Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2024-2025

Conference #4 04 September 2024

CASE I:

Signalment:
4.5 month old, 27 kg neutered male pig (*Sus scrofa domesticus*).

History:
This pig arrived from a facility that had an outbreak of *Staphylococcus hyicus*, with several animals arriving with small lesions on the pinna, or developing lesions after arrival. Lesions progressed the longer the animals were housed in our facility.

Gross Pathology:
There is multifocal, regionally extensive erosion, erythema, and crusting affecting the head, pinna, the lateral left leg, and the lateral rump. There is moderate exudation and hypotrichosis in affected areas, with mild lichenification and fissures in the most severely affected regions.

Laboratory Results:
Gram stains show numerous colonies of gram positive cocci and gram negative bacilli within crusts, and extending into follicles.

Microscopic Description:
Haired skin, pinna and unspecified site: Affecting approximately 90% of the section, there is marked parakeratotic hyperkeratosis, with multifocal intracorneal pustules composed of necrotic cellular debris, necrotic neutrophils, and often individual to colonies of 1

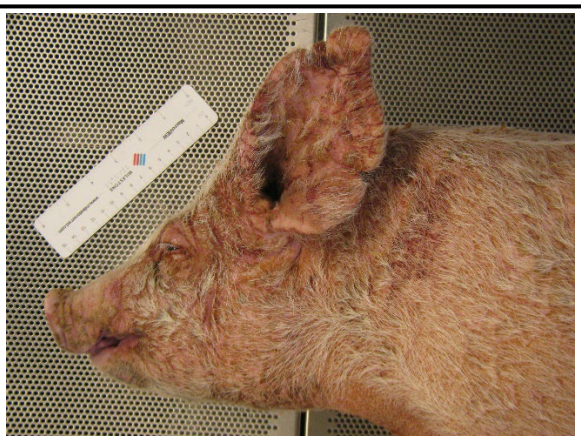

Figure 1-1. Haired skin, pig. There is multifocal to coalescing areas of erythema and crusting on the face, neck and pinna. (*Photo courtesy of:* Walter Reed Army Institute of Research, Department of Pathology, https://www.wrair.army.mil/)

um diameter basophilic cocci. There is multifocal elevation of the necrotic debris, forming a serocellular crust. There is multifocal ulceration, with necrosis of both the epidermis and subjacent dermis. Within the hyperplastic stratum corneum are also multifocal lakes of serum, transmigrating neutrophils, and degenerating and necrotic keratinocytes. Subjacent to the stratum corneum, there is marked acanthosis, with prominent intercellular and intracellular edema, and deep anastomosing rete ridges extending to the mid dermis. Follicles are multifocally dilated with luminal neutrophilic debris admixed with cocci adherent to the inner follicular epithelium. Within the dermis, there are perivascular to superficial histi

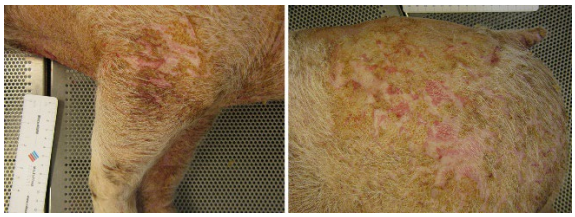

Figure 1-2. Haired skin, pig. There is multifocal to coalescing areas of erythema and crusting on the lateral left leg (left images) and lateral rump (images at right). (*Photo courtesy of:* Walter Reed Army Institute of Research, Department of Pathology, https://www.wrair.army.mil/)

ocytic and eosinophilic infiltrates, fewer lymphocytes and plasma cells, and mildly increased white space between bundles of collagen (edema). Multifocal lymphatics are ectatic, and apocrine glands are ectatic with attenuated epithelium.

Brown and Brenn stain: There are individual and colonies of gram positive cocci within the serocellular crusts, stratum corneum, and adherent to luminal follicular epithelium. There are also gram negative bacilli admixed with gram positive cocci.

Contributor's Morphologic Diagnosis:

1. Haired skin, multiple locations including ear: Epidermitis, exudative and proliferative, diffuse, severe, with intracorneal pustules, rare ulceration, mild chronic active superficial dermatitis, and intracorneal cocci, breed not specified, swine.
2. (Not submitted) Lymph nodes, multiple: No significant findings.

Contributor's Comment:

Exudative epidermitis, or "greasy-pig disease", primarily affects 5-35 day old piglets and is caused by *Staphylococcus hyicus*. The condition is also known as impetigo contagiosa suis, and seborrhea oleosa, and is occasionally caused by *Staphylococcus chromogenes* or *Staphylococcus sciuri*.[6] While this disease can have high morbidity and variable mortality, this is considered an important disease due to economic loss.[6] The bacterium is considered a commensal organism that survives on the skin and within hair follicles, most often not causing disease. However, with compromise to immune function from environmental stressors like overcrowding, transport, poor husbandry, an individual animal's susceptibility to this disease may increase.[8] If not strictly required, it is currently thought that damage to the epidermis from fighting, abrasions from housing, ectoparasitism, or concurrent vesicular disease allows entry of *S. hyicus* for colonization.[2]

The disease is classically categorized into three different presentations. In the acute form, lesions around the eyes, snout, chin, and ears appear rapidly, then spread to the medial aspect of the legs, thorax, abdomen, and coronets. As the stratum corneum of affected regions peels away, moist underlying epidermis and dermis are replaced by greasy, dark brown exudate. In the subacute form, the progression of disease is slower, resulting in thick, wrinkled skin that eventually shows a generalized furrowed appearance. The chronic form affects older piglets, with milder disease, usu-

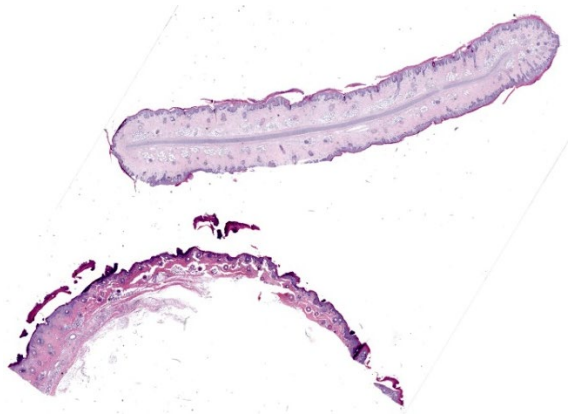

Figure 1-3. Haired skin, pig. Two sections of haired skin are submitted for examination. At this magnification, there is mild diffuse hyperkeratosis and crusting which at the section of bottom, are often detached as a result of processing. (HE, 5X)

ally erythema and waxy brown crusts confined to the head and ears. Older piglets often survive the disease but may have stunted growth.[6]

The most important virulence factors identified to date include a number of exfoliative toxins, *ExhA*, *ExhB*, *ExhC*, *ExhD*, *SHETA*, and *SHETB*. The *Exh* family toxins digest desmoglein-1 in the epidermis, affecting the effectiveness of desmosomes.[6] There are also a number of fibronectin-binding proteins on the surface of the bacteria that allow for adhesion to fibronectin in collagen, fibrin, and heparan sulfate proteoglycans found in the skin. Once the bacteria are established in the dermis, the infection often spreads to hair follicles, causing suppurative dermatitis with sebaceous gland hyperplasia and increased secretion (i.e. the "greasy" aspect of the disease).[7]

Lesions usually progress to exudative dermatitis starting in the groin, axillae, caudal auricular, and traumatized areas. Haired areas are more often affected, but lesions may also arise on the tongue and oral mucosa.[2] While not specific for the disease, a number of histologic features would support the diagnosis, such as subcorneal vesicular to pustular dermatitis, purulent luminal folliculitis, variable acanthosis with elongated rete ridges, intracellular edema of the stratum spinosum, and orthokeratotic and parakeratotic crusts with lakes of serum, accumulations of neutrophils, debris, and colonies of gram-positive cocci.[6]

The virulence and antibiotic resistance of *S. hyicus* continues to change as a function of its environment. A comparison of isolates from Brazilian swine herds in the 1980's and 2012 highlights significant shifts of *ExhA* and *ExhB* expression, as well as different antibiotic resistance profiles.[7] As antimicrobial peptides are investigated to combat increasing antibiotic resistance to macrolides, B-lactams, tetracycline, sulfonamides, and streptomycin, some compounds such as lactoferricin (Lfcin)

