphazardly-arranged clustered groups (schizonts) often

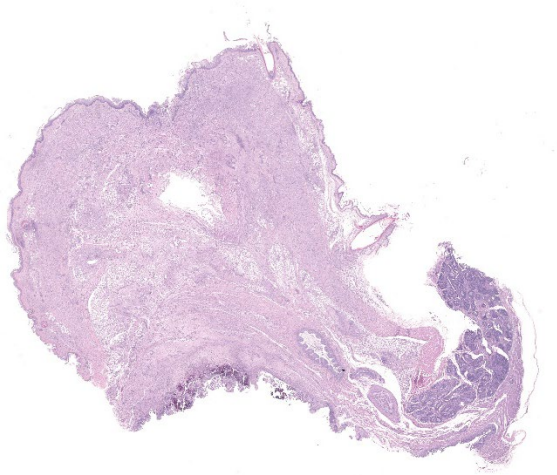

Figure 1-2. Cloaca and associated soft tissues, pigeon. A section of cloaca, associated soft tissues and bursa is submitted for examination. At subgross magnification, the cloaca (at bottom) is necrotic and the bursa (at right) is markedly atrophic and collapsed. (HE, 10X)

containing 6-16 distinct zoites. The intracellular organisms are present in endothelial cells, presumed histiocytes, fibroblasts, and myofibers. Lymphatics and blood vessels throughout the affected region are lined by hypertrophied endothelium (reactive), or are occasionally mildly thickened by intramural leukocytes or eosinophilic debris (vasculitis). The cloacal mucosa is variably moderately to markedly eroded to ulcerated, mineralized (dystrophic), and multifocally bordered by mixed bacteria along the denuded luminal surface. Skeletal myofibers are multifocally moderately fragmented, hypereosinophilic with loss of cross striations, and mineralized. The pericloacal epidermis is multifocally, variably, mildly to moderately hyperplastic, inflamed, and eroded, with intraepidermal leukocytes (heterophils, histiocytes, lymphocytes), intra- and inter-cellular swelling (edema), multifocal keratinocyte apoptosis and separation, and mild superficial dermal mineralization and pigmentary incontinence. Along the skin surface are mild segments of superficial orthokeratotic hyperkeratosis, and mild scattered loose mixed bacteria, occasional budding yeast, and debris (environmental). (A focal artefactual rent into the skin/subcutis is present in some levels).

The Bursa of Fabricius is atrophied with moderate to marked lymphocyte depletion, relative prominence of interstitial stroma, and multifocal dilated intrafollicular spaces containing basophilic fluid and occasional stippled basophilic material (mineral). Low numbers of cells (presumed histiocytes and Bursal epithelial cells) contain botryoid amphophilic intracytoplasmic inclusions (consistent with circoviral inclusions).

Contributor's Morphologic Diagnosis:
1. Cloaca and pericloacal tissue (skeletal muscle, subcutis, skin), Cloacitis and pericloacitis, necrotizing, histiocytic, diffuse, acute to subacute, moderate to marked, with abundant intralesional extra- and intra-cellular protozoal tachyzoites and schizonts, regional cloacal mucosal ulceration, multifocal vasculitis, edema, and mineralization

2. Bursa of Fabricius, Lymphoid depletion (follicle atrophy), chronic, diffuse, moderate to marked with scattered intracellular intracytoplasmic botryoid circoviral inclusions

Contributor's Comment:
Protozoal infection consistent with toxoplasmosis was initially identified by cytologic examination of organ imprints (lung, spleen, and liver) obtained during necropsy. Histologic examination confirmed disseminated protozoal infection with prominent zoite-induced necrotizing hepatitis, pneumonia, and encephalitis amongst other lesions. Infection by *Toxoplasma gondii* (toxoplasmosis) was confirmed by immunohistochemistry and PCR. The cloacal tissue section demonstrated notably florid 'cutaneous' toxoplasmosis in addition to circoviral inclusions captured

within the adjacent segment of Bursa of Fabricius, a not unexpected finding in wild pigeons and possible contributory factor for the extent of disease in this case.

Toxoplasmosis is a common obligate intracellular, apicomplexan, coccidian, protozoal infection affecting warm-blooded animals worldwide, including domestic and wild birds. Clinical toxoplasmosis has been reported in a variety of columbiformes (pigeons, doves), including individual and epizootic cases.[4] Pigeons have shown high sensitivity to natural and experimental infection with high morbidity and mortality,[3] and cases of natural infections are reported in a wide range of domestic and non-domestic pigeon species. Whereas the intermediate hosts of *T. gondii* infection are diverse, only felids are known to serve as definitive hosts for the sexual (coccidian) life stage. Major routes of intermediate and definitive host infection, respectively, are ingestion of infective oocysts, and predation of intermediate hosts by felids.

Reported clinical signs in birds include anorexia, weakness, emaciation, ocular signs (e.g., conjunctivitis, blepharitis, and exudate), dyspnea, and neurologic signs. Protozoal stages are found in many tissues, often including spleen, lung, and brain. In canaries (passeriformes) blindness has been reported as a unique but repeatable clinical presentation of natural infections, with intraocular and/or encephalitic toxoplasmosis seen histologically.[4,11]

Regardless of infected species, typical lesions of active toxoplasmosis consist of necrotizing inflammation with intralesional protozoal organisms. As in this case, the zoites and, less frequently, schizonts can be identified on cytologic tissue impression smears as individualized or clustered, extracellular or intracellular, oval to crescent-shaped pale basophilic organisms with a small nucleus and pale basophilic cytoplasm. Histology with immunohistochemistry and/or molecular testing are commonly employed for definitive diagnosis. Historically, bioassays of infected tissue homogenized and administered into mice were common.

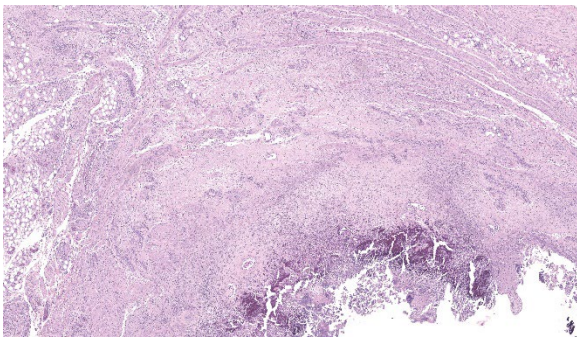

Figure 1-3. Cloaca and associated soft tissues, pigeon. Higher magnification of the necrotic cloaca and extension of inflammation and necrosis into the adjacent pericloacal soft tissues. (HE, 770X)

Another apicomplexan infection, Sarcocystosis, was considered the primarily differential diagnosis in this case, but was excluded by ancillary testing (i.e. PCR and IHC). Other differentiating features of *Toxoplasma gondii* and *Sarcocystis* sp. include: predominant forms of schizogony (endodyogeny for *T. gondii*, in which two zoites are produced, and endopolygeny for *Sarcocystis* sp., in which four or more zoites are produced and can be seen as a radial rosette arrangement); and ultrastructure (in which *Sarcocystis* sp. lack rhoptries, excretory organelles, that are present in *T. gondii*).[4] Although *Neospora caninum* is a classic differential for *T. gondii* cytologically and histologically, *N. caninum* is not known to infect birds. Systemic isosporosis (atoxoplasmosis) is another systemic coccidian infection of birds, but is morphologically distinct and was not a major differential in this case.

The heavy toxoplasmal dermatitis, panniculitis, and myositis around the cloaca in this

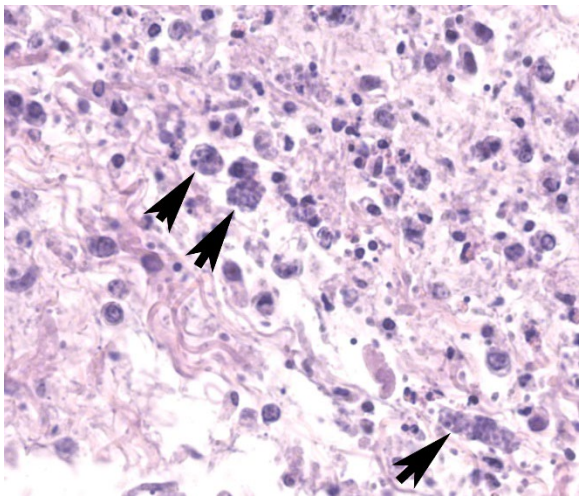

Figure 1-4. Pericloacal soft tissue, pigeon. Within the inflamed soft tissue, numerous cells (likely macrophages) contain numerous apicomplexan zoites (arrows). (HE, 457X)

case prompted a review of cutaneous toxoplasmosis which has been described in humans, few cats, and in rare cases, dogs. This infection can variably present as single localized, or up to many generalized, ulcerative, pustular, or nodular lesions, with or without pruritis. The lesions are histologically characterized by necrotizing and granulomatous or pyogranulomatous dermatitis, panniculitis, and vasculitis with extra-cellular or intra-cellular tachyzoites inhabiting a variety of cell types (e.g., macrophages, fibroblasts, glandular epithelium, and endothelium).[2,6,8] Organism numbers can vary and parasite identification is reported as histologically challenging in some cases.[6] Immunosuppression from concomitant illness and/or therapy (e.g., treatment for immune-mediate disease or transplantation) is a common predisposing factor.[6,8,10]

Basophilic botryoid cytoplasmic inclusions typical of circovirus were identified within the Bursa of Fabricius in this case. Pigeon circovirus is an incompletely described infection of young birds linked to acquired immunosuppression due to lymphocytic depletion in primary and secondary lymphoid tissues. It has been associated with propensity for a variety of secondary infections.[1,13] Circoviral infection has been linked to Young Pigeon Disease Syndrome (Young Bird Syndrome, Swollen Gut Syndrome) in racing and fancy pigeons in parts of Europe.[7] Immunosuppression was a possible contributing factor to the occurrence and severity of toxoplasmosis in this case.

