Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #24 24 April 2024

CASE I:

Signalment:
28-year-old male American Flamingo (*Phoenicopterus ruber*).

History:
This flamingo had a 1-year history of progressive weight loss and a 10-year history of progressive bilateral pododermatitis. Two days prior to death, lethargy and separation from the flock were noted and the flamingo was treated with fluids, Excede, and meloxicam.

Figure 1-1. Feet, flamingo. Flamingo feet, bilateral, January 2013. The plantar aspect of the metatarsal pad of each foot is focally expanded by round, firm tissue with central ulceration ("bumble"). (*Photo courtesy of:* The Ohio State University College of Veterinary Medicine, Department of Veterinary Biosciences, https://vet.osu.edu/biosciences)

Gross Pathology:
On gross examination, the subcutaneous, coelomic, and pericardial fat stores were significantly reduced (BCS 2/5). Bilaterally, there were round, raised, proliferative nodules measuring 4 cm and 2.5 cm in diameter on the right and left metatarsal pads, respectively. There was a small amount of clear to white mucoid material in the lumen of the glottis. Multifocal, pale gray to brown, flat, circular regions measuring 1-5 mm in diameter were present on the tracheal mucosa. There were white to orange, speckled, granular deposits overlying the pericardium at the level of the right ventricle. Within the ventriculus, there was a 3 mm red, circular, area under the koilin membrane. Throughout all lobes of the liver, there were regions of mild pallor. Both kidneys were markedly enlarged, friable, and mottled orange to yellow. Within the right stifle, there was a small amount of white, soft, chalky material.

Laboratory Results:
Severely elevated uric acid and heterophilia were present on serum biochemistry panel and complete blood count. Computed tomography revealed bilaterally enlarged kidneys.

Microscopic Description:
Kidney: Multifocally and randomly expanding and disrupting the interstitium and replacing tubules are foci of brightly eosinophilic

cellular and karyorrhectic or pyknotic nuclear debris (necrosis) surrounded by a thin rim of lymphocytes, plasma cells, and histiocytes. These foci measure approximately 150-250 µm and occasionally contain clear clefts within central areas of necrosis (presumed washed-out uric acid crystals). Moderate numbers of red blood cells extend throughout the adjacent interstitium (hemorrhage). Diffusely and globally, glomerular tufts are expanded by variable amounts of acellular, homogenous, eosinophilic, glassy material (amyloid) that compresses capillaries and distorts glomerular architecture. Glomerular tufts are hypocellular with occasional karyorrhectic or pyknotic nuclear debris (necrosis) and frequently adhere to Bowman's capsule (synechiae). Tubules exhibit one or more of the following changes: swollen epithelium with microvacuolated cytoplasm and large, round vesiculated nuclei (degeneration); hypereosinophilia with pyknotic nuclei (necrosis); increased basophilia with crowded epithelial cells containing hyperchromatic nuclei (regeneration); round, crystalline, deeply basophilic deposits within tubular epithelium (mineral); marked ectasia with flattened, attenuated epithelium and intraluminal eosinophilic to amphophilic proteinaceous fluid or droplets. Multifocally, tubular lumina contain sloughed, necrotic epithelial cells admixed with cellular debris (cellular casts); the basement membranes of affected tubules are often disrupted (tubulorrhexis). Frequently, eosinophilic, homogenous, acellular material (amyloid) expands tubular basement membranes and forms broad cuffs around affected tubules. Multifocally, the tunica media of renal arteries and arterioles is expanded by lightly basophilic to amphophilic amorphous material (amyloid). Mild numbers of lymphocytes and plasma cells expand the renal interstitium.

Figure 1-2. Flamingo feet, bilateral, April 2021. The plantar aspect of the metatarsal pad of each foot is focally expanded by a round to multilobular, firm, mass ("bumble") with central ulceration and accumulation of inspissated material in a central ulceration with formation of numerous exophytic projections of tissue with interspersed deep fissures. Compared to Figure 1-1, masses are approximately 2-3 times larger. (*Photo courtesy of:* The Ohio State University College of Veterinary Medicine, Department of Veterinary Biosciences, https://vet.osu.edu/biosciences)

Great vessels: There are no significant microscopic findings in the great vessels.

Congo Red and Von Kossa histochemical stains: Within glomeruli, tubular basement membranes, and walls of renal arteries and arterioles, there is congophilic pink-orange acellular material that exhibits strong apple-green birefringence when viewed under polarized light. Mineral deposits within renal tubules exhibit strong staining for calcium.

Contributor's Morphologic Diagnosis:

1. Kidney: Diffuse global glomerular amyloidosis with synechiae; moderate to marked renal tubular amyloidosis with degeneration, necrosis, loss, and regeneration with multifocal mineralization; and moderate, chronic, multifocal arteriolar amyloidosis.
2. Kidney: Multifocal urate tophi (presumed) with marked, chronic, multifocal histiocytic and lymphoplasmacytic interstitial nephritis.
3. Kidney: Moderate to marked multifocal tubular ectasia with cellular casts.

Contributor's Comment:

This is a case of confirmed systemic amyloidosis and visceral gout in a flamingo. Amyloidosis in this case is presumably secondary to chronic pododermatitis, as this association has been well-documented in flamingos with this condition. Amyloidosis, pododermatitis, and gout are all reviewed below.

Amyloidosis: Systemic AA (reactive) amyloidosis is an important cause of death in domestic and captive wild birds. AA amyloid is derived from serum amyloid A (SAA), an acute phase lipoprotein produced by hepatocytes. Chronic inflammatory diseases or neoplastic conditions can lead to excessive synthesis of SAA and protein misfolding resulting in amyloidosis.[2,8,11] Several cytokines can induce hepatic synthesis of SAA including IL-1, IL-6, and TNF-α. SAA typically has an immunomodulatory role in the inhibition of fever caused by IL-1β and TNF-α as well as the inhibition of platelet aggregation, suppression of thromboxane synthesis, and serotonin release from platelets.[8] While an increased pool of precursor protein is necessary for the development of amyloidosis, certain amino acid substitutions may favor amyloidogenicity and

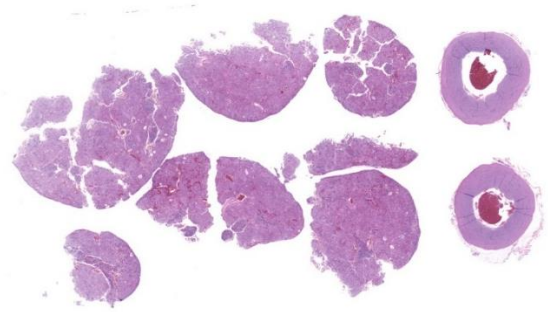

Figure 1-3. Kidney, flamingo. Multiple sections of kidney and sections of artery (right) are submitted for examination. (HE, 4X)

give rise to unstable intermediate proteins that can easily reshape into fibrils.[8]

Two types of amyloidosis have been identified in birds – AA amyloidosis and cerebral Aβ amyloidosis accompanied by cerebral amyloid angiopathy. Avian AA amyloidosis commonly affects waterfowl, small passerine birds, and chickens. Aβ amyloidosis has been reported in great spotted woodpeckers and eagles but not in flamingos.[11]

AA amyloid deposition occurs most frequently in the liver, spleen, kidneys, and small intestine with the proventriculus, large intestine, heart, gonads, and endocrine organs being less affected.[8] While lung, skin, and brain are rarely affected, vascular AA amyloid deposition has been reported in the central and peripheral nervous systems of flamingos.[11]

Grossly, affected organs are swollen, pale, and have a waxy consistency. The presence of amyloid can occasionally be demonstrated at the time of necropsy by applying Lugol's iodine to the cut surface and rinsing with sulfuric acid, rendering amyloid deposits dark brown or black. Amyloid accumulation results in organ dysfunction and may lead to organ rupture due to disruption of tissues by the abnormal protein. Affected tissues are typically friable,

