Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2026-2027

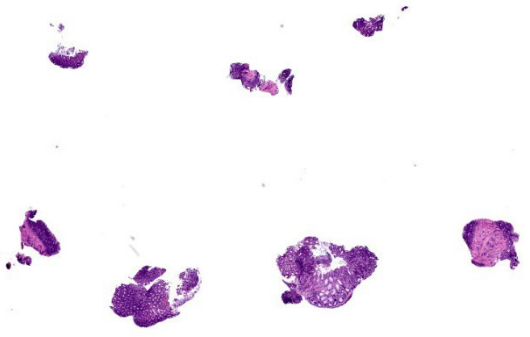
Conference #11 — 4 November 2026

CASE I:

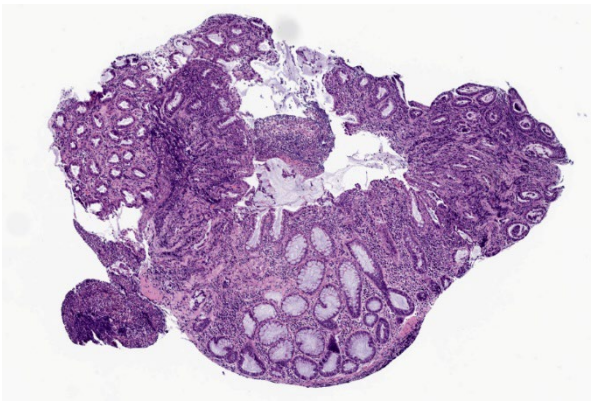
Signalment:
3-year-old intact, female Boxer dog (Canis lupus familiaris)

History:
The patient was reported to have a history of chronic hemorrhagic diarrhea for the past 8 months. In May of 2016 a fecal flotation was performed and results were negative. Giardia/Cryptococcus testing was negative on Immunofluorescence Assay (IFA). In October of 2016 Trypsin-like Immunoreactivity (TLI), Pancreatic Lipase Immunoreactivity (PLI), and Cobalamin/Folate were normal. Fecal cytology revealed moderate neutrophilic inflammation with bacteria.

Gross Pathology:
Endoscopy examination of the stomach and duodenum appeared normal. The colon was ulcerated and inflamed. Multiple punch biopsies of duodenum (7 samples), stomach (7 samples) and colon (9 samples) were collected during endoscopy and submitted.



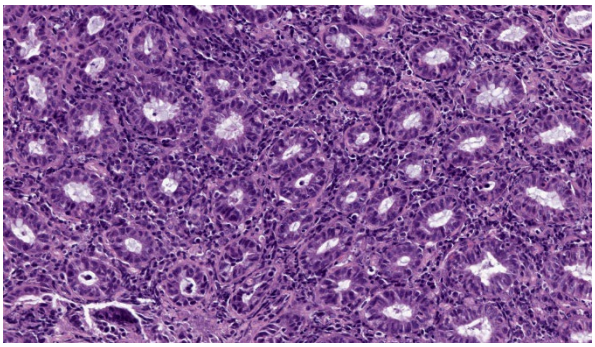
1-1. Colon, dog: Multiple endoscopic biopsies are submitted for examination. (HE, 12X)



1-1. Colon, dog: Within inflamed areas of the mucosa, remnant colonic crypts are separated by an inflammatory infiltrate, and there is a diffuse loss of goblet cells. (HE, 105X)

Laboratory Results:
None available

Microscopic Description:
Colon: The lamina propria contains an infiltrate of numerous neutrophils, eosinophils, with lesser numbers of lymphocytes, and plasma cells. These inflammatory infiltrates are associated with numerous spherical, unicellular, nonbudding organisms which range in size from 6-30 micrometers. These algal forms have a thick, double-layered, refractile wall and dense sporangium. There is often internal septation of the morula-like form of



1-3. Colon, dog: The lamina propria contains numerous macrophages, lymphocytes and plasma cells with separate colonic crypts. (HE, 459X)

sporangia, with endospores arranged symmetrically (endosporulation).

Contributor's Morphologic Diagnoses:

1. Severe suppurative and eosinophilic colitis with intralesional algae organisms (*Prototheca sp.)*
2. Colonic protothecosis

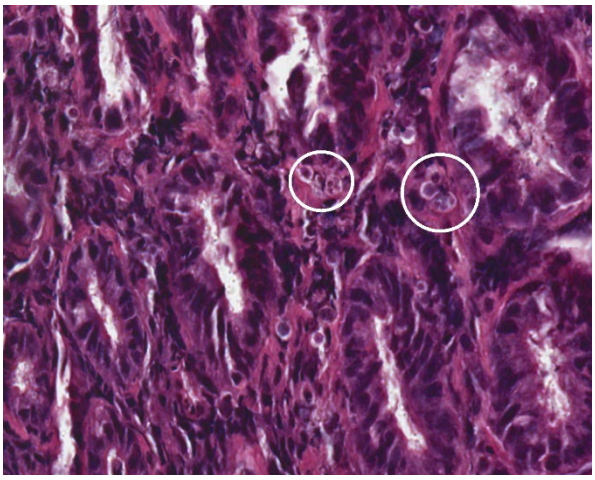
Contributor's Comment:

Examination of several colonic punch biopsy samples finds numerous algal organisms admix with suppurative and eosinophilic inflammation.

Prototheca spp are a colorless algae. They are related to the green algae of the genus *Chlorella*.[3,6] There are three recognized species of Prototheca organisms: *Prototheca zopfii*, *Prototheca wickerhamii*, and *Prototheca stagnora*.[15] A fourth species is also under investigation: *Prototheca salmonis*. Of these four species, only *P. zopfii* and *P. wickerhamii* have been found to be pathogenic in any animal species.[8, 10]

Protothecosis is a rare zoonotic condition with worldwide distribution. It is reported in numerous species including humans, domestic cattle, goats, dogs, cats, roe deer, a beaver, a Cape hyrax, a flying fox, and Atlantic salmon.[3,8,9] The most commonly affected domestic animals are dogs, cats, and cows. Human infections occur as a result of exposure to organisms in the environment and are often associated with underlying immune compromise. *Prototheca* can also be consumed by humans via milk since the organism persists after pasteurization of milk due to their heat-resistant nature.[9]

