Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2025-2026

Conference #2 27 August 2025

CA**SE I:**

Signalment:
28-year-old male castrated domestic pony *(Equus ferus caballus)*

History:
A pony from a zoo in Michigan was diagnosed with choke on January 17th, 2020. On January 22nd, endoscopy was performed, an esophageal tear was suspected, and the animal was treated with Uniprim, procaine penicillin G, and phenylbutazone. The pony became febrile with a 103˚F temperature and had multiple abscesses in the cervical area on ultrasound exam. The pony died with respiratory distress on January 28th when loaded on to the trailer for travel for repeat endoscopy.

Gross Pathology:
There was a 3 cm x 1.5 cm x 3.5 cm focal concretion of caseous material in the right serratus ventralis cervicis muscle. Fifteen to twenty multifocal similar lesions ranging from 6 cm x 0.75 cm x 0.5 cm to 2 mm x 2 mm x 1 mm were scattered throughout the cervical musculature. A 60 cm segment of serosa and tunica muscularis of the mid-esophagus was mottled black to purple. In the center of this affected area was a large cavitary space that extended approximately 10 cm into the surrounding cervical musculature and contained approximately 2 L of a suppurative exudate. The muscle surrounding the cavitary space was multifocally dark black and necrotic. There was no gross evidence of mucosal alterations in the esophagus, and no exudate was observed.

Laboratory Results:
PCR *for Acanthamoeba sp., Balamuthia mandrillaris*, and *Naegleria fowleri* were negative. Aerobic culture yielded growth of moderate

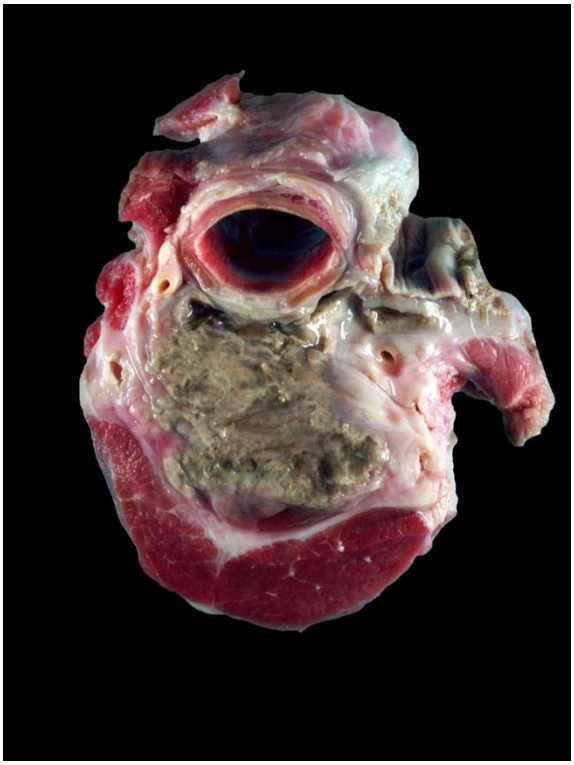

Figure 1-1: Cervical skeletal muscle, pony. There was a 3 cm x 1.5 cm x 3.5 cm focal concretion of caseous material in the right serratus ventralis cervicis muscle. (Photo courtesy of: Michigan State University, Veterinary Diagnostic Laboratory - Michigan State University, https://cvm.msu.edu/pdi; Veterinary Diagnostic Laboratory, https://cvm.msu.edu/vdl).

Actinobacillus spp, few *Escherichia coli*, and numerous *Actinomyces spp*. Anaerobic culture yielded numerous *Bacteroides pyogenes*, moderate *Fusobacterium necrophorum*, and numerous *Porphyromonas sp*. PCR for *Streptococcus equi* was negative.

On cytology, there were large numbers of degenerate neutrophils. A heterogeneous population of extracellular bacterial rods and cocci, including some long filamentous rods, were either individualized or arranged in large aggregates. There were few round to oval organisms measuring approximately 1.5-2X the diameter of a neutrophil with a small purple eccentric nucleus and lightly to moderately basophilic cytoplasm that contained few clear vacuoles.

Microscopic Description:
In multiple sections of cervical skeletal muscle, there were vast areas of liquefactive necrosis comprised of eosinophilic amorphous material, degenerated neutrophils and karyorrhectic debris. Within areas of necrosis were myriads of round amoebic trophozoites that were approximately 15um in diameter with a pale finely vacuolated to granular basophilic cytoplasm and eccentric eosinophilic 2-3um in diameter nucleus with a central karyosome and peripheralized chromatin. These organisms were variably degenerated. There were also numerous colonies of bacterial cocci and bacterial rods. Rimming these areas of necrosis was a band of degenerate neutrophils surrounded by a dense band of mature granulating fibrosis with fewer admixed lymphocytes, plasma cells, and histiocytes. Vessels in these areas were lined by plump reactive endothelium. Bands of fibrosis and lymphoplasmacytic inflammation extended into the skeletal muscle separating myofibers. Myocytes were multifocally shrunken and hypereosinophilic with loss of cross striation.

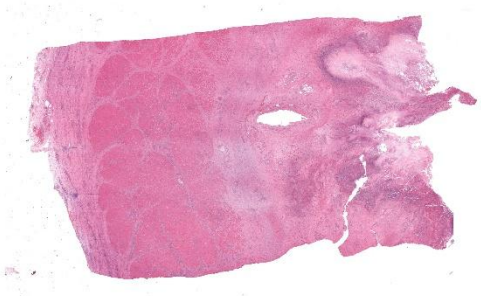

Figure 1-2: Cervical skeletal muscle, pony. A section of skeletal muscle is submitted for examination. There is a large area of coagulative necrosis with viable but highly inflamed necrotic muscle surrounded by a dense fibrous capsular. (HE, 10X)

Contributor's Morphologic Diagnoses:
Cervical muscle: Subacute, severe, locally extensive, necrotizing myositis with intralesional extracellular trophozoites, bacterial cocci, and bacterial rods.

Contributor's Comment:
Amoebic myositis has rarely been reported in humans and animals and has only been associated with *Entamoeba sp*.[2,4,12,16] In the two human cases, rupture of liver abscesses, perforation of intestines, or hematogenous or lymphatic spread of the organisms was the suspected route of infection.[12,16] In these two cases, the amoebic myositis was caused by infections with *Entamoeba histolytica*, and in a single case in a monitor lizard, myositis was caused by *Entamoeba invadens*.[2,12,16] In the monitor lizard, direct infection of a wound and hematogenous spread were determined to be the source of infection.[2] Primary amoebic myositis has not previously been reported in horses and *Entamoeba sp.* has not been demonstrated as a primary pathogen in horses.[3,6]

Entamoeba sp. generally are a nonpathogenic commensal organism; however, they occasionally cause disease in animals that are immune suppressed, have concurrent disease, or experience a dramatic change in their intestinal flora.[16] *Entamoeba equi* has been identified in the intestines of horses; but has not

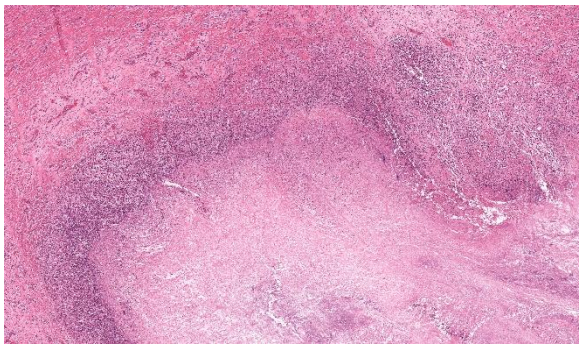

Figure 1-3: Cervical skeletal muscle, pony. An area of coagulative necrosis (right) is surrounded by a dense band of viable and necrotic neutrophils admixed with cellular debris. (HE, 61X)

been associated with clinical disease.[3,6] In animal species, the most commonly reported pathogenic entamoeba species are *Entamoeba histolytica* in primates and rarely dogs and cats, and *Entamoeba invadens* in reptiles.[11,12,17]

In humans and primates, *Entamoeba histolytica* is considered a primary pathogen that causes gastrointestinal disease and is spread via the fecal-oral route.[5] Clinical signs include bloody diarrhea, and the disease is responsible for 70 thousand human deaths a year.[5] The trophozoites are located in the large intestine, and can spread through the intestinal wall leading to hematogenous spread.[5] Entamoebic cysts are excreted in stool, and are ingested by the new host via contaminated food or water.[5] Similarly, *Entamoeba invadens* is a major pathogen in reptilian species and can cause severe necrotizing hepatitis and enterocolitis in various snake species and chelonians.[11,12]