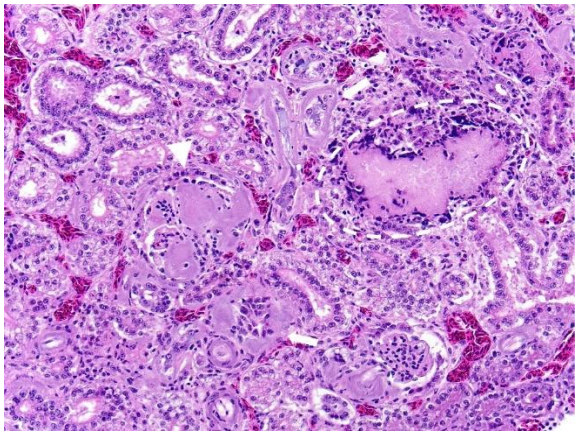

Figure 1-4. Kidney, flamingo. Diffusely and globally, glomerular tufts are expanded by homogenous, acellular, eosinophilic, glassy material (amyloid). (HE, 400X) (*Photo courtesy of:* The Ohio State University College of Veterinary Medicine, Department of Veterinary Biosciences, https://vet.osu.edu/biosciences)

and hepatic fractures with subsequent exsanguination are a common cause of acute death.[1]

Histologically, amyloid is acellular, homogenous, eosinophilic material that commonly accumulates in hepatic sinusoids, renal glomeruli, perifollicular areas of the spleen, lamina propria of the intestines, and vascular walls.[1] The histologic diagnosis must be confirmed with Congo Red staining where amyloid stains peach or pink-orange and exhibits green birefringence under polarized light. AA amyloid can be confirmed via elimination of Congo Red staining affinity by oxidation of tissue sections in potassium permanganate. After staining with thioflavine-T, amyloid exhibits bright yellow fluorescence.[2] Amino acid sequencing and immunohistochemical techniques utilizing monoclonal and polyclonal anti-AA antibodies directed against amyloid fibril proteins can aid in the identification of amyloid.[15] On electron microscopy, amyloid is characterized by nonbranching 7-10 nm-diameter fibrils. [2]

Experimentally, avian amyloidosis can be induced by repeated inflammatory stimuli, such as inoculation with bacterial extracts or vaccination with oil-emulsified bacterins. Thus, antigenic stimulation with bacteria is considered to promote the development of avian AA amyloidosis. In addition, recent studies have suggested that avian amyloidosis is transmissible. Horizontal transmission of amyloidosis via ingestion of amyloid-contaminated feed or feces is suspected to occur among avian species.[10]

Chronic inflammation associated with pododermatitis has been linked to systemic amyloidosis.[10] In this case, systemic amyloidosis is attributed to the patient's history of bilateral pododermatitis. Amyloid deposition was confirmed via Congo Red staining in the kidney, spleen, and pancreas. Arterial mineralization was confirmed in the spleen via Von Kossa staining. Despite the multifocal medial mineralization noted in small blood vessels, there was no evidence of atherosclerosis in the great vessels. It is possible that some of the gross lesions noted in the heart and GI tract at the time of postmortem exam represent foci of mineralization and/or amyloidosis. The grossly described white, chalky material in the stifle may be consistent with articular gout. However, no joint tissue was provided for confirmatory histopathological examination.

Pododermatitis: Pododermatitis (also known as bumblefoot) has only been documented in captive birds, and influencing factors include flooring in water ponds, prolonged periods indoors, environmental temperatures, age, weight, and potentially dietary zinc availability.[12,13,14] Foot lesions are classified as inflammatory or proliferative with inflammatory lesions being more common in younger birds and proliferative lesions more common in older birds.[1] Gross lesions include individual

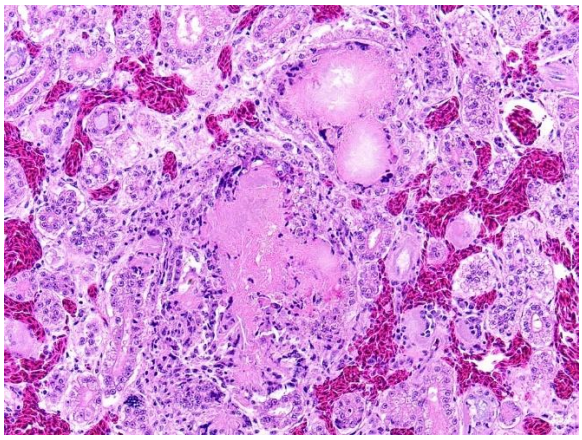

Figure 1-5. Kidney, flamingo. Multifocally, there is brightly eosinophilic cellular and karyorrhectic or pyknotic nuclear debris expanding and disrupting renal tubules and interstitium surrounded by mild to moderate numbers of histiocytes, lymphocytes, and plasma cells. These foci occasionally contain clear clefts (presumed washed-out uric acid crystals). (HE, 400X) (*Photo courtesy of:* The Ohio State University College of Veterinary Medicine, Department of Veterinary Biosciences, https://vet.osu.edu/biosciences)

to coalescing nodules of thickened and hyperkeratotic epidermis with fissures, papillarybgrowths, erosions or ulcers overlying granulation tissue and inflammation. Early lesions include heterophilic dermatitis, hydropic epithelial degeneration, hyperplasia, serocellular crust formation, and pustules. Chronic lesions feature neovascularization, fibrosis, and infiltration by lymphocytes and macrophages.

Secondary bacterial infections with organisms, such as *Fusophorum* spp. and *Staphylococcus aureus* are common and can exacerbate tissue damage and inflammation.[4] Fungal and bacterial agents have not been consistently found and no virus has been isolated in pododermatitis lesions.[12] Poxviral infection is the major differential for pododermatitis lesions.

Gout: Gout occurs in birds, reptiles, great apes, and humans due to the lack of the enzyme uricase, which oxidizes uric acid to allantoin. Uric acid in these species is normally eliminated through a combination of glomerular filtration, reabsorption, and secretion. Urate deposition (gout) usually occurs due to impaired excretion by the kidneys or overproduction of uric acid leading to hyperuricemia and precipitation of monosodium urate crystals (tophi) on visceral and articular surfaces. Comparatively, Dalmatian dogs also cannot oxidize uric acid to allantoin due to a defect in uricase, predisposing them to urate urolithiasis. This defect is an inherited autosomal recessive trait and linked to a mutation in *SLC2A9*, which encodes for a glucose transporter.[2,7]

Gout in birds occurs in acute (visceral) and chronic (articular) forms, which differ in frequency, age of onset, sex predilection, gross and microscopic lesions, pathogenesis and causes.[3] Lesions may appear grossly as white foci, streaks, or sheets on serosal surfaces and viscera. Articular forms of gout include chalky, white deposits in and around joints. Urate crystals are often leached out during tissue processing and may be detected as remnant, wispy, basophilic, “ghost” crystals. Thus, tissues fixed in alcohol rather than formalin may improve detection. Histologically, fine, radiating acicular clefts may be present in the absence of inflammation or associated with histiocytic, granulomatous, or mixed inflammation. Gout should be distinguished from pseudogout, which is composed of nonurate, calcium pyrophosphate crystals.[4]

In birds, acute (visceral) urate deposition is more common than chronic (articular) urate deposition. Acute urate deposition may occur at any age with renal lesions involving white, chalky precipitates. Visceral organs including

liver, myocardium, spleen, and serosal surfaces are commonly affected, whereas soft tissues around the joints may or may not be involved. Microscopically, there is an inflammatory reaction around tophi in the kidney and viscera with usually no inflammation in the synovium. Acute urate deposition is typically due to renal failure with causes including dehydration, nephrotoxicity, nephrotropic infectious agents, vitamin A deficiency, urolithiasis, neoplasia, or immune-mediated glomerulonephritis.[3] Avian astroviruses have been identified as causes of visceral gout in the chicken, duckling, and gosling.[9]

In contrast, chronic urate deposition typically occurs in mature birds with the kidney being unaffected. Soft tissues other than synovium are rarely involved. Joints of the legs, wing, spine, and mandible may be affected. Microscopically, granulomatous inflammation is present in the synovium. Chronic urate deposition is likely due to a metabolic defect in the secretion of urates by renal tubules. Causes include genetics and a high protein diet.[3]

In this case, it is difficult to determine the sequence of events. Presumably, the pododermatitis and chronic inflammation lead to systemic amyloidosis, which impaired renal function. Dehydration and/or impaired excretion of uric acid by the kidneys could have resulted in subsequent deposition of urates. The presumptive urate tophi in the kidney are correlated with the patient's hyperuricemia and bilaterally enlarged kidneys and consistent with acute (visceral) gout.