Contributing Institution:
Wildlife Conservation Society

www.wcs.org

JPC Diagnosis:
1. Cloaca and pericloacal tissue (skeletal muscle, subcutis, skin): Cloacitis and pericloacitis, necrotizing, pleocellular, subacute, marked, with abundant zoites and schizonts

2. Bursa of Fabricius: Lymphoid depletion, chronic, diffuse, moderate, with cystic follicular ectasia and botryoid intracytoplasmic viral inclusions

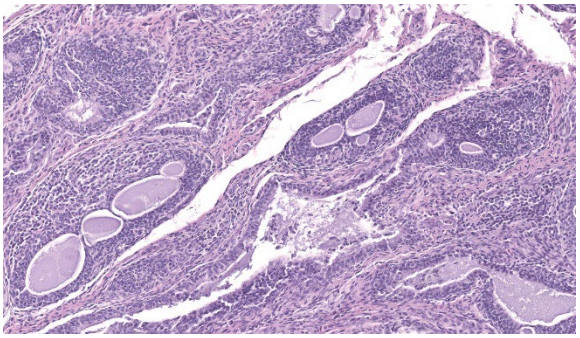

Figure 1-5. Bursa, pigeon. The bursa is markedly atrophic and depleted of B-cells. (HE, 356X)

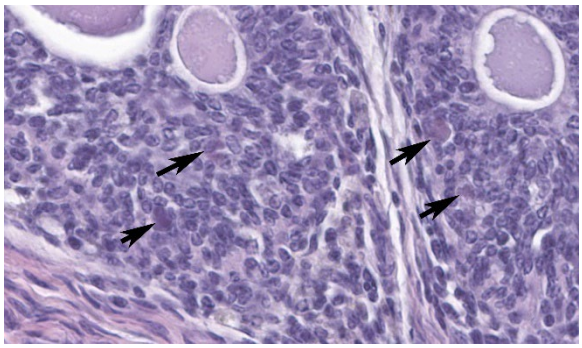

Figure 1-6. Bursa, pigeon. Macrophages adjacent to bursal follicles contain botryoid cytoplasmic inclusions (arrows). (HE 1400)

JPC Comment:
This week's moderator was Dr. Elise LaDouceur who serves as JPC's Chief of Extramural Projects and Research. Her four cases featured birds across separate classes which allowed conference participants to appreciate the role of taxonomy in formulating differential diagnoses for each case.

We thank the contributor for their submission of this neat pigeon double feature. Conference participants reviewed the anatomy of the cloaca which is composed of 3 parts – the coprodeum (continuous with the intestine), the urodeum (entry of ureters and genital ducts), and the proctodeum (terminal portion). There are two lymphoid centers within the cloaca with the larger Bursa of Fabricius overshadowing a smaller dorsal proctodeal 'lymphoglandular ridge' near the terminus proper. The section submitted represents a dorsal sagittal section that captures the bursa, dorsal proctodeum, and external portion of the vent.

We agree that the predominant slide feature was necrotizing inflammation which is characteristic of *Toxoplasma* across species and tissues. We described the cellular infiltrate as 'pleocellular' given the mixed and significant presence of histiocytes, granulocytes, lymphocytes, and plasma cells. Zoites and schizonts were generously present in section. Finding the circovirus inclusions was more difficult, particularly on the scanned slide (one benefit of reviewing glass!) – in this case they are characteristically botryoid though slightly more eosinophilic that typically seen. Dr. LaDouceur emphasized that the loss of bursal lymphocytes with concurrent prominent interstitial tissue and cystic dilatation of follicles is characteristic of lymphoid depletion, which could be caused by multiple viral infections of the bursa; however, the presence of botryoid inclusions is diagnostic for circovirus.

We conclude our case discussion with a brief review of *Isospora* and *Sarcocystis* in birds. Notably, *Isospora* (*Atoxoplasma*) most commonly infects passerine birds, not columbiform birds, such as pigeons. Histologically, it appears as coalescing nodules to sheets of histiocytes (with intracytoplasmic zoites) and lymphocytes which may be confused for lymphoma.[5,12] As the contributor mentions, *Sarcocystis* exhibits schizogony that differs from *Toxoplasma*. Tissue sarcocysts can be a helpful finding if skeletal and/or cardiac muscle is available to review. Schizogony with associated necrosis, lymphohistiocytic inflammation, and free and intracytoplasmic protozoal organisms appears similar to *Toxoplasma,* however.[7]

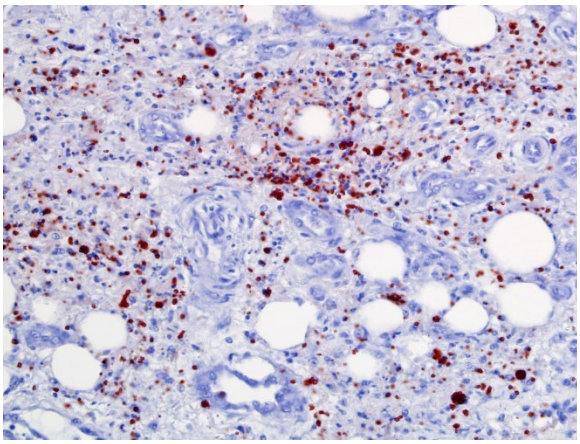

Figure 1-7. Pericloacal soft tissue, pigeon. Cytoplasmic zoites stain strongly positive for animmunomarker agains *Toxoplasma gondii*. (anti-T. gondii, 400X). (*Photo courtesy of*: Wildlife Conservation Society, www.wcs.org)

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CASE II:

Signalment:
4-year-old, female, cockatiel (*Nymphicus hollandicus*)

History:
A 4-year-old, female cockatiel was managed by the Exotics Service over the course of one year for progressive osteolytic lesions. The patient was originally seen in the fall of 2017, for a right ulnar fracture. At that time, radiographs confirmed the ulnar fracture; in addition, mild lytic lesions were noted in multiple other bones. The animal returned in Spring of 2018. At this time, full body radiographs revealed multiple areas of bony lysis; and whole body CT confirmed wide-spread osteolysis. Her owners elected euthanasia given the poor prognosis.

Imaging:

Whole body radiographs: There are multiple radiolucent expansile lesions associated with most of the long bones, with partial destruction of the adjacent cortex. These are suspected to explain the previously observed

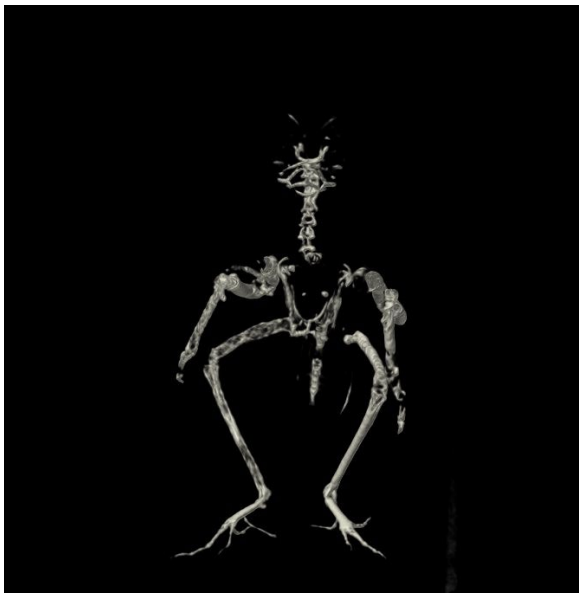

Figure 2-1. Whole body CT scan, cockatiel. Multiple bones exhibit a thin and moth-eaten appearance of the cortices with mild irregular expansion of the bony margins. (*Photo courtesy of: Schwarzman Animal Medical Center, Department of Anatomic Pathology. www.amcny.org*)

distal right ulna fracture, which currently appears mildly displaced, with new bone formation partially bridging it. A minimally displaced fracture of the proximal left ulna is also observed. Minimal soft-tissue swelling is associated with the punctiform lesions of the distal left metacarpophalangeal bones.

Computed tomographic images: The axial skeletal structures are normal. By contrast, multifocal lesions affect the appendicular skeleton (left proximal and distal ulna, left metacarpal bone III and proximal phalanx, right distal ulna, right metacarpal bone III and proximal phalanx, left distal femur, left distal tarsometatarsus, essentially the entire right femur, right proximal and distal tibiotarsus, right proximal and distal tarsometatarsus, questionably right pelvic limb phalanges): these are characterized by a thin and moth-eaten appearance of the cortices with mild irregular expansion of the bony margins. Irregularity of the bone margins is again present in the right distal ulna and left proximal ulna, and the site the previously described pathologic fractures. Medullary enostosis is not detected. Soft tissue swelling is minimal around these lesions.

Imaging Diagnosis:

1. Severe polyostotic aggressive osteolytic lesions of the appendicular skeleton with at least two pathological fractures

Gross Pathology:
The right femur is moderately expanded with a rough cortical surface and easily fractures with minimal manipulation. The right radius has a rough cortical surface and easily fractures with minimal manipulation. The distal aspect of the right ulna is mildly expanded with a rough cortical surface. The proximal left ulna has a rough cortical surface.

After formalin fixation and decalcification, cross sections of multiple bone reveal irregularity and thinning of the bone cortices with expansion of the medullary cavity by a soft, friable, bone tissue. The surrounding skeletal muscle and soft tissues are mottled light and dark brown.