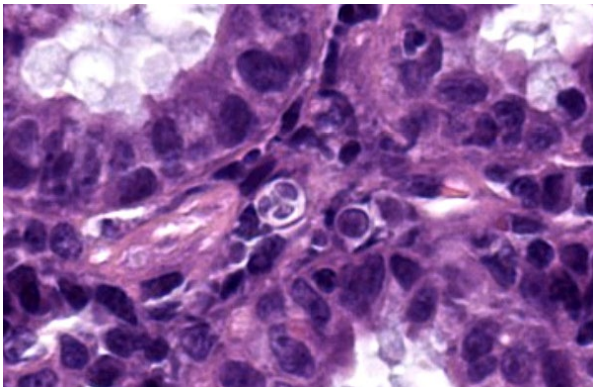
The pathogenesis of protothecosis is largely unknown.[2] It is believed that disease occurs after cutaneous inoculation of organisms into tissues. Another theory suggests that immunosuppressed individuals obtain a possible systemic invasion by organisms that have been ingested or that have colonized the intestinal tract.[1, 12, 10] They are ubiquitous in some environmental niches including raw and treated sewage, water, feces, plant sap, and tree slime. They also can be contaminants of various substrates including cow's milk.[5]



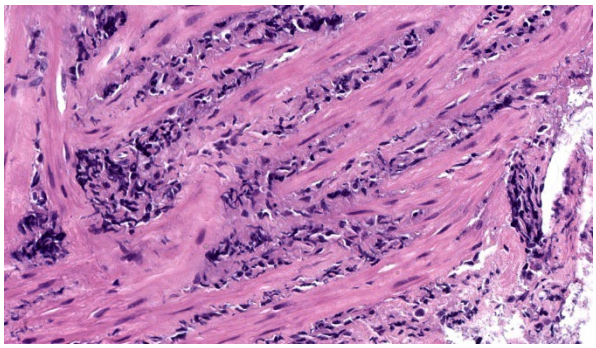
1-4. Colon, dog: Numerous spherical and morula-like organisms (white circle) within the interstitium with multiple segments and range in size from 6-30 micrometers can be seen. (HE, 400X) (*Photo courtesy of:* Tuskegee University College of Veterinary Medicine, https://www.tuskegee.edu/programs-courses/colleges-schools/cvm/cvm-department-of-pathobiology)

The most common clinical presentation of canine protothecosis is protracted colonic hemorrhagic enteritis. Disseminated disease has also been reported. This involves the eyes, ears, skin, skeletal muscles, kidneys, liver, heart, spinal cord, and brain. Gross lesions within the colon are diverse ranging from diffuse reddening of the colonic mucosa to multifocal raised white nodules to coalescing ulcerations accompanied by hemorrhage. Necrosis is rarely observed.[8,10] Histologically intracellular and extracellular algal organisms are observed in variable numbers, depending on the severity of disease in affected tissue. In cases of disseminated disease, granulomas containing organisms can be found in various organs.[10] The presence of multiple wedge-shaped endospores within a single sporangium characterizes *Prototheca* spp.[6] The algae are unicellular, nonbudding, round to oval spherules with a refractile capsule, and range in size from 5-12 µm (*P. wickerhamii*) to 10-25 µm (*P. zopfii*).

The differential diagnosis for prototheca might include *Coccidioides* and *Rhinosporidium*.[7] *Prototheca* in tissue sections stained with hematoxylin and eosin can be difficult to visualize. Periodic acid-Schiff (PAS) or silver stains are helpful. Other potentially pathogenic algal organisms that also undergo endosporulation such as *Chlorella* contain PAS positive cytoplasmic starch granules that are PAS-negative after diastase digestion. This test can be helpful for differentiation of these organisms. Culture, cytology, and fluorescent antibody tests are also helpful ancillary diagnostic techniques.[10]



1-5. Colon, dog: The wedge-shaped tripartite endosporulating algae (center) is a classic morphology of *Prototheca*, with pathologists observing either a "peace sign" or the "logo of a Mercedes-Benz" depending on their particular outlook. (HE, 1779X)

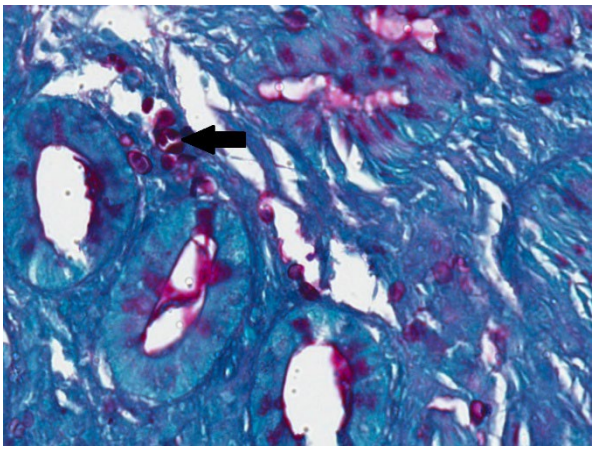


1-6. Colon, dog: The lymphoplasmacytic and histiocytic infiltrate extends down into the submucosa. (HE, 459X)

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JPC Diagnoses:
Colon, mucosa and submucosa: Colitis, histiocytic and lymphoplasmacytic, chronic, diffuse, moderate, with extracellular and intrahistiocytic algae.

JPC Comment:
This week's moderator is MAJ Katie Scott, an alumnus of the DODVPR and the current chief of our department's diagnostic pathology service.



1-7. Colon, dog. These algal forms have a thick, double-layered, refractile wall and dense sporangium. There is often internal septation of the morula-like form of sporangia (black arrow), with endospores arranged symmetrically (endosporulation). (*Photo courtesy of:* Tuskegee University College of Veterinary Medicine, https://www.tuskegee.edu/programs-courses/colleges-schools/cvm/cvm-department-of-pathobiology) (Periodic acid-Schiff, 400X)

The contributor has provided a concise yet informative review of protothecosis in veterinary species. The algae were first discovered

in 1894 by Kruger, who studied them as yeasts and was unaware of their true nature or their ability to cause disease.[11] The first case of protothecosis in veterinary species was documented in a case of bovine mastitis in 1952. The first human case of infection in a foot wound was described in 1964.[11] The number of known *Prototheca* species is currently 18 (and likely increasing).[12] In addition to the discovery of additional new pathogenic species of *Prototheca*, including *P. cutis* (human wound infections), *P. miyajii* (systemic human infections in Japan), and *P. blaschkeae,* which has been isolated from cases of bovine mastitis.[12,13] In addition, for those fighting the good fight to keep up with the endless name changes in the world of microbiology, *P. zopfii* genotype 1 has been renamed to *P. ciferrii*, and *P. zopfii* genotype 2 is now *P. bovis*.[13]