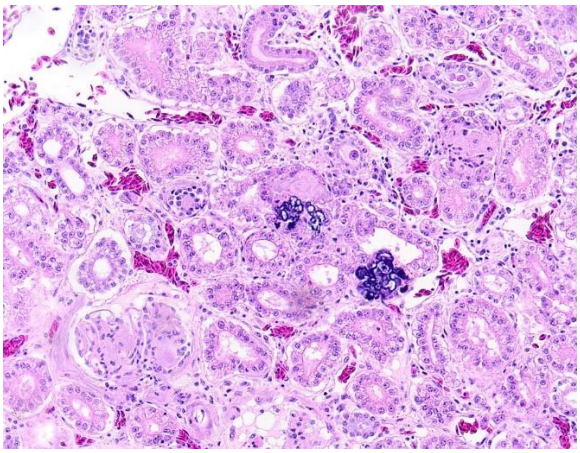

Figure 1-6. Kidney, flamingo. Renal tubules occasionally contain deeply basophilic, crystalline material (mineral). (HE, 400X) (*Photo courtesy of:* The Ohio State University College of Veterinary Medicine, Department of Veterinary Biosciences, https://vet.osu.edu/biosciences)

Contributing Institution:
The Ohio State University
College of Veterinary Medicine
Department of Veterinary Biosciences
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JPC Diagnosis:
1. Kidney, glomeruli: Amyloidosis, segmental to global, diffuse, marked.
2. Kidney, arteries, and tubular basement membranes: Amyloidosis, diffuse, marked.
3. Kidney, tubules: Urate tophi, multiple.
4. Kidney: Nephritis, interstitial, chronic and lymphocytic, diffuse, mild to moderate.

JPC Comment:
Flamingos and amyloid are a classic combination, and the blessing continue in this case with the addition of visceral gout and a history of pododermatitis. The contributor does an admirable job of summarizing these three components of the flamingo party pack.

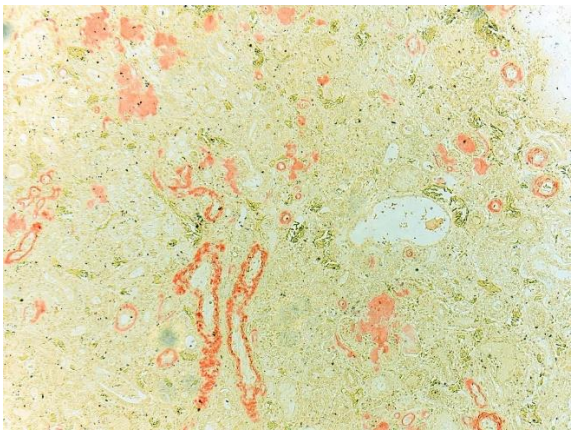

Figure 1-7. Kidney, flamingo. Congophilic, pink-orange, acellular material is present within glomeruli, tubular basement membranes, and the walls of renal arteries and arterioles. When viewed under polarized light (not shown), this material exhibits apple-green birefringence (consistent with amyloid). (*Photo courtesy of:* The Ohio State University College of Veterinary Medicine, Department of Veterinary Biosciences, https://vet.osu.edu/biosciences)

Pododermatitis, the presumed antigenic stimulus for the systemic amyloidosis in this case, has been reported in many birds, including raptors, water fowl, penguins, cocaktiels and, of course, flamingos.[12] Varying degrees of plantar lesions were found in up to 100% of captive flamingos in one study, and these lesions were the reason for euthanasia or a secondary finding in 95% of flamingos in another.[12] In contrast to classic bumblefoot lesions, which consist of nodules with central ulceration, flamingo pododermatitis often includes varying degrees of hyperkeratosis, fissues of varying depth, nodular lesions with or without ulceration, and papillomatous growths.[12]

A 2015 study attempted to characterized the pododermatitis lesions in a group of 19 flamingos from a zoological collection. Viral PCR assays for papillomaviruses and herpesviruses were performed and were all negative for papillomaviruses; unknown herpesviral DNA was detected in samples from three birds, but the inconsistent presence of the virus made this unlikely to be the sole etiologic agent.

An intriguing result of this study was the identification of *Micrococcus*-like bacteria invading the stratum corneum of juvenile flamingos. The bacteria had zoospores and segmented, branching pseudohyphae that were PAS positive and Gram negative.[12] In addition to the invading bacteria, the epidermis was characterized by heterophilic exocytosis, serocellular crust formation, and hydropic degeneration of the keratinocytes, particularly within the stratum granulosum.[12] The subjacent dermis was characterized by accumulations of heterophis and lymphocytes, increased vascular profiles, and increased intercellular matrix. Invasion of *Micrococcus*-like bacteria was present in all examined juvenile flamingos (except two severely autolyzed carcasses), but the bacteria were rare in biopsies taken from adult animals.[12]

The bacteria found in the diseased plantar skin was isolated, subjected to 16S rRNA sequencing, and found to be a novel species within the genus *Arsenicicoccus*, which was subsequently named *A. dermatophilus*.[5] The *Arsenicoccus* genus comprises gram-positive, facultatively anaerobic, catalase-posivie, nonspore forming bacteria that have been isolated both from the environment and from animal sources.[6]

In the 2015 study, histologic lesions were detected in both non-lesional and lesional skin, and the authors suggest that the proliferative lesions of flamingo pododermatisis are a reaction to the inflammation caused by *A. dermatophius*, which is given a leg up by a skin

barrier weaked by nutritional deficiencies and inappropriate husbandry practices.[12]

Conference participants were tickled pink with this visual feast of a slide. Leading discussion was this week's moderator, Dr. Neel Aziz, Supervisory Veterinary Medical Director and Head of the Diagnostic Pathology Unit at the Smithsonian National Zoological Park and Conservation Biology Institute. Dr. Aziz began by noting that diseases seen in zoological collections differ in kind and severity than thos seen in the wild, as this case illustrates. As noted by the contributor, pododermatisis is a disease of animal under human care; it is not seen in the wild. And this individual animal, at 28 years old, is an aged animal that has had time to collect more pathology than a typical wild flamingo.

Some conference participants were initially confused by the appearance of the amyloid on this slide which had a more basophilic hue than typica amyloid. Dr. Aziz noted that this amyloid can take on a variety of appearance, appearing more typically eosinophilic or more amphophilic, even within the same animal.

Dr. Aziz discussed the typical appearance of gout, which is characterized by the deposition of needle-like monosodium urate crystals that typically do not survive processing and leave behind only negative, ghost tophi shapes in histologic sections. By contrast, the gout appreciated in this case is consisted of amphophilic, amorphous material which the moderator speculated might represent an early stage lesion. Dr. Aziz noted that the terminology of "visceral" and "articular" should be reserved for the reptiles; in birds, "acute" versus "chronic" is the preferred terminology, with acute urate deposition occurring in the viscera

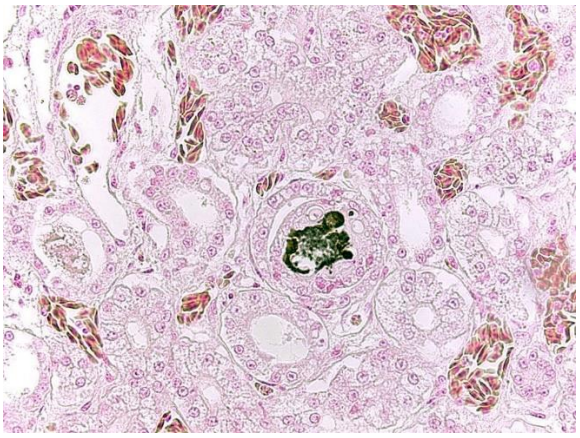

Figure 1-8. Kidney, flamingo. Brown to black granular deposits within renal tubules exhibit positive staining for calcium. (HE, Von Kossa)

of birds and chronic urate deposition occurring in the joints.

In an embarrassment of riches, Dr. Karen Terio, Clinical Professor in the Zoological Pathology Program at the University of Illinois, was also in attendance at conference this week. Dr. Terio noted that it can be overwhelming to describe a slide with this complexity. She recommended organizing such descriptions by pathogenesis and would start a description of this slide with glomerular changes, as the observed tubular changes are likely secondary to the glomerular amyloidosis.

Conference discussion produced a flock of morphologic diagnoses largely in agreement with the contributor. Participants discussed the interstitial nephritis at length and whether it was only in response to the urate deposition or if the inflammation also appeared in areas of the interstitium untouched by tophi. Participants thought it likely that the interstitial nephritis could not be completely explained by the urate deposition and thus preferred to describe it in a separate morphologic diagnosis.

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CASE II:

Signalment:
6-week-old male leopard tortoise (*Stigmochelys pardalis*).