Laboratory Results:
Touch imprint cytology, Bone, right femur, ulna, and radius: Changes varied in severity between tissue imprints and one sample is comprised of blood. There is a relative mild to moderate increase in the numbers of erythroid precursors. Touch imprints contain small to moderate numbers of osteoclasts and few macrophages.

Microscopic Description:
Long Bones (multiple sites): The medullary cavity is variably replaced and expanded by a dense inflammatory cell infiltrate, comprised

of macrophages (including epithelioid morphology) and multinucleated giant cells mixed with heterophils, necrotic cellular debris, and lesser lymphocytes and plasma cells. In some sections, this inflammatory infiltrate entirely effaces the medullary cavity. Inflammation surrounds islands of woven bone (lined by osteoblasts) or irregular, resorbing cortical bone. The cortical bone is irregular and multifocally discontinuous where it is invaded by large numbers of inflammatory cells. The endosteal surface is scalloped and are bordered by increased numbers of osteoclasts within Howship's lacunae. The bone margin is multifocally comprised of woven bone and there are multiple reversal lines. The outer cortical surface is irregular with prominent scalloping and the periosteum is predominantly expanded by a large numbers of similar inflammatory cells and small numbers of pleomorphic fibroblasts. Where present the subchondral bone is often irregular, thinned, and infiltrated by a similar inflammatory cell population and the joint capsule is variably infiltrated. The surrounding and intra-articular adipose tissue contains small hemorrhages and islands of a similar inflammatory infiltrate. Surrounding myofibers are variably degenerate and invaded by inflammation.

The overlying dermis contains small numbers of lymphocytes and plasma cells that commonly dissect between collagen fibrils. The epidermis is diffusely moderately expanded by compact orthokeratotic hyperkeratosis.

Fite-Faraco stains confirm small numbers of acid-fast positive, intrahistiocytic bacilli. In addition, the liver and small intestine have few, small granulomas.

Contributor's Morphologic Diagnosis:
Cortical bone wih joints, right and left wings and legs: Osteomyelitis, periostitis, and synovitis, granulomatous, heterophilic, chronic, multifocal to locally extensive, severe with

Figure 2-2. Long bone, cockatiel. The right femur has a roughened cortical surface (as does the right radius and left and right ulna (not shown). (*Photo courtesy of: Schwarzman Animal Medical Center, Department of Anatomic Pathology. www.amcny.org*)

few intralesional, intrahistiocytic acid-fast positive, intrahistiocytic bacilli (consistent with Mycobacterial infection), bone resorption, multifocal, severe, remodeling, mild and periosteal fibroplasia, multifocal

Skeletal muscle, right and left wings and legs:

1) Myositis, granulomatous, heterophilic, chronic, multifocal, mild to moderate
2) Myofiber degeneration, chronic, multifocal moderate with mild, multifocal myofiber regeneration and multifocal hemorrhages

Contributor's Comment:
Clinical presentation, as well as the gross and histologic findings supportive a chronic osteomyelitis with evidence of disseminated granulomatous inflammation. The presence of acid-fast positive bacilli was highly suggestive of avian mycobacteriosis, although this was not confirmed with culture or molecular testing. In birds differentials for osteomyelitis are vast and include trauma, neoplasia, lymphoproliferative disease, and infectious etiologies.[10-12] Reported neoplasms in the bones of birds include osteosarcoma,

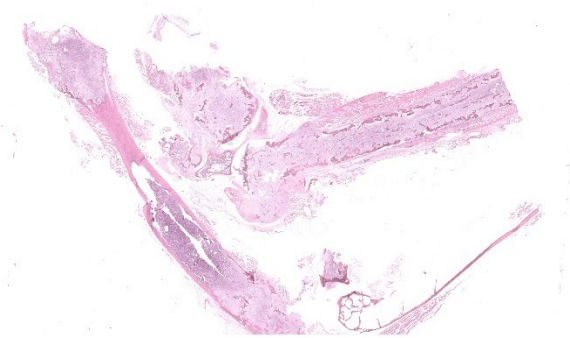

Figure 2-3. Long bone, cockatiel. Multiple long bones are submitted for examination. In each of the submitted bones, the marrow and medullary cavity is multifocally effaced by a cellular infiltrate that multifocally extends through the cortex into the adjacent soft tissue. (HE, 10X)

giant cell tumor of bone, air sac carcinoma, fibrosarcoma, and hemangiosarcoma, as well as metastatic disease.[10-12] Infectious osteomyelitis can be caused by aerobic and anaerobic bacteria (including mycobacteriosis) and fungi (e.g., aspergillosis, candidiasis cryptococcosis, and histoplasmosis).[11] In this case, the presence of few acid-fast positive, intrahistiocytic bacilli was consistent with a paucibacillary mycobacterial osteomyelitis. As in this case, skeletal mycobacteriosis commonly presents as soft tissue swelling and bone irregularity.[11] As the disease progresses, pathologic factures can occur.[11]

Mycobacterial infections are a significant cause of morbidity and mortality in numerous species, including humans.[6] This diverse group of bacteria contains organisms that range from environmental saprophytes and opportunistic pathogens to obligate pathogens.[14] Obligate pathogens include the tuberculosis group (*M. tuberculosis* and *M. bovis*) and the leprosy group (*M. lepraemurium*). Opportunistic pathogens include the saprophytes (*M. fortuitum*, *M. smegmatis*, *M. chelonae*, *M. abscessus*, and *M. thermoresistibile*) and the slow-growing (atypical)[8] organisms (*M. avium-intracellulare* complex, *M. kansasii*, and *M. ulcerans*). By convention, tuberculosis refers to infections with organisms in the tuberculosis complex, while mycobacteriosis refers to those caused by atypical or opportunistic forms.[6,15] Differentiation of mycobacterial species requires a combination of bacterial culture, biochemical tests, molecular techniques (PCR with subsequent DNA sequencing or Interferon-Gamma Release Assays (IGRAs)), and pigment production.[6,12,13]

Mycobacteria are weakly gram-positive, acid-fast positive bacilli.[1-16] Mycobacteria's lipid-rich walls make them hydrophobic, which allows them to survive in adverse environmental conditions.[10,11,14,16] In other words, it is endemic worldwide, stable in the environment, and difficult to eradicate once established.[1,4,11,16] Modes of transmission include the skin (typically at areas of skin barrier breakdown), inhalation, and ingestion.[4, 10] In birds, oral ingestion is considered the most common.[11] Infections in most species tend to be protracted and associated with a chronic wasting syndrome.[11,14-16]

Granulomatous inflammation is a distinct form of chronic inflammation, that is typically the result of a poorly degradable and persistent antigen, specific host responses (e.g., Th and macrophage responses), and the interplay of various pro- and anti-inflammatory mediators.[15] Mycobacterium species employ multiple mechanisms to ensure survival and typically do so by entering and persisting within tissue macrophages.[14,15] These include disruption of the phagosome-lysosome complex (i.e. inhibit acidification of the phagosome, phagosome-lysosome fusion), interference of cytokine synthesis and function (i.e. block injury from toxic oxygen and nitrogen intermediates), or inactivation of lysosomal enzymes.[14,15] In general mycobacterial species can suppress the ability of macrophages to be activated by cytokines, especially IFN-

gamma.[15] Complement receptors on tissue macrophages (e.g., mannose and CD14 receptors) are the major receptors responsible for mycobacterial phagocytosis.[15] Other receptors (e.g., integrin receptors, TLRs, Ig Fc receptors, CD14 receptors, scavenger receptors, etc.) are involved in the early recognition and cell signaling in response to the bacteria, which eventually leads to antimicrobial chemokine, cytokine, and metabolite synthesis.[15] Mycobacteria highjack these pathways and attenuate macrophage activation in response to IFN-gamma.[15] *Mycobacterium avium*, in particular, has a unique cell wall that prevents fusion of the phagosome to the lysosome, effectively down-regulating the killing mechanisms of macrophages.[12,15] A similar mechanism is described in *Mycobacterium tuberculosis*.[15] Migration of infected macrophages allow for discrimination of infection.[14] These infections are characterized by a strong cell mediated response, where macrophage recruitment and proliferation accelerates under the influence of cytokines produced by T-lymphocytes.[14,15] The chronic presentation of these cases and resulting tissue destruction are due to a combination of organism persistence and cell-mediated response and other host responses.[12,15]

The type of pathology and clinical disease depends on the infecting mycobacterial species, host's immune response, host's genetic susceptibility, dose of infection, mode of transmission, and infection stage.[11] Infection is typified by granulomatous inflammation.[1-16] This can manifest as diffuse visceral enlargement and/or discrete granulomas, which correlate to infiltration by a pleocellular, macrophage heavy inflammatory infiltrate.[1-15] Affected macrophages can have a large amount of amphophilic cytoplasm with a fine cytoplasmic granularity but acid-fast stains are required for confirmation of intracellular bacilli.[11] The number of bacteria can vary considerable and ranges from paucibacillary

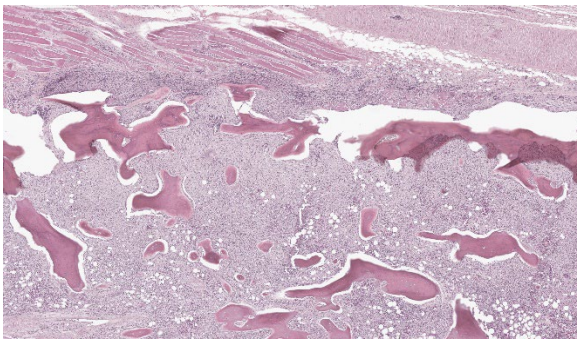

Figure 2-4. Long bone, cockatiel. The cellular infiltrate effaces bone marrow, results in lysis of hematopoetic and cortical bone, and extends into the adjacent skeletal muscle. (HE, 88X)

to abundant/florid.[11,12,15] There are two main pathologic forms of mycobacterial infection: a tubercle and lepromatous form.[11,15] The tubercle form is a Th1-biased immune response and is characterized by granuloma formation with a region of central necrosis surrounded by inflammation.[11,15] Meanwhile, the lepromatous form is a Th-2-biased immune response and is characterized by diffuse inflammation without necrosis.[11,15]