Prototheca sp. are a significant global pathogen of cattle, and cases are frequent in regions with high temperature and humidity, which support its growth in the environment. It is a chronic, often clinically silent disease that reduces milk production and increases somatic cell counts. Clinical cases, characterized by a hard, swollen udder and watery milk with white flakes, are less common. *P. bovis* is the most common isolate, with *P. blaschkeae* being more recently but less commonly isolated. Like most other causes of mastitis, *Prototheca* enters through the teat orifice, typically from contaminated milking or infected substrate. Prevalence in affected herds ranges from 10-30%; accurate rates of infected animals are difficult to determine due to intermittent shedding. The algae often survive the drying-off period and recrudesce during subsequent lactations.[13]

In cats, nodular skin lesions at the site of cutaneous wounds are most common, with *P. wickerhamii* being the most commonly isolated species.

In humans, the skin is the typical portal of infection, and immunosuppressed humans (or those taking immunosuppressive drugs) are at higher risk. The disease is most common in the elderly, and less common in people over 30, but childhood infections have been described. Workers in rice paddies (the first described case of human *Prototheca* infection was identified in a paddy worker), farmers, fishermen, and aquarium staff are at an increased risk. Humans have been described with cutaneous lesions, olecranon bursitis, and systemic infections, with olecranon bursitis typically oc-

curring in immunocompetent individuals. Cutaneous infections account for half of all cases, with immunosuppressed individuals predominating.[14] Cutaneous lesions are typically vesicobullous and ulcerated and take several weeks to develop. Rarely, *Prototheca* algae may be shed in the feces of asymptomatic human carriers.[11]

Antifungal drugs are commonly used to treat *Prototheca* infections in humans and animals. Amphotericin B is considered most effective; however, its nephrotoxicity makes it an uncommon choice in animal species. A recent publication details the effective use of posaconazole in affected dogs.[12]

References:

1. Ginel, P. J., Perez, J., Molleda, J. M., Lucena, R., & Mozos, E. (1997). Cutaneous protothecosis in a dog. Veterinary Record, 140(25), 651-653. doi:10.1136/vr.140.25.651
2. Kwiecinski J. Biofilm formation by pathogenic Prototheca algae. Lett Appl Microbiol. 2015 Dec;61(6):511-7. doi: 10.1111/lam.12497. Epub 2015 Nov 2. PMID: 26394169.
3. Macedo JT, Riet-Correa F, Dantas AF, Simões SV. Cutaneous and nasal protothecosis in a goat. Vet Pathol. 2008 May;45(3):352-4. doi: 10.1354/vp.45-3-352. PMID: 18487492.
4. Mohabeer AJ, Kaplan PJ, Southern PM Jr, Gander RM. Algaemia due to *Prototheca wickerhamii* in a patient with myasthenia gravis. J Clin Microbiol. 1997 Dec;35(12):3305-7. doi: 10.1128/JCM.35.12.3305-3307.1997. PMID: 9399541; PMCID: PMC230169.
5. Pressler BM, Gookin JL, Sykes JE, Wolf AM, Vaden SL. Urinary tract manifestations of protothecosis in dogs. J Vet Intern Med. 2005 Jan-Feb;19(1):115-9. doi: 10.1892/0891-6640(2005)19<115:utmopi>2.0.co;2. PMID: 15715059.
6. Rahman, Md.Tanvir. (2014). Protothecosis: an emerging algal disease of humans and animals. International Journal of Life Sciences Biotechnology and Pharma Research.. 3. 1-13.
7. Stenner VJ, Mackay B, King T, Barrs VR, Irwin P, Abraham L, Swift N, Langer N, Bernays M, Hampson E, Martin P, Krockenberger MB, Bosward K, Latter M, Malik R. Protothecosis in 17 Australian dogs and a review of the canine literature. Med Mycol. 2007 May;45(3):249-66. doi: 10.1080/13693780601187158. PMID: 17464846.
8. Stockinger BG, Doster AR. DISSEMINATED PROTOTHECOSIS IN A RUWENZORI LONG-HAIRED FRUIT BAT (ROUSETTUS LANOSUS). J Zoo Wildl Med. 2017 Dec;48(4):1260-1263. doi: 10.1638/2017-0070R.1. PMID: 29297807.
9. Uzal FA, Platner BL, Hostetter JM. Prototheca enterocolitis. In: Maxie MG, ed. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. Vol 2. 6th ed. St. Louis, MO: Elsevier; 2016:203-204.
10. Vince AR, Pinard C, Ogilvie AT, Tan EO, Abrams-Ogg AC. Protothecosis in a dog. Can Vet J. 2014 Oct;55(10):950-4. PMID: 25320382; PMCID: PMC4187369. Vince AR, Pinard C, Ogilvie AT, Tan EO, Abrams-Ogg AC. Protothecosis in a dog. Can Vet J. 2014 Oct;55(10):950-4.
11. Libisch B, Picot C, Ceballos-Garzon A, Moravkova M, Klimesová M, Telkes G, Chuang ST, Le Pape P. Prototheca Infections and Ecology from a One Health Perspective. Microorganisms. 2022 Apr 29;10(5):938.

12. Langner KFA, Higgins A, Roser M, Bunn TA, Lam A, Proskurnicka A, Jagielski T, Malik R. Prolonged survival of 3 dogs with *Prototheca bovis* infection treated with posaconazole. J Vet Intern Med. 2026 Jul 1;40(4):aalag170.

13. Marques S, Silva E, Kraft C, Carvalheira J, Videira A et al. Bovine mastitis associated with *Prototheca blaschkeae*. J Clin Microbiol 2008;46:1941–1945.