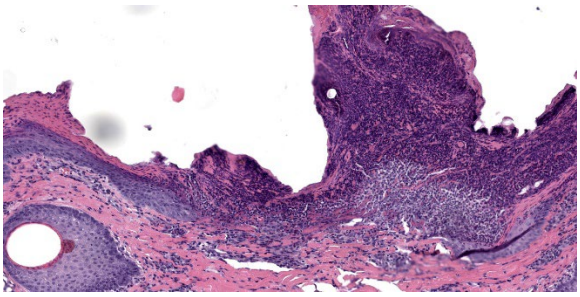

Figure 1-4. Haired skin, pig. Two sections of haired skin are submitted for examination. At this magnification, there is mild diffuse hyperkeratosis and crusting which at the section of bottom, are often detached as a result of processing. (HE, 5x)

have recently shown effectiveness in treating *S. hyicus* in a mouse model. Lfcin disrupts the bacterial cell wall and was effective in reducing both the bacterial load and levels of proinflammatory cytokines TNF-a, IL-6, and IL-1B.[4] Other biologics with efficacy have been recently investigated as well, such as the insect defensin DLP4 from *Hermetia illucens* (black soldier fly),[5] fungal defensin NZL from *Pseudoplectania nigrella*,[3] and *Siphoviridae*-like bacteriophages.[9]

Contributing Institution:
Walter Reed Army Institute of Research
Department of Pathology
https://www.wrair.army.mil/

JPC Diagnosis:
Haired skin: Epidermitis and folliculitis, suppurative, subacute, diffuse, severe, with multifocal ulceration, pustules, and intracorneal cocci.

JPC Comment:
The moderator for this week's conference was Major Kelsey Fiddes who serves as the Chief of Resident Training at the JPC. Each year, conference 4 is the annual rite of passage for our new first-year residents, presenting their first WSC case. This first case is a classic entity that we have covered in WSC before (Case 1, Conference 9, 2008-2009 and

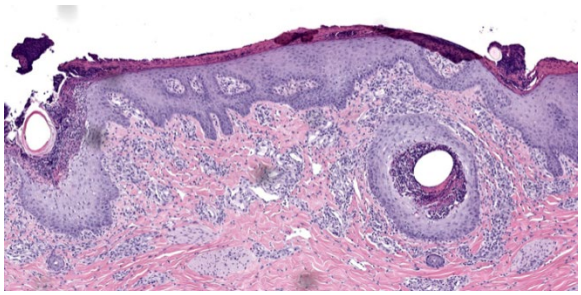

Figure 1-5. Haired, skin, pig. The hyperkeratosis and pustule formation extends down into the follicular ostia. (HE, 99X)

Case 3, Conference 8, 2009-2010) and the supplied section supplied is diagnostically rewarding.

As the contributor notes, the sebaceous gland hyperplasia elicited by *S. hyicus* may be the most visually obvious clue of this disease grossly, but there are several histologic features that should not be overlooked (Figures 1-4 and 1-5). In particular, the changes within the follicular infundibulum spanning the lumen and wall led us to include folliculitis with our morphologic diagnosis. In addition, the morphologic diagnosis focuses on epidermal changes, as the underlying dermis lacks significant changes. Gram stains (not particularly necessary in this case) highlight cocci within the serocellular crusts, stratum corneum, and adherent to luminal follicular epithelium similar to what the contributor reports.

As the contributor summarizes, the exfoliative toxins of *S. hyicus* play an important role in the pathogenesis of this disease. Other staphylococci such as *S. aureus* also produce serine protease exfoliative toxins;[1] these have been associated with human cases of bullous impetigo and staphylococcal "scalded skin syndrome"[6] which bear some similarity to exudative epidermitis of pigs. Microscopic lesions may be seen in other tissues due to exfoliative toxins. Renal lesions, including degeneration and/or exfoliation of the tubular epithelium, are a common sequela and do not require concurrent bacteremia. In cases of bacteremia, purulent pyelonephritis is a common finding[6] though it should be distinguished from other potential causes (e.g. *Actinobacillus)* that have a different pathogenesis.

References:

1. Bukowski M, Wladyka B, Dubin G. Exfoliative Toxins of Staphylococcus aureus. *Toxins*. 2010; 2(5):1148-1165.
2. Helke KL, Ezell PC, Duran-Struuck R, Swindle MM. Biology and Diseases of Swine. In: Fox JG, et al. eds. *Laboratory Animal Medicine*. 3rd ed. San Diego, CA: Elsevier. 2015:742-743.
3. Lui H, Yang N, Teng D, et al. Design and pharmacodynamics of recombinant fungus defensin NZL with improved activity against *Staphylococcus hyicus in vitro* and *in vivo*. *Int. J. Mol. Sci*. 2021; 22:5435.
4. Liu H, Yang N, Teng D, et al. Fatty acid modified-antimicrobial peptide analogues with potent antimicrobial activity and topical therapeutic efficacy against *Staphylococcus hyicus*. *Applied Microbiology and Biotechnology*. 2021; 105: 5845–5859.
5. Ma X, Yang N, Mao R, et al. The pharmacodynamics study of insect defensin DLP4 against toxigenic *Staphylococcus hyicus* ACCC 61734 *in vitro* and *vivo*. *Front Cell Infect Microbiol*. 2021; 11:638598.
6. Mauldin EA, Peters-Kennedy J. Integumentary System. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, Vol 3. 6th ed. Philadelphia, PA: Saunders Elsevier; 2016:630-632.
7. Moreno AM, Moreno LZ, Poor AP, et al. Antimicrobial resistance profile of *Staphylococcus hyicus* strains isolated from Brazilian swine herds. *Antibiotics*. 2022; 11(2):205.

8. Stanton JB, Zachary JF. Mechanisms of Microbial Infections. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 6th ed. St. Louis, MO: Elsevier; 2017:235.
9. Tetens J, Sprotte S, Thimm G, et al. First molecular characterization of *Siphoviridae*-like bacteriophages infecting *Staphylococcus h*yicus in a case of exudative epidermitis. *Front. Microbiol*. 2021; 12:653501.

CASE II:

Signalment:
Adult, female, file-eared tree frog (*Polypedates otilophus*)

History:
A captive, adult, female file-eared tree frog (*Polypedates otilophus*) was found dead.

Gross Pathology:
Approximately 70% of the left cornea and 80% of the right cornea contains white, multifocal to coalescing, irregularly marginated, and opaque material.

Figure 2-1. Eye, tree frog. Approximately 80% of the cornea is expanded by a dense opaque white material (lipid). (Wildlife Conservation Society, Zoological Health Program; https://oneworldonehealth.wcs.org, www.wcs.org)

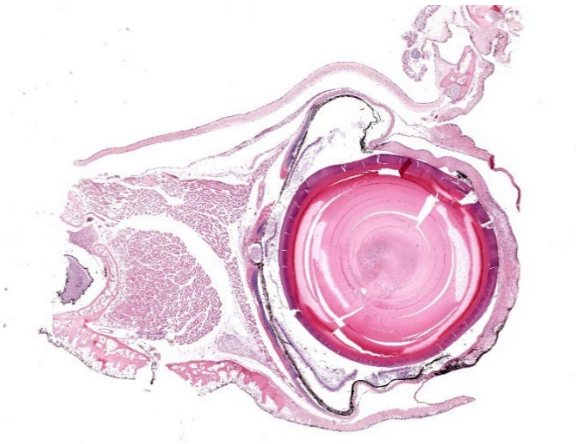

Figure 2-2. Eye, tree frog. One section of eye with attached lids is submitted for examination. At subgross magnification, the cornea is markedly expanded by clear space and at the ventral aspect, abundant pigment. (HE, 5X)

Microscopic Description:
Diffusely expanding the corneal stroma, extending to the limbus, and mildly elevating the overlying corneal epithelium are hundreds of coalescing, clear, acicular clefts (cholesterol clefts), interspersed with fewer individual lymphocytes and histiocytes. Histocytes are occasionally expanded by small to moderate amounts of intracytoplasmic finely vacuolated and clear material (lipid), with an eccentrically displaced nucleus. The corneal stroma occasionally contains small-caliber blood vessels (neovascularization) interspersed with inflammation and rare individual extravasated erythrocytes. The corneal epithelium is multifocally thickened up to 5 cells thick (epithelial hyperplasia), attenuated, and rarely absent. An adjacent nerve within the section is infiltrated by small numbers of scattered lymphocytes and rare individual histiocytes.

Contributor's Morphologic Diagnosis:
Eye, Keratitis, xanthomatous, chronic, diffuse, severe, with cholesterol clefts and neovascularization.