The pathophysiology of Entamoeba-associated diseases is linked to the important virulence factor in *Entamoeba sp.,* the galactose and *N*-acetyl D-galactosamine (Gal-GalNAc) adherence lectin. This lectin allows the parasite to bind to the exposed Gal-GalNAc residues on the target cell glycoproteins.[5] *Entamoeba sp.* also form an amebapore, which is a channel-forming peptide.[5] Once inserted into the target cell, this pore allows water and ions to enter the cell leading to lysis. *Entamoeba sp.* also produces cysteine proteases,[5] which break down the extracellular matrix allowing for invasion of the organism.[5]

The suspected source of infection in the submitted case is a contaminated wound or injection site. The grossly observed white, inspissated material in multifocal areas of cervical muscle appeared microscopically as pools of pale eosinophilic crystalline material and most likely represent old injection sites. Clinically, an esophageal tear secondary to choke was suspected which may have represented a possible site of infection; however, no evidence of an acute or healing tear was observed grossly. Opportunistic infections of free-living amoeba have been reported in horses causing pneumonia, encephalitis, and placentitis.[1,7,8] Considering the unusual location of the infection, a free-living amoeba was suspected as the cause of myositis; however, *Acanthamoeba sp.*, *Balamuthia mandrillaris*, and *Naegleria fowleri* were excluded by PCR on formalin fixed paraffin embedded tissue.[10] The water from the facility was also tested for free-living amoeba as there was concern for potential infections of other exotic species. No organisms were found in the tested water sample. Regardless, the histologic appearance of the trophozoites is most consistent with *Entamoeba sp.* as the nuclei have a central karyosome and peripheralized chromatin clumps.[17] We were unable to confirm this by PCR as the available test was limited to fresh material due to the large size of

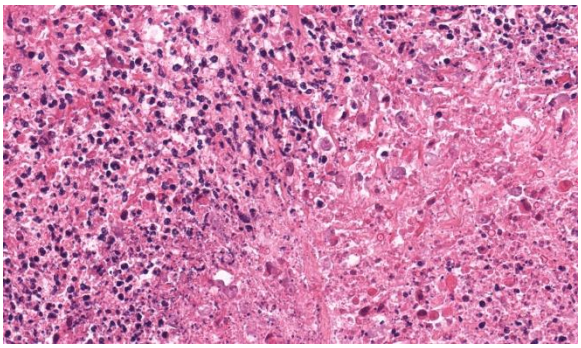

Figure 1-4: Cervical skeletal muscle, pony. Within the area of coagulative necrosis, there are numerous amebic trophozoites (arrows). (HE, 766X)

the PCR target. Further characterization with additional primers or submission of fresh material for additional testing could not be performed due to the COVID-19 pandemic. A large component of the infection in this case was also bacterial. Organisms cultured in this case such as *Fusobacterium sp., Bacteroides sp. and Actinomyces sp.* are commonly associated with necrotizing myositis.[4] Overall, the necrotizing myositis is suspected to be due to a combination of the effects of these bacteria and the *Entamoeba sp.*

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JPC Diagnoses:
Skeletal muscle: Rhabdomyositis, necrotizing, chronic-active, focally extensive, severe, with numerous amoebic trophozoites.

JPC Comment:
Kicking off the second conference in this year's lineup is the Joint Pathology Center's very own Dr. Bruce Williams! (Please hold your applause until the end.) Supplemented by an excellent contributor write-up and an AI-generated image of a psychedelic "ant-amoeba" (courtesy of Dr. Williams; please don't hesitate to contact him for this image as it is truly… something), this case made for a tough diagnostic challenge for conference participants. Who would have thought *Entamoeba sp.* in a pony's neck!?

Conference discussion focused on the five "famous amoebae" that pathologists should have mentally filed away for a rainy day, which are *Entamoeba histolytica, Entamoeba invadens, Balamuthia mandrillaris, Naegleria fowleri*, and *Acanthamoeba spp*. In addition, participants were put through their paces to name the primary species affected, organs affected, and diseases caused by these five. The main morphologic differences between a protozoal trophozoite and a protozoal cyst were covered and can be distilled down to trophozoites having more indistinct cell borders, a single nucleus, and exhibiting phagocytosis whereas cysts are round with distinct borders and 4+ nuclei. A rather unfortunate reference was made to the fact that, in humans, amoebic abscesses used to be called "anchovy paste abscesses", which is something that absolutely no one needed to be informed of…. ever. The last point of discussion was generated by a question from a conference participant asking why a karyosome is called such when it so closely resembles a nucleolus? To answer that inquiry, a nucleolus is a membrane-bound organelle in the nucleus of a cell responsible for making ribosomal RNA (rRNA) and producing ribosomes. A karyosome, by contrast, is a clustered bunch of chromatin and protein found within the nucleus and is often associated with the nucleolus. Hypothetically, they should sometimes both be able to be seen histologically within amoebic trophozoites.

There are scattered reports of amoebic infection in horses primarily involving "free-living" amoeba species, in particular *Acanthamoeba* and *Balamuthia* spp.[8] However, *Entamoeba histolytica* and *E. moshkovskii* have also been reported to cause severe disease, and these have both been isolated in rare cases from horses. *E. histolytica*, the most widely studied member of its species

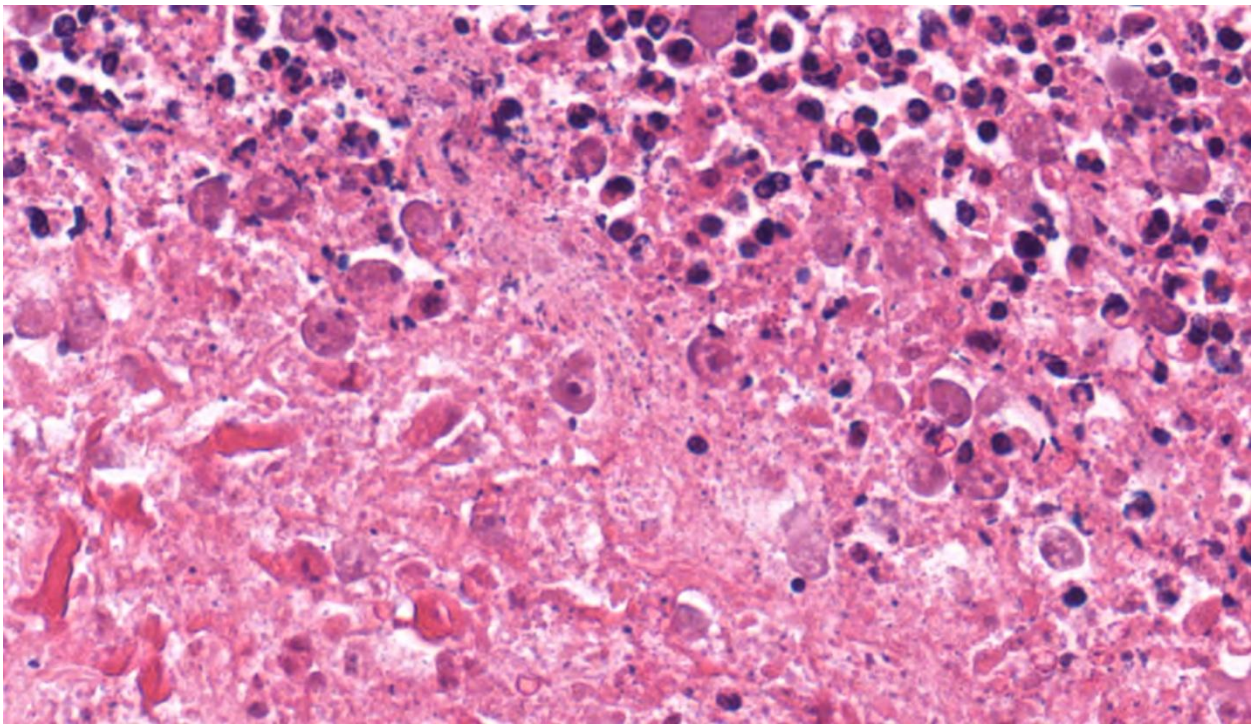

Figure 1-5: Cervical skeletal muscle, pony. High magnification of amebic trophozoites within the area of necrosis. (HE, 1316X)

that most notably affects humans and non-human primates, is equipped with a variety of nasty virulence factors that enable it to cause devastating disease in its host. As a generality, most *Entamoeba sp.* is non-pathogenic, lacking the same virulence factors as pathogenic *Entamoeba sp*. Even pathogenic *Entamoeba sp*. doesn't generally cause issues unless there is a drastic change in the host's gastrointestinal microflora, which are the primary nutrient source for *Entamoeba sp.* within the host. If that happens, all bets are off. Think of these pathogenic *Entamoeba sp.* as tiny, living bombs with anger management problems that are kept docile only by giving them their favorite foods to eat…if they no longer can feed on those preferred bacteria, they exist the GI lumen by invading the intestinal walls and, in a blind rage, start blowing up everything in their paths. From there, they can go wherever the vascular system takes them.