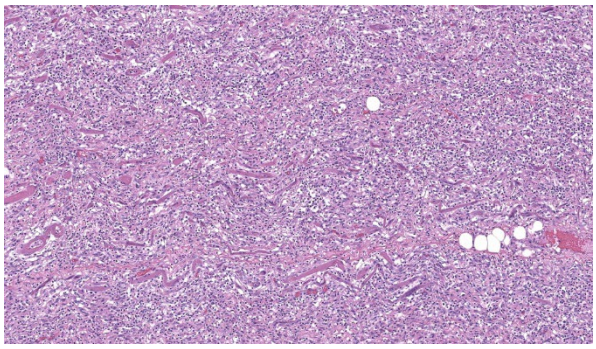
CASE II:

Signalment:
5-year-old, male castrated Italian Spinone dog (*Canis lupus familiaris*)

History:
Presented to referring DVM for dysphagia and dysphonia that progressed to weakness, lethargy and dropped jaw over a few weeks' duration. On physical examination, the patient easily became cyanotic. A subepiglottal abscess with a stick foreign body and mild aspiration pneumonia were diagnosed via a CT scan. After recovery from surgical debridement of the abscess, the patient developed progressive stridor, dysphagia, and left-sided facial paralysis, followed by acute respiratory arrest and death 48 hours after surgery.



2-1. Diaphragm and peripheral nerve, dog: One section of diaphragm and one section of peripheral nerve are submitted for examination. (HE, 80X)

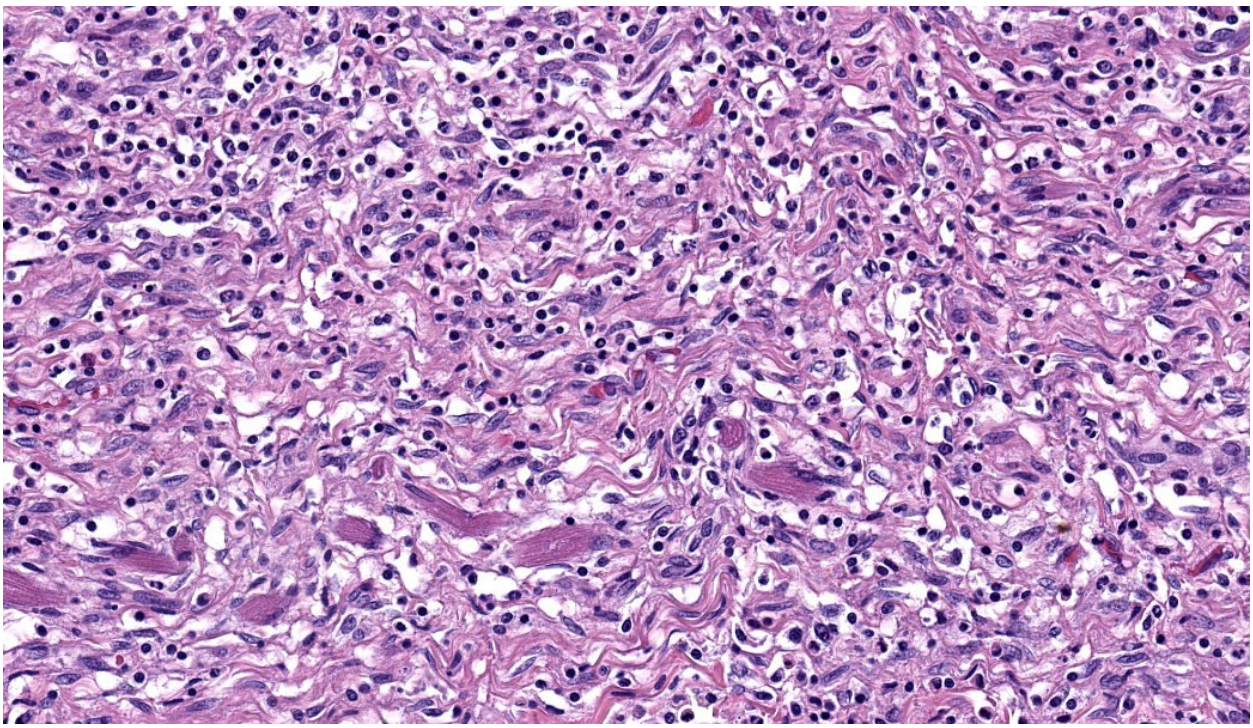


2-2. Diaphragm, dog: The diaphragmatic skeletal muscle is infiltrated by innumerable lymphocytes with fewer macrophages and plasma cells. There is marked loss of atrophy of myofibers. (HE, 174X)

Gross Pathology:
The sub-epiglottal soft tissue is minimally thickened. The cricoarytenoideus dorsalis muscles are bilaterally flattened to sunken (atrophied). Dozens of pinpoint dark red foci are scattered along the thoracic diaphragmatic surface. The cervical and appendicular muscles are multifocally dark red and friable.

Laboratory Results:
None available

Microscopic Description:
Skeletal muscle, diaphragm: Throughout the section, myofibers are effaced by an inflammatory infiltrate composed of lymphocytes and macrophages with fewer neutrophils. There is acute degeneration and necrosis, characterized by fragmented, swollen, hypereosinophilic myofibers that exhibit a loss of cross-striation, and are often infiltrated by numerous macrophages. Occasionally, some myofibers are mineralized. Other myofibers exhibit evidence of attempted regeneration, characterized by increased basophilia and chains of large, centralized nuclei. Myofibers



2-3. Diaphragm, dog: There is marked infiltration by lymphocytes with fewer macrophages and plasma cells. Skeletal myofibers are shrunken and atrophic, and there is moderate fibrosis. (HE, 558X)

are frequently lined by plump, activated satellite cells. Numerous proliferating fibroblasts and wavy collagen fibers separate the affected myofibers.

Also on this slide is the recurrent laryngeal nerve. All examined nerves were unremarkable.

Contributor's Morphologic Diagnoses:

Severe diffuse polyphasic lymphohistiocytic and neutrophilic myositis with myofiber necrosis, mineralization, regeneration, and fibrosis

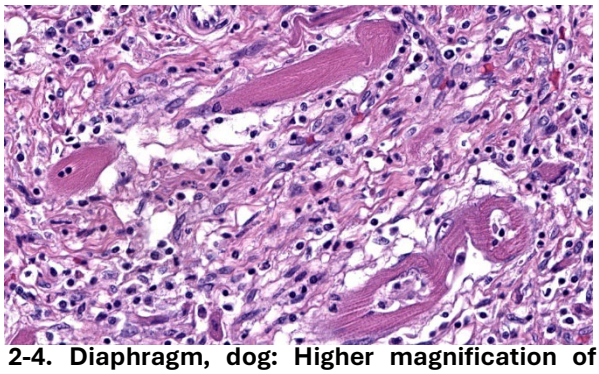
Contributor's Comment:

This case provides an example of canine polymyositis (PM) resulting in respiratory arrest, presumably attributed to dysfunction secondary to severe inflammation of the diaphragm. There was also significant involvement of the subepiglottal tongue, cricoarytenoideus dorsalis, and appendicular musculature, explaining the patient's dysphagia, dysphonia, and weakness, respectively. No bacterial, fungal, or protozoal organisms were identified on H&E, Gram, PAS, or GMS stains. While protozoal disease (*Toxoplasma, Neospora*) cannot be definitively ruled out in the absence of serology or immunohistochemistry given the constellation of clinical signs and lesion distribution, immune-mediated polymyositis is highly prioritized.

