Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE
2021-2022

Conference 3

1 September, 2021

Joint Pathology Center
Silver Spring, Maryland

CASE I: 288/09 (JPC 41120035-00)

Signalment:
24-month old, male, Nelore (*Bos taurus indicus*), bovine.

Figure 1-1. ***Presentation, ox: Affected animals demonstrate significant hindlimb weakness and ataxia. (Photo courtesy of: Laboratório de Patologia Veterinária, Universidade Federal de Mato Grosso. http://www1.ufmt.br/ufmt/unidade/?l=ppgvet*)**

History:
The disease occurred in January-February 2010, in cattle held on a farm in the state of Pará Norh Brazil. Twenty five out of 3,000 cattle were affected by a neurological disorder 60 days after being vaccinated against the foot and mouth disease (FMD). The affected cattle were of different ages and fair nutritional condition. They presented a 5cm diameter nodular protuberance, commonly on the left side of the lumbar region midline, involving muscle and subcutaneous tissue. The clinical signs were consistent with lumbar spinal cord compression syndrome with progressive paralysis of the hind limbs, characterized by ataxia with signs of dragging the hooves, crossing of hindlimbs when walking, "dog sitting" position with spread hindlimbs, urinary incontinence, and stumbling and falling. These signs lasted for 2-5 months and evolved to sternal recumbency with difficulty in rising. The sensory and motor reflexes of the forelimbs were normal.

Gross Pathology:
There were areas of grayish yellow discoloration in the *longissimus dorsi* muscle. These areas were firm and infiltrated muscle bundles and communicated with a roughly fusiform cylindrical 10x3cm white-yellowish mass located in the epidural space extending from L1 to L4 and adhered both to the periosteum of the vertebral body and to the dura mater (Fig. 2). In this area, whitish oily fluid, resembling the consistency of FMD vaccine, was infiltrated the intervertebral spaces. On cut surface (Fig. 3), the epidural mass was white and firm and had multifocal to coalescing, soft, fairly nodular yellow foci ranging in size from 0.2 to 2 cm in diameter.

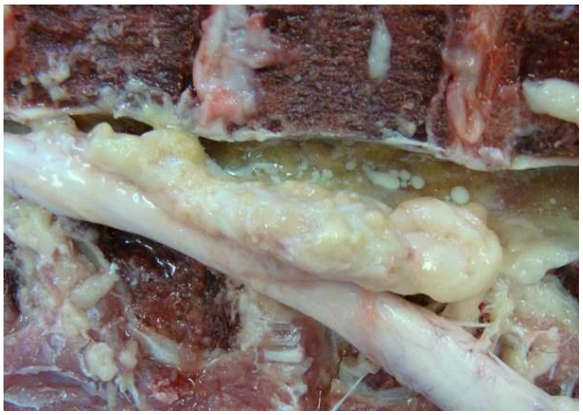

Figure 1-2. ***Spinal cord, ox. The epidural fat adjacent to the lumbar cord is hard with yellow-white mottling throughout. (Photo courtesy of: Laboratório de Patologia Veterinária, Universidade Federal de Mato Grosso. http://www1.ufmt.br/ufmt/unidade/?l=ppgvet)***

Laboratory Results:
A myelography of the lumbosacral revealed contrast retention at the level of L4 vertebra and an intradural mass extending from L1 to L3. No bacterial growth was obtained from the bacterial culture of spinal cord samples from this region.

Microscopic Description:
The subcutaneous tissue of the lumbar area and muscle was expanded and partially effaced by extensive pyogranulomatous inflammation and fibrosis. There was hyaline and floccular degeneration of the striated muscle in the area. The mass in the epidural space consisted of the same type of pyogranulomatous inflammation. Some of the pyogranulomas consisted of a necrotic and mineralized with numerous neutrophils surrounded by foamy and epithelioid macrophages (occasionally forming multinucleated giant cells), lymphocytes, and plasma cells. Clear spaces ranging from 20 to 200μm were seen in central necrotic areas. The dura mater was thickened by fibrosis and the epidural space obliterated the pyogranulomatous mass. Neuronal chromatolysis were observed mainly in the dorsal horns of the gray matter of the spinal cord, and mild to severe Wallerian degeneration in the dorsal funiculi.

Samples from the granulomatous mass and spinal cord were stained using Steiner's silver and Ziehl-Neelsen acid-fast techniques.

Contributor's Morphologic Diagnosis:
1. Severe, focally extensive, pyogranulomatous panniculitis; myositis with intralesional vacuoles.

2. Spinal cord pyogranulomatous pachymeningitis with intralesional vacuoles (consistent with oil adjuvant droplets).

3. Myelomalacia, secondary to spinal cord compression (consistent with oil adjuvant droplets).

Contributor's Comment:
The findings described here characterize cases of spinal cord compression caused by granulomatous inflammation caused by incorrect inoculation of the FMD vaccine. Vaccines against FMD contain an oil adjuvant. Adjuvants act non-specifically by increasing the immune response against antigens and are a key component of vaccines. They can cause adverse effects such as anaphylaxis, infection, granulomas at the injection site, and tumors.[4]

The cases of spinal cord compression described here were attributed to improper vaccination. There is limited information about economic losses due to vaccine reactions in cattle in Brazil. The practice of mass vaccination, mainly in beef cattle, has led to the appearance of abscesses in the injection site because of improper sharing of needles between individuals and antisepsis procedures during the vaccination.

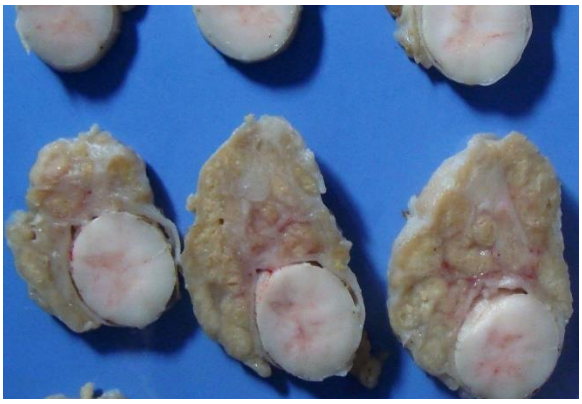

Figure 1-3. ***Sectioning of the spinal cord discloses fibrosis and numerous foci of inflammation within the epidural fat. (Photo courtesy of: Laboratório de Patologia Veterinária, Universidade Federal de Mato Grosso. http://www1.ufmt.br/ufmt/unidade/?l=ppgvet)***

The most remarkable histopathological findings in this case were somewhat similar to those observed in others studies.[3,5,6,7] Demonstration of lipid-like material surrounding or within the center of pyogranulomas, coupled with the absence of bacterial infection, indicated that the reaction was most likely directed toward a component in the vaccine, in association with the practice of improper procedures

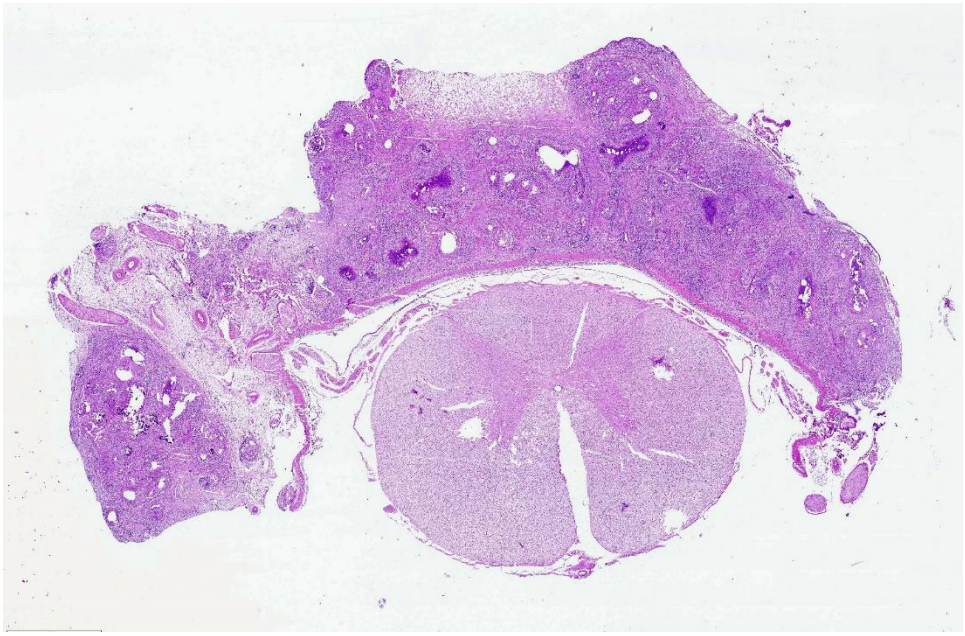

Figure ***1-4. Spinal cord, ox: Subgross magnification of a section of spinal cord demonstrates numerous well-formed pyogranulomas centered on clear vacuoles within the largely effaced epidural fat. (HE, 7X)***