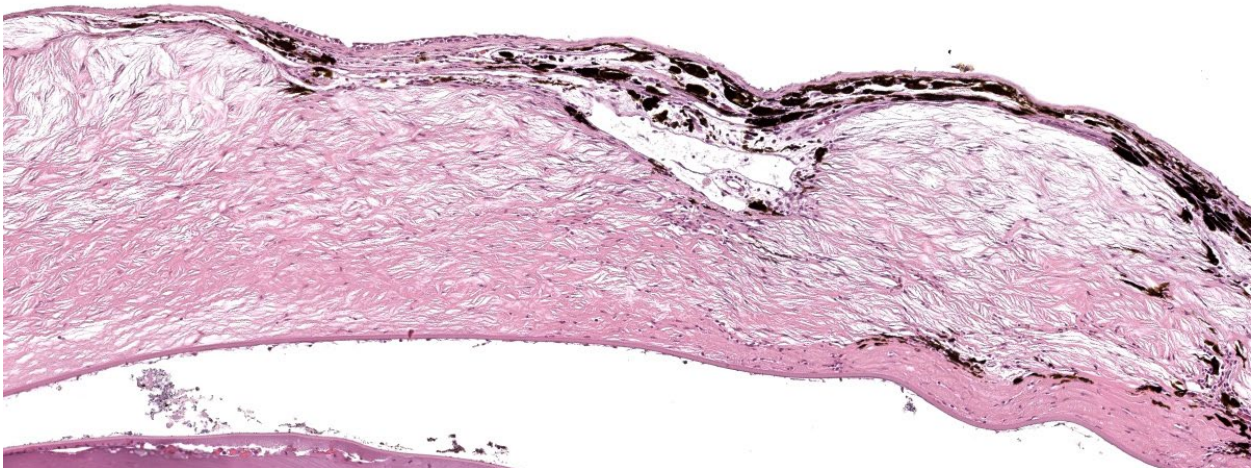

Figure 2-3. Eye, tree frog. The corneal stroma is expanded by innumerable clear acicular clefts (lipid). The overlying epithelium is infiltrated by large numbers of melanin-containing macrophages which occasionally infiltrate the underlying corneal stroma. (HE, 106X)

Contributor's Comment:
Bilateral corneal opacities noted at gross necropsy in this captive, adult, female file-eared tree frog (*Polypedates otilophus*) correlated to microscopic evidence of severe corneal lipid deposition. Given the species affected and the apparent absence of underlying ocular pathology, a diagnosis of lipid keratopathy was favored.

Corneal lipid deposition, also referred to as lipid keratopathy or corneal lipidosis, is a commonly encountered ocular disorder of captive amphibians.[2] While initially reported in Cuban tree frogs, lipid keratopathy has since been identified in multiple anuran species.[1,2,5,6] The disease presents clinically as a circumferential accumulation of white infiltrative material extending across the cornea over the course of weeks to months.[6] Lesions begin as small white foci at the corneal limbus and as disease advances can extend centrally to involve up to one-half of the corneal circumference.[5,6] Vision may become compromised in severe disease, affecting thermoregulation and ability to detect food.[8] In this case, the frog remained in good body condition, suggesting it was still able to find food despite the presence of corneal lesions.

Corneal lipid deposition is typically bilateral, and the corneal surface may become raised, thickened, and irregular from lipid deposition, grossly appearing as white plaques or nodules.[3,8,9] Early lesions are characterized histologically as small numbers of cholesterol clefts and foamy macrophages within the corneal stroma. As disease progresses, lipid accumulation can become associated with variable degrees of inflammation, neovascularization, and fibrosis.[4] Underlying ocular disease is typically not identified within affected cases.[6] Special stains including oil red O and Sudan black B can be used to highlight lipid within the corneal stroma.[6]

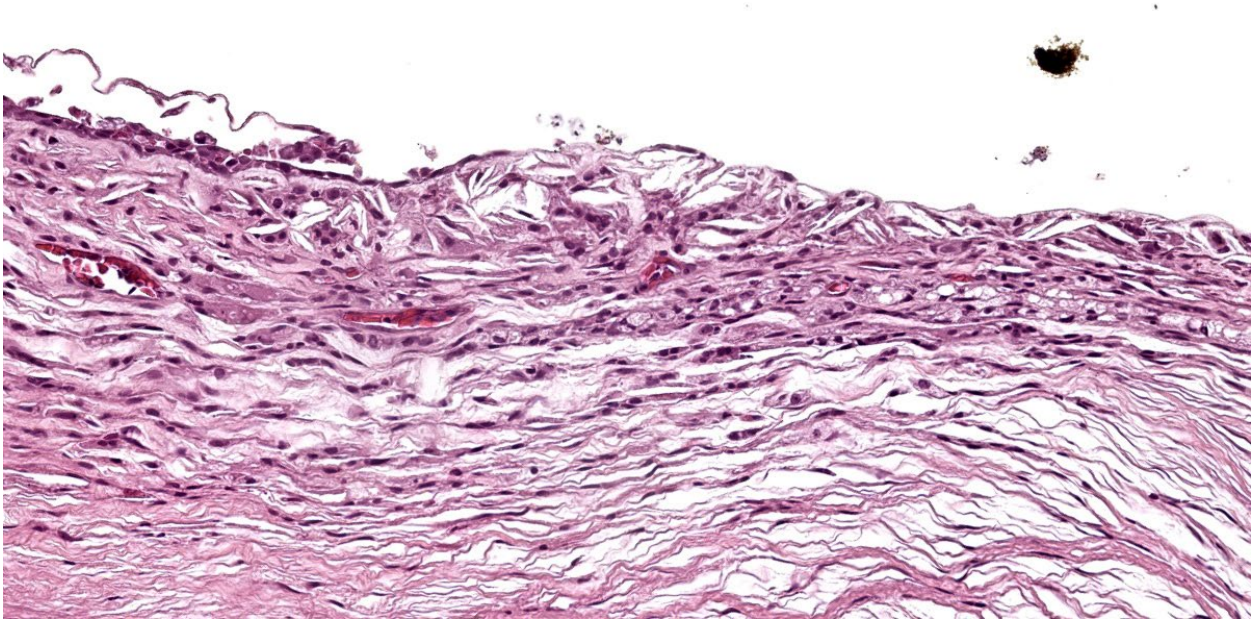

Figure 2-4. Eye, tree frog. In areas in which the corneal epithelium is ulcerated, there is vascularization of the underlying lipid-laden stroma with infiltration of low numbers of granulocytes. (HE, 240X)

The inciting cause for corneal lipid deposition in captive anurans is suspected to be nutritional and associated with variations in diet lipid composition. In one experimental study, corneal lipid deposition was found to be more prevalent in frogs fed high-cholesterol diets.[6] Furthermore, captive frogs fed both normal and high-cholesterol diets were found to have a higher serum total cholesterol than wild frogs, suggesting there may be an association with diets in captivity and disease development. Some reports also identify an increased prevalence of corneal lipid deposition in female anurans.[2,5]

In some species of amphibians, corneal lipid deposition has been reported in conjunction with disseminated xanthomatosis.[1] Xanthomas are non-neoplastic masses comprised of cholesterol and associated granulomatous inflammation, which form via extravasation of lipids.[10] Xanthomas have been reported in a wide range of taxa, can occur in multiple tissues, and can be associated with elevations in serum cholesterol.[5] Anurans diagnosed with xanthomatosis have been reported to have associated lesions involving the cornea, central nervous system, peripheral nerves, multiple visceral organs, and periarticular and digital soft tissues.[2,4] In this case, a xanthoma was identified in the choroid plexus.

Contributing Institution:
Wildlife Conservation Society, Zoological Health Program
https://oneworldonehealth.wcs.org
www.wcs.org

JPC Diagnosis:
Eye, cornea: Lipidosis, chronic, focally extensive, severe, with multifocal ulceration, vascularization, and pigmentation.

JPC Comment:
This second case is descriptively simple which admittedly is a rarity for many of the eyeballs we cover in conference. The numerous clear acicular clefts within the cornea is an obvious feature of this case which nicely correlate to the submitted gross image.. The migration of pigmented epithelial cells from the limbus is

both a response to ulceration and an adaptive response to chronic irritation. Conference participants looked carefully through this section, but found little else of note. Changes in other parts of the globe were largely ascribed to autolysis, although a focal area of hyperplasia of the retinal pigmented epithelium suggests an area of antemortem retinal detachment. Although hemorrhage was not a feature corneal neovascularization in this case, it has been documented in this entity in other frog species.[3]

Although lipid keratopathy is an important differential in this species, there are other differentials for corneal opacity (or 'white eye' in general) across species that merit brief discussion.[7] Corneal dystrophy may be acquired or inherited and reflects abnormal lipid metabolism of corneal endothelial cells, stromal, cells, or epithelial cells (keratocytes). Affected animals may not have an underlying serum lipid abnormality; this has been described in dogs (among others, beagles, Siberian huskies, and collies) and rabbits (New Zealand White). Familial hyperlipidemia as a cause of lipid deposition is well known in Schnauzers and Watanabe rabbits. Other potential rule outs include hypopyon, and rarely, anterior staphyloma. Finally, corneal granulation tissue and/or mineralization secondary to inflammation and hypercalcemia should also be considered.