History:
The animal presented with anorexia, lethargy, and yellow bumps along the skin. The animal was found deceased approximately 48 hours after onset of symptoms. There were 6 other clutch mates housed in the same enclosure with similar skin lesions, but without behavioral changes.

Gross Pathology:
The bilateral inguinal and gular skin is moderately thickened with fissures; ventrally and laterally, there are multifocal, pale tan-yellow, pinpoint, raised, nodular foci. No additional significant findings were observed.

Laboratory Results:
16S PCR and bidirectional Sanger sequencing identified the presence of *Austwickia chelonae* within the affected skin.

Microscopic Description:
The epidermis of the distal hind limb is multifocally overlain by lamellar, compact keratin admixed with dense mats of primarily filamentous but also fewer coccoid bacteria. The filamentous bacteria, along with fewer cocci, invade into the dermis and superficial skeletal muscle. Multifocally, the epidermis is eroded to ulcerated with sloughing and epidermal-dermal junctional clefting of the extant epidermis. The affected epidermis is often homogenously eosinophilic with pale cellular outlines (coagulative necrosis) and contains variably prominent intracellular and intercellular vacuolation (edema). Similar regions of coagulative necrosis are present within the dermis with low numbers of extravasated erythrocytes, scattered granulocytes, and occasional histiocytes. Venules and arterioles in these regions are often disrupted and necrotic with invasion of filamentous bacteria transmurally into the vascular lumen. Similar findings are seen within the pericloacal, gular, and cephalic skin.

Contributor's Morphologic Diagnosis:
Dermatitis, necrotizing, multifocal to coalescing, severe, chronic with abundant intralesional, gram-positive filamentous bacteria (morphology consistent with *Austwickia chelonae*) and fewer cocci.

Contributor's Comment:
Both the gross and histologic findings in this leopard tortoise are consistent with the PCR identification of *Austwickia chelonae*. *A. chelonae* is a gram-positive, filamentous, facultative anaerobic actinobacterium that can cause disease (austwickiosis) in both captive and free-ranging reptiles. Prior to 2010, this organism was categorized within the same genus as perhaps the best known member of the Dermatophilaceae, *Dermatophilus congolensis*.[3,5] First described in 1995 as *Dermatophilus chelonae*, the organism was later reassigned to the

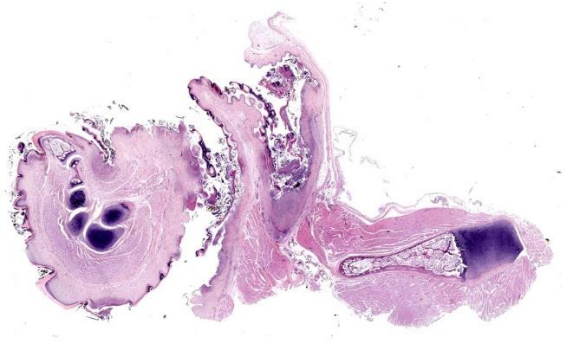

Figure 2-1. Leg, tortoise. There are areas of basophilic lamellar hyperkeratosis, with ulceration and accumulation of keratin debris within a fissure of the hyperplastic epithelium at top center. (HE, 4X)

newly created genus, *Austwickia* (Family Dermatophilaceae).[3,8] As part of its initial characterization using samples collected from captive chelonians in a western Australian Zoo, *A. chelonae* showed many unique features in comparison to *D. congolensis.* These included different biochemical characteristics, distinct morphology, and specific culture requirements, including optimal growth occurring at 27° C compared to 37° C for *D. congolensis*.[8] Most commonly, gross findings in chelonids consist of dermatitis with hyperkeratosis and progressive epidermal necrosis and ulceration often resulting in the formation of yellow to white nodules that can extend into the subcutis.[1,3] Although the extent of the potential host range of *A. chelonae* has not been fully determined, infections have been reported in numerous chelonian species, including captive desert (*Gopherus agassizii*), speckled cape (*Homopus signatus*), and sulcata (*Centrochelys sulcata*) tortoises.[3,7,9] Less frequently, *A. chelonae* has been implicated as a cause of septic arthritis and visceral granulomas in captive tortoises.[1,3] In captive, juvenile *C. sulcata*, *A. chelonae* infections were associated with extensive bone involvement with destruction of the nasal cavity and mandible.[9] In 2022, *A. chelonae* was documented for the first time in free-ranging chelonians in North America as a cause of cutaneous granulocytic granulomas in a wild gopher tortoise (*G. polyphemus*).[7]

Though the exact reservoir of *A. chelonae* has not been determined, the organism has been isolated from environmental soil and water sources.[4] It is suspected that initial local trauma to the skin, such as from tick bites or embedded foreign material, or inappropriate captive husbandry (high humidity or excessive soaking), are necessary to permit austwickiosis.[7,9] *A. chelonae* has recently been

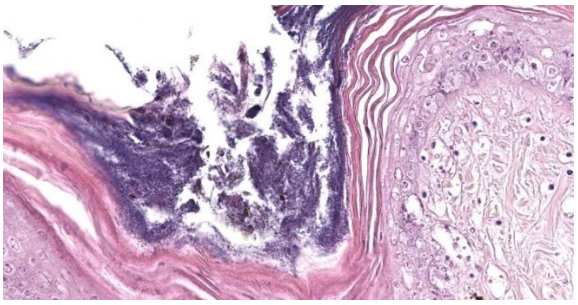

Figure 2-2. Skin of leg, tortoise. The most superficial layers of the dense lamellar keratin are replete with filamentous bacilli (and few cocci) that give it a dense blue color. (HE, 481X)

documented to encode a gene for a toxin similar to diphtheria toxin that had been previously only described within the genus *Corynebacterium*.[7] Diphtheria toxin terminates RNA synthesis and has been shown to notably interact with the host's immune system.[7] A presumptive diagnosis of austwickiosis may be made based on the combination of signalment combined with gross and histologic features using routine and special histochemical (Gram, GMS) stains. A definitive diagnosis of *A. chelonae*, however, requires PCR, culture, or metagenomic next-generation sequencing (mNGS).[3,4]

Austwickiosis is not restricted to chelonians. Captive inland bearded dragons *(Pogona vitticeps*) coinfected with *A. chelonae* and ranavirus presented with multifocal superficial necrotizing dermatitis.[10] The reported histopathologic changes in the skin of the affected bearded dragon were similar to those reported in this case.[9] Recurrent granulomatous dermatitis associated with PCR confirmed *A. chelonae* (syn. *D. chelonae*), was described in a king cobra (*Ophiophagus hannah*) in 2004.[6,11] In 2019, a study of the endangered Chinese crocodile lizard (*Shinisaurus crocodilurus*) revealed cutaneous lesions associated with *A.*

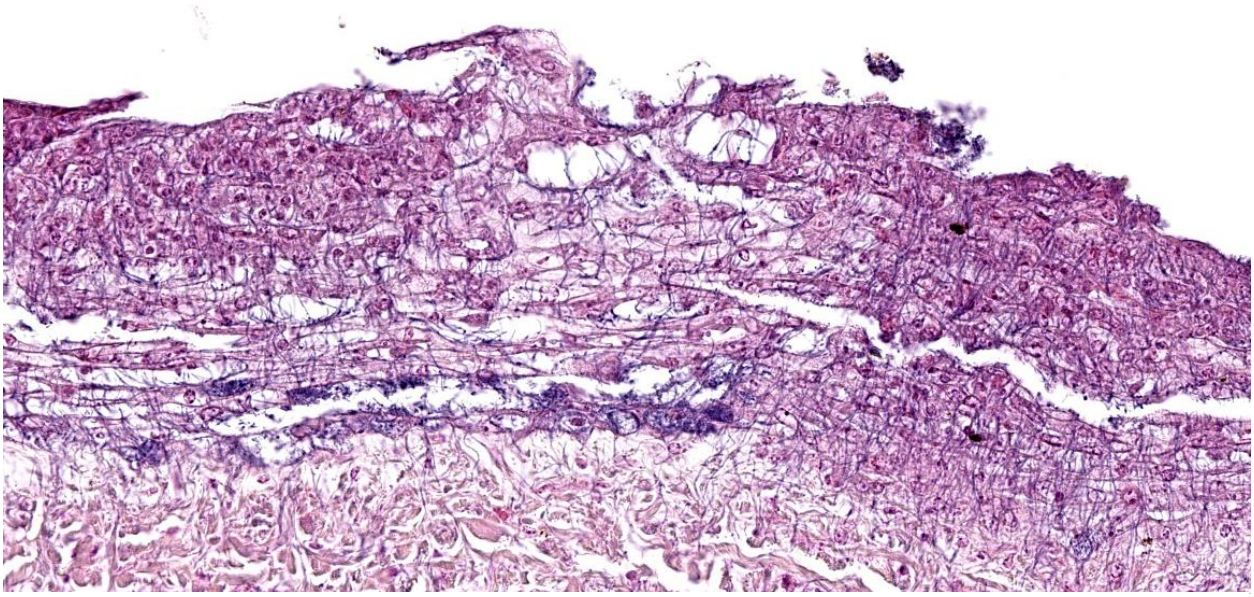

Figure 2-3. Skin, tortoise. There are areas of epidermal necrosis and ulceration with numerous filamentous bacilli penetrating into the underlying dermis. (HE, 91X)

chelonae.[4] Interestingly, experimental inoculation of the bacteria resulted in austwickiosis not only in Chinese skinks (*Plestiodon chinensis*), but also in domestic sheep, rabbits, and guinea pigs.[4] This suggests that *A. chelonae* has the potential to infect a wider range of species, including mammals, though natural infections have not been reported.