Mycobacteriosis is common in various bird species, including domestic fowl, as well as pet and exotic birds and wildlife.[12] Although all species are susceptible, captive waterfowl, collection birds, tropical and ring-necked doves, Amazon parrots, brotogerids, pionus parrots, finches, and canaries are commonly reported.[11] The disease can have severe economic impact and is associated with high levels of mortality and morbidity and reduction in egg production.[16] In one recent studying in backyard chickens, 42% of birds died from bacterial disease.[3] In this report *E. coli* and *Mycoplasma gallisepticum* or *M. synoviae* were the most common, but mycobacteriosis was reported in 6 cases.[3] Oral route appears to be the primary means of infection in birds, with lesions typically involving the gastrointestinal tract and/or liver.[11,12] Airborne and cutaneous infections can be seen to a lesser

degree.[12] *Mycobacterium avium-intracellulare* complex organism infect the intestine and due to a lack of lymph nodes, the infection subsequently disseminates easily.[12] Therefore, infection is typified by a chronic, disseminated granulomatous disease in semimature to mature birds, although localized disease can occur (i.e. dermal or intestinal mycobacteriosis).[11,12] Gallinaceous birds typically develop discrete granulomas, while psittacine and passerine species tend to develop the lepromatous form.[12] The two most common mycobacterial species to affect birds are *Mycobacterium avium-intracellulare* complex and *Mycobacterium genavense*.[9,10,11,12,16] Other reported species include *Mycobacterium tuberculosis, M. bovis, M. gordonae, M. nonchromogenicum, M. fortuitum, M. peregrinum, M. intermedium, M. celatum, M. africanum, M. simiae, M. arupense, M.* URHd0023*,* and *M. vulneris*, in addition to others.[9,12] The lesions created by these species are indistinguishable from one another and co-infection can occur; thus definitive diagnosis requires culture and/or molecular testing.[9,16]

Avian bacterial osteomyelitis can be caused by a number of ubiquitous and opportunistic bacteria, including *Mycobacterium* spp., *Staphylococcus aureus*, *E. coli*, *Salmonella* spp., *Pasteurella multocida*, *Streptococcus* spp., *Enterococcus* spp., *Pseudomonas* spp., and *Aeromonas* spp.[1] Mycobacteriosis in birds commonly involves the bone, and in one report 93% of avian mycobacteriosis cases had bone lesions.[1, 11] Infection is clinically characterized by osteolysis (as in this case), sclerosis, or bone cysts that are most commonly located in the metaphysis of long bones, ribs, and/or sternum with or without pathologic fractures.[1,12] Bacterial toxins and localized ischemia can lead to bone necrosis with sequestra formation.[1] Histologic findings include severe myeloid hyperplasia and granulomatous inflammation with eventual

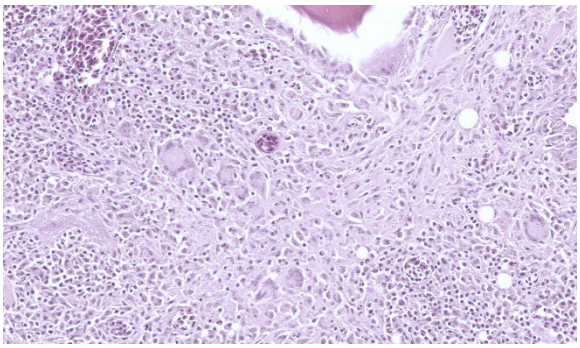

Figure 2-5. Long bone, cockatiel. The mixed cellular infiltrate is predominantly composed of epithelioid macrophages, and fewer Langhans and foreign body type giant cell macrophages, heterophils, lymphocytes and plasma cells. (HE, 556X)

bone marrow effacement and extension into the surrounding bone and soft tissues.[11, 12] Avian granulomas associated with mycobacteriosis tend not to mineralize.[1,11,12]

Mycobacterium avium-intracellulare complex organisms can cause sporadic, opportunistic disease in numerous species and is typically associated with immunosuppression.[14] Other important Mycobacteria of this complex include *M. avium paratuberculosis* (Johne's disease), *M. avium silvaticum* (wood pigeon mycobacterium), *M. avium hominissuis* (pig and human infection), and *M. intracellulare*.[10,14,16] Other important mycobacterial species in veterinary medicine include *M. tuberculosis* (human and elephants), *M. bovis* (domestic and wild animals, humans), *M. microti* (small rodents, hyraxes, llamas, pigs, and ferrets), *M. africanum* (rare in humans, cattle, and pigs), and *M. marinum, M. chelonae, M. xenopi*, and *M. liflandii* (reptile and amphibian species).[6,7,8,15] The clustering of infections in Bassett hounds, Miniature Schnauzer, Siamese cats, Somali cats, and Abyssinian cats suggest a genetic predisposition.[4,8] The cause for this predisposition is unclear but may be related to a cell-mediated immunodeficiency in either T-cells or macrophages.[4] Failure to regrow hair is a unique

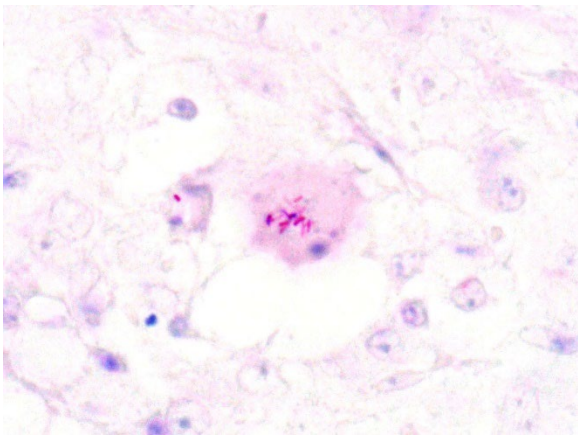

Figure 2-6. Long bone, cockatiel. Rare epithelioid macrophages contain acid-fast bacilli within their cytoplasm. (Fite-Furaco, 400X). (*Photo courtesy of: Schwarzman Animal Medical Center, Department of Anatomic Pathology. www.amcny.org*)

presentation in Abyssinian cats.[8] Important veterinary mycobacterial disease include Bovine cutaneous opportunistic mycobacteriosis (caused by atypical mycobacterial species), feline leprosy (*M. lepraemurium, M. visibilis*, and others), ulcerative dermatitis in marsupials (*M. ulcerans*), bovine tuberculosis (*M. bovis*), feline ocular mycobacteriosis (*M. bovis, M. microti, M tuberculosis, M avium-intracellulare* complex), and Johne's disease (*M. avium paratuberculosis*).[6,8,10,13,14,15] The two most common etiologic diagnoses in weedy and leafy seadragons is mycobacteriosis and scuticociliatosis, where mycobacterial infection is associated with chronic erosive and proliferative skin lesions that typically involve the snout.[2] Dissemination can occur and typically involves the liver, kidney, heart, gill, and skin.[2] The cause of canine leproid granuloma syndrome is unknown but mycobacterial infection is suspected given the presence of acid-fast bacilli in lesions.[15] Most mycobacterial infections require treatment with long course antibiotics and/or surgical excision of affected tissues.[13]

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JPC Diagnosis:
1. Hematopoietic bones and surrounding soft tissue: Osteomyelitis, synovitis, myositis, and cellulitis, granulomatous, chronic, multifocal, severe with infractions

2. Bone marrow: Granulocytic hyperplasia, chronic, diffuse, severe

JPC Comment:
The contributor provides an outstanding summary of avian mycobacteriosis to accompany an interesting case presentation. The polyostotic nature of the lesion led some to consider neoplasia or metabolic bone disease for this case. Dr. LaDouceur noted that metabolic disease such as fibrous osteodystrophy may affect multiple bones, but more importantly is not an inflammatory lesion (inflammation was abundant in this case) and would not be expected to extend into the surrounding soft tissue. Similarly, a multifocal distribution would be unusual for a primary bony neoplasia, although neoplasia is a rule out for osteolytic lesions. Metastatic carcinomas and air sac carcinomas have also been reported in the bone of birds,[17] though these are morphologically distinct from the mixed inflammatory cell population seen here.

There are a number of ancillary changes that should not be missed in this case. Although the bony changes are impressive, there are numerous small, non-displaced fractures (infractions) and degenerative changes to the joint. The adjacent joints have fibrovascular membranes that span the articular surface (pannus) that accompany villonodular proliferation of the synovium and loss of glycosaminoglycans. The hyperplastic bone marrow with marked expansion of myeloid precursors

is a common concurrent change in mycobacteriosis – we estimated a myeloid:erythroid ratio of at least 4:1 where as a normal is 0.4:1 (i.e. 10x times expanded in this case). Other rule outs for extreme myeloid hyperplasia include *Aspergillus* sp. (see Case 4 of this conference) and avian chlamydia. Additionally, we emphasize "hematopoietic bone" in our morphologic diagnosis as there is a good example of a pneumatized (and unaffected) bone on this slide as well.