In domestic animals, which exhibit posterior ataxia and paralysis, several differential diagnoses such as fractures, neoplasms (mainly lymphosarcoma), rabies, and botulism should be considered in this region of Brazil. However, most of them have clinical, macroscopic and microscopic different aspects, which were ruled out. In this case, the history of incorrect vaccination procedures was essential to the diagnosis.[7]

The neck is the usual inoculation site for the FMD vaccine in cattle in Brazil. Paravertebral intramuscular injection sites should be avoided for vaccination purposes when potentially irritating biological products are used.

Contributing Institution:

Laboratório de Patologia Veterinária, Universidade Federal de Mato Grosso
http://www1.ufmt.br/ufmt/unidade/?l=ppgvet

JPC Diagnosis:
Spinal cord, meninges and epidural fat: Pyogranulomas, multiple, with numerous clear vacuoles (consistent with oil droplets) and marked fibrosis.

JPC Comment:
The contributor's comment provides a concise overview of adjuvant's role within vaccines and potential consequences of inappropriate administration.

Adjuvants (Latin: adjuvare "to help") mimic microbial antigens detected by antigen presenting cell pattern recognition receptors, increasing the innate immune system's response and subsequently facilitating the development of adaptive immunity. Antigen presenting cells subsequently express of molecules called costimulators and secrete cytokines that stimulate the proliferation and differentiation of T lymphocytes in peripheral lymphoid organs which then facilitate development of the adaptive immune system (i.e. cell mediated and humoral immunity).[2,5]

Water-in-oil adjuvanted vaccines are commonly used in food animals worldwide, including the United States, but are no longer used in human formulations due to their irritancy in tissue, particularly in regard to more slowly absorbed mineral oil mixtures.[5]

Scar tissue formation is an undesirable side effect of vaccination site reactions in food animals, reported to cost the United States cattle industry

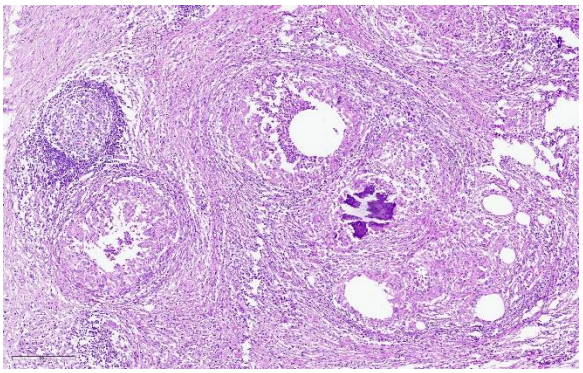

Figure 1-5. ***Spinal cord, ox: Higher magnification discloses the variation in pyogranuloma formation. Pyogranulomas are centered on clear vacuoles, which are also present in their walls. The number of neutrophils at the center decreases in more mature lesions, which also contain crystalline mineral. (HE, 360X).***

$55 million dollars annually (2005 value) due to trim, affecting 10% of carcasses in 1998.[5] Producer organizations in North America therefore recommend biological, antibiotic, vitamin, and mineral products be formulated for subcutaneous use whenever possible and administered in the neck. However, these products may ultimately be administered elsewhere, such as in epaxial or hip muscles for reasons such as operator safety, ease of administration, and in some cases lack of a manufacturer statement advising against injection in these locations.[5]

Aqueous based (often combined aluminum hydroxide [$Al(OH)_3$] and saponin) adjuvants are also commonly used in veterinary medicine and have also been implicated in the formation of immune mediated granulomas.[1,8]

One study in sheep found approximately 92% of lambs injected with an aluminum based adjuvant without vaccine solution developed immune mediated granulomas. The presence of aluminum within granulomas (and lymph nodes) was confirmed using fluorescence microscopy with lumogallion staining and electron microscopy.[1]

The moderator discussed additional causes of compressive myelopathy in ruminants, including abscesses, trauma, malformation, and neoplasia.

References:

1. Asín J, Molín J, Pérez M, et al. Granulomas Following Subcutaneous Injection With Aluminum Adjuvant-Containing Products in Sheep. *Vet Pathol*. 2019;56(3):418-428.
2. Diseases of the immune system. In: Kumar V, Abbas AK, Aster JC, eds. *Robbins and Cotran Pathologic Basis of Disease*. 9th ed. Philadelphia, PA: Elsevier; 2015:189-204.
3. Kleinman NR, Kier AB, Diaconu E, et al. Posterior paresis induced by Freund's adjuvant in guinea pigs. *Lab Anim Sci*. 1993;43(4):364-366
4. O'Toole D, McAllister MM, Griggs K. Iatrogenic compressive lumbar myelopathy and radiculopathy in adult cattle following injection of an adjuvanted bacterin into loin muscle: Histopathology and ultrastructure. *J. Vet Diagn Invest* 1995;7(2):237-244
5. O'Toole D, Steadman L, Raisbeck M, et al. Myositis, lameness, and recumbency after use of water-in-oil adjuvanted vaccines in near-term beef cattle. *J Vet Diagn Invest*. 2005; 17(1):23-31
6. Panziera W, Rissi DR, Galiza GJN, et al. Pathology in practice. *J Vet Med Assoc*. 2016; 249 (5): 483-485.
7. Ubiali DG, Cruz RAS, Lana MVC, et al. Spinal cord compression in cattle after the use of an oily vaccine. *Pesq Vet Bras*. 2011; 31(11): 997-999.
8. Ulziibat G, Maygmarsuren O, Khishgee B, et al. Immunogenicity of imported foot-and-mouth vaccines in different species in Mongolia. *Vaccine*. 2020;38(7):1708-1714.

CASE II: 14W4636 (JPC 4068606-00)

Signalment:
Juvenile, male, Rocky mountain elk (*Cervus canadensis nelsoni).*

History:
Forty-four free-ranging elk calves died on a Wyoming Game and Fish Department winter feedground over the previous month (March). The winter was atypical, even for Wyoming: unusually heavy amounts of wet snow, followed by bouts of above freezing temperatures.

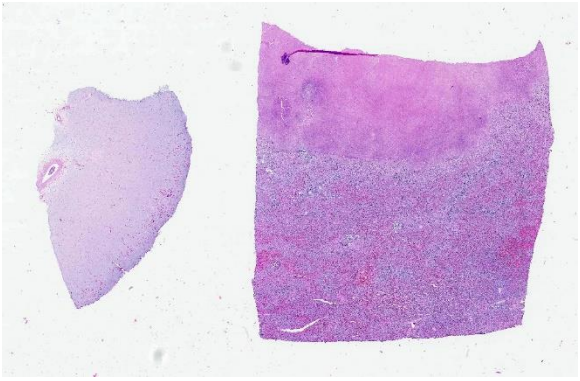

Figure 2-1. ***Bone marrow, liver, elk: At subgross magnification, the section of bone marrow lacks distinct lobules of adipocytes and marrow elements. A large area of necrosis comprises approximately 33% of the section. (HE, 5X)***

Biologists described calves as losing condition and becoming recumbent. This calf was euthanized by gunshot and submitted for necropsy.

Gross Pathology:
The carcass was that of a juvenile male elk in poor nutritional condition. Dead weight: 110 kg. There was serous atrophy of fat throughout. Femoral bone marrow was red and gelatinous. Approximately 25% of the liver had sharply-demarcated caseous necrosis (from pathologist's notes). Interdigital lesions were recorded as present but 'minimal'.