References:

1. Carpenter JL, Bachrach A Jr, Albert DM, Vainisi SJ, Goldstein MA. Xanthomatous keratitis, disseminated xanthomatosis, and atherosclerosis in Cuban tree frogs. *Vet Pathol.* 1986; 23(3):337-339.
2. Holmberg BJ. Ophthalmology of exotic pets. In: Maggs DJ, Miller PE, Ofri R, eds. *Slatter's Fundamentals of Veterinary Ophthalmology.* 4th ed. St. Louis, MO, USA: Elsevier; 2008: 427-441.
3. Moore BA, Gjeltema J. Once in a blue moon: Lipid keratopathy and intrastromal hemorrhage in a Mission golden-eyed tree frog (Trachycephalus resinifictrix). *Vet Ophthalmol*. 2019 Nov;22(6):933-936.
4. Pessier AP. Amphibia. In: Terio KA, McAloose D, St. Leger J, eds. *Pathology of Wildlife and Zoo Animals*. 1st ed. London, UK: Elsevier; 2018:921-951.
5. Russell WC, Edwards DL, Stair EL, Hubner DC. Corneal lipidosis, disseminated xanthomatosis, and hypercholesterolemia in Cuban tree frogs (*Osteopilus septentrionalis*). *J Zoo Wildl Med*. 1990; 21(1):99-104.
6. Shilton CM, Smith DA, Crawshaw GJ, et al. Corneal lipid deposition in Cuban tree frogs (*Osteopilus septentrionalis*) and its relationship to serum lipids: an experimental study. *J Zoo Wildl Med*. 2001; 32(3):305-319.
7. Wilcock BP, Njaa BL. Special Senses. In: Maxie MG, ed. *Jubb, Kennedy & Palmer's Pathology of Domestic Animals*. Vol 1. 6th ed. St. Louis, MO: Elsevier; 2016:407-508.
8. Williams DL, Whitaker BR. The amphibian eye: a clinical review. *J Zoo Wildl Med*. 1994; 25(1):18-28.
9. Wright K. Cholesterol, corneal lipidosis, and xanthomatosis in amphibians. *Vet Clin North Am Exot Anim Pract*. 2003;6(1):155-67.
10. Zak A, Zeman M, Slaby A, Vecka M. Xanthomas: clinical and pathophysiological relations. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2014; 158(2):181-188.

CASE III:

Signalment:

7-months-old, male, pit bull terrier, *Canis lupus familiaris*, canine.

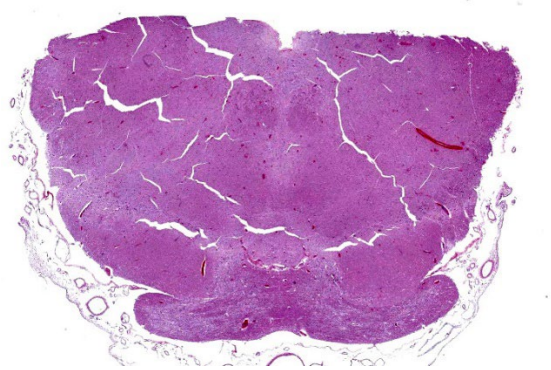

Figure 3-1. Brainstem, dog. One section of brainstem at the level of the pons is submitted for examination. (HE, 5X)

History:
The dog was taken into the animal shelter as a stray. It was found dead in the kennel the next morning.

Gross Pathology:
The dog was in good body condition with mild postmortem decomposition. The lungs were congested, heavy, and wet. The liver and spleen were congested.

Laboratory Results:
The affected blood vessels in the brainstem contained intact and degenerate endothelial cells that were positive for canine adenovirus using immunohistochemistry.

Microscopic Description:
There are multifocal hemorrhages in the neuropil of the brainstem. The capillaries and venules are dilated. The affected capillaries and venules are often lined by swollen endothelial cells and karyorrhectic endothelial cells. Multifocally, there are intranuclear inclusion bodies in the vascular endothelial cells. In a few areas, the tunicae media of the blood vessels are expanded by brightly eosinophilic, homogenous to beaded material admixed with scattered pyknotic and karyorrhectic debris (fibrinoid necrosis) and infiltrated by lymphocytes, and macrophages(vasculitis).

Contributor's Morphologic Diagnosis:
Brainstem – Vasculitis with endothelial cell necrosis, intranuclear inclusion bodies in endothelial cells, and perivascular hemorrhage; etiology, canine adenovirus - 1

Contributor's Comment:
Infectious canine hepatitis (ICH) is an uncommon disease of dogs that is caused by canine adenovirus-1 (CAV-1).[2-7] CAV-1 is a non-enveloped, icosahedral, double-stranded DNA virus.[6,7] CAV-1 can infect and cause disease in domestic dogs, wild canids, skunks, and bears.[2-7] In domestic dogs, ICH is uncommon due to the routine vaccination of dogs, but ICH can be seen in unvaccinated dogs.[2-7] ICH is typically seen in young dogs less than 1 to 2-years-old, but any dog not vaccinated for canine adenovirus can develop ICH. CAV-1 is antigenically related to canine adenovirus-2, which causes respiratory disease in dogs.
CAV-1 is secreted in the saliva, urine, and feces of infected dogs.[2,-7] Transmission to a naïve dog is by oronasal exposure from dog-to-dog contact or from the contaminated environment. After oronasal exposure, CAV-1 infects the tonsils causing tonsillitis. The virus will then spread to the regional lymph nodes and then to the blood causing viremia 4 – 9 days after exposure, which corresponds to the incubation period of ICH. Viremia of CAV-1 results in dissemination to the hepatocytes, endothelial cells, and mesothelial cells. Disease caused by CAV-1 can be divided into three syndromes.[4,6,7] Peracute disease that occurs within a brief illness that ranges from 3 – 48 hours and is characterized by circulatory collapse and death. Acute ICH is characterized by fever, depression, anorexia, vomiting, diarrhea, petechiae on the mucus membranes, pale mucus membranes, and mild icterus. Acute ICH can be fatal. The last syndrome of ICH is a mild chronic disease where the infected dog has partially immunity and may recover or die weeks to months later due to chronic liver disease.

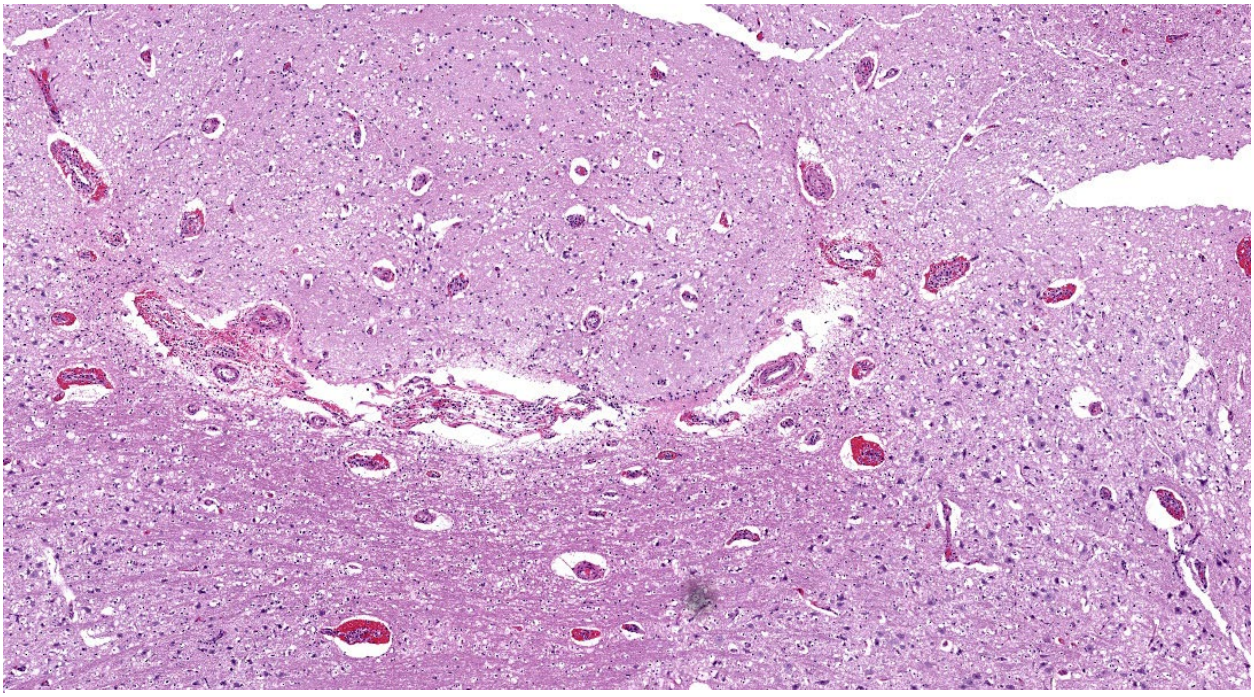

Figure 3-2. Brainstem, dog. Venules within the brainstem exhibit mural hemorrhage and extramural hemorrhage and edema. throughout the section. (HE, 44X)