Infections by other members of the family Dermatophilaceae have been reported in reptiles, mammals, birds, and humans.[4] In crocodiles, reports of ventrally located brown skin lesions, colloquially called "brown spot disease", has been reported in young captive crocodiles (*Crocodylus porosus and C. novaeguineae*) in Papua New Guinea, farmed saltwater crocodiles (*C. porosus*) in Australia, and farmed American alligators (*Alligator mississippiensis*) in the southeastern United States.[2,3] Of the saltwater crocodiles evaluated in Australia, approximately 30% of animals with clinical dermatitis contained intralesional filamentous, *Dermatophilus*-like bacteria.[2,3] In lizards, dermatophilosis has been reported in captive bearded dragons confirmed as *D. congolensis* on culture. Dermatophilus-like organisms have been observed by light microscopy in a number of other species, including bush anoles (*Polychrus marmoratus*), collared lizards (*Crotaphytus collaris*), green iguanas (*Iguana iguana*), Senegal chameleons (*Chamaeleo senegalensis*), savannah monitors (*Varanus exanthematicus*), and panther chameleons (*Furcifer pardalis*).[3]

Contributing Institution:
University of Florida
College of Veterinary Medicine
Department of Comparative, Diagnostic, and Population Medicine
Gainesville, Florida
https://cdpm.vetmed.ufl.edu/

JPC Diagnosis:
Skin: Dermatitis, necrotizing, multifocal to coalescing, with hyperkeratosis and numerous filamentous bacilli.

JPC Comment:
The contributor provides an excellent, thorough summary of austwickiosis in reptiles and notes an unusual feature of this organism: the presence of a toxin, similar to diptheria toxin, previously unknown outside of the *Corynebacterium* genus.

Diptheria toxin (DT), produced by specific species of *Corynebacterium*, is the main virulence factor responsible for the respiratory, neurologic, and cardiopulmonary symptoms that characterize diptheria in humans.[12] DT is encoded on a bacteriophage which is integrated into the bacterial genome at specific sites. Regulation of DT expression is reliant on bacterial chromosome encoded diptheria toxin repressor genes, whose products can bind bacterial DNA sequences and block transcription and expression of DT.[12]

DT is considered the first example of the A-B class of toxins, an evolutionarily conserved motif found in almost all intracellular toxins.[12] DT is produced as an inactive proenzyme that is cleaved to its active form on encountering a target cell. The two resulting peptides are DT-A, the catalytic portion of the toxin, and DT-B, which is required for receptor binding and subsequent translocation of the toxin into the target cell by receptor-mediated endocytosis.[12]

Once inside the cell, the acidic environment of the endosome causes a conformational change in DT, which causes the DT-A catalytic portion of the toxin to insert into the endosome membrane, exposing DT-A to the cytosol.[12] DT-A catalyzes the transfer of an ADP compound from NAD+ to a histidine residue on elongation factor-2 (EF-2). EF-2 catalyzes the movement of ribosomes along mRNA during translation, making it essential for protein synthesis. When EF-2 is modified by the action of DT-A, the ribosome is unable to move, translation is halted, and the cell ultimately dies.[12] DT-A disables EF-2 in all eukaryotic species except mice and rats, and one DT-A molecule exposed to the cytosol is enough to kill a cell.[12]

Diptheria-toxin like genes were identified in 2018 in several bacteria outside of the *Corynebacterium* genus, including *Austwickia* and *Streptomyces* spp.[13] The key features of the toxin, including the catalytic and translocation domains, are conserved, but the receptor-binding domains are different, accounting for the different host ranges and cellular tropisms of the DT-like toxin in non-*Corynebacterium* organisms.[12] In addition, DT-like toxin is encoded in the bacterial genome of the new hosts rather than on bacteriophages, suggesting an alternate method of lateral gene transfer has occurred in these species.[12]

It is unclear if DT-like toxin has a role in the pathogenesis of *A. chelonae* infection; however, abundant necrosis is a hallmark of this disease in all species, raising the possibility that DT-like toxin causes the same cellular injury via the same mechanism as its DT ancestor.

Discussion of this case initially centered on the abundant autolysis which caused interpretive challenges for conference participants. Participants noted that the abundant filamentous bacteria were seemingly not accompanied by the a robust inflammatory reaction, prompting a lengthy discussion about whether the bacteria could represent post-mortem bacterial overgrowth rather than true pathogenic bacteria. On closer inspection, however, participant believed that some of the ghost cells

in the tissue are presumptive heterophils that have been rendered inapparent due to autolysis.

The organism itself cuts a striking filamentous figure in this slide. The moderator noted that this morphologic feature can be used to narrow the differential list considerably to the most commonly-encountered filamentous bacteria: *Actinomyces*, *Nocardia*, *Dermatophilus*, and *Streptobacillus*. The moderator also reminded participants not to neglect the bone marrow The bone marrow in this case appears mildly hypocellular, which comports with the observed mild inflammation. Dr. Terio noted that ambient temperature has a profound impact on the ability of poikilotherms to mount inflammatory responses and speculated that low body temperature might explain the disconnect between the level of inflammation and the extent of bacterial infection observed in this animal.

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CASE III:

Signalment:
1-year-old, gender not specified Nile crocodile (*Crocodiles niloticus*).

History:
Biopsy from a farmed yearling Nile crocodile (*Crocodiles niloticus*) that presented with multifocal to coalescing 3-5 mm ovoid ulcerated lesions on the abdominal ventrum. There were no associated clinical abnormalities.

Gross Pathology:
Multifocal to coalescing 3-5 mm ovoid ulcerated lesions on the abdominal ventrum.

Laboratory Results:
Lesions were PCR positive for crocodile pox.

Microscopic Description:
Focal erosion, ulceration and hyperplasia of the epidermis with thick adherent serocellular crust. Ballooning degeneration of keratinocytes is a feature and numerous eosinophilic intracytoplasmic inclusions are observed. There are marked perivascular to more widespread accumulations of admixed heterophils, lymphocytes, and macrophages within the subjacent superficial dermis.

Contributor's Morphologic Diagnosis:
Skin: Dermatitis, necrotizing and proliferative with ballooning degeneration and intracytoplasmic, eosinophilic inclusions consistent with poxvirus infection, Nile crocodile (*Crocodiles niloticus*), reptile.