Laboratory Results:
- PCR on liver *Fusobacterium necrophorum*: detected
- *Clostridium perfringens* and *Trueperella pyogenes* isolated (liver)
- Negative on culture for *Brucella abortus* (multiple lymph nodes)
- Negative IHC for chronic wasting disease (obex; palatine tonsil; medial retropharyngeal LN)

Microscopic Description:
Two tissues are presented. The femoral bone marrow exhibits marked serous atrophy of fat, with atrophic adipocytes separated by expanded eosinophilic intercellular matrix. The section of liver encompasses an abrupt margin between necrotic and viable hepatic tissue. There is fibrosis, disorganization of hepatic plates, and lymphocytic-histiocytic inflammation with neutrophils throughout. Bacteria are present in necrotic tissue close to viable tissue margins. A Gram's stain demonstrates that bacteria are filamentous and Gram-negative. No *Clostridium* sp. bacteria are seen in special stains.

Contributor's Morphologic Diagnosis:
1. Serous atrophy of fat, severe, diffuse.

2. Necrotizing hepatitis, severe, subacute, locally extensive, with intralesional filamentous Gram-negative bacteria.

Contributor's Comment:
Fusobacterium necrophorum is a common opportunistic pathogen familiar to diagnostic pathologists from its involvement in footrot in cattle, interdigital dermatitis in sheep, calf diphtheria, necrotic rhinitis of pigs, and hepatic necrosis in feedlot cattle fed a 'hot' carbohydrate-rich ration. Necrobacillosis is recurrent among free-ranging elk on Wyoming's winter feedgrounds.[1] Typically it affects juveniles, which develop a combination of digital and internal lesions. Digital skin and the mouth are presumed portals of entry. The association with crowding elk on winter feedgrounds (in Jackson Hole, WY) was made in the late 1920s by pioneering elk biologist Olaus Murie.[2] The cumulative death toll on the feedground in late winter 2014 was 80 elk, but in some years it can be in the several 100s. Other ungulates, when stressed and crowded, are similarly susceptible. They include white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), pronghorn (*Antilocapra americana*), wild and semi-domesticated tundra reindeer (*Rangifer tarandus tarandus*), and wisent (*Bison bonasus*);[3,4] Wobeser et al's paper has excellent illustrations of the lesions.[4] A memorably catastrophic episode involved pronghorn trapped in Wyoming and, for their sins, sent to Texas.[5]

The overall pattern of losses in elk was typical of necrobacillosis. Elk calves became sick in part due to the wet weather. *F. necrophorum* is a normal inhabitant of the gastrointestinal system and is present in feces. It thrives in wet anaerobic environments. Multiple freeze/thaw cycles resulted in crusting of snow with sharp, jagged ice and mud. Such conditions predispose to digital

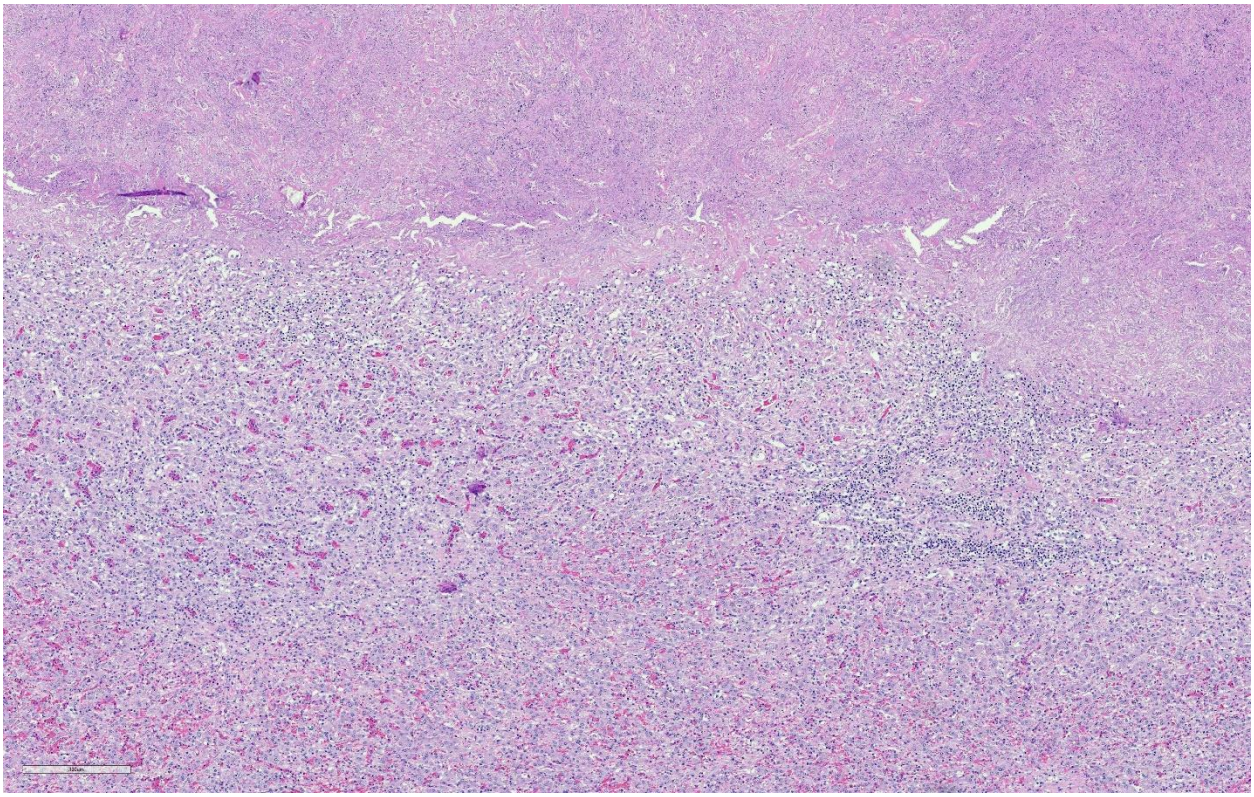

Figure 2-2. ***Liver, elk. There is diffuse lytic necrosis of hepatic parenchyma (top), a transitional zone of hepatocellular degeneration and necrosis with infiltration of numerous neutrophils and fewer macrophages (middle), and normal hepatic parenchyma (bottom). (HE, 96X)***

dermatitis in elk. Extension to liver is a common sequel, resulting in death. Calves and yearlings are typically most susceptible, but adults can be affected.

Trueperella pyogenes was also cultured from liver. This is a common concurrent synergistic infection in necrobacillosis. *T. pyogenes* supplies a heat-labile factor which stimulates replication of *F. necrophorum*. Returning the favor, *F. necrophorum* produces a leukotoxin which aids survival of *T. pyogenes*.

There is no vaccine for the disease in wildlife, at least in the United States, partly due to the modest immunological response. Control is by avoiding concentration of animals in environments heavily contaminated with feces; preventing cutaneous or oral lacerations; reducing stress; and avoiding feeding practices that promote oral lesions or ruminal acidosis.

Contributing Institution:
Wyoming State Veterinary Laboratory – http://www.uwyo.edu/wyovet/

JPC Diagnosis:
1. Liver: Hepatitis, necrotizing, focally extensive, severe, with filamentous bacteria.
2. Bone marrow: Serous atrophy of fat, diffuse, severe with filamentous bacteria.

JPC Comment:
The contributor provides an excellent review of *Fusobacterium necrophorum*'s pathogenesis in elk and in addition to other domestic and wildlife species.