CAV-1 infection of its target cells corresponds to the lesions seen grossly.[2-7] There can be enlargement and reddening of the tonsils and lymph nodes in the area. Petechiae on serosal surfaces and clear fluid in the peritoneal and surfaces can occur. The liver can be swollen, turgid, and friable with fine yellow mottling to a distinct accentuated reticular pattern. There can be fibrin on the surface of the liver. The gallbladder can be variably edematous, and gallbladder edema is considered to be pathognomonic of ICH. Icterus is mild when present. There can be hemorrhagic infarcts in the renal cortex as well as hemorrhages in the lungs. Hemorrhage and necrosis can occur at the metaphysis of long bones. The brainstem and midbrain can have hemorrhages in a small percentage of cases.[1-7] The late development of corneal edema due to a type III hypersensitivity reaction corresponding to increasing neutralizing antibodies can occur with ICH.[2,3,4,6,7]

The microscopic lesions of ICH in the liver are of centrilobular necrosis with intranuclear inclusion bodies in hepatocytes adjacent to the necrotic foci.[2-7] The necrotic foci contain small numbers of infiltrating leukocytes mainly neutrophils heterophils. The microscopic lesions in the other organs are the result of the endothelial cell injury secondary to infection with CAV-1. CAV-1 infection of endothelial cells in the renal glomeruli can cause glomerulonephritis, and infection of renal tubules cells can result in virus shedding in the urine. Pulmonary hemorrhages are secondary to vascular damage in the lung. Corneal edema is secondary to the immune response to CAV-1 infection of the corneal endothelium. When brain lesions due occur, there tend to occur in the brainstem and midbrain and consist of hemorrhages secondary to endothelial damage.[1,-7] Widespread endothelial damage can result in disseminated intravascular coagulopathy, which can also result in widespread

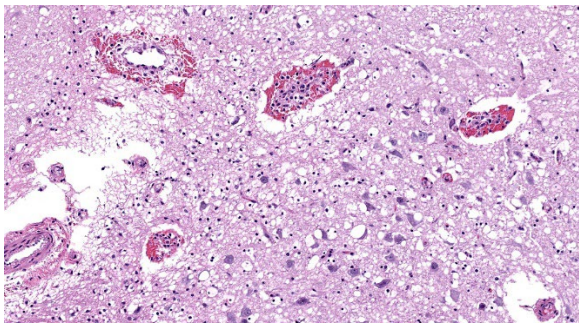

Figure 3-3. Brainstem, dog. Higher magnification of affected vessels. The extensive edema in the perivascular space has extended into the adjacent neuroparenchyma, resulting in spongiosis. (HE, 99X)

petechiae in serosal surface and multiple organs and death of the dog.[2-7]

Contributing Institution:
New Mexico Department of Agriculture Veterinary Diagnostic Services

https://nmdeptag.nmsu.edu/labs/veterinary-diagnostic-services.html

JPC Diagnosis:
Pons: Vasculitis, necrotizing, acute, diffuse, moderate, with rare neuronal necrosis, mild gliosis, and endothelial intranuclear viral inclusions.

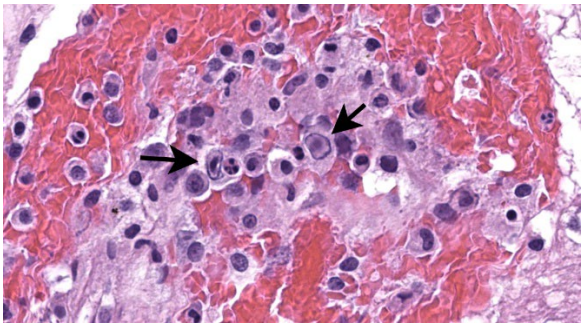

Figure 3-4. Brainstem, dog. Endothelial cells occasionally contain a large basophilic viral inclusion surrounded by a halo (arrow). (HE, 650X)

JPC Comment:
The contributor provides a succinct summary of CAV-1 and ICH. Connecting the dots to the present case, this section features diffuse vascular mural and transmural hemorrhage and edema attributable to endothelial intranuclear adenoviral inclusions. IHC for adenovirus performed by the contributor strongly and specifically labeled these endothelial cells, confirming the diagnosis. Conference participants felt that there was also microglial activation resulting from early parenchymal inflammation . These microglial cells were nicely highlighted by IBA1 (ionized calcium-binding adapter molecule 1) immunostain, which highlights dendritic cells, macrophages, and in the neuroparenchyma, microglia. Early characterization of this protein was performed in rat brain microglia.[9] In our experience, IBA1 has generally worked well across species and expression seems to be reasonably conserved.

Neurologic manifestation of CAV-1 is rare in domestic canines[1,5]; the animal in this case was reportedly a stray with an unknown vaccination history.

References:

1. Caudell D, Confer AW, Fulton RW, Better A, Saliki JT, Fent GM, Ritchey JR. Diagnosis of infectious canine hepatitis virus (CAV-1) in puppies with encephalopathy. *J Vet Diagn Invest*. 2005;17:58-61.
2. Cullen JM and Stalker MJ. Liver and biliary system. In: Maxie GM, ed. *Jubb Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. vol 2. Elsevier; 2016.
3. De Jonge B, Van Brantegem L, Chiers K. Infectious canine hepatitis, not only in the textbook: a brief review and three case reports. *Vlaams Diergeneeskd Tijdschr*. 2020;89: 284-291.
4. Green CE. Infectious canine hepatitis and canine acidophil cell hepatitis. In: Greene CE, ed. *Infectious Diseases of the Dog and Cat*. 4th ed. Elsevier; 2012.

5. Hornsey SJ, Philibert H, Godson DL, Snead ECR. Canine adenovirus type 1 causing neurological signs in a 5-week-old puppy. *BMC Vet Res*. 2019 Nov 21;15(1):418.
6. Knowles DP. Adenoviridae. In: MacLachlan NJ and Dubovi EJ, eds. *Fenner's Veterinary Virology*. 4th ed. Elsevier; 2011.
7. Sykes JE. Infectious canine hepatitis. In: Sykes JE, ed. *Canine and Feline Infectious Diseases*. 1st ed. Elsevier; 2014.
8. Van Wettere AJ and Brown DL. Hepatobiliary system and exocrine pancreas. In: Zachary JF, ed. *Pathologic Basis of Veterinary Disease*. Elsevier; 2022.
9. Ito D, Imai Y, Ohsawa K, et al. Microglia-specific localisation of a novel calcium binding protein, Iba1. *Molecular Brain Research* 1998; 57(1): 1-9.

CASE IV:

Signalment:
8 years old, female, *Correlophus ciliatus* (formerly *Rhacodactylus ciliatus*), crested gecko

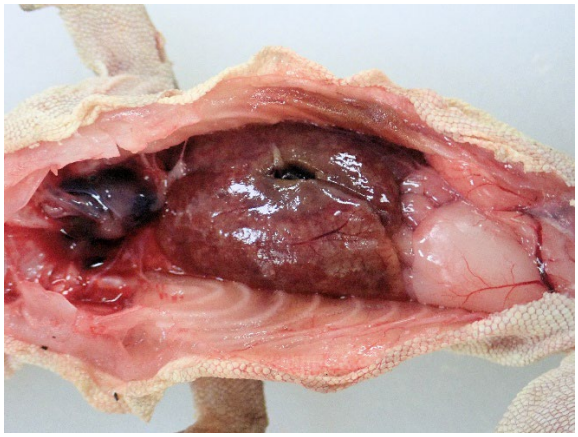

Figure 4-1. Liver, gecko. The liver was markedly enlarged, pale, firm and contained numerous coalescing variably sized tan foci (*Photo courtesy of:* New Mexico Department of Agriculture Veterinary Diagnostic Services, www.nmda.nmsu.edu/vds)

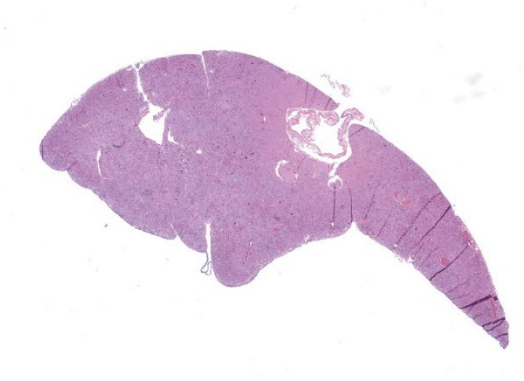

Figure 4-2. Liver, gecko. One section of liver is submitted for examination. (HE, 6X)

History:
The gecko was lethargic and sitting on the floor of the terrarium for prolonged periods instead of its normal climbing activity on the terrarium's vegetation. The owner noticed cutaneous ulcers on the gecko, which the owner treated topically. The gecko was found dead two days after clinical signs started.