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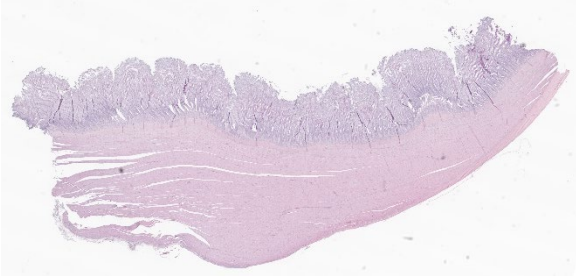

Figure 3-1. Ventriculus, tinamou. One section of ventriculus is submitted for examination. (HE, 7X)

CASE III:

Signalment:
13-year-old male elegant crested tinamou (*Eudromia elegans.*

History:
This elegant crested tinamou (*Eudromia elegans*) was housed in a zoological collection, and was euthanized due to marked weight loss and declining clinical condition. He had a history of chronic articular gout of the right third digit and mild lateral deviation of the rhinotheca resulting in malocclusion ("scissor beak").

Gross Pathology:
Gross examination confirmed beak malocclusion and a focal swelling over the proximal aspect of the right third digit that exuded white chalky to pasty material from cut surfaces. The animal was in thin body condition, with scant subcutaneous and intracavitary adipose tissue stores. The crop was moderately distended with fresh vegetables, the proventriculus was empty, and the ventriculus was filled with vegetable ingesta, small pebbles, and a small amount of green, pasty material. The intestines and ceca contained a small amount of green-brown, pasty digesta.

Laboratory Results:
Cytologic evaluation of liver, spleen, and lung impression smears was unremarkable.

Microscopic Description:
Ventriculus: Throughout the section, the koilin layer is diffusely, superficially disrupted and separated by a dense mat of elongate yeasts. The yeasts are approximately 2-3 micrometers wide and 20-40 micrometers long. They aggregate in haphazard orientations on the surface, and also stream into ventricular glands in roughly parallel-oriented, 'logjam-like' arrangements, rarely reaching the deepest portions of the glands. The lamina propria contains a scant infiltrate of granulocytes and rare lymphocytes that rarely extends into the mucosal epithelium. Small, subepithelial pools of eosinophilic to amphophilic, smudgy, acellular material are scattered throughout the lamina propria. Small-caliber blood vessels in the mucosa and tunica muscularis are multifocally surrounded by low numbers of mononuclear cells. Arterioles throughout the tunica muscularis and subtending the serosal surface are variably expanded by smudgy, eosinophilic, acellular material that partially or wholly obscures normal cellular detail in the arteriolar walls.

Contributor's Morphologic Diagnosis:
Ventriculus: Ventriculitis, heterophilic and lymphocytic, chronic, multifocal, mild, with superficial koilin disruption and myriad surface-associated yeasts

Ventriculus: Amyloidosis, arteriolar and subepithelial, chronic, multifocal, mild to moderate

Contributor's Comment:
Histopathology in this elegant crested tinamou revealed florid superficial colonization of the mucosal surface of the proventricular-ventricular isthmus and ventricular koilin by elongate yeasts. The morphology and tissue distribution of the yeasts are characteristic of *Macrorhabdus ornithogaster*. Arteriolar and

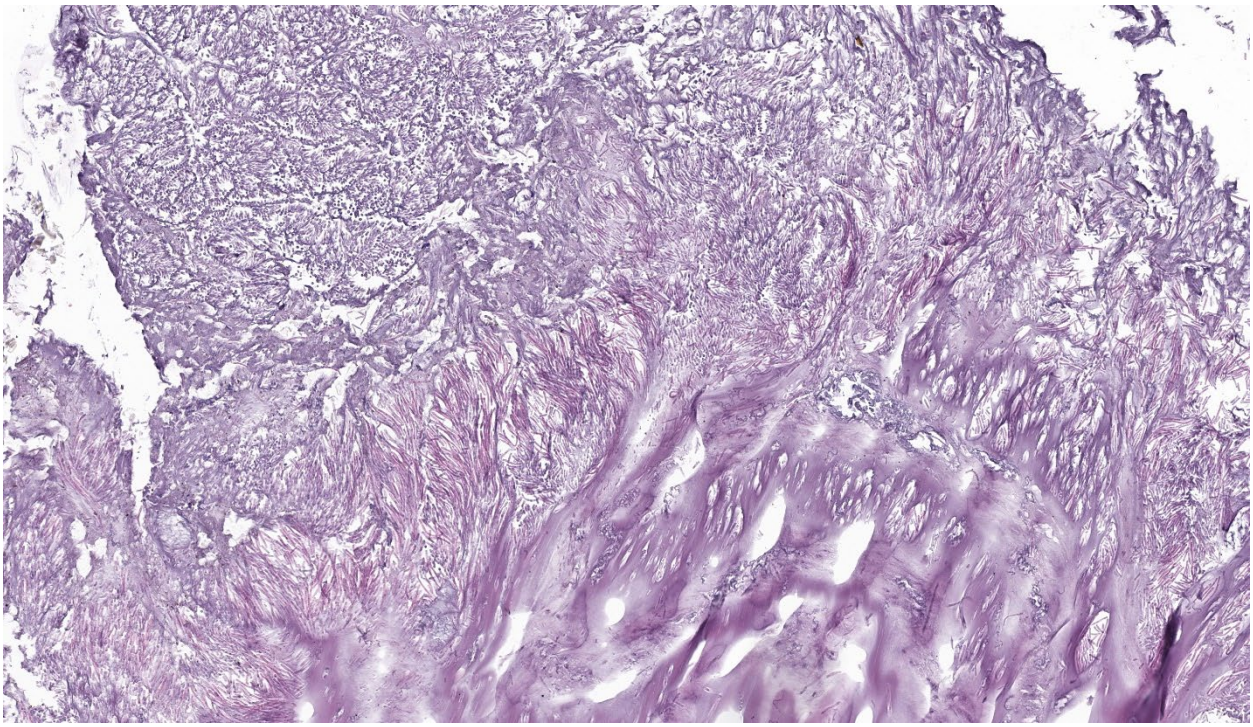

Figure 3-2. Ventriculus, tinamou. Within the superficial 2/3 of the koilin layer, there are numerous 1x2um wide, elongate filamentous yeast. (HE, 234X)

subepithelial amyloid deposition, while relatively mild in sections of the gastrointestinal tract, were manifestations of multi-organ amyloidosis that was considered to be the most important factor in this bird's clinical decline.

Macrorhabdiosis, or 'avian gastric yeast' infection, is a well-recognized condition in various avian species. The causative organism, *Macrorhabdus ornithogaster*, was famously misinterpreted to be a large bacterium ("megabacteria") after its discovery but is now recognized as an ascomycetous yeast.[9] The yeasts have a distinctive elongate morphology, measuring approximately 2-3 micrometers wide and 20-40 micrometers long, and are often arranged in densely packed clusters or streams, sometimes referred to as 'haystack,' 'matchstick,' or 'logjam' arrangements.[7,9] Infections occur at the mucosal surface of the proventricular-ventricular isthmus, with organisms sometimes penetrating into isthmus glands and extending into the koilin layer of the ventriculus. Organisms can be detected in cytologic preparations of feces or scrapings from the isthmus. Although staining characteristics can be variable, they are typically gram-positive and stain dark blue with rapid Romanowsky stains (e.g., Diff-Quik).[4] In histologic sections, the yeasts are eosinophilic and often readily identifiable with routine hematoxylin and eosin staining. They also stain positively with silver stains and Periodic acid-Schiff (PAS) stain.

Gross lesions can include emaciation, excessive proventricular mucus production, and mucosal erosions or ulcerations with hemorrhage. Histologic examination reveals variable associated inflammation and goblet cell hyperplasia in addition to the characteristic organisms. In the ventriculus, colonization can be associated with marked disruption and attenuation of the koilin layer, with variable

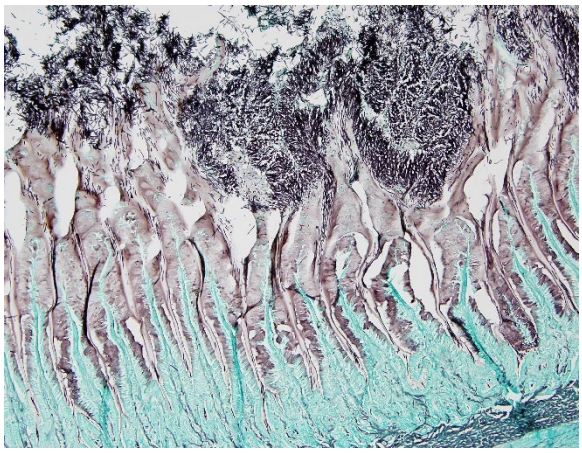

Figure 3-3. Ventriculus, tinamou. A silver stain demonstrates the morphology of these elongate yeasts. (GMS, 400X) (*Photo courtesy of:* Wildlife Conservation Society, Zoological Health Program; https://oneworldonehealth.wcs.org, www.wcs.org)

inflammation. An association with proventricular adenocarcinoma in budgerigars (*Melopsittacus undulates*) has been proposed, but the mechanism of this association is not well understood.[5]

Macrorhabdiosis can be subclinical or can be associated with a chronic, progressive, and fatal wasting syndrome.[7] Clinically significant or fatal disease associated with *Macrorhabdus ornithogaster* infection is most often diagnosed in psittacine, passerine, and gallinaceous species, but has also been reported in paleognaths such as ostriches and rheas.[1,2,3,6,8,10] While not, to our knowledge, previously reported in tinamous (also paleognaths), macrorhabdiosis has been diagnosed in one previous case from our zoological collection.

In some cases, an underlying stressor may be needed to trigger the development of clinically significant disease.[6,7] Yeasts were abundant in multiple sections of the orad ventriculus from this case, but no clear indications of chronicity, such as chronic inflammation or goblet cell hyperplasia, were observed. We suspect that the yeast overgrowth may have occurred in a relatively short period prior to death, potentially as a consequence of debilitation due to underlying disease. This tinamou's clinical decline was attributed primarily to multi-organ amyloidosis involving the liver, spleen, kidneys, myocardium, and arteries in multiple tissues, confirmed by Congo red staining of selected tissues. While suspected to be a sequela of chronic inflammation, a specific inciting cause for amyloidosis was not determined. The contribution of macrorhabdiosis to this tinamou's clinical decline is unclear.