F. necrophorum is an aerotolerant-to-anaerobic, non-motile, non-spore forming, pleomorphic, Gram-negative bacterium that tends to form filaments. The organism is a normal inhabitant of the mammalian gastrointestinal tract, particularly within the rumen, and fecal contamination contributes to the pathogen's distribution and concentration in the environment.[1,6] Unable to penetrate healthy epithelium, the organism requires a breach in the epidermal barrier in

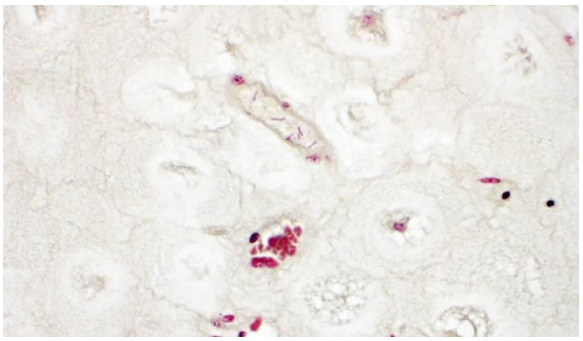

Figure 2-3. ***Liver, elk. Scattered throughout thearea of necrosis, are small aggregates gram-negative filamentous bacilli consistent with Fusobacterium necrophorum. Brown-Brenn, 400X).***

addition to an anaerobic environment to establish infection. Trauma, ischemia, parasitic and viral infections are common predisposing factors.[1]

Multiple virulence factors contribute to the pathogenicity of *F. necrophorum*, including a potent leukotoxin, high levels of endotoxin, hemagglutinin, and a collagenolytic cell wall component responsible for dermotoxic activity. Once established in tissue, *F. necrophorum* proliferates and causes extensive coagulative necrosis linked to vasculitis, thrombosis, and ischemia, likely induced by these potent toxins.[1,6] As mentioned by the contributor, concurrent bacterial infections are facilitated by *F. necrophorum*'s leukotoxin, which induces apoptosis of ruminant leukocytes. Pathogens such as *Truperella pyogenes and Staphylococcus aureus* lower the oxygen tension and redox potential of the tissue, resulting in a synergistic infection.[1]

Hepatic necrobacillosis is commonly reported in cattle as the result of ruminal acidosis followed by primary infection of *F. necrophorum* within the rumen wall and subsequent portal spread. In domestic ruminants, digital necrobacillosis does not commonly result in a septicemia and pedal lesions and are often restricted to one foot.[7]

One study following an outbreak of digital necrobacillosis in a herd of wild Norwegian reindeer found most lesions were localized to a single foot, similar to as described in cattle.[6] Significant pain is associated with this disease and lesions are often severe and chronic. Affected animals are therefore more likely to suffer from poor nutrition, as seen in this case (serous atrophy of fat), and/or predation.[6] However, another study of 40 wild-caught pronghorns with either primary pododermatitis or necrotic stomatitis caused by *F. necrophorum* progressed to produce fatal septicemia with metastatic lesions found in the forestomachs, lung, liver, and cecum in 38 animals.[7]

During the 1800s and early 1900s, digital necrobacillosis was the most serious problem seen in Norwegian reindeer and was linked to intensive herding practices. High numbers of animals were regularly restricted to small, muddy, and feces-contaminated areas for milking and other activities that ultimately resulted in favorable conditions for transmission. The disease has more or less disappeared from reindeer herds since more extensive hearing practices have been adopted, although outbreaks continue to be reported wild Norwegian reindeer.[6]

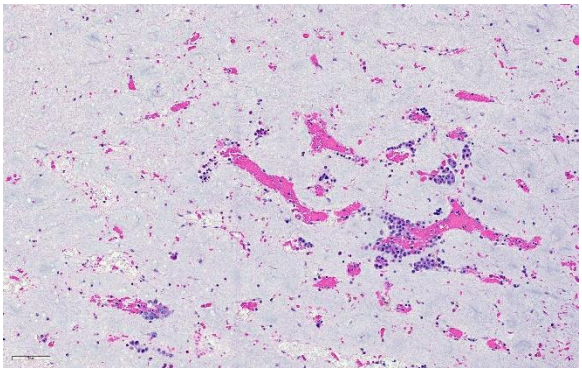

Figure 2-4. ***Bone marrow, elk. There is diffuse severe serous atrophy of fat with markedly decreased marrow elements. (HE, 120X).***

References:

1. Carvallo FR, Uzal FA, Flores C, et al. Alimentary necrobacillosis in alpacas. *J Vet Diagn Invest*. 2020;32(2):339-343.
2. Kreeger TJ, Cornish T, Creekmore TE, Edwards WH, Tate C. Foot rot. In: *Field Guide to Diseases of Wyoming Wildlife, pp. 117-119. Wyoming Game and Fish Department*. Cheyenne, WY: 2011.
3. Murie, OJ. An epizootic disease of elk. *J. Mammal*. 1930;1:214-222
4. Wobeser G, Runge W, Noble D. Necrobacillosis in deer and pronghorn

antelope in Saskatchewan. *Can Vet J*. 1975;16(1):3-9.
5. Handeland K. *Fusobacterium necrophorum* infection. Gavier-Widén D, Duff JP, Meredith A, eds. onIn: *Infectious Diseases of Wild Mammals and Birds in Europe.* Wiley-Blackwell, Chichester, West Sussex; 2012:428-430.
6. Handeland K, Boye M, Bergsjø B, Bondal H, Isaksen K, Agerholm JS. Digital necrobacillosis in Norwegian wild tundra reindeer (Rangifer tarandus tarandus). *J Comp Pathol*. 2010;143(1):29-38.
7. Edwards JF, Davis DS, Roffe TJ, Ramiro-Ibañez F, Elzer PH: 2001, Fusobacteriosis in captive wild-caught pronghorns (*Antilocapra americana*). *Vet Pathol.* 38(5):549-552.

CASE III: 17-145 (JPC 4117030-00)

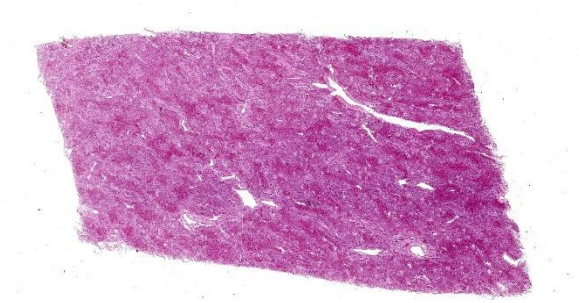

Figure 3-1. ***Liver, ox. At subgross magnification, there is a retiform pattern of lobular hemorrhage present diffusely throughout the section. (HE, 5X)***

Signalment:
2.5-year-old Hereford heifer, *Bos taurus.*

History:
42 of 187 grazing beef cattle died between June 28 and July 2017 in a farm in Florida department, Uruguay. Morbidity rate was 22.9%, mortality 22.5% and lethality 97.7% (the farmer indicated that one affected cow recovered). The herd had 42 cows, 70 2.5-year-old heifers, and 75 yearlings (heifers and steers). At the end of the outbreak dead animals included 3 cows (7.1%), 11 heifers (15.7%) and 28 yearlings (37.3%). Clinical signs included stupor or aggressiveness and drooling. The animals were found dead or died after a clinical course of up to three days. Numerous groups of insect larvae identified as *Perreyia flavipes* (sawfly) were found in the pasture.

Gross Pathology:
Hepatic changes included diffuse enhanced lobular pattern with yellowish edema expanding the gall bladder wall. Other necropsy findings were abdominal and pericardial effusion and petechiae and ecchymoses in the pericardium, peritoneum and pleura. Diffuse hemorrhages were observed on the capsular surface of the spleen. The omasal content was dry. Body fragments and heads of *Perreyia* sp. larvae were found in the content of the rumen and omasum.

Laboratory results:
Not applicable.

Microscopic Description:
Liver: subgrossly, there is diffuse enhancement of the lobular pattern, the lobular histoarchitecture is effaced and hepatic lobules are collapsed. There is necrosis and loss of hepatocytes in the centrilobular and midzonal areas, and replacement by abundant extravasated erythrocytes (hemorrhage). Remaining hepatocytes are either rounded, swollen and detached from the hepatic cords, or have angular borders, pyknotic nucleus and shrunken hypereosinophilic cytoplasm (necrosis). In the periportal areas, remaining viable hepatocytes are swollen and vacuolated (hydropic and lipid degeneration), and there are occasional bi- or multi-nucleated hepatocytes (regeneration). There are increased numbers of histiocytes and neutrophils in the sinusoids, and Kupffer cell hypertrophy and hyperplasia. There is mild bile duct hyperplasia, and portal tracts are multifocally infiltrated by inflammatory cells, including histiocytes, lymphocytes, fewer neutrophils and eosinophils, and expanded by mature collagen bundles (fibrosis); similar changes extend to the hepatic capsule in some sections (not included in all slides).