Diffuse inflammatory myopathies in dogs encompass those caused by infectious organisms, mainly protozoa (i.e. *Neospora, Toxoplasma*), and bacteria (i.e. *Borrelia* and rickettsial diseases), and PM, an immune-mediated disorder (1,2). PM is characterized by lymphocytic infiltrates in skeletal muscle, elevated CK, abnormal electromyography, and negative serologic tests for infectious disease[1,2]. Breed specific variants of polymyositis have been identified, though not in Italian Spinones. Breed associations have been described in Newfoundlands and Boxers, in which antibodies to an unidentified sarcolemmal antigen. A breed-specific variant in Hungarian

Vizlas is associated with a specific MHC II haplotype [4,5]. An association between a deletion located upstream of the IL21/IL2 locus in Dutch Kooiker Dogs has recently been identified[6,7]. The lymphocytic infiltrate in most cases of canine PM is predominantly CD 8+ T-cells, suggesting a cell-mediated immune response [1,2,8], though the reported form in Vizslas is associated with a predominantly CD4+ T-cell infiltrate. Many cases of PM also show upregulation of MHC I [9]. As such, immunohistochemistry for CD8, CD4 and MHC I can aid in accurate diagnosis of PM. Polymyositis has also been identified as a pre-neoplastic change, particularly in Boxers, associated with lymphoma [1,2].

Clinical signs of PM vary depending on the affected musculature, mainly ranging from weakness, stilted gait and muscle atrophy to dysphagia, dysphonia, and megaesophagus. Respiratory arrest secondary to diaphragmatic involvement is an uncommon presentation, but a case of PM involving the diaphragm, in which dyspnea was the presenting complaint, was recently reported. In that case, interstitial pneumonia was also reported, a constellation more commonly seen in humans with PM [10].
Other inflammatory myopathies in dogs include focal inflammatory myopathies, mainly masticatory muscle myositis (MMM) and extraocular myositis (EOM), as well as dermatomyositis, an inherited inflammatory disease of Collies and Shetland Sheepdogs that affects muscle, skin and vasculature[1]. MMM is an inflammatory myopathy localized to the masticatory muscles and is associated with serum antibodies to the unique type 2M form of myosin in these muscles[2, 11]. Polymyositis in dogs may also involve masticatory muscles, and few cases of "overlap syndrome", characterized by both masticatory and generalized muscle involvement and positive serology for antibodies to type 2M myofibers, have been described [2]. In our case, the masticatory muscles were unaffected.



2-4. Diaphragm, dog: Higher magnification of fragmented and atrophic myofibers. Several myofibers are infiltrated by lymphocytes or macrophages, resulting in myofibrillolysis. (HE, 800X)

Contributing Institution:
PennVet Diagnostic Laboratories
Department of Pathobiology
University of Pennsylvania School of Veterinary Medicine
https://www.vet.upenn.edu/ryan-hospital/diagnostic-labs/

JPC Diagnoses:
Diaphragm: Rhabdomyositis, lymphocytic, polyphasic, chronic, diffuse, severe, with myocyte degeneration, necrosis, regeneration, atrophy, and loss.

JPC Comment:
The contributor provided an excellent review of the recent literature, particularly breed-specific forms of polymyositis. Describing breed-related polymyositis is important, as it mirrors genetic predispositions in humans for various types of inflammatory myopathy based on familial clustering of affected individuals.[5] The relatively non-specific term of "polymyositis", which has been in evidence in the human (and subsequently veterinary) literature since the first descriptions in the 1890's, has largely been replaced by the term "inflammatory myopathy". Today, these diseases (which

share a common overarching theme of a sterile, autoimmune attack on skeletal muscle fibers) are subclassified in human medicine because of the heterogeneous syndromes with which they present, as well as varying epidemiologic profiles, clinical manifestations, immunopathologic signatures (including the specific autoantibodies they produce), and long-term prognosis.[12,13]

In humans, inflammatory myopathies have been classified into five major subtypes, each with specific phenotypes, affected tissues, and autoantibodies. These conditions are most commonly seen in females, except inclusion body myositis. They may occur at any age, but most often appear around 60 years of age (except inclusion body myositis, which is male-predominant and typically appears around 35 years of age). [12,13]

These conditions are subdivided into:

- Inclusion body myositis
- Immune-mediated necrotizing myopathies
- Anti-synthetase syndrome
- Overlapping myositis
- Dermatomyositis (myositis occurring in the spectrum of other connective tissue diseases)
- Overlapping myositis

A recent article details a case report of overlapping myositis with multiple immune-mediated conditions in a 4-year-old female mixed-breed dog. The affected dog was diagnosed with concurrent polymyositis, masticatory myositis, and lymphoid thyroiditis.[14]

References:

1. Cooper BJ, Valentine BA. Muscle and tendon. In: Maxie MG, ed. Jubb, Kennedy and Palmer's Pathology of Domestic Animals. 6th edition, vol 1. Elsevier Ltd; 2016
2. Evans J, Levesque D, Shelton GD. Canine Inflammatory Myopathies: A Clinicopathologic Review of 200 Cases. J Vet Intern Med. 2004;**18**(5):679-691.
3. Hankel S, Shelton GD, Engvall E. Sarcolemma-specific autoantibodies in canine inflammatory myopathy. Vet Immunol Immunopathol. 2006;**113**(1-2):1-10.
4. Massey J, Rothwell S, Rusbridge C, et al. Association of an MHC Class II Haplotype with Increased Risk of Polymyositis in Hungarian Vizsla Dogs. PLoS One. 2013;8(2):e56490.
5. Haley A, Platt S, Kent M, et al. Breed-Specific Polymyositis in Hungarian Vizsla Dogs. J Vet Intern Med. 2011;**25**(2):393-397.
6. Alf V, Opmeer Y, Shelton GD, et al. Pathologic Changes in and Immunophenotyping of Polymyositis in the Dutch Kooiker Dog. Animals (Basel). 2024;**14**(17):2519.
7. Opmeer Y, van Steenbeek FG, Rozendom C, et al. Polymyositis in Kooiker dogs is associated with a 39 kb deletion upstream of the canine IL21/IL2 locus. PLoS Genet. 2025;**21**(1):e1011538.
8. Pumarola M, Moore PF, Shelton GD. Canine inflammatory myopathy: Analysis of cellular infiltrates. Muscle Nerve. 2004;**29**(6):782-789.
9. Neumann J, Bilzer T. Evidence for MHC I-restricted CD8+ T-cell-mediated immunopathology in canine masticatory muscle myositis and polymyositis. Muscle Nerve. 2006 Feb;33(2):215-24.
10. Daentl K, Umana V, DeMonaco S. Polymyositis associated with dyspnoea and in-