Contributing Institution:
Wildlife Conservation Society, Zoological Health Program

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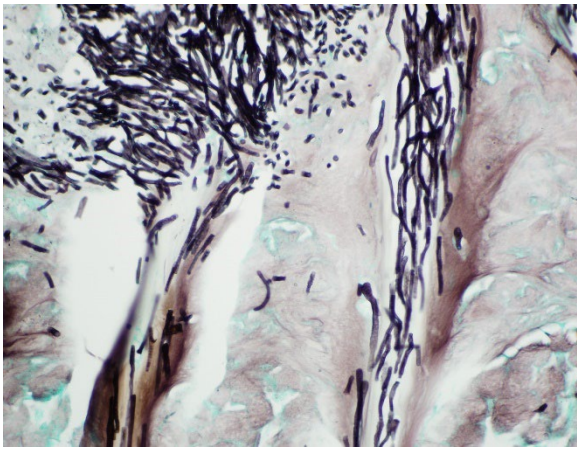

Figure 3-4. Ventriculus, tinamou. A higher magnification of the silver stain demonstrating the morphology of these elongate yeasts. (GMS, 1000X) (*Photo courtesy of:* Wildlife Conservation Society, Zoological Health Program; https://oneworldonehealth.wcs.org, www.wcs.org)

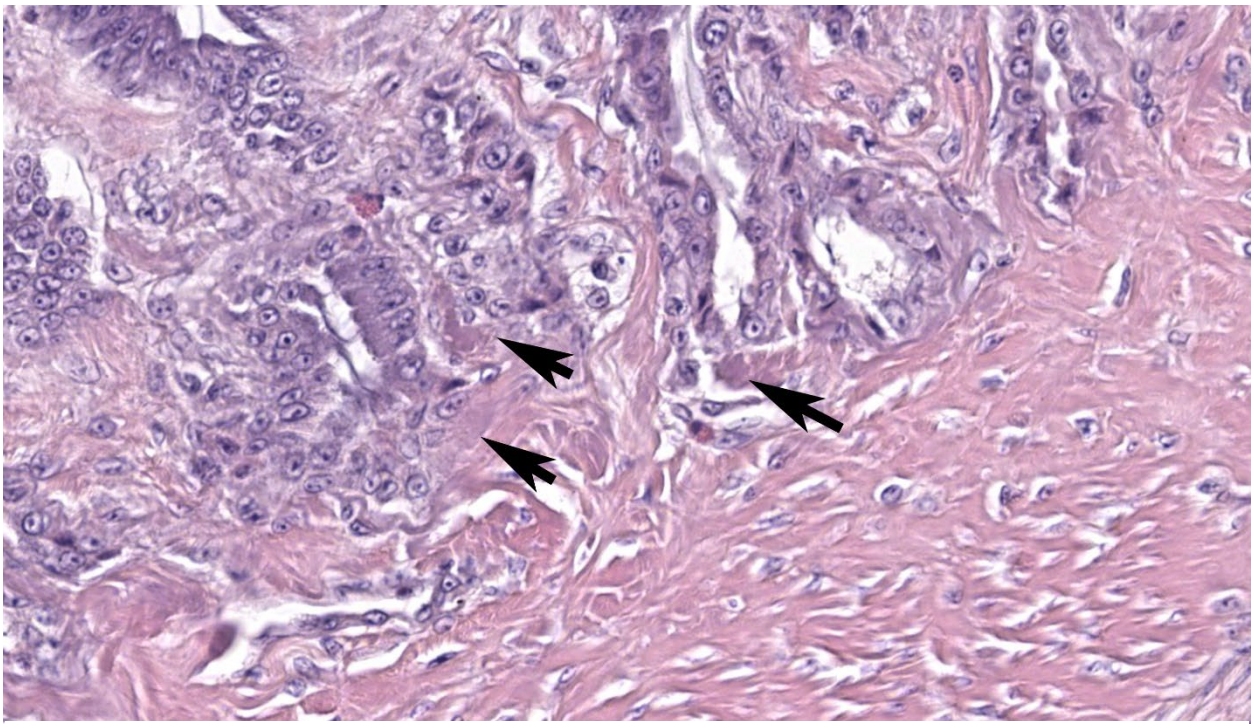

Figure 3-5. Ventriculus, tinamou. Small aggregates of amyloid are present within subepithelial areas in the lamina propria (arrows). (HE, 979X)

JPC Diagnosis:
1. Ventriculus: Koilin loss, diffuse, severe with myriad elongate yeast and mild granulocytic ventriculitis

2. Ventriculus (lamina propria and blood vessels): Amyloidosis, chronic, multifocal, mild

3. Adipose tissue: Atrophy, subacute, diffuse, mild

JPC Comment:
Case 3 offers a synopsis of classic avian lesions. We differ slightly from the contributor in identifying the primary lesion as koilin loss (which was substantial) though we agree that there is mild inflammation of the ventriculus. We added an additional morphologic diagnosis for the atrophy of fat (best observed at the serosal surface) as adipocytes are small with variably sized lipid vacuoles. Amyloid deposition in this case is subtle and easily overlooked on H&E. On this slide, amyloid is most discernable within sagittal sections of larger vessels and multifocally within the lamina propria as lightly eosinophilic to amphophilic material. In birds, common locations for amyloid deposition include the kidney (glomerulus and basement membrane), the liver (within the space of Disse), and the spleen. While we searched for a urate tophus (i.e. gout) to connect back to the clinical picture outlined by the contributor, we were unable to make this case a 'four-fer'.

The long sagittal sections of peripheral nerves (likely a nerve plexus) prompted a short group discussion. In a vacuum, this might resemble ganglioneuritis (i.e. avian bornavirus). As Dr. Ladouceur noted, avian nervous tissue is highly cellular and satellite cells could be confused for infiltrating T-lymphocytes. Once again, considering avian type is helpful as avian bornavirus affects psittacine birds (and rarely passerines) and

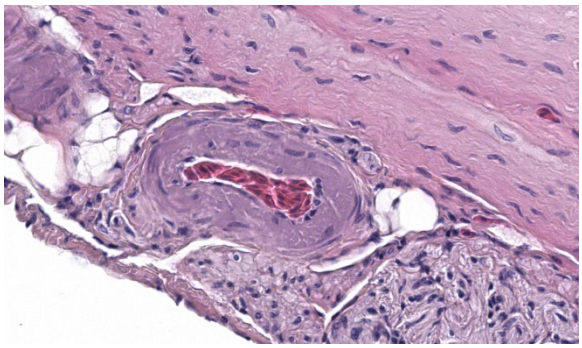

Figure 3-6. Ventriculus, tinamou. The walls of arterioles are also expanded by amyloid. (HE, 1090X)

not tinamiforms such as this tinamou. If this were a psittacine, CD3 IHC can be useful in differentiating satellite cells from infiltrating T lymphocytes in cases of suspected avian bornavirus. Screening HE sections for plasma cells is also useful (none were noted in the nerves in this case).

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CASE IV:

Signalment:
12-year-old, male, blue fronted Amazon parrot (*Amazona aestiva*).

History:
Two-week history of not acting right. One week history of acute

decline, weight loss, ataxia, increased respiratory effort/rate and loss of deep pain over 24 hours. Radiographs revealed evidence of airsacculitis, and bloodwork showed severe heterophilia. The patient's condition deteriorated despite antibiotic administration. Euthanasia was elected.

Gross Pathology:
The right and left abdominal air sacs are severely thickened and tan to yellow, with shaggy, irregular projections (airsacculitis). The coelomic cavity contains small amounts (approximately 1 mL) of tan to yellow effusion. Focal regions of yellow to tan thickening are noted over the body wall in the regions of the right and left femur. The right lung is mottled dark red, light red and pale tan. A 0.5 cm diameter, firm, tan nodule is present in the middle section of the lung lobe. Samples from this lobe sink in formalin. The left lung is also mottled light and dark red to brown and has a focal, 0.3 cm diameter tan nodule (presumed granulomatous heterophilic pneumonia). A firm region in the middle section of the lung lobe is observed. Sections from this lobe float in formalin.

The spinal cord is removed in situ, is fixed, decalcified and sectioned. In the lumbar spine, there is a focal, white, firm nodule in the bone. This nodule overlies and is just caudal to the glycogen body of the spinal cord.

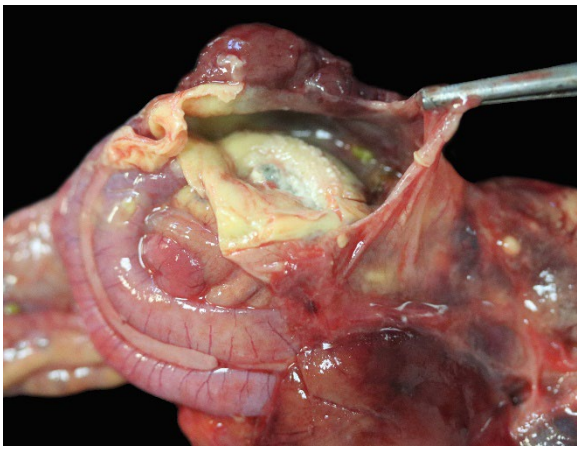

Figure 4-1. Airsac, Amazon parrot. There is yellow exudate within the airsac. (*Photo courtesy of: Schwarzman Animal Medical Center, Department of Anatomic Pathology. www.amcny.org*)

Laboratory Results:
CBC: Hematocrit 38 (range 39-54%), WBC 56.5 K/µL (range 5-15 K/µL), Heterophils 44 K/µL (range 1.5-11 K/µL), Heterophil bands 5.1 K/µL (0 K/µL), Lymphocytes 2.3 K/µL (range 1-10 K/µL), Monocytes 5.1 K/µL (range 0-0.4 K/µL), Eosinophils 0 K/µL (range 0-0.3 K/µL), Basophils 0 K/µL (range 0-0.4 K/µL), thrombocytes adequate, polychromasia slight, no parasites seen.