Contributor's Morphologic Diagnosis:
1. Liver: severe diffuse acute centrilobular and midzonal hepatocellular degeneration, necrosis, loss and hemorrhage, *Bos taurus*.

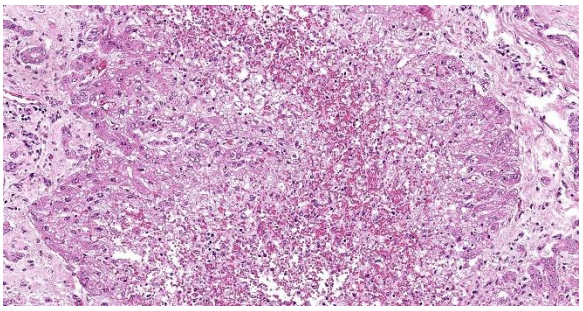

Figure 3-2. ***Liver, ox. There is loss of hepatocytes within the central and midzonal areas of the lobule with replacement by acute hemorrhage. Remaining periportal hepatocytes are swollen by an accumulation of numerous cytoplasmic lipid droplets. (HE, 192X)***

2. Liver: mild portal fibrosis and bile duct hyperplasia, with lymphohistiocytic portal hepatitis, *Bos taurus*.

Contributor's Comment:
The diagnosis of *Parreyia flavipes* intoxication in this outbreak was based on the observation of severe acute centrilobular necrosis, the presence of large amounts of *Perreyia flavipes* larvae in the pasture, and the presence of Hymenoptera (sawfly) larval body fragments and heads in the contents of the forestomachs of the necropsied cattle. No hepatotoxic plants were found in the pasture. Speciation of different *Parreyia* spp. under field conditions is challenging, because of the lack of dichotomous keys for species identification. Identification of adult specimens is necessary to confirm the sawfly species. However, based on the regional literature, we speculate that the species involved in the outbreak was *Perreyia flavipes.*[4]

It is worth mentioning that there is evidence of chronic damage in the submitted sections of liver, including portal fibrosis, mild bile duct hyperplasia and portal hepatitis. We interpreted these as incidental pre-existing lesions, which has no direct relationship with the acute hepatotoxic condition associated with the death. Probably these chronic lesions could represent sequelae of ingestion of sublethal amounts of pyrrolizidine alkaloids, contained in some plants such as *Senecio* spp., or hepatic fascioliasis, which are well documented causes of chronic liver damage in the geographical area where the outbreak occurred.[2,11]

The consumption of larvae of insects in the order Hymenoptera ("sawfly"), should be considered as a differential diagnosis in cases of acute hepatotoxicity in cattle, sheep and pigs.[2] For more than half a century cases of sawfly intoxication have been described in different parts of the world. In Australia, the intoxication is a consequence of the consumption of *Lophyrotoma interrupta* (family: *Pergidae*). The outbreaks are limited to areas with large forests of *Eucalyptus melanophloia*, host tree of the larva.[7] In Denmark sawfly poisoning is reported in sheep due to the consumption of *Arge pullata* (family: *Argidae*), on pastures in birch forests (*Betula pendula*).[16] In Brazil (states of Rio Grande do Sul and Santa Catarina), outbreaks had been reported in pigs, sheep and cattle, due to the consumption of larvae of *Perreyia lepida* (family: *Pergidae*), colloquially known as "mata porco".[12,13,15] In Uruguay, Dutra et al. (1997) described naturally-occurring cases associated with the ingestion of *Perreyia flavipes*, including epidemiological aspects, clinical signs and pathology. In addition, the author reproduced the poisoning experimentally in sheep and calves and described some aspects of the biology of the adult insect.[4,5]

Since the discovery of the toxic effect of sawfly larvae, additional information has been generated about the biology of these insects. Adults of *P. flavipes* are wasps with a short life-span (<72 h), emerging in February/March.[5,14] They are easily differentiated from other *Perreyia* species due to morphological characteristics: bright orange leg parts and 13–15 antennal articles.[8] The adults do not feed. Females have little activity and fly only short distances, usually staying on the top of grasses or trying to reach an elevated position. Eggs are laid in the soil in clusters of 100–700. Eggs are oblong and yellowish-white, although once inseminated develop a blackish-yellow color.[5] The incubation period is limited to 4–8 weeks, larvae start to hatch in March/April (late summer and early autumn in the Southern hemisphere), and develop through the autumn and winter.[14] During the winter season, from June to September, aggregates of *P. flavipes* larvae are commonly found in open grasslands, forming an orderly moving column of approximately 100 individuals, particularly after rainfalls and on

cloudy days. Occasionally, large numbers of these insect masses are seen moving around the ground, while other days very few or no groups are found.[4]

The sawfly toxin responsible for deaths of livestock had been at first reported as a hepatotoxic octopeptide containing 4 D-aminoacids, called lophyrotomine. This compound has been isolated from larvae of *L. interrupta* and *A. pullata* at concentrations of up to 0.07% of the dry matter, with a lethal dose 50 (LD50) of 2 mg/kg by intraperitoneal injection in mice.[9] Subsequently, a closely related octapeptide (LGlu) and 3 heptapeptides were discovered in other sawfly species: pergidin (Perg), 4 valinepergidin (VPerg), and dephosphorylated pergidin (dpPerg).[4,10] The heptapeptides contain 5 D-amino acids, and Perg and VPerg also contain phosphoserine. These compounds are highly toxic for vertebrates and have been detected and quantified in 4 sawfly species: *L. interrupta*, *L. zonalis*, *P. flavipes* (*Pergidae*), and *Arge pullata* (*Argidae*).[6,10] The main toxin in *P. flavipes* is pergidin, a heptapeptide containing a phosphoseryl residue, although small amounts of lophyrotomin are also present.[1,9] All these sawfly species can be found in large masses in the field, which facilitated the investigation and detection of the toxins. Recent studies, using a new extraction procedure that allowed testing single larvae by liquid chromatography–mass spectrometry (LC-MS) analyses, also identified the toxins in related but nonpullulating sawfly species. The study found deviations from the published species-specific chemical profiles in 3 of 4 Hymenoptera species.[1] The authors concluded that intraspecific variation in the peptides analyzed, but also the methods used for chemical extractions and analyses, are possible explanations for these differing results.[1]

Contributing Institution:
"Plataforma de Investigación en Salud Animal, Instituto Nacional de Investigación Agropecuaria" (INIA), La Estanzuela, Uruguay.

JPC Diagnosis:
Liver: Necrosis, centrilobular and midzonal, diffuse, with hemorrhage.

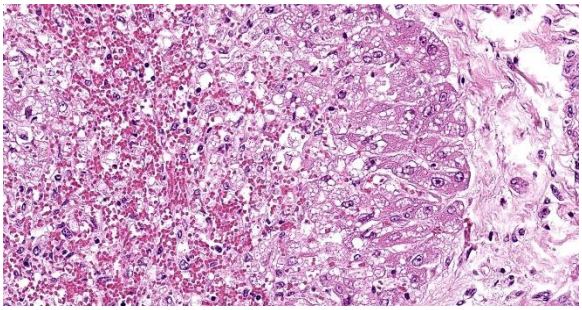

Figure 3-3. ***Liver, ox. Higher magnification of necrotic centrilobular and midzonal hepatocytes and degenerating, lipid-filled periportal hepatocytes. (HE, 387X).***

JPC Comment:
The contributor provides an excellent and interesting review of sawfly larva toxicity, a condition that affects cattle and small ruminants in various geographic locations, including Australia, Denmark, and South America.[2] A similar 2012 WSC case was presented in conference 15, case 1.