Contributor's Comment:
The pathognomonic features of keratinocyte ballooning and eosinophilic intracytoplasmic inclusions, taken together with the clinical appearance and positive PCR result, are consistent with a diagnosis of crocodile poxvirus infection. Infection typically results in proliferative skin lesions approximately 1-3 mm diameter, grey to brown, and sessile or slightly depressed in contour. Lesions are variously encrusted, ulcerated or, less frequently, exophytic and wart-like and can occur over the entire body.[3, 4] Sequelae can include opportunistic infections by *Dermatophilus*-like bacteria or by fungi. The presence of pox virions can

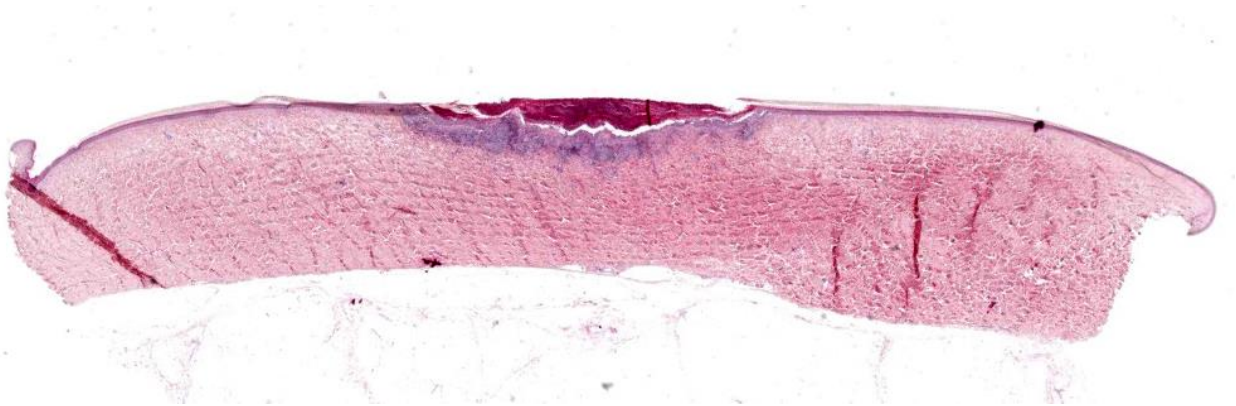

Figure 3-1. Skin, crocodile. A section of skin with a central area of necrosis is submitted for examination. (HE, 4X) (*Photo courtesy of*: Veterinary Sciences Centre, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland, http://www.ucd.ie/vetmed/)

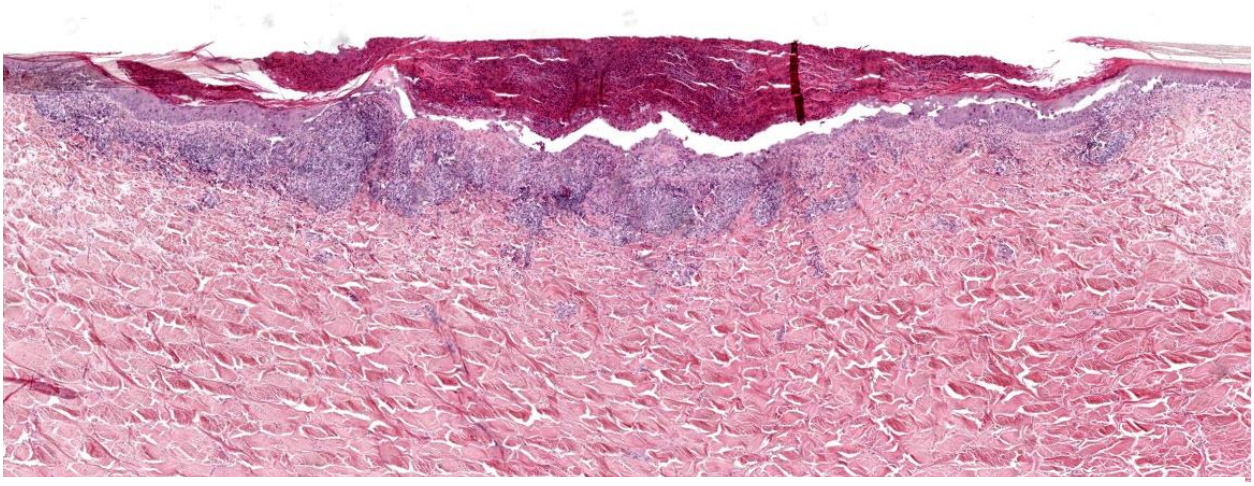

Figure 3-2. Skin, crocodile. There is full thickness necrosis of the epidermis in the center of the lesion. This area of necrosis is covered with a serocellular crust and there is a marked inflammatory infiltrate in the immediately subjacent superficial dermis. The crust extends over the periphery of the lesion. (HE, 33X)

be confirmed by electron microscopy with visualization of characteristic 100 nm by 200 nm virions with a dumbbell-shape with cross striations.[2,4]

While some members of the poxvirus family are host specific, others can infect many species.[5] The *Poxviridae* family is subdivided into the *Entomopoxvirinae* and *Chordopoxvirinae* subfamilies and members of the latter subfamily cause disease where skin lesions are the predominant clinical sign. Currently, 29 members of this subfamily are known to infect mammals, 10 are found in birds and one species is associated with disease in reptiles including several crocodilian species.[5]

Poxvirus-associated disease in reptiles has been described in caimans (*Caiman crocodilus*), Nile crocodiles (*Crocodilus niloticus*), and in both saltwater (*Crocodylus porosus*) and freshwater (*Crocodilus johnstoni*) crocodiles.[2] Although found in crocodilians worldwide, only three crocodile poxvirus genomes have been published to date and the source of infection remains unclear, although mosquitos have been suggested as potential vectors following infection of saltwater crocodiles.[1,6]

Contributing Institution:
Veterinary Sciences Centre
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JPC Diagnosis:
Skin: Dermatitis, necrotizing and proliferative, focal, moderate with epidermal ballooning degeneration and intracytoplasmic viral inclusions.

JPC Comment:
This small piece of tissue packs a big punch, managing to illustrate both the characteristic histologic lesions of poxviral dermatitis and the wide variety of hosts in which the disease can occur. While the *Poxviridae* family is familiar to most, it likely comes a surprise to some that the family includes a small, evolutionarily divergent group of poxviruses in the

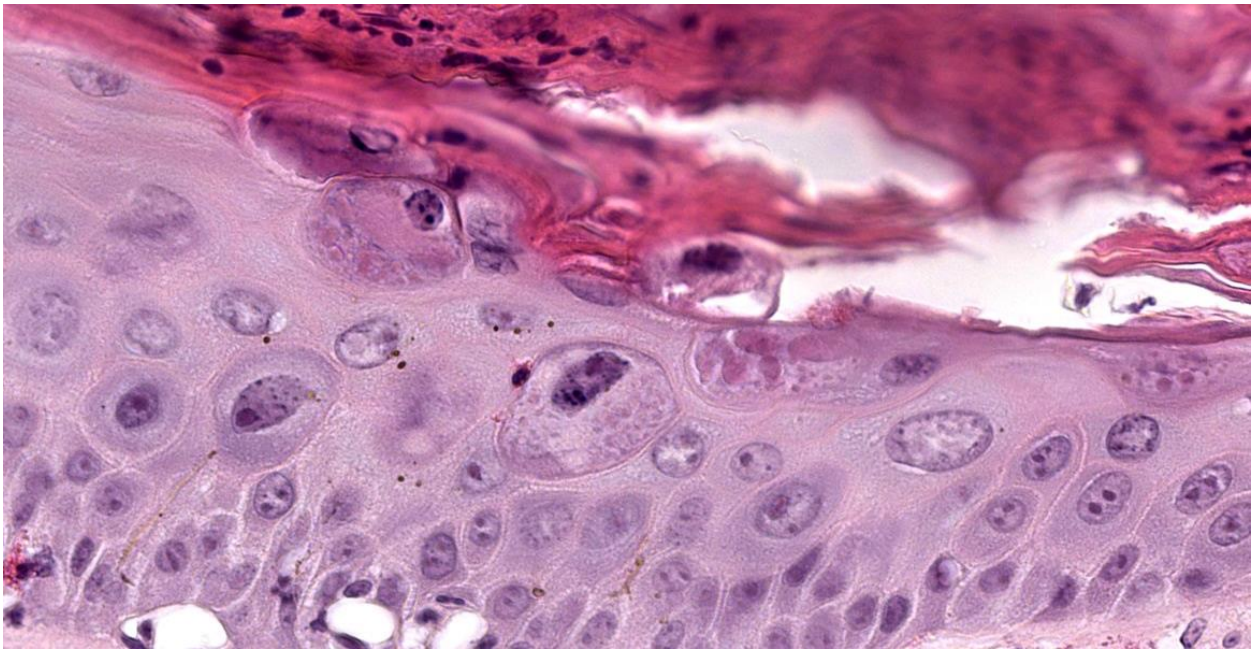

Figure 3-3. Skin, crocodile. Within the intact epithelium at the periphery of the lesion, keratinocytes within the stratum spongiosum are swollen with cytoplasmic clearing (ballooning degeneration), prominent nucleoli with prominent nucleoli, and several cells contain numerous pink cytoplasmic viral inclusions, consistent with poxvirus. (HE, 652X)

genus *Crocodylidpoxvirus* that affects the order Crocodylia worldwide.[5] The currently-characterized members of this group include Nile crocodilepox virus-1 (CPV) and saltwater crocodilepox viruses 1 and 2.[6]

CPV most commonly causes a nonfatal dermatitis with complete recovery which is mostly of economic importance to the commercial crocodile leather farming industry. Occasionally, however, more severe disease can develop with clinical signs of ophthalmia, rhinitis resulting in asphyxia, and debilitating illness with stunting and high mortality.[1] Histopathologic features are characteristic of poxviral infection generally (necrosis, hyperkeratosis, ballooning degeneration, and intracytoplasmic inclusion bodies) as nicely illustrated by this histologic section.