Microscopic Description:
A section of lumbosacral vertebral column and spinal cord are examined. Within the bone of the lumbar vertebral column, a densely cellular region of inflammation is present. The cellular infiltrate comprises predominantly heterophils including degenerate heterophils and fewer macrophages, lymphocytes and plasma cells. Multinucleate giant cells and epithelioid macrophages are occasionally observed, with formation of heterophilic granulomas. Inflammatory populations result in lysis of adjacent bone, with irregular, scalloped margins of attenuated vertebral bone. Additional foci of inflammation are multifocally distributed throughout the vertebral bone

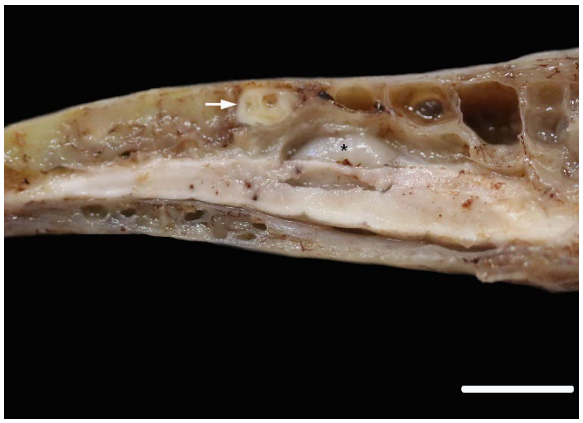

Figure 4-2. Synsacrum, Amazon parrot. There is a firm white nodule in the vertebral body dorsal to the glycogen body. (*Photo courtesy of: Schwarzman Animal Medical Center, Department of Anatomic Pathology. www.amcny.org*)

within the section. The marrow and pneumatized cavities contain lightly eosinophilic fluid (edema). The inflammation extends to the spinal cord, with heterophils and macrophages infiltrating through the meninges and into the spinal cord parenchyma. In these regions, the spinal cord exhibits myelin vacuolation with regional gliosis including Gitter cells. Axons are enlarged and homogeneously eosinophilic (spheroids). Inflammatory cells are intermixed with fungal hyphae. Hyphae occasionally surround and infiltrate vessel walls, which contain inflammatory populations, hyaline, eosinophilic, acellular material (fibrinoid change) and are surrounded by wispy, loosely arranged, eosinophilic material (edema). Fungal hyphae have parallel walls with infrequent septations, range from approximately 3 to 6 µm in width, and demonstrate acute angle, dichotomous branching, highlighted with a Gomori's methenamine silver (GMS) stain. Heterophilic, histiocytic inflammation and fungal hyphae are observed at the periphery of and minimally infiltrating into the glycogen body, which also contains small foci of hemorrhage.

Contributor's Morphologic Diagnosis:
Lumbosacral spine and spinal cord: Osteomyelitis and myelitis, heterophilic,granulomatous, lymphoplasmacytic with myriad fungal hyphae, regional myelin vacuolation with gliosis and spheroid formation, vasculitis with fibrinoid degeneration and edema.

Abdominal airsacs (not included on slide): Airsacculitis, severe, necrotizing, heterophilic, granulomatous with myriad fungal hyphae and conidiophores (morphology consistent with *Aspergillus* spp.).

Contributor's Comment:
This psittacine bird had a disseminated fungal infection, involving multiple organ systems including the lungs, air sacs, vertebral column, spinal cord, skeletal muscles, coelomic cavity, kidneys, liver, intestines, adrenal glands and thyroid glands. The most severely affected organ system was the respiratory system. Myriad fungal hyphae were present within foci of inflammation. These hyphae contained the morphologic features of

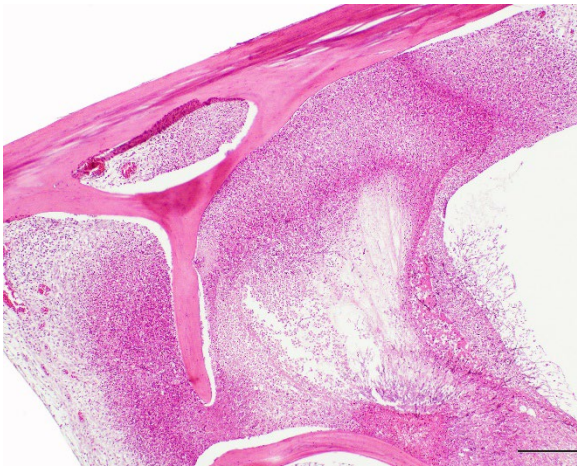

Figure 4-3. Synsacrum, Amazon parrot. There is a large focus of heterophilic inflammation within the vertebral body. Fungal hyphae extend from the inflammation at right. (HE, 200X) (*Photo courtesy of: Schwarzman Animal Medical Center, Department of Anatomic Pathology. www.amcny.org*)

Aspergillus species, including narrow width (3-6 µm), parallel walls with septation, dichotomous, acute angle branching and a tendency to be oriented in the same direction.[2,4]

In some locations, conidiophores were observed. Distinctive conidiophores (asexual fruiting bodies) occur on surfaces or cavities exposed to air, such as the air sacs.[2,7] Conidiophores consist of a stalk protruding from a mycelial foot cell, with a terminal globose, hemispherical or flask shaped vesicle. Peg-like phialides (formerly sterigmata) arise from the vesicle and may be directly connected and arranged in a single layer (uniseriate) or connected by supporting cells (metulae) and arranged in a double layer (biseriate).[2,7] Unbranched chains of conidia are formed from the distal ends of the phialides.[2,7] The conidiophores in this case appeared to be uniseriate, which is a feature of the *Aspergillus fumigatus* group, however, definitive diagnosis requires ancillary testing, as there is overlap in histomorphologic features of *Aspergillus* conidiophore morphology (see below).

A sequel to the fungal infection in this case was osteomyelitis of the lumbosacral spine with extension to the spinal cord. Frequent sites of secondary involvement in birds include the coelomic cavity, central nervous system (CNS), liver, intestines, kidney, pneumonic bone, adrenal glands and vertebral column.[8] In the submitted case, osteomyelitis was most severe in the lumbosacral portion of the spine (in the region of the corpus gelatinosum or glycogen body), but inflammation was also found in the thoracic and cervical segments. Inflammation of the lumbar vertebral column led to osteomyelitis and regional myelitis, with involvement of the glycogen body. Inflammation of the glycogen body is of uncertain significance in the context of the clinical signs. The glycogen body (corpus gelatinosum) is an ovoid, circumventricular, ge-

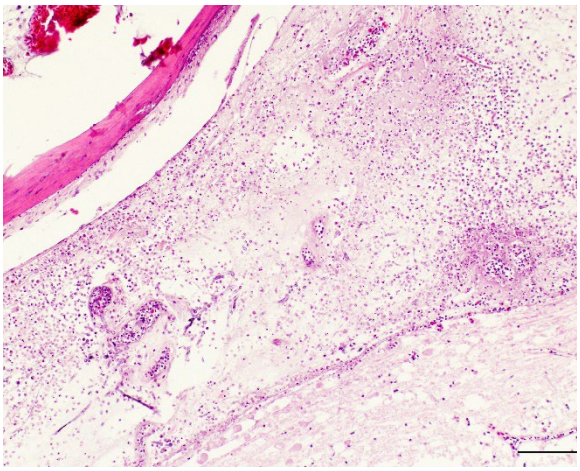

Figure 4-4. Spinal cord, Amazon parrot. Inflammation extends into the spinal cord; there are fungal hyphae and necrotic vessels at right (HE, 200X) (*Photo courtesy of: Schwarzman Animal Medical Center, Department of Anatomic Pathology. www.amcny.org*)

latinous structure of unknown function embedded in the lumbosacral region of the spinal cord, occupying the rhomboid sinus.[5] The corpus gelatinosum is dorsal to the spinal cord and the ventral portion encloses the central canal. Peripheral and central cellular zones have been described. Cells of this structure contain abundant, pale, vacuolated cytoplasm and peripheralized nuclei. These glycogen containing cells are hypothesized to arise from specialized, modified glial cells (astrocytes).[5] Although the function is unknown, hypotheses include an energy source for the central nervous system (CNS), transmission of hydrostatic pressure changes during movement, roles in neuron metabolism, and myelin formation.[5] Extension of the fungal infection from the abdominal airsacs to the skeletal muscle of the proximal limbs also likely contributed to the ataxia noted in the history.