The first reliable report of sawfly toxicity in cattle was in the Maranoa district of Queensland, Australia in 1911, although the first suspected poisoning was reported in 1887, approximately two decades after cattle were introduced to the region. A 1982 survey of farms within the same region found the estimated annual cost to graziers to be the equivalent of $3.2 million US dollars when adjusted for inflation and current exchange rates.[3]

Participants discussed patterns of zonal necrosis, including centrilobular (zone 3), midzonal (zone 2), periportal (zone 1), and massive (entire lobule).[2] Direct acting toxins typically cause periportal necrosis due to these hepatocytes being the first exposed. Meanwhile, centrilobular necrosis (in cases of toxicity) occurs due to the biotransformation of xenobiotics into toxic intermediates as the result of hepatocytes in this region having the highest concentration of cytochrome P450 enzymes. The moderator provided the following table of agents associated with acute hepatotoxicity in cattle:

Name	Toxic Principle
Blue-green algae	Microcystin-LR
Mushrooms: Amanita, Phalloides and others	Amatoxins
Cycads (Zamia sp.)Cycads (Zamia sp.)	Methylazoxymethanol
Solanacae (Cestrum sp.)	Atractyloside
Compositae (Xanthium-cockleburr)	Carboxyatractyloside
Ulmaceae (Trema sp.-Poison peach)	Trematoxin
Myoporacae (Myoporum)	Ngaione (periportal)
Iron	
Sawfly larvae (Lophyrotoma sp.)	Lophyrotomin/pergidin

Prior to death, clinical signs consistent with liver failure caused by a severe necrotizing hepatopathy may be observed and include acute hepatic encephalopathy, hemorrhage, jaundice, and photosensitization. Acute hepatic encephalopathy occurs due to increased plasma ammonia levels and hypoglycemia, manifesting as weakness, depression, and stupor, although some animals may become highly excited and demonstrate aggressive behavior as observed in this affected herd. Widespread hemorrhage has been reported to be variably prominent in field cases; however, studies of experimentally infected calves have found increased prothrombin and activated thromboplastin times and reduced fibrinogen concentrations. Jaundice and photosensitization are rarely observed due to the short course of the disease.[4]

There is bridging portal fibrosis and biliary hyperplasia within the section, as noted by the contributor. The significance of this finding was discussed amongst participants with consensus that the lesion is likely pre-existing and unrelated to sawfly larvae ingestion.

Finally, there was spirited discussion amongst participants in regard to use of the word "diffuse" in the morphologic diagnosis due to risk of confusion with massive necrosis, which was not seen in this case. Massive necrosis refers to necrosis of an entire hepatic lobule, not necessary the liver as a whole.[2] Therefore, when describing zonal necrosis, one should strive to avoid such confusion by including a zonal distribution modifier in addition to the overall distribution within the examined section.

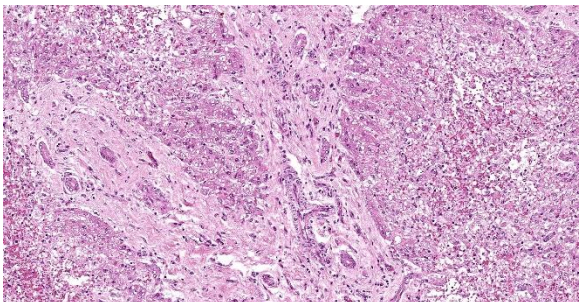

Figure 3-4. ***Liver, ox. There is moderate fibrosis of portal areas, and mild biliary hyperplasia. Bile duct epithelium is hypertrophic. (HE, 157X)***

References:

1. Boevé JL, Rozenberg R, Shinohara A, Schmidt S. Toxic Peptides Occur Frequently in Pergid and Argid Sawfly Larvae. PLOS ONE. 2014. 9(8): e105301. https://doi.org/10.1371/journal.pone.0105301
2. Cullen JM, Stalker MJ. Liver and biliary system. In: Maxie MG. ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 6th ed. Vol 2. Saunders Elsevier. 2016:284, 330-332.
3. Dadswell LP, Abbott WD, McKenzie RA. The occurrence, cost and control of sawfly larval (Lophyrotoma interrupta) poisoning of cattle in Queensland 1972-81. *Aust Vet J*. 1985;62(3):94-97.
4. Dutra F, Riet-Correa F, Mendez MC, Paiva N. Poisoning of cattle and sheep in Uruguay by sawfly (Perreyia flavipes) larvae. *Vet Hum Toxicol*. 1997;39(5):281-286.
5. Dutra F. Intoxicación por larvas de *Perreyia flavipes* en bovinos y ovinos. Caracterización de la enfermedad y biología del insecto. *Veterinaria (Montevideo)*. 2003;38 (152-153):7-24. In Spanish.
6. MacLeod JK, Braybrook C, Simmonds PM, Oelrichs PB. A unique toxic peptide from the larvae of the South American

sawfly, *Perreyia flavipes*. *Aust J Chem*. 2000;53:293–297.
7. McKenzie RA, Dunster PJ, Twist JO, Dimmlock CK, Oelrichs PB, Rogers RJ, Reichmann, KG. The toxicity of sawfly larvae (*Lophyrotoma interrupta*) to cattle. Qld. Dept. Prim. Ind. Bull. (1985). QB85001.
8. Neves, F.M., Pie, M.R. On the Adult Behavioral Repertoire of the Sawfly *Perreyia* flavipes Konow, 1899 (Hymenoptera: Pergidae): Movement, Mating, and Thanatosis. *Neotrop Entomol*. 2018;47:46–52.
9. Oelrichs PB, MacLeod JK, Seawright AA, et al. Unique toxic peptides isolated from sawfly larvae in three continents. *Toxicon*. 1999;37(3):537-544.
10. Oelrichs PB, MacLeod JK, Seawright AA, Grace PB. Isolation and identification of the toxic peptides from Lophyrotoma zonalis (Pergidae) sawfly larvae. *Toxicon*. 2001;39(12):1933-1936.
11. Preliasco M, Rivero R. Poisoning of Cattle by *Senecio* spp. in Uruguay. In: *Poisoning by plants, mycotoxins, and related toxins*. 2009;30:198-207.
12. Raymundo DL, Bezerra Jr. PS, Bandarra PM, Pedroso PMO, de Oliveira EC, Pescador CA, Driemeier D. Spontaneous poisoning by larvae of *Perreyia flavipes* (*Pergidae*) in sheep. Pesq. Vet. Bras. 2008. 28 (1): 19-22.
13. Raymundo DL, Bezerra Jr. PS, Bandarra PM, Dalto AGD, Soares MP, da Cruz CEF, Driemeier D. *Perreyia flavipes* larvae toxicity. *Pesq. Vet. Bras*. 2012; 32(8):735-738.
14. Soares MP, Riet-Correa F, Smith D, Pereira Soares M, Mendez MC, Brandolt AL. Experimental intoxication by larvae of *Perreyia flavipes* Konow, 1899 (Hymenoptera: *Pergidae*) in pigs and some aspects on its biology. *Toxicon*. 2001; 39:669–678.
15. Soares MP, Quevedo PS, Schild AL. Intoxicacao por larvas de *Perreyia flavipes* em bovinos na regiao sul do Rio Grande do Sul. *Pesq. Vet. Bras*. 2008;28(3):169-173. In Portuguese.
16. Thamsborg SM, Jørgensen RJ, Brummerstedt E. Sawfly poisoning in sheep and goats. *Vet Rec*. 1987;121(11):253-255.

CASE IV: N17-459 (JPC 4142728-00)

Signalment:
4-year-old, spayed-female, mixed breed dog (*Canis familiaris*).

Figure *4-1. Presentation, dog. Dorsal to the rectum there is a broadly ulcerated to deeply cavitated, 6.5x5.5 cm defect lined by pale tan to tan, soft to firm, irregular tissue that extends deep into the underlying subcutaneous tissue and connects with the dorsal pelvic canal. (Photo courtesy of: University of Florida, College of Veterinary Medicine, Veterinary Diagnostic Laboratory, P.O. Box 100123, Gainesville, FL 32610, http://labs.vetmed.ufl.edu)*

History:
This dog presented for a history of perineal lesions. The tail was amputated by the referring veterinarian, after which *Lagenidium* sp. infection was confirmed via PCR. MRI revealed sublumbar lymphadenopathy and regional soft tissue involvement. The patient became fecally incontinent. On presentation to the University of Florida, there was thickening of the rectal wall and pain on rectal palpation. There was an initial dramatic reduction of the lesion size with therapy. However, lesions returned and worsened, with necrosis of perineal tissue. Euthanasia was elected.