terstitial lung disease in a dog (Canis familiaris). Vet Rec Case Rep. 2025;**13**:e70031

11. Shelton GD, Cardinet GH III, Bandman E, Cuddon P. Fiber type-specific autoantibodies in a dog with eosinophilic myositis. Muscle Nerve 1985;8:783–790.
12. Allenbach Y, Benveniste O. Inflammatory Myopathies. N Engl J Med. 2026 May 14;394(19):1925-1938.
13. Wang G, Cui S, Yang L, McCullagh KJA, Ma C, Liu A. Animal Models of Idiopathic Inflammatory Myopathies: Bridging Mechanistic Insights and Clinical Translation. Int J Mol Sci. 2026 Jul 27;27(15):6697.
14. Crawford-Jennings MI, Moreno RC, Shelton GD, Fowler KM, Vezza CR, Shinn RL, Parker RL, Wilkinson AR. Case Report: Overlap syndrome with concurrent polymyositis, masticatory myositis, and lymphocytic thyroiditis in a dog. Front Vet Sci. 2026 Jun 4;13:1807901. doi: 10.3389/fvets.2026.1807901. PMID: 42328063; PMCID: PMC13275230.

CASE III:

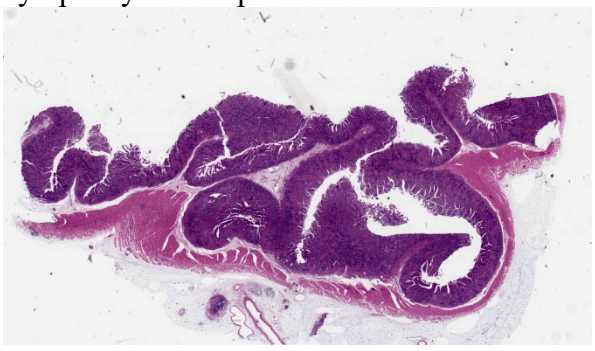
Signalment:
Bos taurus (Cow), female, 12-year-old, Angus

History:
Cow was an embryo recipient. She deteriorated in late pregnancy with twins. Profuse diarrhea and hind end weakness were observed. She bounced back with Draxxin and Sulfa treatments but declined after delivery. Owner has noticed this same scenario in a few cows over the last 2 years. All eventually die despite treatment. This cow had a pyometra at necropsy but delivered 2 thriving calves 2 weeks before mortality.

Gross Pathology:
A necropsy evaluation was performed by the referring veterinarian and samples collected during evaluation were forwarded to the anatomic pathologist for histological evaluation. Based on the history in the submittal, the rDVM reported observing pyometra during gross evaluation

Laboratory Results:
PCR positive for Mycobacterium avium subsp. paratuberculosis

Microscopic Description:
Slide 1: One section of small intestine is evaluated (jejunum). In regionally extensive areas, the mucosal lamina propria is significantly expanded by abundant macrophages, moderate numbers of lymphocytes and plasma cells, and small numbers of eosinophils, markedly displacing and sometimes replacing the crypt architecture. Macrophage nuclei are frequently peripheralized by prominent, hazy intracytoplasmic material. Epithelioid macrophages are also present.
Lymphocytes and plasma cells in the mucosa



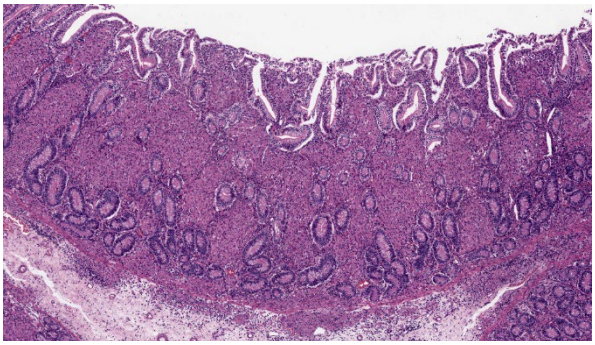
3-1. Jejunum, ox. A section of jejunum is submitted for examination. The mucosa is thickened at subgross magnification, and inflamed serosa lymphatics are visible at this magnification. (HE, 10X)

are frequently concentrated at the interface between remaining crypts and the interstitium. Rarely, few crypts are significantly dilated containing abundant amounts eosinophilic cellular debris and lined by flattened epithelia (inspissated glands). Extending beyond the lamina propria through the muscularis mucosae and into the submucosa and tunica muscularis, are regionally extensive areas with small to moderate numbers of aforementioned inflammatory infiltrates, frequently forming discrete aggregates and sheets.

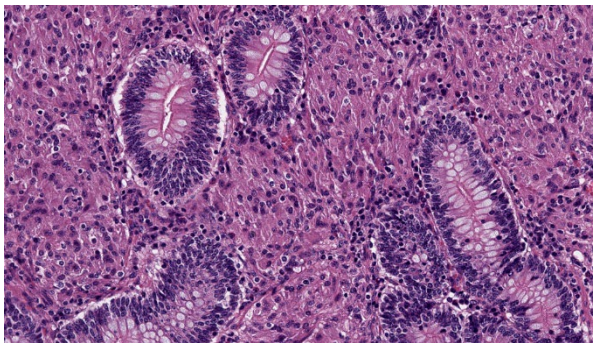
Slide 2: Fites Acid fast stain: Within numerous macrophages in the lamina propria and submucosa are intracytoplasmic, acid-fast positive bacilli bacteria.