Aspergillosis is a non-contagious fungal disease that affects captive and wild birds.[1,3] The fungus is ubiquitous, opportunistic and saprophytic, and exposure to large

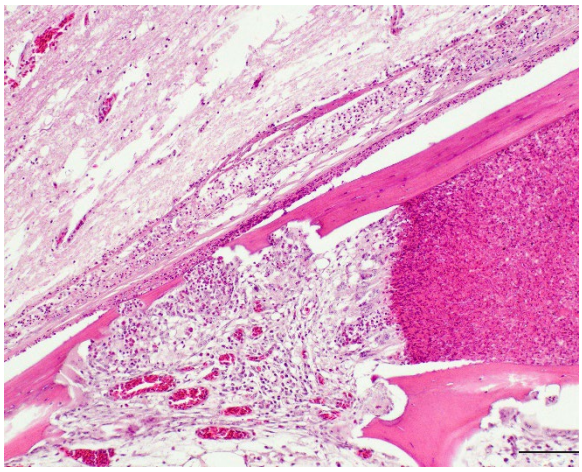

Figure 4-5. Spinal cord, Amazon parrot. Fungal hyphae are 3-4um wide, have parallel walls, sepatations, and acute angle dichotomous branching. (HE, 400X) (*Photo courtesy of: Schwarzman Animal Medical Center, Department of Anatomic Pathology. www.amcny.org*)

numbers of fungal spores can result in infection. In birds, aspergillosis is most commonly caused by *Aspergillus fumigatus*, and less commonly *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nidulans, Aspergillus glaucus, Aspergillus oryzae* and others.[1,3,7–9] A recent report detailed a case of *Aspergillus pseudoviridinutans* (a cryptic species of *Aspergillus* within section *Fumigati*) causing rhinitis in an orange winged Amazon parrot.[6] Some avian species are thought to be predisposed including *Amazona* sp., *Psittacus erithacus*, *Pionus* sp., as well as species that originate from arid environments.[6,9] Other predisposing factors include increased concentrations of environmental spores, a warm environment, improper humidity, poor ventilation, poor sanitation, and long-term storage of feed. Impaired immunity can also predispose to development of Aspergillosis, and has been reported with steroid administration, metabolic bone disease, inadequate diet, overcrowding, shipping, starvation, thermal discomfort, infections, toxins and trauma, among others.[1,3,6,8,9]

The main route of infection is thought to be inhalation. As the spores of *Aspergillus fumigatus* are smaller than spores of other *Aspergillus* spp, some may reach the lungs and air sacs.[1,6] Similar to this case, the air sacs are a common site of primary infection (inhaled air reaches caudal thoracic and abdominal air sacs before the pulmonary epithelial surfaces).[1] Leukocytosis is a common clinical feature, and is often severe (20,000 to more than 100,000 white blood cells per microliter) with a left shift, monocytosis and lymphopenia. Leukocytosis in this case was 56.5 K/µL, with heterophilia, a left shift, and monocytosis. Serological tests for aspergillus have been developed, however, false negative results can occur.[1] Serum protein electrophoresis may reflect an acute phase response, often observed with aspergillosis, which includes increased globulins with reduced albumin, increases in alpha 2 globulins and increased serum amyloid A levels.[3]

Gross lesions are variable, but generally comprise granulomas of the lungs and air sacs. In acute infections, miliary granulomas may be distributed throughout the respiratory tract.[1,8,9] White to yellow plaques (scutulae) may be seen within the respiratory tract or over serosal surfaces. Air sacs are often thickened and may contain caseous exudate or visible mycelial formation.[8,9] Nodules within the lungs may have caseous, consolidated or necrotic centers. Histologically, large numbers of degenerate heterophils are typically present, accompanied by necrosis, multinucleated giant cells, epithelioid macrophages, and variable numbers of fungal organisms.[8,9] In aerated organs, aggregates of radiating hyphae (the sunburst pattern) frequently occur.[1,2] If the infection is not eliminated in the respiratory tract, disseminated infection can occur, both by direct extension through the air sac wall and hematogenous spread.[1] More information is needed regarding virulence factors of avian aspergillosis.

Figure 4-6. Spinal cord, Amazon parrot. A silver stain demonstrates the morphology of the hyphae. (GMS, 400X) (*Photo courtesy of: Schwarzman Animal Medical Center, Department of Anatomic Pathology. www.amcny.org*)

Conidia of *Aspergillus fumigatus* may be resistant to respiratory macrophage-induced killing.[1] Invasion of blood vessels is a frequently reported sequel,[1,2] and was observed in this case.

Deposition of calcium oxalate crystals in association with *Aspergillus* spp has been documented in domestic animals and humans.[2,7] *Aspergillus* spp synthesize oxalic acid as a product of the tricarboxylic acid cycle, resulting in precipitation of calcium oxalate crystals, which may cause tissue damage.[7] The crystals are needle-like and may have wheatsheaf or rosette arrangements, are clear to pale yellow and strongly birefringent under polarized light.[2,7] In one study, cases of avian aspergillosis with oxalate crystal formation occurred commonly in respiratory tissues that interfaced with air (nasal sinus, trachea, syrinx, lung, air sac).[7] Sparse or colorless crystals can be difficult to identify without routine polarization. In the aforementioned study, calcium oxalate crystals were found in 11 of 16 avian cases after retrospective review, however, few were identified on the initial report.[7] Thus, routine polarization of slides in cases with *Aspergillus* infections in respiratory tissues is recommended.[7]

Definitive diagnosis of aspergillosis in birds is based on fungal identification and documentation of fungal organisms within the associated lesions. Hyphal morphology of *Aspergillus* is similar to that of other fungi in the hyalohyphomycosis group. Some studies found that hyphae morphologically consistent with *Aspergillus* spp were identified as other genera with culture or PCR.[4] The presence of conidiophores allows definitive diagnosis of the genus *Aspergillus*, however, there is substantial overlap in the morphologic features of the conidiophores of different Aspergillus species, including seriation, phialides arrangement and pigmentation.[7] Thus, conidia morphology cannot be relied upon for speciation of *Aspergillus*.[2,7] Fungal culture from clinical samples is considered to be the gold standard for fungal identification, however, the time frame for these cultures is often long. Additional options include use of mo-

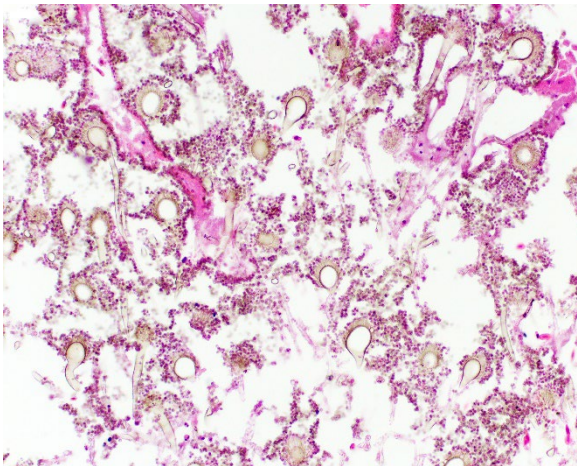

Figure 4-7. Abdominal air sacs, Amazon parrot. Conidia are present within the abdominal air sacs. (HE, 1000X) (*Photo courtesy of: Schwarzman Animal Medical Center, Department of Anatomic Pathology. www.amcny.org*)

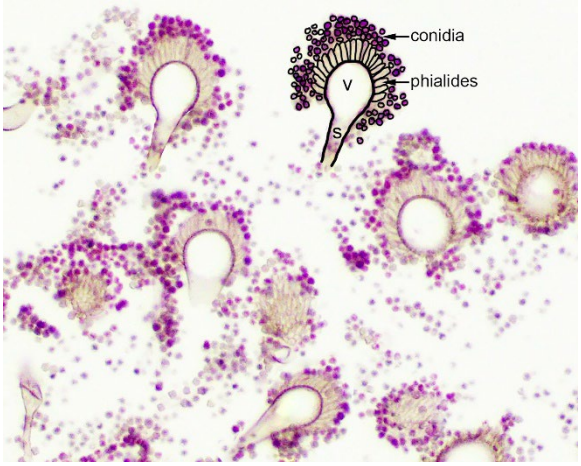

Figure 4-8. Abdominal air sacs, Amazon parrot. Diagram of diagnostic features of fungal conidia. (HE, 1000X) (*Photo courtesy of: Schwarzman Animal Medical Center, Department of Anatomic Pathology. www.amcny.org*)

lecular assays, immunohistochemistry and in-situ hybridization from formalin fixed, paraffin-embedded (FFPE) tissues and cytology slides. PCR using primers that target and amplify conserved portions of the fungal genome (internal transcribed spacers and the 5.8S rRNA gene) followed by sequencing is another potential method of identification.[3,4,6] High quality fungal DNA was able to be obtained from FFPE tissues in 70% of cases in one study, which correlated with the histological identification in 62% of cases. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is an additional potentially useful tool, however, is currently only available for some *Aspergillus fumigatus* related species.[6] Given the presence of conidiophores, additional ancillary tests for speciation were not requested in this case.

Contributing Institution:
www.amcny.org

JPC Diagnosis:
Synsacrum, lumbosacral spinal cord: Osteitis and meningomyelitis, heterophilic, necrotizing, acute, multifocal, severe with vasculitis and fungal hyphae

JPC Comment:
The contributor provides an excellent summary of the case along with many helpful figures. The framing of the entire synsacrum and glycogen body on the slide that mirrors the gross image is simply a bonus for us.

We approached our morphologic diagnosis slightly differently and emphasized (meningo)myelitis and osteitis separately. In contrast to Case 2, the bone in this section is pneumatic and lacks marrow elements. As osteomyelitis best describes inflammation of the osseus medulla (including the marrow), we made this distinction. In our review of the slides we did not observe oxalate crystals, though this may reflect a lack of free calcium to utilize in the TCA cycle (e.g. from adjacent hemorrhage and cell loss).

We covered *Aspergillus* in the guttural pouch of a horse this year in Conference 1, Case 3. Both cases feature vascular lesions, though the degree of infarction and secondary changes are much more pronounced in that horse than in this parrot. This likely reflects the route of entry via extension of the air sac versus direct hematogenous entry.

Conference participants should have been able to recognize this histologic section as "pelvis" or "synsacrum" based on either of two histologic features: the presence of the glycogen body, which is located in the lumbosacral spinal cord, or the fusion of the vertebral bodies, which represents the synsacrum.

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