The virulence factors that allow them to do so include the Gal/GalNAc lectin, which is a protein that allows the *Entamoeba* to adhere to host cells, including erythrocytes and GI epithelial cells, and the protective mucus layer of the GI tract.[17] The Gal/GalNAc lectin is the primary determinant in *Entamoeba*'s ability to invade tissues[9]. Additionally, it has pore-forming peptides known as "amoebapores" that the *Entamoeba* shoves into 7host cell membranes, resulting in cell lysis.[9,17] Amoebapores are basically the offensive, single-protein, protozoal version of our own multi-protein, defensive complement cascade. This amoebapore enables the *Entamoeba* to kill host cells and invade tissues.[9] Once within the

tissue, *E. histolytica* secretes a variety of cysteine protease (CPs) enzymes that degrade host tissue components, including intestinal mucus and extracellular matrix.[9] Specific CPs have been linked to pathogenicity, such as CP5 in humans, which enables the induction of apoptosis in host target cells. In the face of all this destruction, the host immune system utilizes, among other things, oxidative stress to try and fight off the invaders. However, *Entamoeba histolytica* has an uncanny ability to resist oxidative stress by using a combination of antioxidant enzymes, such as peroxiredoxin and superoxide dismutase, and by making metabolic shifts towards glycerol and chitin biosynthesis, both of which are additionally protective against free O_2 radicals[13]. Finally, as if this protozoon needed any additional advantages, *Entamoeba histolytica* engages in "trogocytosis", in which the trophozoite ingests small "bites" of living host cells. It then processes and displays host cell membrane proteins on its own surface, which function like a cloaking device and gives the *Entamoeba* a +10 stealth boost against host immune cells. With all these virulence factors primed for pure devastation, it should come as no surprise

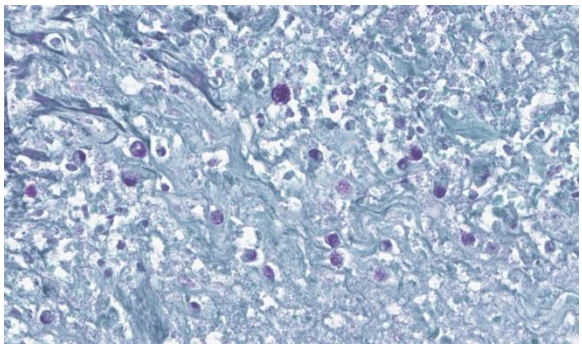

Figure 1-6: Cerebrum, horse: A PAS stain helps to highlight the location of amebic trophozoites. (PAS with malachite green counterstain 1271X)

that the National Institute of Allergy and Infectious Diseases (NIAID) classifies *E. histo lytica* as a category B priority biodefense pathogen.

The contributor made note that they also cultured three different bacteria from this case: *Fusobacterium necrophorum*, *Actinomyces sp.*, and *Bacillus fragilis*. Each of these bacteria is considered an opportunistic commensal, and conference participants largely regarded them and their potential effects as secondary to the damage wrought by the *Entamoeba*. However, they're worth briefly discussing.

F. necrophorum, once inoculated into an anaerobic environment (such as an area of necrosis), can cause devastating lesions via multiple virulence factors, including a leukotoxin, endotoxin, hemolysin, haemagglutinin, and adhesin. Among these, leukotoxin and endotoxin are believed to be the more important toxins in overcoming the host's defenses[15]. *F. necrophorum* is frequently seen in mixed infections and synergisms between it and other pathogens are considered important in causing disease. *Bacillus fragilis*, now known as *Bacteroides fragilis*, which usually resides in the intestines, is a common cause of peritonitis and sepsis from intestinal integrity issues or diseases, including post-operative complications.[19] It, too, has multiple virulence factors at its disposal, such as fragilysin (also known as BFT, or *Bacteroides fragilis* toxin), capsular polysaccharides that promote abscess formation, make the bacteria more resistant to degradation, and increasing bacterial aggregation, as well as several enzymes, including hyaluronidase, chondroitin sulfatase, deoxyribonuclease, proteases, phosphatases, and lipases, which allow attack and penetration of the

host's extracellular matrix.[19] The production of hemolysins is thought to facilitate access of *B. fragilis* to iron and heme *in vivo* via damaged host cells and erythrocytes. Lastly, and showing up empty-handed to the community potluck of devastation, is *Actinomyces sp*. *Actinomycosis* infections are usually polymicrobial and they are generally considered opportunistic bystanders, with few notable exceptions, who don't really contribute much but get to reap the benefits of just being there. All in all, it was the opinion of conference participants that *Entamoeba* was the star of this show, followed around by three enthusiastic bacterial groupies to cause the worst case of "not-actually-choke" choke ever.

References:

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CASE II:

Signalment:
10 year old male rhesus macaque (*Macaca mulatta*)

History:
This animal was received from a U.C. Davis colony in October of 2018, and was subsequently used on malaria and dengue studies. The last routine physical performed in December 2023, where it was noted that this animal was missing the tips of the left and right pinna, and there was unspecified dental disease.

During 02 February 2024 cage-side rounds examination, this animal was noted to be staring at a resident too intently, and a sedated exam was elected to investigate further. Moderate abdominal distention was noted when the animal was placed in dorsal recumbency. A firm bladder was palpated on physical exam, and animal caretakers noted that there was low urine production for this animal. Ultrasound and radiographs showed a large bladder with no obvious blockage, and medical management was elected over the weekend.

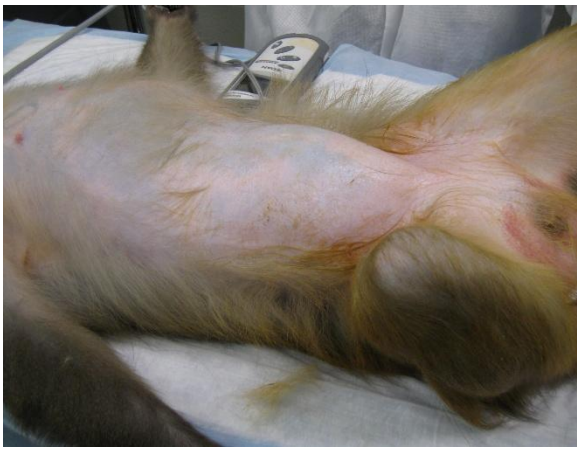

Figure 2-1: Presentation, rhesus macaque. The animal had significant abdominal distention. (Photo courtesy of: Walter Reed Army Institute of Research).

On 05 February 2024, this animal's clinical signs persisted, and a cystotomy was performed. A large amount of friable, tan-brown irregularly shaped to molded round material was removed from the urinary bladder. The material had a foul odor and was suspected to have a significant bacterial component. The luminal material was sent for culture and sensitivity, a section was used to make a smear cytology, and H&E slides. The results of preliminary diagnostics are below.

This animal recovered, and approximately one month later was euthanized as part of a protocol and was submitted for necropsy. The submitted slides were created from materials collected at necropsy.