Contributor's Morphologic Diagnoses:
Small Intestine; jejunum, mucosa and submucosa: Enteritis, granulomatous, regionally extensive, chronic, severe with abundant intrahistiocytic, acid-fast positive bacteria (consistent with Mycobacterium avium subsp. paratuberculosis)

Contributor's Comment:
Granulomatous enteritis with intrahistiocytic acid-fast positive bacterial microorganisms in



3-2. Jejunum, ox. The lamina propria is markedly expanded by innumerable histiocytes which surround and efface crypts, and extend through the muscularis mucosa into the underlying edematous submucosa. (HE, 96X)

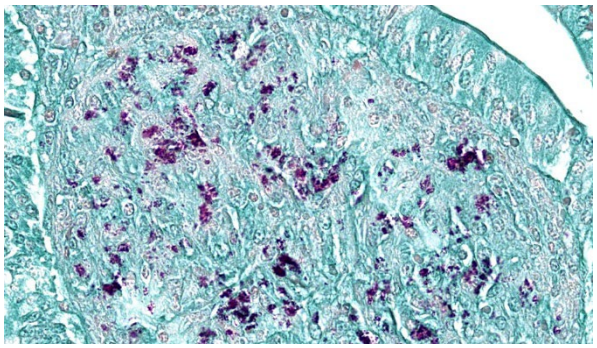


3-3. Jejunum, ox. High magnification of the histiocytic infiltrate within the lamina propria. (HE, 459X)

Bovidae, has been associated with Mycobacterium avium subsp. paratuberculosis (MAP) infection, the causative agent of Johne's disease. Johne's disease is a chronic disease, and clinical signs are reported in infected cattle over 2 years of age, likely following infection during calfhood[12] . Johne's disease has been reported to significantly impact the dairy industry with over $198 million USO in losses annually[1].

Mycobacterium avium subsp. paratuberculosis belongs to the family Mycobacteriaceae, classified as 'non-tuberculous mycobacteria' and part of the Mycobacterium avium complex. Prior to the onset of clinical signs in ruminants, incubating bacterium are shed through varying mechanisms contaminating the environment. This contaminated environment is the likely source for disease spread to other ruminants and humans[5] via suspected routes of transmission include transmammary, *in utero* and through lactation routes[8,13].

Four different stages of this disease process have been identified in cattle: subclinical nondetectable infection (Stage 1 or silent infection), subclinical shedders (Stage 2 or subclinical disease), clinical Johne's disease (Stage 3), and severe emaciation and diarrhea



3-4. Jejunum, ox. Histiocytes within the lamina propria contain numerous acid-fast bacilli. (Fite-Faraco, 1159X)

(Stage 4). Cattle in stages 1 or 2 are prominent transmitters of the disease and can infect other ruminants when introduced into new herds[4,14].

The most common clinical sign associated with Johne's disease is chronic diarrhea. After ingestion, the bacterium reaches the intestinal tract, where it translocates through the intestinal mucosa via M cells and enterocytes[2,3]. The invading bacterium is transported to local lymphoid tissue such as Peyer's patches, which leads to bacterial dissemination through lymphatics[4].

Subsequently, either immediately after intestinal translocation or during dissemination, activation of the innate immune response results in phagocytosis of these bacteria, predominantly by macrophages. This macrophage uptake has been reported as preferentially facilitated by the complement system[1,2]. Following uptake of the pathogen, macrophage functions are significantly hampered by a wide array of mechanisms, such as inhibition of phagosome maturation, inhibition of phagosome-lysosome fusion, impairment of phagosome function by disrupting phagosome membrane functions[1,7] etc., leading to bacterial survival within macrophages.

With multiple bacteria surviving within macrophages, a TH2 response is observed where more macrophages are recruited leading to the histological presentation of granulomatous inflammation characterized by macrophages in large numbers, frequently arranged in sheets, including epithelioid macrophages. Along with macrophages are small numbers of lymphocytes and plasma cells within poorly demarcated regions in the intestinal tract[6].

Significant gross lesions in cattle are restricted to intestinal tract, frequently either segmental or diffuse in presentation. The mucosal surfaces are markedly thickened transversely, forming distinctly raised folds (transverse rugae). Frequently alongside thickened mucosae are prominent serosal edema, enlarged ileocecal and mesenteric lymph nodes and infrequently prominent mesenteric vasculature[15]. Histologically, as in this presentation, a transmural granulomatous inflammation with prominent lymphadenitis and lymphangitis, along with varying amounts of intralesional, intrahistiocytic acid-fast positive bacteria[9, 15]

Besides PCR-based molecular detection and histological evaluation with special staining, serological testing strategies such as Agar Gel Immuno-Diffusion (AGID), Complement fixation and Enzyme-linked Immunosorbent Assays (ELISAs) have also been used for screening and diagnosis of this pathogen in cattle[4, 5, 14].

M. avium subsp. paratuberculosis has also been reported in other species including sheep, goats, non-domesticated ruminants, non-human primates, rabbits, with rare reports of occurrence in dogs, pigs, horses and

few other laboratory animals[4].

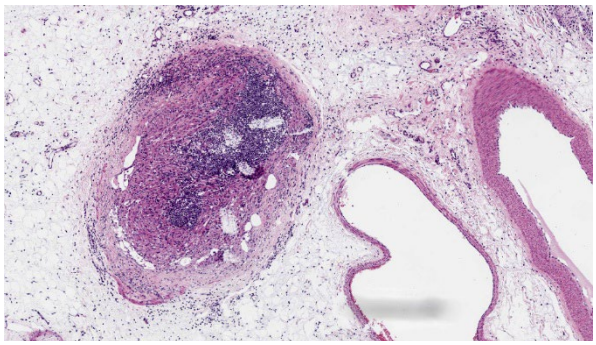
Johne's disease has been well documented and frequently studied. Significant attributes of the disease are environmental contamination, long incubation periods, significant economic losses annually, possibilities for cross-species transmission and the potential for zoonosis. Subsequent screening, culling of positive animals, and other biosecurity protocols will be needed to minimize the spread of this pathogen amongst cattle and other domestic species.

The main findings in this case are: granulomatous enteritis, the presence of intrahistiocytic acid-fast positive bacterial microorganisms, and PCR detection of viral antigen (Mycobacterium avium subsp. paratuberculosis). Altogether, these features are consistent with Johne's disease in this cow.

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JPC Diagnoses:
Jejunum: Enteritis, granulomatous, chronic, diffuse, marked, with villar blunting, crypt abscesses and loss, and lymphohistiocytic lymphangitis.