Gross Pathology:
The carcass presented for postmortem examination was an 8 years old, 31.5 grams, female, crested gecko. The gecko was slightly underweight (normal weight of an adult female crested gecko is 35-55 grams) with moderate amounts of fat in the coelomic fat pads. The subcutis and organs were dry and tacky suggesting dehydration. The liver was markedly enlarged, pale, firm and contained numerous coalescing variably sized tan foci (Figure 1).

Microscopic Description:
The liver contained numerous small adult and larval rhabditid nematodes that were often surrounded by variably sized coalescing foci of necrosis admixed with macrophages, multinucleated giant cells, lymphocytes, and granulocytes. A few nematodes were fragmented and mineralized. The musculature of the nematodes was indistinguishable due to their small size. The nematodes had a rhabditiform esophagus with a corpus, isthmus and bulb (Figure

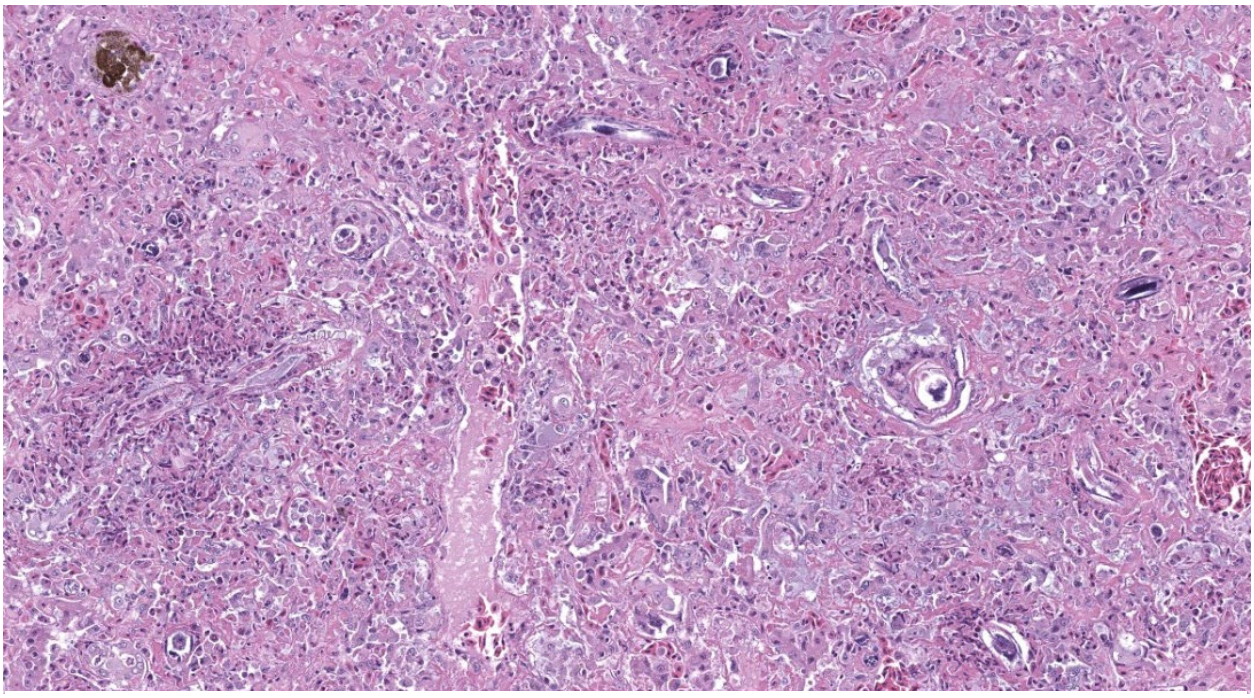

Figure 4-3. Liver, gecko. There is diffuse loss of normal plate architecture. Plate architecture is distorted by profound inflammation, moderate fibrosis, and numerous migrating rhabditoid adults, larvae, and rare eggs. (HE, 114X)

2). They had a single intestine and a paired genital tract (Figure 3). The genital tract contained large uninucleate ova as well as multinucleated ova (Figure 4). There were rare free multinucleated ova within the hepatic parenchyma (Figure 5). There was bile duct hyperplasia in the portal areas that were within the affected foci. The capsule was multifocally mineralized. In addition to the liver, rhabditid nematodes could be seen intravascular and within the parenchyma of the kidney (Figure 6), lung (Figure 7), brain (Figure 8), intestine, spleen, pancreas and ovary. These organs were not submitted.

Contributor's Morphologic Diagnosis:
Liver: Hepatitis, necrotizing, granulocytic and granulomatous, multifocal to coalescing with intralesional adult and larval rhabditid nematodes most consistent with *Strongyloides* species.

Contributor's Comment:
Endoparasitism is a frequent disorder of reptiles.[2,18] They are host to a large number of parasites including protozoa, trematodes, cestodes, nematodes, acanthocephalans, and pentastomes.[2,7,11,12,18] The most common two genera of rhabditiform nematodes to infect reptiles are *Rhabdias* and *Strongyloides* .[12,18] *Rhabdias* species are pulmonary parasites of anurans and reptiles.[1,12, 18] *Entomelas* species are rhabditid parasites of the lungs of some species of lizards.[18] *Strongyloides* are intestinal parasites of a large number of vertebrate species including mammals, birds, reptiles and amphibians.[1,12,14,16,17] *Rhabdias* species can be identified histopathologically by their intestine that has pigmented intestinal cells, vacuolated lateral chords, and females can have larvated ova in their paired uteri.[5] *Strongyloides* species also have a paired genital tract, do not have pigmented intestinal cells, and most species lay uninucleate ova that develop in the tissue or intestine.[5] The ova of

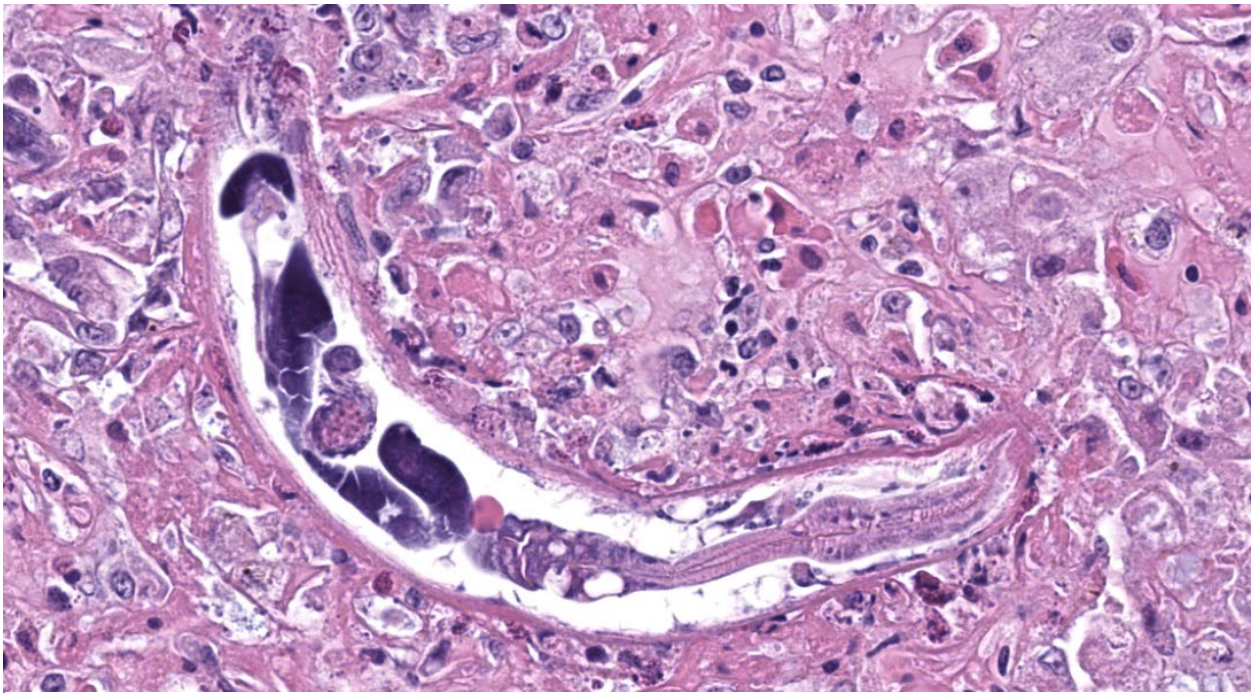

Figure 4-4. Liver, gecko. Tangential section of an adult rhabditoid nematode with esophagus with prominent corpus and bulb and a more distal triradiate esophagus and multiple sections of a deeply basophilic uterus. (HE, 572X)