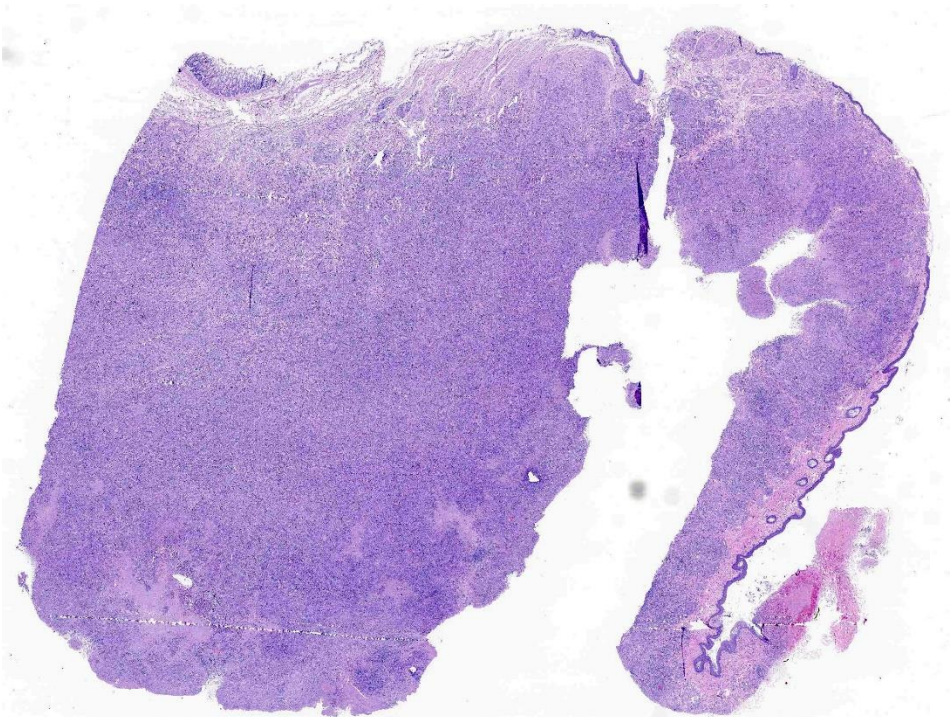

Figure *4-2. Rectum, anus, perianal tissue, dog. A large inflammatory mass containing rectum (top left), anus (top middle) and perianal tissue is submitted for examination. The inflammatory mass encompasses the submucosa of the rectum and adjacent tissues, and extends to effaces tissues deep to the anus and perianal skin. (HE, 32X)*

Gross Pathology:
There is moderate, diffuse expansion of the subcutis of the left hind limb with yellow, gelatinous material (edema), with the proximal limb to hock region twice the circumference of the contralateral limb. There is a 12x5x3 cm nodule within the left inguinal region that is centrally cavitated and contains brown-green, watery to gelatinous material. The surrounding tissue is brown to purple, firm and irregular, and approximately 3 mm to 1 cm thick. Dorsal to the rectum there is a broadly ulcerated to deeply cavitated, 6.5x5.5 cm defect lined by pale tan to tan, soft to firm, irregular tissue that extends deep into the underlying subcutaneous tissue and connects with the dorsal pelvic canal. Adjacent to the rectum and attached to the rectal wall there are three pale tan, soft to firm, nodular to confluent masses measuring up to 7x3x3 cm within the pelvic canal as described above. On cut section these nodules are solid and mottled pale tan to tan with a granular appearance.

Laboratory results:
PCR via the RDVM detected *Laginidium* sp. DNA.

Microscopic Description:
Within the dermis and subcutis of the perineum, there is a multinodular to confluent inflammatory infiltrate with a large tract lined by inflamed granulation tissue. The epidermis adjacent to the tract is ulcerated. A similar inflammatory infiltrate is present in the perianal dermis and subcutis and anal skeletal muscle, mesorectum/mesocolon and rectal muscularis, with minimal extension into the anorectal submucosa. A similar inflammatory infiltrate effaces about half of a small lymph node in the mesorectum/mesocolon. A tract is also present in the perianal tissue, with much of the tract lined by fibrin with enmeshed cellular debris and neutrophils. In one area of the tract, the defect is lined by stratified squamous epithelium. The inflammatory infiltrate is composed primarily of macrophages and multinucleated giant cells with fewer lymphocytes, plasma cells and sometimes few to low numbers of neutrophils and/or eosinophils. Within the inflammatory infiltrate, and often within the cytoplasm of multinucleated giant cells, there are large numbers of approximately 4-12 µm diameter septate, branching hyphae with non-parallel walls. Vessels within the inflammatory infiltrate sometimes to often exhibit fibrinoid necrosis, contain cell debris and/or inflammatory cells within the vessel walls, recanalization, and/or contain fibrin thrombi. There are multifocal

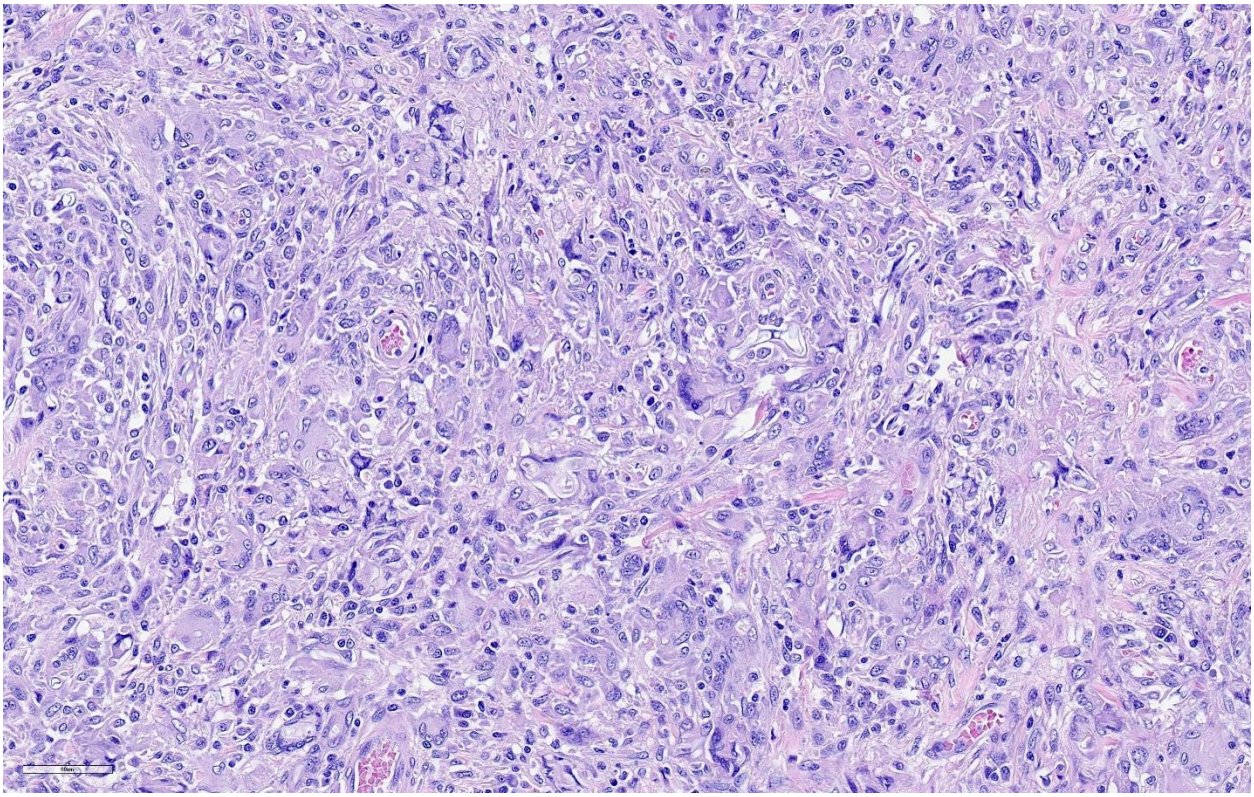

Figure 4-3. ***Perirectal soft tissue, dog. The tissue is effaced by a disorganized mass of innumerable epithelioid macrophages, multinucleated giant cell macrophages, lymphocytes, plasma cells and fewer neutrophils. Within the cytoplasm, there are numerous cross sections of fungal hyphae (arrows), measuring up to 8um in diameter. (HE, 400X)***

variably sized areas of necrosis within the inflammatory infiltrate, often adjacent to vessels with vasculitis or fibrinoid necrosis. There are occasional small areas of necrosis central within nodular granulomatous infiltrates.