Gross Pathology:
There is moderate abdominal distension in dorsal recumbency. Lateral radiographs show a moderate amount of heterogenous material at the cranial aspect of the urinary bladder, having irregular margins and poor contrast against surrounding tissue. Urinary catheter placement was difficult due to suspected narrowing of the urethra. Contrast material

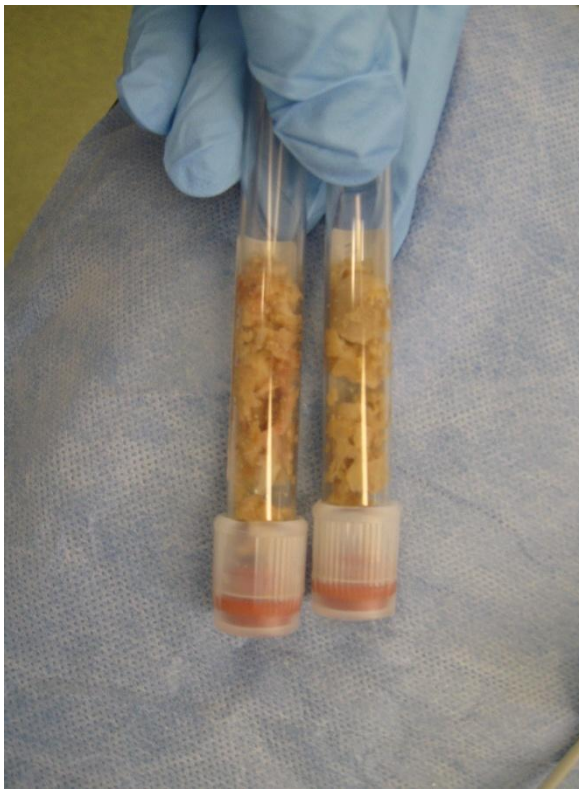

Figure 2-2: Urinary bladder, rhesus macaque. At cystotomy, a large amount of friable material was removed from the bladder. (Photo courtesy of: Walter Reed Army Institute of Research).

did not uniformly highlight the margins of the urinary bladder. A 60 cc syringe was used to remove normal colored urine from the distended urinary bladder.

Gross examination of the urinary bladder during exploratory surgery revealed congested serosal vessels, and mild erythema of the detrusor muscle. The urinary bladder wall is markedly thickened at approximately 1 cm thickness. There is multifocal friable, irregularly tan-brown material in the lumen of the urinary bladder, which emits an odor consistent with bacterial infection.

On subsequent necropsy, there is approximately 20 mL of blood in the abdomen. There are multifocal adhesions from the lateral and ventral aspect of the urinary bladder to the body wall. The urinary bladder appears mildly thickened. There is tan-brown irregular friable material within the lumen of the urinary bladder, and the urothelial mucosa is characterized by mild multifocal petechial haemorrhage.

Laboratory Results:

Cytology: These good quality, moderately cellular cytologic specimens contain mature sperm, rafts of 1-2 µm basophilic cocci, 1-2 µm x 1 µm bacilli, aggregates of extracellular matrix, infrequent macrophages, and necrotic material, overlying a faintly basophilic proteinaceous background. Sperm most often consist of only the head, with acrosome surrounding a variably intact nucleus, and measuring approximately 3 µm x 4 µm, with a polar oriented basophilia. Numerous sperm also contain the centriole and tail intact. Macrophages most often have abundant cytoplasm densely packed with cocci and bacilli, and few have multiple peripheralized nuclei (multinucleated giant cells).

A coagulum of material was processed for routine H&E evaluation. Multifocal colonies

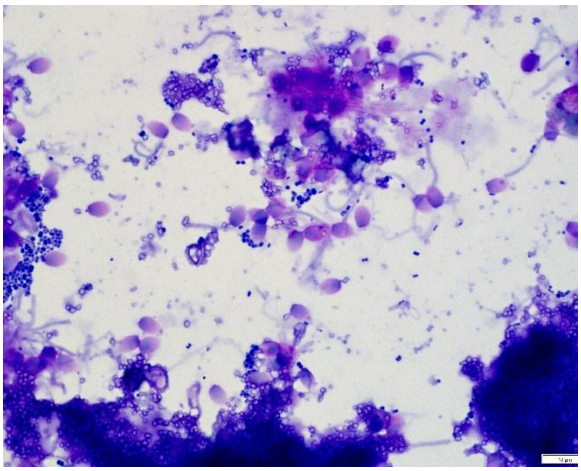

Figure 2-3: Cytology specimen, urinary bladder contents, rhesus macaque. Specimens contain mature sperm, rafts of 1-2 µm basophilic cocci, 1-2 µm x 1 µm bacilli, aggregates of extracellular matrix, infrequent macrophages, and necrotic material, on a faintly basophilic proteinaceous background. Sperm most often consist of only the head. (*Photo courtesy of:* WRAIR).

of gram-positive coccobacilli are visualized embedded in the extracellular proteinaceous material.

Culture and initial identification were performed using MALDI. The isolate was small, pleomorphic, gram positive bacilli that are catalase positive and oxidase negative. An initial identification of *Corynebacterium confusum* was made.

Sensitivity testing indicated the following:

Antimicrobial	Result
Tetracycline	S
Levofloxacin	R
Amoxicillin Clavulanic Acid	S
Erythromycin	S
Chloramphenicol	NA
Nitrofurantoin	NA
Sulfamethoxazole Trimethoprim	NA
Oxacillin	R
Ciprofloxacin	R
Vancomycin	S
Cefazolin	S
Clindamycin	NA
Gentamicin	S
Polymyxin B	I

S: Sensitive; I: Intermediate; R: Resistant

After whole genome sequencing, the isolate was identified as *Corynebacterium renale*.

Microscopic Description:
Occupying approximately 90% of the markedly dilated prostatic urethra is a round, 13 mm diameter cross section of a heterogenous plug, composed of eosinophilic extracellular protein, variably mature fibrin, collagen, and numerous macrophages surrounding or centered on spermatids. The fibrin and collagen vary from compact, to basketweave appearance, and there is multifocal mineralization.

The prostatic urothelium is attenuated, with multifocal ulceration. Multifocal degenerating urothelial cells are characterized by moderate to marked cytoplasmic vacuolation, with neutrophils transmigrating the urothelium. The underlying lamina propria has multifocal to coalescing hemorrhage, edema, and perivascular to diffuse neutrophilic urethritis. Multifocal myocytes are characterized by hypereosinophilia, sarcoplasmic vacuolation, and/or pyknotic nuclei.

The glands of the ventral prostate are multifocally ectatic, occasionally filled with eosinophilic proteinaceous material admixed with necrotic neutrophils and macrophages. Glandular epithelial cells are multifocally attenuated, vacuolated (degenerate), or necrotic, with multifocal neutrophilic inflammation surrounding glands and overlying regions of increased fibrosis. There are multiple corpora amylacea within glands.

Contributor's Morphologic Diagnoses:
1. Prostatic urethra: Plug, proteinaceous, with urethral dilatation, moderate neutrophilic urethritis, moderate multifocal hemorrhage, and smooth muscle degeneration and necrosis.
2. (Not submitted) Urinary bladder: Cystitis, neutrophilic and eosinophilic, multifocal to coalescing, acute, mild, with urothelial de

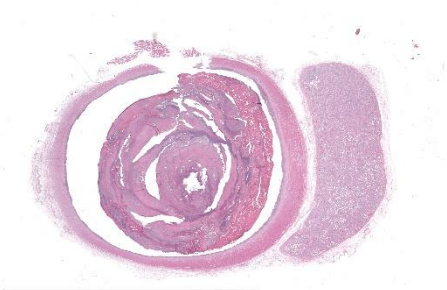

Figure 2-4: Prostatic urethra, rhesus macaque. The prostatic urethra is markedly dilated and occluded by a non-adherent lamellated proteinaceous plug. (HE, 8X)

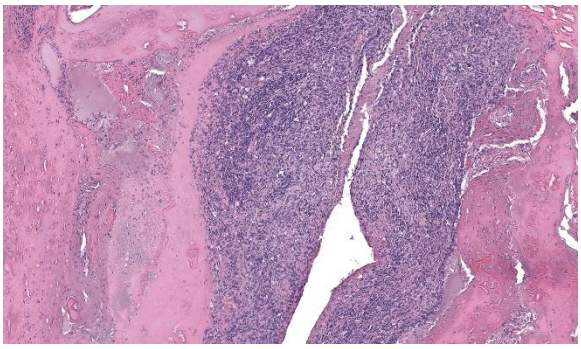

Figure 2-5: Prostatic urethra, rhesus macaque: The plug is composed of islands of intact and degenerating spermatids, inflammatory cells and abundant eosinophilic protein and fibrin. (HE, 311X)

generation and erosion, edema, and retrograde ejaculation coagulum.
3. (Not submitted) Seminal vesicle: Dilation, multifocal, mild, with multifocal aggregates of sperm.
4. (Not submitted) Kidney, left; kidney, right: Dilation, renal pelvis, moderate, with papillary necrosis, tubular epithelial necrosis, and dilation of Bowman's space.
5. Ventral prostate: Prostatitis, lymphocytic and neutrophilic, multifocal, moderate, with multifocal corpora amylacea, and regional glandular ectasia.
6. (Not submitted) Testis, seminiferous tubules: Degeneration, multifocal, moderate, with moderate ectasia.