The progression of poxviral dermatitis lesions has been detailed in the saltwater crocodile and classified into four stages: early active, active, expulsion, and healing.[4] Early active lesions are 1-3 mm in diameter, white to grey foci of pinpoint of keratin damage. Early active lesions are histologically characterized by epidermal hyperplasia and hypertrophy with visible intracytoplasmic inclusion bodies and in intact overlying kertain layer.[4] With progression to the active stage, affected surface area increases and lesions develop a distinct raised outer contour with a depressed central core of abnormal keratin. The stage is characterized histologically by disruption of the superficial keratin layer and the development of a mild perivascular lymphocytic dermal infiltrate.[4]

In the expulsion stage, the central core of necrotic cells is released revealing a crust of necrotic inflammatory cells that given the gross lesion an orange/tan color; the underlying epidermis at this stage is of normal thickenss and

cellular morphology.[4] In the final healing phase, the lesion surface area decreases, with the surface keratin appearing abnormal, though with the color of normal skin. The lesions slowly become less visible during this phase, eventually returning to an almost normal macroscopic appearance.[4]

CPV is most phylogenetically related to molluscum contagiosum virus, but is rather distinct from other *Chordopoxviruses*.[1] CPV lacks many recognizable virulcence genes common to other *Chrodopoxviruses*, including interferon responses, intracellular signaling, and host immune response modulation. The CPV genome contains many putative genes which are speculated to perform these functions in novel ways, though none have yet been characterized.[1] CPV remains a bit mysterious, with seemingly familiar gross and histomorphologic lesions that belie a unique, recently diverged, and largely unknown biological armamentarium.

Conference participants had no difficult identifying the poxviral etiology for these lesions, though there was some discussion about the size, quantity, and character of the intracytoplasmic viral inclusions, which participants felt looked slightly different from normal poxviral inclusions. The moderator, who has extensive experience with crocodile pathology and husbandry, noted that this histologic section is an excellent, quality example of crocodile skin. The moderator also directed participants to the lateral edges of the section to appreciate normal crocodile skin, which typically has a very thin epidermis. Comparing the normal skin to the lesional skin highlights the degree hyperkeratosis that is typical of poxviral dermatitis in any species.

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CASE IV:

Signalment:

Adult female koala **(***Phascolarctos cinereus)*.

History:

A free-ranging koala was found in poor body condition, surrendered to a wildlife hospital, and euthanised on welfare grounds.

Gross Pathology:

Consolidation of the left caudal lung lobe with pleural adhesions.

Laboratory Results:

Heavy growth of anaerobic bacteria from the left caudal lung lobe. Cultured bacteria were identified by bacterial 16S rRNA gene as a novel *Actinomyces* spp.

Microscopic Description:
Lung: The normal lung parenchyma is effaced and replaced by branching coarse fibrocollagenous connective tissue (fibrosis), which dissects between multifocal to coalescing densely cellular infiltrates of neutrophils, macrophages, and lymphocytes. Inflammatory infiltrates often cluster around lakes of amorphous necrotic debris and intensely eosinophilic radiating material which surround or are admixed with fine filamentous bacteria (pyogranulomas with central club colonies and Splendore-Hoeppli material). There is moderate to marked irregular expansion of the pleura by immature granulation tissue, haemorrhage, fibrin, and leukocytes.

Bacteria are gram-positive, Ziehl-Neelsen and modified Ziehl-Neelsen negative. No organisms are visualized with PAS or Alcian blue stains.

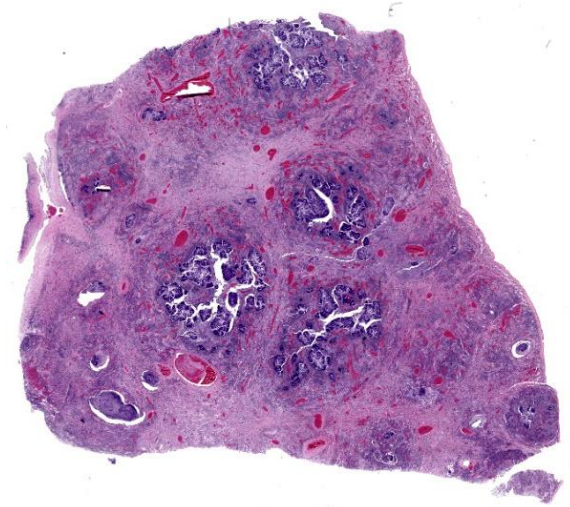

Figure 4-1. Lung, koala. One section of lung is submitted for examination. There are bacterial colonies and resulting inflammation within and effacing airways. (HE, 5X)

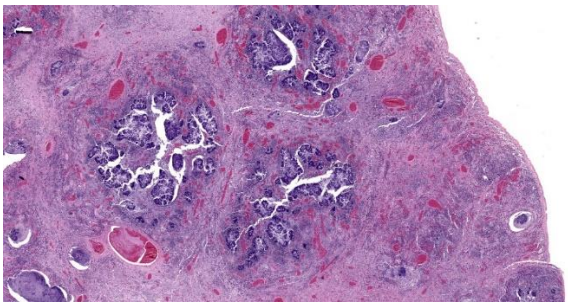

Figure 4-2. Lung, koala. Higher magnification of inflamed airways. (HE, 10X)

Contributor's Morphologic Diagnosis:
Lung: Severe chronic fibrosing pyogranulomatous pleuropneumonia with intralesional filamentous bacteria and Splendore-Hoeppli phenomenon.

Contributor's Comment:
In recent years, free-ranging and captive koalas (*Phascolarctos cinereus*) from the Mount Lofty Ranges of South Australia have been identified with chronic pyogranulomatous bronchopneumonia or lobar pneumonia, most frequently involving the left caudal lung lobe.[17] Within lesions, numerous gram-positive or gram-variable, non-acid-fast filamentous bacteria are observed in association with Splendore-Hoeppli phenomenon. Culture in this case yielded growth of anaerobic bacteria, subsequently identified by molecular techniques as a novel *Actinomyces* species (pulmonary actinomycosis).

Actinomyces is an anaerobic or facultative aerobic, gram-positive, filamentous bacteria, which is non-spore forming and non-motile.[14] *Actinomyces* species can be found in the normal healthy microbiota of the human oropharynx and gastrointestinal tract and on nasal, oral, or oropharyngeal mucosal surfaces of other animals and are most often associated with opportunistic infections.[4,14,18,20] Pulmonary actinomycosis is rare in animals but has