Rhabdias species and *Strongyloides* species are embryonated in the feces of the host, and it is common to find free first stage rhabditiform larvae in the feces of infected hosts.[12,18] The embryonated ova and larvae of both species in the feces of the host are morphologically similar and difficult to impossible to distinguish from one another.[12,18]

As stated previously, *Rhabdias* species are lung parasites of anurans and reptiles.[1,9,12] The third stage larvae of *Rhabdias* species infect the host percutaneously or orally.[9] Oral infection mediated by eating infected prey is most likely important in reptiles with dry and scaly skin. After penetration into the connective tissue, the parasites molt into fourth stage larvae and enter the body cavity. The worms migrate to and enter the lungs as adult where they feed on the host's blood. The parasitic worms are female with some species reproducing by parthenogenesis or some species are hermaphrodites.[1] *Rhabdias* species ova can be laid by the female or the embryonated ova can develop within the body cavity of the female worm being release when she dies (matricidal endotoky). *Rhabdias* ova passed in the feces can develop by heterogony (first stage larvae molt twice to form third stage larvae of both sexes to become adults, mate and produce ova) or homogony (first stage larvae molt twice to become filariform third stage larvae that infect the host and mature into parthenogenetic females). Infected animals can exhibit respiratory distress, which can be severe.[12,18]

The intestinal parasite *Strongyloides* species can infect numerous vertebrate hosts.[1,14,16,17] The genus has been best studied in mammals. There appears to be some degree of host specificity with the different *Strongyloides* species, but some species like *S. stercoralis* can infect multiple mammalian species and have the potential to be zoonotic particularly between dogs and nonhuman primates and humans.[10,14] However, molecular techniques

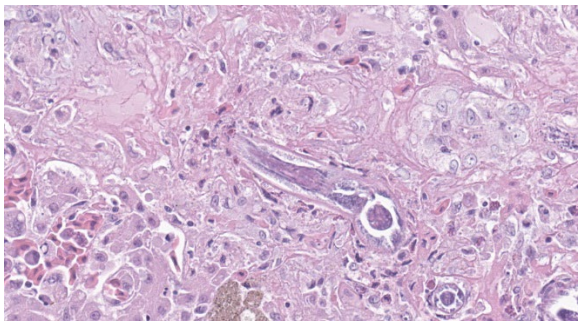

Figure 4-5. Liver, gecko. A single larva demonstrates the typical rhabditoid esophagus as well, with corpus, isthmus, and bulb. (HE, 600X) (*Photo courtesy of:* New Mexico Department of Agriculture Veterinary Diagnostic Services, www.nmda.nmsu.edu/vds)

have demonstrated that there are differences between the human and dog strains of *S. stercoralis* raising the question of whether the dog strains of *S. stercoralis* are truly zoonotic.[10,14]

The life cycle of *Strongyloides* species is similar to that of *Rhabdias* with one major difference; some *Strongyloides* species (particularly *S. stercoralis*, *S. fuelleborni*, and *S. fuelleborni kellyi* in humans) have the potential to autoinfect the host that can lead to infections that last decades.[8,10,13,15,16,17,19] The parasitic form of *Strongyloides* are parthenogenetic females. The female nematodes mostly live burrowed in the mucosa of the small intestine with some exceptions notably *S. tumefaciens* and occasionally *S. stercoralis* where the females live in nodules in the colon of cats.[14,19] The ova laid by the females embryonate in the mucosa and lumen of the small intestine and are passed in the feces as embryonated eggs or first stage rhabditiform larvae. The first stage rhabditiform larvae in the environment will molt twice to become third stage filariform larvae. The filariform larvae can then either penetrate the skin or mucosa of the mouth to infect the host or molt two more times to form adult free-living male and female worms. The adult free-living male and female worms can mate and their female offspring will molt to the infective filariform larvae to percutaneously or orally infect the host. Once inside the host, the filariform larvae can migrate to the small intestine or enter the vasculature where they migrate through the heart to the lungs. The filariform larvae in the lungs are coughed up and swallowed to reach the small intestine. When in the small intestine, the filariform larvae will mature into adult females. In autoinfection, the first stage rhabditiform larvae will molt twice in the large intestine to the infective third stage filariform larvae. The filariform larvae will penetrate the intestinal mucosa or perianal skin and migrate to the small intestine, which in some cases occurs through random organs. Humans and animals are initially exposed to infective filariform larvae of *Strongyloides* in a contaminated environment particularly in endemic areas.[17] The environment (water, foodstuffs, soil, and unwashed body parts) is contaminated by feces from an infected human or animal.[17] In addition, insects can mechanically carry ova of *Strongyloides*.[17]

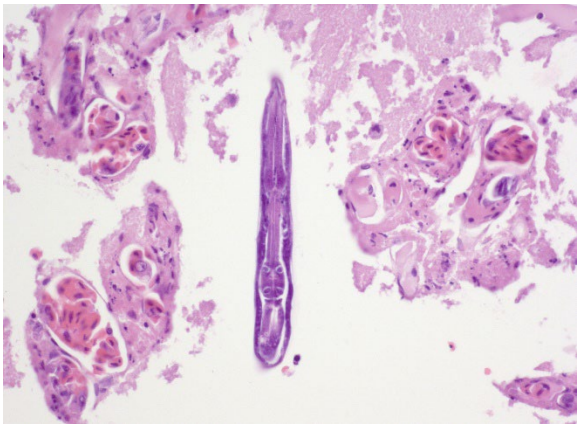

Figure 4-6. Liver, gecko. A single isolated larva demonstrates the typical rhabditoid esophagus as well, with corpus, isthmus, and bulb. (HE, 600X) (*Photo courtesy of:* New Mexico Department of Agriculture Veterinary Diagnostic Services, www.nmda.nmsu.edu/vds)

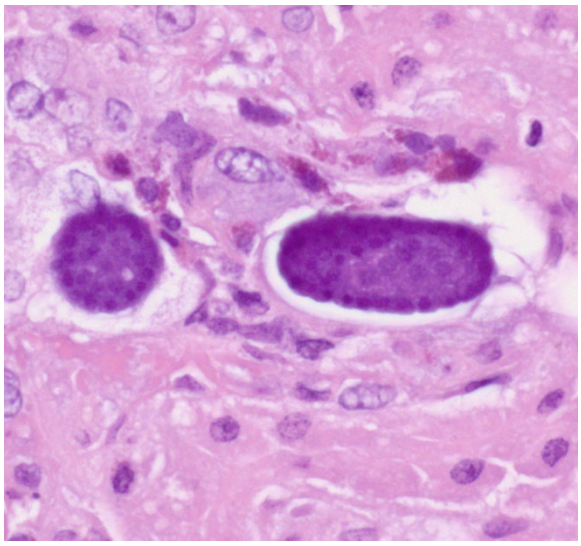

Figure 4-7. Liver, gecko. There are rhabditid eggs free within the parenchyma. (HE, 600X) (*Photo courtesy of:* New Mexico Department of Agriculture Veterinary Diagnostic Services, www.nmda.nmsu.edu/vds)

Clinical disease caused by *Strongyloides* species is typically mild and self-limiting.[13,14,15,16,19] It usually consists of diarrhea and respiratory disease that is most prevalent in individuals when they are initially exposed to the parasites. Naïve individuals exposed to infective filariform larvae can also develop dermatitis due to the parasites migrating through the skin. In domestic animals, disease caused by *Strongyloides* typically occurs in neonatal animals. In reptiles, particularly snakes, disease caused by *Strongyloides* can manifest as diarrhea, anorexia, weight loss and lethargy.[12,18] There are, however, cases of severe disease caused by *Strongyloides* species that have been described in humans and rarely dogs.[3,4,7,8,13,15,16] Severe disease caused by *Strongyloides* has been divided into two syndromes termed hyperinfection syndrome and disseminated disease, but some consider the syndromes to be the same.[8,13,15,16] Hyperinfection syndrome can occur in any infected individual, but is most often seen in immunocompromised individuals. Disseminated disease occurs in immunocompromised individuals. Both of these syndromes are often fatal. Hyperinfection syndrome is defined as a severe infection with *Strongyloides* nematodes in the normal locations of the parasites: the skin, the intestine, and the lungs. The time of completion of the life cycle of *Strongyloides* in hyperinfections is shortened. Disseminated strongyloidiasis occurs when *Strongyloides* parasites are found in organs outside of the normal life cycle (i.e., organs other than the skin, intestine or lungs). The gecko in this case most likely had disseminated strongyloidiasis.