Contributor's Comment:

Retrograde ejaculation cystolithiasis is an uncommon, but previously reported, affliction of nonhuman primates. This condition has been associated with repeated electroejaculation events; however, spontaneous disease has also been reported, as in this case.[3] In most cases, clinical signs include stranguria, dysuria, hematuria, lethargy, and distended abdomen. This animal was fortunate to have forged a close relationship with the residents and communicated his distress non-verbally.

In the 1985 seminal work on electrostimulation and penile erection, it was shown that in canines and macaques (pigtail and Rhesus) the spinal nucleus responsible for erection is the mediolateral autonomic neurons in the T_{12}-L_3, and S_1-S_3 locations. However, stimulation of these nerves does not generally result in ejaculation.[5] During normal ejaculation, there are two physiologically synchronized events controlled by sympathetic nerves. Emission and expulsion of ejaculate are distinct processes, and even minor alterations may adversely affect the animal. The expulsion process requires both urethral and penile muscle tissue to contract, allowing for the correct flow of fluid. If the internal urethral sphincter in the urinary bladder does not contract completely, reflux of fluid may occur and is called retrograde ejaculation. Retrograde ejaculation has been reported in numerous domestic species, as well as non-human primates. However, in non-human primates, the condition has most often been associated with electroejaculation, likely caused by asynchronous stimulation of nerve bundles. In these primates, the deposition of coagulum in the posterior urethra results in continued retrograde flow of semen into the urinary bladder. This has been reported in Rhesus macaques (*Macaca mulatta*) and cynomolgus macaques (*Macaca fascicularis*)[1], though usually without cystolithiasis in cynomolgus monkeys.[4]

The composition of semen impacts the possible degree of compromise in these cases. Ejaculate is comprised of sperm suspended in a fibrinous material produced by the accessory sex glands. The consistency of ejaculate changes based on the balance of coagulation and fibrinolytic factors present, and different ratios exist for different species. In humans,

the balance of fibrinogen, factor VIII:c, plasminogen, antithrombin III, fibrin monomers, and plasminogen activator inhibitor-1 is critical, and altered ratios of D-dimer/thrombin-antithrombin-III ratios have been detected post-vasectomy and in some cases of involuntary infertility.[8] For the human patient, after discharge from the urethra, semen normally takes on a gelatinous consistency, but will turn to liquid again after 3 to 30 minutes.[7] This is due to fibrinolysin contributed by the prostate, and this degradation does not occur in Rhesus macaques due to lower fibrinolytic activity and no fibrinolysin.[3]

In this animal, there was no history of electroejaculation. However, also considered were conditions that affect the competency of the urethral sphincter, and included congenital malformation, spinal cord damage or disease, previous urinary bladder surgery, or chronic inflammation. This event appears to be idiopathic, with no predisposing cause identified.

Unfortunately for this animal, a concurrent bacterial infection colonized the proteinaceous coagulum. There was spirited debate about the order in which events took place, and a primary bacterial infection leading to retrograde ejaculation cystolithiasis cannot be definitively excluded. However, the specific location of the plug and accompanying clinical signs support a primary retrograde ejaculation cystolithiasis with secondary bacterial infection. Regardless of event order, the observed cystitis and hydronephrosis are common findings accompanying cystolithiasis and urinary obstruction.

Contributing Institution:
Walter Reed Army Institute of Research
Silver Spring, MD

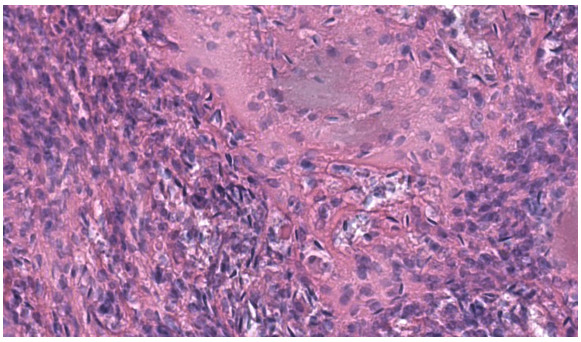

Figure 2-6: Prostatic urethra, rhesus macaque. High magnification of spermatozoa embedded within the urethral plug. (HE, 1500)

JPC Diagnoses:
Prostatic urethra: Seminal plug, chronic, with diffuse, mild, neutrophilic and eosinophilic urethritis and skeletal muscle atrophy.

JPC Comment:
The contributor provides a thorough overview of this entity in non-human primates in their comment! Conference discussion touched on several important topics mentioned in the contributor's write-up, as well as provided a review of the differences in terminology between spermatids, spermatozoa, and sperm. Spermatids are the developing, immature form of spermatozoa. Spermatozoa are the mature form that has an acrosome and a tail. The term "sperm" refers to the whole ejaculate that contains spermatozoa and other fluid components.

Another species in which a similar condition occurs is mice. Known as Mouse Urologic Syndrome (MUS), it is characterized as an obstructive genitourinary condition in male mice resulting from a urethral blockage composed of a white-yellow protein-rich plug resembling seminal vesicular gland secretions (the copulatory plug). Histologically, these plugs are composed of a proteinaceous coagulum with mixed inflammatory cells, spermatozoa,

and sloughed urothelial cells, which can become impacted within the urethral bulb, urethra, or urinary bladder. This can lead to urinary bladder distension, edema, complete pelvic or penile urethral blockage, paraphimosis, penile ulceration, and/or lethal hydronephrosis.[6]

The flexed anatomy of the penis in male mice, along with the pressure placed on the urethra from surrounding tissues and the ability of the seminal fluids to rapidly coagulate to form the copulatory plug, are thought to be contributory to the formation of MUS.[6] Histologically, seminal coagulum plugs have been associated with urothelial erosion, congestion, and inflammation, which are consistent with prolonged pressure injury.[9] Certain strains of mice are more predisposed to the development of MUS than others, including the male epileptic EL strain, STR/1N[10] strain, and obese diabetic KK-A^{y} strain.[9] This study also found that there was an increased incidence of MUS with medetomidine-ketamine anesthesia in C57/B6 and mixed genetic background strains due to the release of seminal fluids during anesthetic events with these medications. When their protocol was altered to xylazine-ketamine, the mice did not develop MUS.[9]

Something to be aware of when diagnosing MUS is that careful consideration must be given to the presence of other confirmatory lesions outside of the presence of a urethral plug. It is well known that mice will also release agonal nonobstructive plugs during death, so differentiating these from obstructive antemortem plugs is crucial in the diagnosis of MUS.[6,9] In an effort to standardize their criteria, one study utilized the presence of histologic urethritis, clinical azotemia, and gross/histologic hydronephrosis or renal cortical dilation to help them diagnose MUS vs agonal secretion.[9]

As a closing "fun fact", one of Dr. Williams' rules of engagement for today's conference was that each case presenter had to bring him a fact about their assigned entity that he did not know. The presenter for this case successfully found such a fact and stated that other species that produce a copulatory plug include butterflies, spiders, scorpions, cats, kangaroos, and Madagascar hissing cockroaches. The more you know…

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CASE III:

Signalment:
3-year-old, male, African hedgehog, *Atelerix albiventris*

History:
The hedgehog was lethargic, with a history of decrease in water intake, respiratory distress, and anorexia. At clinical examination, the hedgehog was bradycardic, hypothermic, and with agonal breathing. Supportive care and treatment, including external warming, subcutaneous fluids and atropine, were given, without success. The hedgehog died of cardiac arrest and was submitted for postmortem examination.