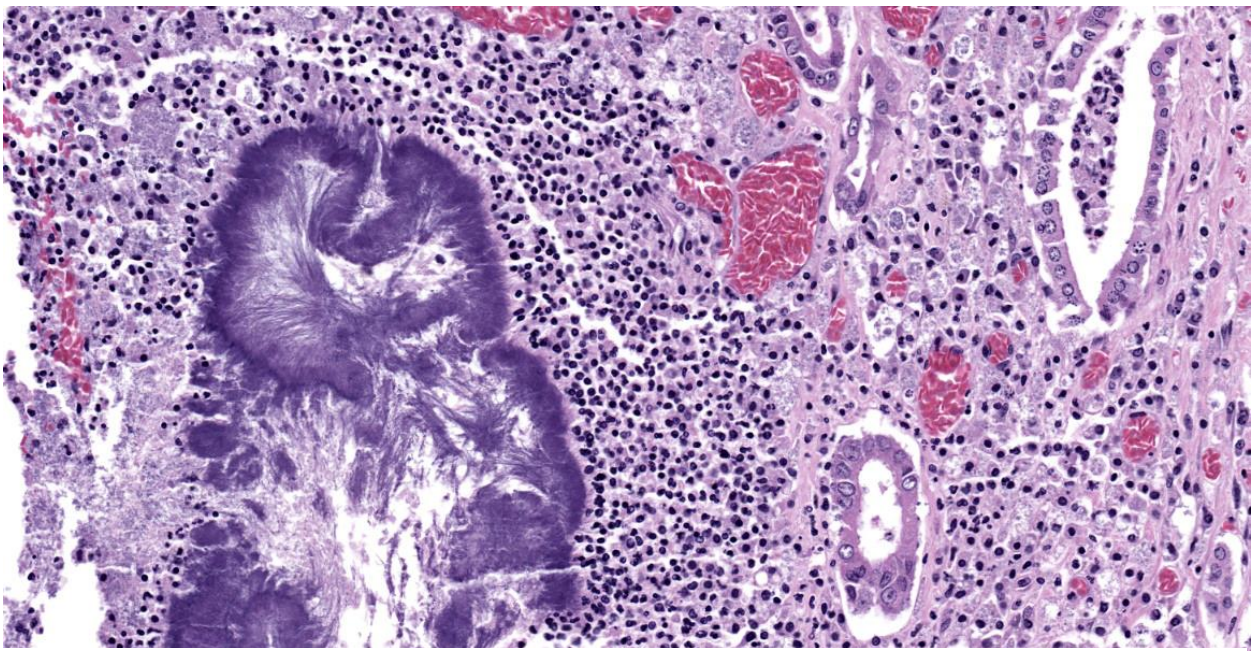

Figure 4-3. Lung, koala. High magnification of aggregates of filamentous bacteria surrounding by neutrophils and cellular debris. (HE, 252X)

been described in a small number of free-ranging species including two chamois (*Rupicapra rupicapra*),[15] a black-tufted marmoset (*Callithrix penicillata*),[16] and in a captive red kangaroo (*Osphranter rufus*).[8]

Actinomyces spp. have been reported as commensals of the oral microbiome and associated with similar pulmonary lesions in other species. Examination of resin lung casts from healthy koalas suggests greater laminar flow of air to the left caudal lung lobe in koalas.

Considering the predilection for involvement of the left caudal lung lobe observed in multiple in koalas with this condition, aspiration is suggested as the likely cause in at least some cases of pulmonary actinomycosis in koalas.

Other pathogens reported to cause pneumonia in koalas include *Bordetella bronchiseptica*,[3,11] *Chlamydia* spp.,[1,5,9] *Cryptococcus gattii* (previously *Cryptococcus neoformans* var. *gattii*),[3,7] *Pseudomonas aeruginosa*,[2,13] *Nocardia asteroides*,[19] *Staphylococcus epidermidis*,[19] *Mycobacterium ulcerans*,[12] and parasitic pneumonia associated with *Marsupistrongylus* sp.[10]

Contributing Institution:
Veterinary Diagnostic Laboratory
School of Animal and Veterinary Sciences
University of Adelaide
Roseworthy, South Australia
https://sciences.adelaide.edu.au/animal-veterinary-sciences/

JPC Diagnosis:
Lung: Bronchopneumonia, pyogranulomatous, chronic, diffuse, severe, with colonies of filamentous bacilli and Splendore-Hoeppli material.

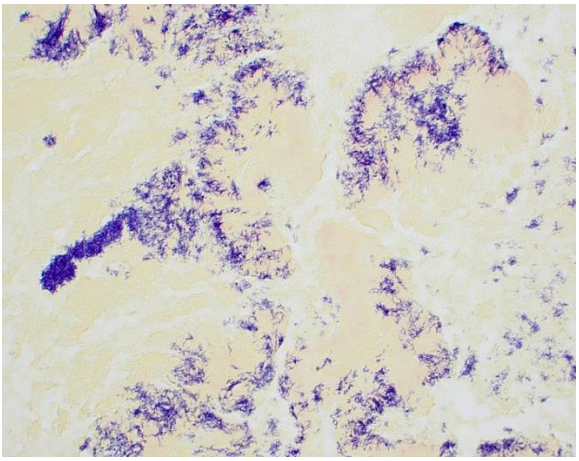

Figure 4-4. Lung, koala. Filamentous bacteria are gram-positive. (BB, 200X)

JPC Comment:

This stunning histologic slide is a remarkable example of bacterial pneumonia caused by *Actinomyces*, one of the members (along with *Yersinia, Actinobacillus, Corynebacterium, Streptococcus, and Staphylococcus* spp.) of the "YAACSS" large colony forming bacteria. As the contributor nicely describes, pulmonary actinomycosis is an increasingly recognized disease of koalas, and the history and gross and histologic findings described in this case are characteristic.

The contributor provides an excellent summary here; a more fulsome discussion is provided in the published case series from which this case was taken.[17] Pulmonary actinomycosis in koalas affects the left caudal lung lobe in 82% of cases, and is the only affected lobe in 21% of cases.[17] In 43% of cases, both the left caudal and the right middle lung lobes are affected.[17] Resin cast studies provided a possible answer for this apparent site predilection as the left primary bronchus in the koala follows a relatively straight caudal path without branching into lobar bronchi until deep in the pulmonary parenchyma. In contrast, the right primary bronchus is less linear, branches from the trachea in a more lateral direction, and branches into lobar bronchi much earlier than the left primary bronchus.[17] Researchers speculate that the more linear bifurcation pattern creates greater laminar flow, essentially creating a more direct path for bacterial colonization of the left caudal lobe.[17]

Microbial culture and isolation were performed on five koalas from this case series and a variety of anaerobic agents were identified. PCR amplification and sequencing of the 16S rRNA gene matched most closely with *Actinomyces timonensis*, though due to only 95% sequence homology with the reference *A. timonensis* genome, researchers speculate that the etiologic agent could be a novel *Actinomyces* species.[17]

Pulmonary actinomycosis in South Australian koalas is occasionally accompanied by hypertrophic osteopathy.[6] In a recent case study describing this combination of lesions, imaging findings included periosteal reaction on multiple appendicular skeletal bones, including the scapula, humerus, ulna, radius, femur, tibia, fibula, and carpal bones. Gross findings included thick, roughened periosteum on the metaphyses and diaphysis of long bones, and histologic findings included proliferative trabecular bony spicules oriented perpendicular to the cortical bone.[6]

The moderator began discussion of this case by noting that tissue identification is difficult since the pulmonary architecture is almost entirely obliterated by the florid pyogranulomatous inflammation. The moderator noted that there are many clues to pathogen identity here, including the filamentous morphology of the bacteria, the pyogranulomatous nature of the inflammatory reaction, and the presence of Splendore-Hoeppli material.

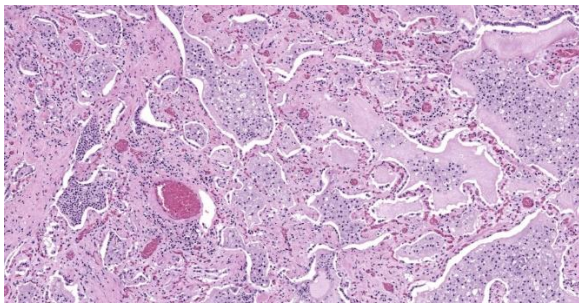

Figure 4-5. Lung, koala. The extensive fibrosis within the adjacent alveolar parenchyma has resulted in ectasia and an irregular shape to remaining alveoli ("traction emphysema") which are filled with alveolar macrophages and fewer neutrophils. (HE, 87X)

As discussed in the previous case of *Austwickia chelonae* dermatitis, the most commonly encountered filamentous bacteria are *Actinomyces*, *Nocardia*, *Dermatophilus*, and *Streptobacillus.* The moderator also noted a variety of conditions that are typically associated with Splendore-Hoeppli reaction, including fungal infections (sporotrichosis, zygomycosis, candidiasis, aspergillosis, blastomycosis, orbital pythiosis, pityrosporum folliculitis), bacterial infections (botryomycosis, nocardiosis, and actinomycosis), and parasitic infections (strongyloidiasis, schistosomiasis, and cutaneous larval migrans). The intersection of these two differential lists raises a clinical suspicion of actinomycosis even before the inevitable google reveals the susceptibility of koalas to pulmonary actinomycosis. Nevertheless, the moderator noted that *Chlamydia* spp. is an important rule-out in this case, and participants reviewed a Giemsa stained section that was convincingly negative. Participants were amazed at the florid koality of the inflammation in this case which, even for a koala, is pretty severe, causing participants to spectulate that the koala might be immunosuppressed.

Discussion of the morphologic diagnosis was straight-forward, with a short aside dedicated to whether the process could be described as necrotizing. In this case, the necrosis is due to inflammatory by-stander damage and not to virulence factors deployed by *Actinomyces*, so participants preferred a morphologic diagnosis of pyogranulomatous bronchopneumonia.

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