Contributing Institution:
New Mexico Department of Agriculture Veterinary Diagnostic Services

www.nmda.nmsu.edu/vds

JPC Diagnosis:
Liver: Hepatitis, necrotizing and granulomatous, chronic, diffuse, marked, with intraparenchymal rhabditoid adults, larvae, and eggs.

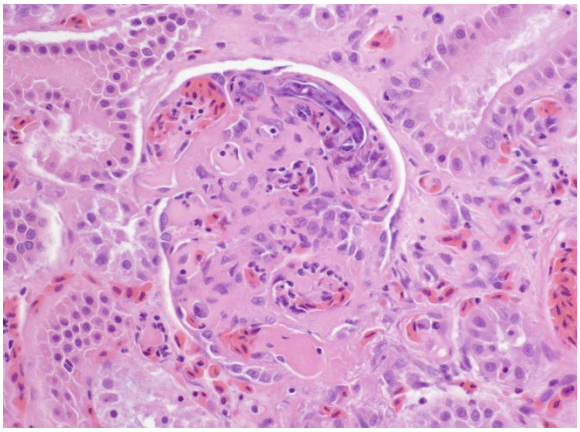

Figure 4-8. Kidney, gecko. A rhabditid larva is present within a renal glomerulus. (HE, 600X) (*Photo courtesy of:* New Mexico Department of Agriculture Veterinary Diagnostic Services, www.nmda.nmsu.edu/vds)

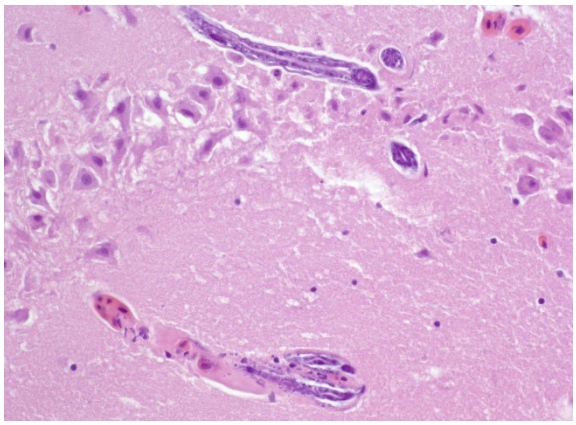

Figure 4-9. Brain, gecko. Rhabditid larva are present within the neuroparenchyma. (HE, 600X) (*Photo courtesy of:* New Mexico Department of Agriculture Veterinary Diagnostic Services, www.nmda.nmsu.edu/vds)

JPC Comment:
This conference concludes with a wonderful parasite case. We previously showed this slide to Dr. Chris Gardiner who confirmed the features of this nematode in histologic section and remarked that he had never seen a case like this one in over 50 years as a parasitologist! The presence of *Strongyloides* within the liver is unusual as they are typically encountered within the epithelial layer of the enteron, or less commonly, within the mucosa of the urinary bladder or the skin and lungs as the contributor notes.

Conference participants were able to appreciate most of the major features of adult and larval rhabditid nematodes. Careful examination of the slide is required to identify the few eggs scattered within the parenchyma.

Finally, we commend the contributor for their exhaustive discussion of *Strongyloides*. We have little to add on save for mentioning a recent *Veterinary Pathology* article concerning proliferative strongyloidiasis in a colony of colubrid snakes.[6] The histologic features of *Strongyloides* in these snakes was similar to the conference case, aberrant migration to the reproductive, respiratory and upper alimentary tracts, as well as the eye.[6] These findings in this paper highlight the utility of speciating the *Strongyloides* via molecular techniques to characterize novel species in the face of unexpected tissue distribution.

References:

1. Anderson RC. Nematode transmission patterns. *J Parasitol*. 1988;74(1):30-45.
2. Benson KG. Reptilian gastrointestinal diseases. *Semin Avian Exot Pet Med*. 1999;8(2):90-91.
3. Cervone M, Giannelli A, Otranto D, Perrucci. *Strongyloides stercoralis* hyperinfection in an immunosuppressed dog from France. *Revue Vétérinaire Clinique*. 2016;51(2):55-59.
4. Dillard KJ, Saari SAM, Marjukka A. *Strongyloides stercoralis* infection in a Finnish kennel. *Acta Vet Scand*. 2007;49,37.
5. Gardiner CH, Poynton SL. Rhabditoids. In: *An Atlas of Metazoan Parasites in Animal Tissues*. Armed Forces Institute of Pathology; 2006.
6. Graham EA, Los Kamp EW, Thompson NM, et al. Proliferative strongyloidiasis in a colony of colubrid snakes. *Veterinary Pathology.* 2024;61(1):109-118.
7. Graham JA, Sato M, Moore AR, *et al*. Disseminated *Strongyloids stercoralis* infection in a dog following long-term treatment with budesonide. *J Am Vet Med Assoc*. 2015;254(8):974-978.
8. Ippen R, Zwart P. Infectious and parasitic diseases of captive reptiles and amphibians with special emphasis on husbandry practices which prevent or promote diseases. *Rev Sci Tech Off Int Epiz*. 1996;15(1):43-54.
9. Kassalik M, Mönkemüller K. *Strongyloides stercoralis* hyperinfection syndrome and disseminated disease. *Gastroenterol Hepatol*. 2011;7(11): 766-768.

10. Langford GJ, Janovy Jr. J. Comparative life cycles and life histories of North American *Rhabdias* spp. (Nematoda: Rhabdiasidae): lungworms from snakes and anurans. *J Parasitol*. 2009;95(5):1145-1155.
11. Page W, Judd JA, Bradbury RS. The unique life cycle of *Strongyloides stercoralis* and implications for public health action. *Trop Med Infect Dis*. 2018; 3(2), 53;
12. Ratai AV, Lindtner-Knific R, Vlahović K, *et al*. Parasites in pet reptiles. *Acta Vet Scand*. 2011;53,33.
13. Šlapeta J, Modrý D, Johnson R. Reptile parasitology in health and disease. In: Doneley B, Monks D, Johnson R, Carmel B, eds. *Reptile Medicine and Surgery in Clinical Practice*. 1st ed. John Wiley & Sons Ltd.; 2018.
14. Tefé-Silva C, Machado ER, Faccioli LH, Ramos SG. Hyperinfection syndrome in strongyloidiasis. In: Rodriquez-Morales AJ, ed. *Current Topics in Tropical Medicine*. InTech; 2019.
15. Thamsborg SM, Ketzis J, Horii Y, Matthews JB. *Strongyloides* spp. infections of veterinary importance. *Parasitol*. 2017;144:274-284.
16. Vasquez-Rios G, Pineda-Reyes R, Pineda-Reyes J, *et al*. *Strongyloides stercoralis* hyperinfection syndrome: a deeper understanding of a neglected disease. *J Parasit Dis*. 2019;43(2):167-175.
17. Viney ME, Lok JB. *Strongyloides* spp. In: *WormBook*. 1-15; doi:10.1895/wormbook.1.141.1.
18. White MAF, Whiley H, Ross KE. A review of *Strongyloides* spp. environmental sources worldwide. *Pathogens*. 2019;8,91; doi:10.3390/pathogens803009.
19. Wilson SC, Carpenter JW. Endoparasitic diseases of reptiles. *Semin Avian Exot Pet Med*. 1996;5(2):64-74.
20. Wulcan JM, Dennis MM, Ketzis JK, *et al*. *Strongyloides* spp. in cats: a review of the literature and the first report of zoonotic *Strongyloides stercoralis* in colonic epithelial nodular hyperplasia in cats. *Parasit Vectors*. 2019;12,349.