Gross Pathology:
The hedgehog was in good body condition, with pale mucous membranes and distended abdomen. The spleen was severely enlarged, measuring 7.0 x 3.5 x 2.0 cm and weighing 6.52% of total body weight (reference interval: 0.1-2.47% of the body weight)[3], with multifocal to coalescent pale yellow to green areas (necrosis) interspersed with dark red areas (hemorrhage). The liver was also enlarged, with round borders, diffuse pale-yellow discoloration, and accentuated lobular pattern . The liver samples floated in 10% formalin. The kidneys exhibited multifocal well delimited and irregular tan

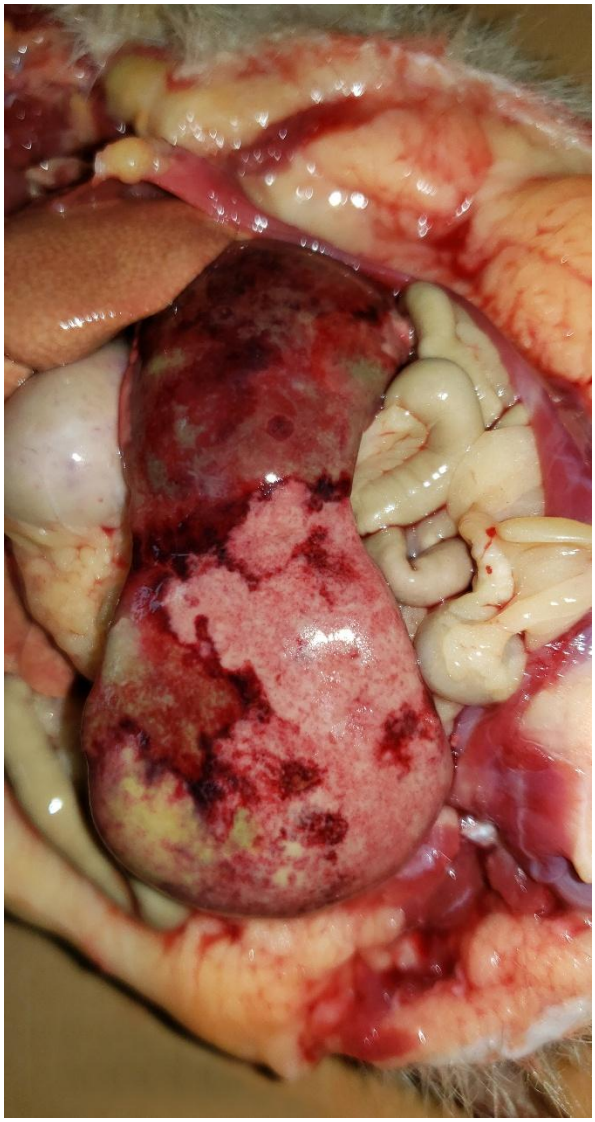

Figure 3-1: Abdominal viscera, hedgehog. The spleen is severely enlarged, weighing 6.52% of total body weight (reference interval: 0.1-2.47% BW), and pale with multifocal hemorrhage. (Photo courtesy of: Louisiana Animal Disease Diagnostic Laboratory)

areas. In the distal region of the colon, a 2 mm diameter ulcer was observed in the mucosa, at 5 cm from the rectum.

Laboratory Results:
N/A

Microscopic Description:
The submitted slide has a section of lung, a section of kidney, and a section of liver. In the lung, a neoplastic population of immature and mature eosinophils expands the peribronchiolar and perivascular interstitial tissue and is present in the lumen of blood vessels including both larger vessels and capillaries in the alveolar septa. The neoplastic cells are of intermediate size, measuring approximately 1.5 to 2 times the size of erythrocytes, and have abundant lightly basophilic cytoplasm containing variable quantities of small, round, eosinophilic granules. The nucleus of the immature eosinophils ranges from round to reniform, with open chromatin and one prominent nucleolus. The mature eosinophils have similar cytoplasm, multilobulated nuclei, and inconspicuous nucleoli. There is mild anisocytosis and anisokaryosis. Mitoses are rare (1 to 2 figures per high power field). The pleura is lined by mildly hypertrophic mesothelial cells. In the renal interstitium, there are multifocal to coalescing, predominantly perivascular sheets of immature eosinophils and fewer mature eosinophils like those described in the lung. A few immature eosinophils are entrapped in glomerular capillaries. The renal tubules have occasionally attenuated epithelium and intraluminal eosinophilic proteinaceous fluid or casts. Multifocal mineral deposits are present in renal medullary tubules. An aggregate of immature and mature eosinophils is seen in the perirenal adipose tissue. In the liver, hepatocytes are diffusely swollen, with clear cytoplasmic vacuoles (lipid-type vacuolar degeneration). The sinusoids are often expanded by predominantly immature and fewer mature eosinophils like those present in the lung and kidney. Perivascular areas (predominantly in the portal regions) have aggregates of similar cells, which also infiltrate multiple other tissues, including the bone marrow (sections not submitted). The cytoplasmic granules in both the immature and mature eosinophils stain strongly with the Luna stain.

Contributor's Morphologic Diagnoses:
Lung, kidney, and liver: Eosinophilic leukemia

Liver: Lipidosis, diffuse, severe

Contributor's Comment:
Spontaneous neoplasia is common in adult African hedgehogs without sex predilection. The median age of affected animals is 3.5 years.[11] The most common sites of tumor development in these animals are skin (especially mammary gland), lymphoid, gastrointestinal, endocrine, and reproductive systems. Most of these tumors are malignant and tend to have a poor prognosis.[11] Hematopoietic tumors can account for up to 11% of the tumors in hedgehogs. Lymphoma is the most common of this type of tumor, predominantly of the multicentric and alimentary forms, while leukemia in hedgehogs is considered rare.[9,11] Leukemias in hedgehogs include eosinophilic leukemia and acute leukemia/lymphoma.[7,9,10]

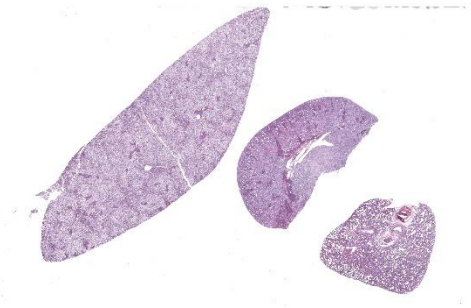

Figure 3-2: Liver, kidney, lung, hedgehog. One section of each tissue is submitted for examination. At subgross magnification, marked hepatocellular lipidosis can be discerned. (HE, 10X)

Eosinophilic leukemia is a variant of granulocytic leukemia and has been described in cats and humans.[3,4] This type of neoplasm is considered to be chronic with an indolent course, and the cells are usually immature with eosinophilic differentiation. Some reports suggest that hedgehogs may have a unique genetic susceptibility to this specific type of leukemia, and that the behavior of the neoplasm is more aggressive in this species.[9] Differential diagnoses include idiopathic hypereosinophilic syndrome and paraneoplastic hypereosinophilia, both described as increase of solely mature eosinophils in blood and tissues.[4,7,9] The diagnosis of idiopathic hypereosinophilic syndrome is made by exclusion, in the absence of an eosinophilic neoplasm, like leukemia, or by determining primary causes of reactive increase of eosinophils, such as allergies, autoimmune disease, or parasitic infestation.[7,9,10]

The diagnosis of leukemia can be performed through clinical pathologic findings, especially marked leukocytosis, composed mostly of immature eosinophils, which can be observed in the peripheral blood smear. Animals with myelogenous leukemia can present concurrent cytopenias, like non-regenerative anemia and thrombocytopenia. Serum biochemistry findings depend on the organs affected by the neoplastic cells. The literature describes a predominance of liver injury, leading to hypoalbuminemia.[9] Histologically, it is common to visualize neoplastic cell infiltrate in multiple organs, including the bone marrow, pancreas, liver, kidney, gastrointestinal tract, lungs, lymph nodes, heart, and spleen.[7,9] Although eosinophils can be easily recognized cytologically or histologically, the identification of immature cells can be a challenge. Histochemistry and/or immunohistochemistry may be

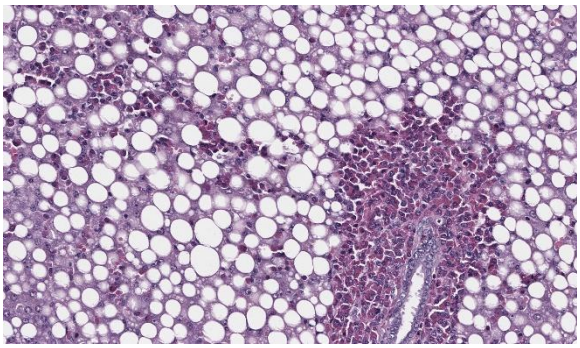

Figure 3-3: Liver, hedgehog. Neoplastic eosinophiles form aggregates within portal areas and within sinusoids. Diffusely, hepatocytes contain large cytoplasmic lipid droplets. (HE, 318X)

helpful in these cases to confirm the lineage of the leukemia cells. In cases of eosinophilic leukemia, Luna stain is the most consistent stain utilized to highlight the granules of both immature and mature eosinophils.[10] Cytochemical staining for myeloperoxidase (MPO), alkaline phosphatase (ALP), and Sudan Black B can also be performed to demonstrate the granules of eosinophils in blood smears.[9]