Contributor's Morphologic Diagnoses: Cellulitis, perianal/perineal dermatitis, anoproctitis, and lymphadenitis, granulomatous to pyogranulomatous to eosinophilic and granulomatous, chronic, locally extensive, severe, with intralesional hyphae

Contributor's Comment:

Lagenidium is one of a number of pathogenic oomycetes, with several species infecting mammals, insects, and nematodes, with the prevalence of *Lagenidium* infections in mammals increasing over the last several years.[8] *Lagenidium giganticum* was also approved for several years as a biological control agent for mosquitoes; subsequent studies have found significant molecular similarities between mammalian *Lagenidium* strains and those approved for mosquito control[9], although biocontrol strains did not grow well at 37˚C.

Oomycetes are aquatic dimorphic water molds, and include the genera *Lagenidium* and *Pythium*, as well as a number of significant plant pathogens, such as *Phytophthora infestans*, the organism that caused the Irish potato famine in the mid 19th century.[1] *Pythium* causes two syndromes; the first is cutaneous, which is more common in horses and produces spongy masses that ulcerate and become necrotic[5], while the second is gastrointestinal. Hyphae are GMS-positive but PAS-negative, as they do not produce chitin.

Lagenidium produces similar lesions and has a similar geographic distribution, which makes differentiation of pythiosis and lagenidiosis difficult. However, unlike the fairly wide host range of *Pythium*, *Lagenidium* appears to be only a pathogen of dogs. Lesions tend to be on the

limbs, mammary, and inguinal areas[5], with spread to regional lymph nodes. *Lagenidium* also has a predisposition for invasion of great vessels, with one study finding 70% of cases had vascular invasion.[3] Histologic lesions typically are pyogranulomatous with significant numbers of eosinophils.

Treatment of these cases is difficult, and typically consists of surgical resection, chemotherapy, and supportive care.[7] Prognosis is guarded and medical therapy is difficult, as one of the main antimycotic targets, ergosterol, is missing in oomycetes.[2]

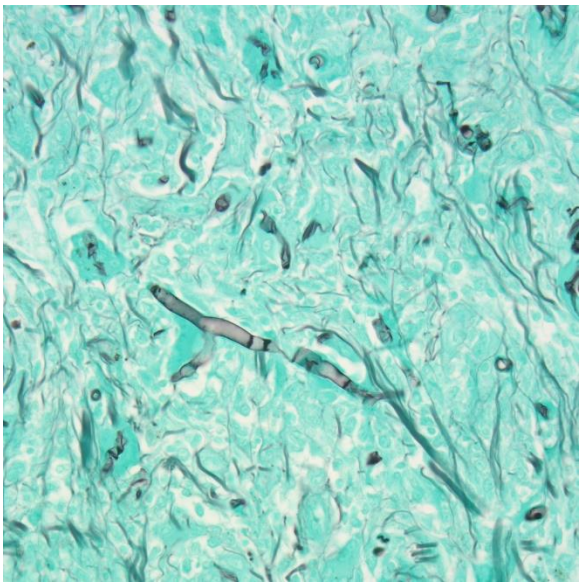

Figure 4-4. ***Perirectal soft tissue, dog. Numerous pauciseptate hyphae measuring up to 8um in diamtere are present within the cytoplasm of macrophages and multinucleated giant cell macrophages. (GMS, 400X)***

Contributing Institution:
University of Florida
College of Veterinary Medicine
Veterinary Diagnostic Laboratory
P.O. Box 100123
Gainesville, FL 32610 http://labs.vetmed.ufl.edu

JPC Diagnosis:
Rectum, anus, perianal tissue: Perianal dermatitis and cellulitis, granulomatous, chronic, focally extensive, severe, with numerous intra- and extracellular hyphae.

JPC Comment:
The contributor provides an excellent review of both of pythiosis and lagenidiosis.

Both agents share similar clinical signs and characteristics within cytologic and histologic sections, including large diameter, non-parallel, rarely septate hyphae associated with eosinophilic granulomatous to pyogranulomatous inflammation.[6] Although PCR assay or ribosomal DNA sequencing should be used for definitive differentiation, an important histologic feature that may help to differentiate lagenidiosis from pythiosis is hyphal diameter in tissue. *Lagenidum* spp. is typically larger and more variable (7 to 25 μm) compared to *P. insidiosum* (2 to 7 μm). This feature is repeatable when cultured, with *Lagenidium sp*. (25 to 40 μm) compared to *Pythium sp*. (4 to 10 μm).[6]

Participants discussed an additional differential diagnosis of mucormycosis (formerly known as zygomycosis), caused by etiologies within the order Mucorales such as *Rhizipus, Mucor, Lichtheima* (*Absidia),* and *Saksenaea*. As with *Pythium* and *Lagenidium*¸ these etiologies are histologically indistinguishable and definitive identification requires culture, PCR, or immunohistochemistry.[5]

Lagenidium giganteum's transient historical use as a biopesticide for mosquito control stems from the ability of its biflagellate zoospores to selectively attach and penetrate the cuticle of mosquito larvae and proliferate within, killing the host in less than three days. Under ideal conditions the mycelia differentiate into asexual zoospores to amplify the initial infection but can also remain dormant for days to years until conditions again favor mosquito breeding and germination of spores. Recycling following a single application has been documented for up to 8-10 years, which made its use as a biopesticide an attractive option.[4]

L. giganteum was registered as a biologic control agent with the US Environmental Health Protection Agency in 1995 under the trade name Laginx, but was later deregistered at the manufacturer's request in 2011. A subsequent 2013-2014 phylogenic study of 21 mammalian

Lagendium strains found 11 could not be differentiated from strains the US Environmental Protection Agency had approved for biological control of mosquitoes; these strains were later unregistered and are no longer available. Although the phylogenically indistinguishable biological control and mammalian strains both grew well at 25ºC, only the mammalian strains grew well at 37ºC.[9]

References:

1. Fry WE, Grünwald NJ: Introduction to Oomycetes. *In*: The Plant Health Instructor. 2010
2. Grooters AM. Pythiosis, lagenidiosis, and zygomycosis in small animals. *Vet Clin North Am Small Anim Pract*. 2003;33**:** 695-720, v.
3. Grooters AM, Hodgin EC, Bauer RW, Detrisac CJ, Znajda NR, Thomas RC. Clinicopathologic findings associated with Lagenidium sp. infection in 6 dogs: initial description of an emerging oomycosis. *J Vet Intern Med*. 2003;17**:** 637-646.
4. Kerwin JL. Oomycetes: Lagenidium giganteum. *J Am Mosq Control Assoc*. 2007;23(2 Suppl):50-57.
5. Mauldin EA, Peters-Kennedy J. Integumentary system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol 1. 6th ed. St. Louis, MO: Elsevier; 2016:657-660.
6. Rajeev S, Ilha MR, Harrison JM, et al. Pathology in practice. Lagenidium infection. *J Am Vet Med Assoc*. 2012;241(4):447-449.
7. Schmiedt CW, Stratton-Phelps M, Torres BT, et al. Treatment of intestinal pythiosis in a dog with a combination of marginal excision, chemotherapy, and immunotherapy. *J Am Vet Med Assoc*. 2012;241**:** 358-363.
8. Spies CFJ, Grooters AM, Levesque CA, et al. Molecular phylogeny and taxonomy of Lagenidium-like oomycetes pathogenic to mammals. *Fungal Biol*. 2016;120**:** 931-947.
9. Vilela R, Taylor JW, Walker ED, Mendoza L. Lagenidium giganteum pathogenicity in mammals. *Emerg Infect Dis*. 2015;21**:** 290-297.