In the current case, variable degrees of neoplastic cell infiltrate were observed in the bone marrow, lungs, trachea, nasal cavity, heart, kidneys, liver, spleen, pancreas, gastrointestinal tract, mesenteric lymph node, skin, eyes, eyelids, lacrimal glands, sciatic nerve, and cerebral meninges. Blood vessels were filled with immature eosinophils, which indicates abundant neoplastic eosinophils were in circulation, consistent with leukemia. Causes of hypereosinophilia, such as parasitism or allergy, were ruled out based on the history and lack of gross or microscopic evidence. The severely enlarged spleen (6.52% of total body weight; reference interval: 0.1-2.47% body weight)[5] had multifocal to coalescent, large areas of necrosis within extensive sheets of neoplastic cell infiltrate; little of the normal parenchyma

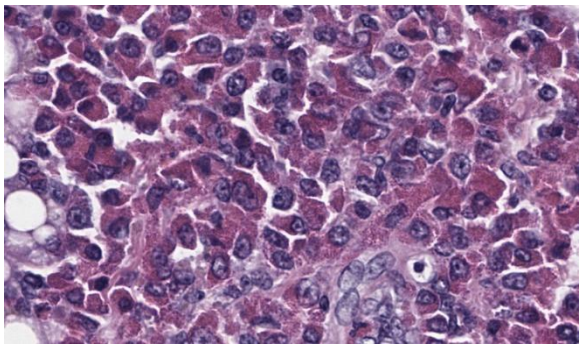

Figure 3-4: Liver, hedgehog. High magnification of neoplastic eosinophils. While some mature forms are bilobed, the majority have large round nuclei. (HE, 1450X)

remained. The bone marrow was markedly hypercellular; 70-80% of the cellularity was composed of immature forms of eosinophils, with 2 mitotic figures per 10 high power fields. Megakaryocytes and erythroid precursor cells were markedly reduced in numbers. The cause of death was presumed to be from multiorgan dysfunction due to the severe neoplastic cell infiltrate in all organs, further complicated by the severe hepatic lipidosis.

There is no scientific evidence of the efficacy of chemotherapy for the treatment of eosinophilic leukemia in hedgehogs. Drugs like cytarabine, used for the treatment of cats and dogs with hypereosinophilic syndrome, were tested in hedgehogs.[10] No treatment has been successfully established for this condition in hedgehogs, however. Affected animals usually die shortly after diagnosis.[8-10]

Contributing Institution:
Louisiana Animal Disease Diagnostic Laboratory (LADDL), School of Veterinary Medicine, Louisiana State University
(http://www1.vetmed.lsu.edu/laddl/index. html)

JPC Diagnoses:
1. Liver, kidney, lung: Myeloproliferative neoplasm, favor chronic eosinophilic leukemia.
2. Liver, hepatocytes: Lipidosis, diffuse, severe.

JPC Comment:
Of the four entities seen in today's conference, this is the only one that has been seen in a Wednesday Slide Conference before (WSC 2020-2021, Conf 19, Case 2). In what initially was thought to be a straightforward case, some conference participants engaged in a spirited debate about whether to call this acute vs chronic eosinophilic leukemia and if it also classified as an eosinophilic sarcoma due to extravasation of the neoplastic epithelial cells into the surrounding tissues. As such, a CD34 immunohistochemical marker was performed post-conference to determine maturity of the eosinophils, which revealed that the neoplastic cells were not immunoreactive to CD34. This allowed participants to conclude that the leukemic portion of the diagnosis was chronic. Additionally, this case was sent for human hematopathology consult with the Walter Reed National Military Medical Center, who expressed that they would diagnose this case as chronic eosinophilic leukemia based on the degree of granularity of the eosinophils, which they stated is a feature of maturity. However, they also mentioned that "myeloproliferative neoplasm" would be a more appropriate term according to the WHO, as there was no clinical documentation to demonstrate a peripheral eosinophilia over time in this patient and there was no clonality performed. Both of these are considered diagnostic criteria according to the most updated World Health Organization classification for leukemias.[2] All factors considered, conference participants agreed with the diagnosis of "myeloproliferative neoplasm, favor chronic eosinophilic leukemia" in line with the WHO classification.

The eosinophil was discovered by Paul Erlich in 1879 when he found distinctive properties of a particular subset of leukocytes that exhibited a pink color when stained with eosin dye.[1] Eosin dye is made from fluorescein, which is a coal tar derivative, that is treated in an ethanol solution with bromine to form tetrabromofluorescein.[8] This compound, which is acidic, is then neutralized into a salt, resulting in a water (or ethanol) soluble dye. Eosin is negatively charged, and thus stains positively charged tissues, including cytoplasmic elements, extracellular matrix, and connective tissues such as collagen.[8] Additionally, it stains basic proteins, including Major Basic Protein found in the granules of eosinophils, which is what causes them to stain so strongly with eosin and gives them their characteristic name.[8]

Eosinophils originate in the bone marrow from multipotent hematopoietic stem cells (HSC) that differentiate down the myeloid lineage. The common CD34+ myeloid progenitor (CMP) gives rise to myeloblasts, which are enter granulopoiesis towards one of three cell types: neutrophils, basophils, and eosinophils. The differentiation and maturation of eosinophils through eosinophilic lineage are dependent on timely expression and presence of several transcription factors and cytokines, such as interleukin-5 (IL-5).[12] IL-5 is the most important factor promoting eosinophil differentiation, maturation, and survival. Other cytokines, including IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) also contribute to this process.[12]

Mature eosinophils contain granules rich in major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase

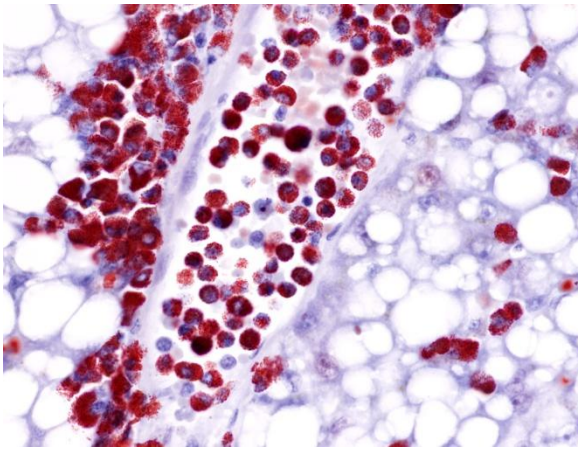

Figure 3-5: Liver, hedgehog. A Luna mast cell stain highlights the eosinophilic granules within the cytoplasm. Additionally numerous neoplastic eosinophils are present within vessels. (Luna mast, 1000X) (Photo courtesy of: Louisiana Animal Disease Diagnostic Laboratory).

(EPO/EPX), and eosinophil-derived neurotoxin (EDN), among other things.[12] Eosinophil granules are cytotoxic and are released in response to a variety of infections, particularly parasitic infection, as well as to allergen exposure. Normally, in response to infection or allergen, Type 2 inflammatory (Th2) cells that are in proximity to the stressor produce high levels of IL-5, which triggers eosinophil infiltration into tissues and activation.[12] Activated eosinophils release a variety of substances, including cytokines, chemokines, and granule proteins such as those mentioned above. These mediators, if not regulated, contribute to the tissue damage and dysfunction seen in the affected organs. Eosinophil peroxidase, in particular, impart a characteristic green hue to tissues it affects due to its high iron concentration, which at one point led to the use of the now-retired terms “chloroleukemia” and “chloroma” to describe associated lesions of eosinophilic leukemias grossly.

Now that some basics eosinophil origin and function have been discussed, what about eosinophilic leukemias? Specific mutations in the CD34+ myeloid progenitor cells can lead to primary eosinophilic disorders, including leukemias and hypereosinophilic syndromes, where eosinophils are part of the clonal expansion. The FIP1L1-PDGFRA fusion gene is a well-studied example of such a mutation associated with chronic eosinophilic leukemia (CEL) in humans.[6] This gene is not normally present and arises from a chromosomal deletion that results in the production of an activated tyrosine kinase that drives uncontrolled growth and division of myeloid cells, particularly eosinophils.[6]

In veterinary species, this disease is rare with only scattered reports in African pygmy hedgehogs, cats, dogs, and one report in a tiger salamander. Despite the growing body of information that exists about this entity in humans, studying the lineage and genetics of eosinophilic leukemia in veterinary species has proven to be challenging due to the lack of cross-reactive or species-specific reagents and immunohistochemical markers.[9] Until that changes and someone develops species-specific reagents to study eosinophilic leukemia in African pygmy hedgehogs (or other affected species, I'm sure there's a Ph.D. niche there), further understanding of this disease in animals will come with time....and funding.

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CASE IV:

Signalment:
7-year-old male neutered Domestic Shorthair Cat.

History:
A red plaque from the right upper conjunctiva was submitted for biopsy.

Gross Pathology:
One irregular piece of pale tan, soft to firm tissue with no natural borders was received.

Laboratory Results:
N/A

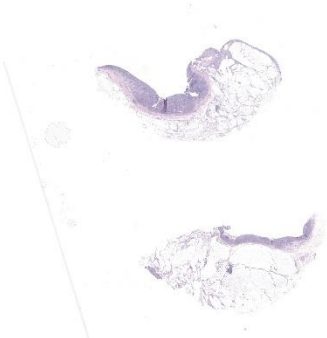

Figure 4-1: Eyelid, cat. Two sections of eyelid are submitted for examination. At this magnification, marked inflammation of the dermis is evident, as is significant expansion of the underlying tissue by numerous variably sized clear spaces. (HE, 10X)

Microscopic Description:
Skin (right upper palpebral conjunctiva). One bisected tissue is examined. The substantia propria is markedly expanded by coalescing, variably sized clear spaces (presumptive lipid), occasionally containing low numbers of macrophages, and surrounded by a small amount of fibrosis, few macrophages, and rare multinucleated giant cells. Larger aggregates of macrophages are present superficially. The superficial substantia propria is moderately expanded by low numbers of lymphocytes and few plasma cells.

Contributor's Morphologic Diagnoses:
Skin (right upper palpebral conjunctiva):
Marked chronic lipogranulomatous conjunctivitis

Contributor's Comment:
These findings are consistent with the uncommon condition known as Feline Lipogranulomatous Conjunctivitis. This condition results in nodular lesions, most commonly affecting the palpebral conjunctiva, and it occurs secondary to leakage of lipid-rich material into the tissue. Cats with sparse periocular pigment (such as white or orange cats) may be predisposed. One source reports that nearly a quarter of cats with this condition also had concurrent periocular neoplasia; there was no evidence of neoplasia in these sections. Complete excision is expected to be curative for this benign lesion.

Contributing Institution:
University of Tennessee College of Veterinary Medicine
Department of Biomedical and Diagnostic Sciences
https://vetmed.tennessee.edu/academics/biomedical-and-diagnostic-sciences/

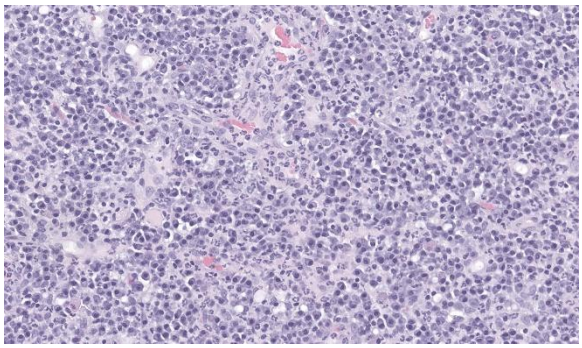

Figure 4-2: Eyelid, cat. The superficial dermis, particularly that underneath the ulcerated mucosal epithelium, is infiltration by large number of plasma cells, with fewer lymphocytes, neutro-phils, and macrophages. HE, 522X)

JPC Diagnoses:
Conjunctiva: Conjunctivitis, lipogranulomatous and lymphoplasmacytic, chronic, diffuse, severe.

JPC Comment:
The last conference case of the day represented a unique feline entity that lent itself to a difficult tissue ID for conference participants. Many thanks to our contributor for this unique case! Conference participants waffled on anatomic location initially but eventually settled on either conjunctiva or mucocutaneous junction of some kind. Dr. Williams steered participants in the right direction to conjunctiva and drew special attention to the large lakes of clear space within the subconjunctival tissues. He turned those lakes into yet another lesson about paying special attention to what is NOT present. He stated that, when there is clear space, it should make the pathologist consider, "Is this air (emphysema), clear fluid, or fat?" In this case, those clear spaces were what was left of large lakes of lipid, characteristic in this condition, following tissue processing.

Feline lipogranulomatous conjunctivitis includes characteristic non-ulcerated, smooth, white, conjunctival nodules within the palpebral conjunctiva. These are almost always adjacent to the eyelid margin and usually involve the meibomian glands.[1,3] These nodules often cluster, are usually bilateral, and may be present on both upper and lower eyelids.[1,3] However, lesions are usually more severe on the upper lid than the lower. This condition doesn't generally respond well to treatment and surgery is usually recommended if there is irritation. Luckily, these cats typically have a good prognosis post-op. Feline lipogranulomatous conjunctivitis is similar to chalazia in dogs, except that chalazia are usually the result of Meibomian gland neoplasia and do not tend to cluster.[3] Differential diagnoses for conjunctival inflammation with nodule formation in cats should include meibomian gland infections (i.e. marginal blepharitis or styes), eosinophilic keratoconjunctivitis, feline herpesvirus 1 (FHV-1), *Chlamydia felis*, feline calicivirus (FCV), and *Mycoplasma spp*. Conjunctival neoplasia should also be considered in these cases, as approximately 25% of cats with lipogranulomatous conjunctivitis have had concurrent neoplasms, usually squamous cell carcinomas.[2,3]

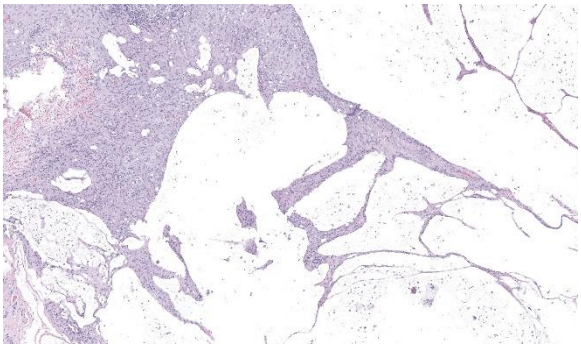

Figure 4-3: Eyelid, cat. Deeper areas of the dermis are expanded by large, irregularly shaped clear spances which are separated by fibrous stroma that is infiltrated by regionally variable numbers of vacuolated epithelioid macrophages. (HE, 106X)

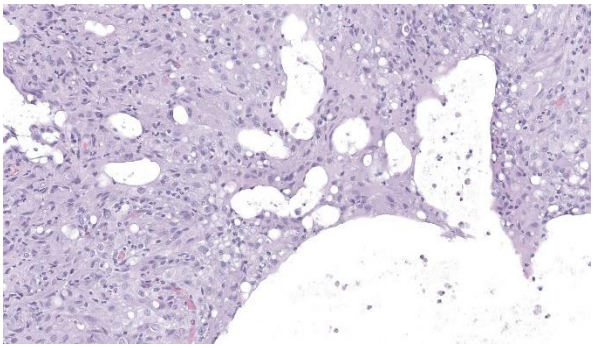

Figure 4-4: Eyelid, cat. Higher magnification of the infiltrate of vacuolated macrophages which border clear spaces. The clear spaces contain few macrophages as well. (HE, 381X)

Inflammation within the Meibomian glands is usually minimal, but abnormal squamous epithelium may be seen within ducts. Usually, there is fibrosis surrounding the gland.[3]

The pathogenesis is currently thought to involve actinic (UV or solar) radiation-induced damage to the Meibomian glands, resulting in blockage of normal glandular secretion and leakage of sebaceous material into the surrounding tissues, which incites a granulomatous inflammatory response.

It is thought that the UV radiation leads to DNA damage and oxidative stress, followed by cytokine release and immune activation with subsequent damage to the gland. This might help explain why cats with low levels of periocular pigment are more susceptible to the condition, as well as why a quarter of these cats also have concurrent UV-related neoplasms.

On microscopic examination, the nodules are characterized by variably sized lipid lakes within the submucosal connective tissue. These lakes are usually surrounded by macrophages, multinucleated giant cells, and low numbers of plasma cells and lymphocytes.[3] Macrophages frequently will have phagocytosed lipid droplets and/or cholesterol crystals.

References:

1. Dubielzig, RR, Ketring, K, McLellen, GJ, Albert, DM. Veterinary Ocular Pathology. Elsevier; 2010. pp. 174, 179.

2. Foster TM, Newbold GM, Miller EJ, Jeong YJ, Premanandan C, Husbands BD. Periocular fibrosarcoma with lipogranulomatous conjunctivitis in a cat. Vet Ophthalmol. 2025;28(3):646-650.

3. Read, RA, Lucas, J. Lipogranulomatous conjunctivitis: clinical findings from 21 eyes in 13 cats. Veterinary Ophthalmology. 2001;4:93-98.