JPC Comment:
The contributor has provided an outstanding review of *Mycobacterium avium* var. *tuberculosis* infection (Johne's disease) in cattle. Johne's disease is also one of the most frequently submitted diseases to the WSC (befitting its status as a disease of major economic



3-5. Jejunum, ox. There is marked granulomatous lymphangitis within the edematous serosa. (HE, 111X)

importance in ruminants), with 33(!) submissions since 1970.

This is not a historical disease in which research tends to tail off over time; research and discovery continue today at a frenetic pace. One recent development is the reclassification of MAP strains based on molecular typing methods. Today, MAP is classified into three major genotypes: the cattle strain (C type; type II), the sheep type (S type; type I/III), and the bison type (B type). Unlike the cattle and bison types, the sheep strain (S type) has a more restricted host range limited to sheep and goats, while the C and B types infect a wide range of ruminants and other domestic species.[15]

Recent years have brought significant advances in understanding mycobacterial infection and disease pathogenesis at the subcellular level. A complete review of these advances is beyond the scope of this document; however, an excellent review shows that bacterial attachment is mediated by the bacterium's fibronectin-attachment protein, which binds to a beta-1-integrin receptor on the M cell. This complex activates the host's CDC42 signaling to trigger actin rearrangement and bacterial internalization. Another compound that facilitates bacterial invasion is a mycobacterial major membrane protein, whose transcription is

upregulated in the hypoxic and hypoosmolar environment of the intestine. This antigen also participates in antigen presentation to CD4-positive and CD8-positive T cells, setting up early host immune responses. Another facilitator of bacterial invasion is the predisposition of wild-type MAP strains to form organized biofilms, which prolong MAP contact with M cells. Multiple stages of pathogenesis of Yoni's disease are available in the review article by Tao et al.[15]. The initial point of entry for MAP is the M cells (M is short for microfold, which rarely appears in literature on this disease), which are located over Peyer's patches in the distal ileum.[15]

In humans, MAP has been controversially associated with several chronic inflammatory diseases. The purported source of MAP in humans is likely consumption of contaminated food or water, including unpasteurized dairy products. These associations are, at best, controversial. [15]

The least tenuous association is between MAP and Crohn's disease in humans, a chronic inflammatory disorder of the ileum characterized by granulomatous inflammation. MAP RNA has been detected in ileal biopsies from patients with Crohn's disease, and host immune responses directed against MAP antigens have been identified. Additionally, antimicrobial therapies have shown some success in patients with CD. Critics of this correlation cite the widespread environmental distribution of MAP and the possibility that it may represent an intestinal commensal in these patients rather than a primary pathogen.[15]

MAP has also been putatively linked with many other inflammatory or autoimmune conditions in humans, including multiple sclerosis and ALS. This link is based on the presence of anti-MAP antibodies in MS patients from geographically confined cohorts, as well as some structural resemblance between MAP antigens and human myelin proteins. Molecular mimicry is also the basis of a link between MAP and diabetes, with some evidence suggesting that MAP may act as a trigger for type 1 diabetes mellitus and latent autoimmune diabetes in adults. Other conditions that have been linked to MAP include sarcoidosis, uveal melanoma, colorectal tumors, glioblastoma, and more traditional autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus. Significant research is ongoing to establish any causality between MAP and these diseases.[15]

A quick note on the difference between the contributor's morphologic diagnosis and the JPC morphologic diagnosis: as only HE slides are provided to participants in advance of the conference, our morphologic diagnoses will traditionally only include features that are visualized on the HE section. In this case, as an acid-fast stain is not provided to participants, we traditionally will not include the findings of an acid-fast stain in the JPC morphologic diagnosis. (We do, however, expect our residents to identify the relatively classic lesion of Johne's disease in the absence of an acid-fast stain in this case.)

References:

1. Arsenault RJ, Maattanen P, Daigle J, Potter A, Griebel P, Napper S. From mouth to macrophage: mechanisms of innate immune subversion by Mycobacterium avium subsp. paratuberculosis. Vet Res. 2014;45(1):54.
2. Arsenault R, Menge C, Barletta RG, et al. Pathogenesis, Molecular Genetics, and Genomics of Mycobacterium avium subsp. paratuberculosis, the etiologic Agent of

Johne's Disease. 2017;4:187.

3. Bermudez LE, Petrofsky M, Sommer S, Barletta RG. Peyer's patch-deficient mice demonstrate that Mycobacterium avium subsp. paratuberculosis translocates across the mucosal barrier via both M cells and enterocytes but has inefficient dissemination. Infect lmmun. 2010;78(8):3570-3577.
4. Council NR. Johne's Disease in Domesticated and Wild Animals. In: Diagnosis and Control of Johne's Disease. National Academies Press (US) 2003:
5. Huntley JFJ, Whitlock RH, Bannantine JP, Stabel JR. Comparison of Diagnostic Detection Methods for Mycobacterium avium subsp. paratuberculosis in North American Bison. Vet Pathol. 2005;42(1):42-51.
6. James F. Zachary. Diffuse (Leprematous) Granulomas (TH2 - biased Granulomas). In: Pathologic Basis of Veterinary Disease. Elsevier 2022:145-145.
7. Karakousis PC, Bishai WR, Dorman SE. Mycobacterium tuberculosis cell envelope lipids and the host immune response. Cell Microbiol. 2004;6(2):105-116.
8. Kumar A, Patyal A. An insight on microbial flora of milk and milk products. In: The Microbiology, Pathogenesis and Zoonosis of Milk Borne Diseases. Elsevier 2024:69-94.

8. McGavin MD, Zachary JF. Pathologic basis of veterinary disease. Elsevier Health Sciences 2006.

9. Radam sura. Pathological Impact of Johne's Disease in Cattle: A Review Article. 2021;0(0):0-0.

10. Rasmussen P, Barkema HW, Mason S, Beaulieu E, Hall DC. Economic losses due to Johne's disease (paratuberculosis) in dairy cattle. J Dairy Sci. 2021;104(3):3123-3143.
11. Stabel JR. Johne's Disease: A Hidden Threat. J Dairy Sci. 1998;81(1):283-288.

12. Sweeney RW, Collins MT, Koets AP, Mcguirk SM, Roussel AJ. Paratuberculosis (Johne's Disease) in Cattle and Other Susceptible Species. J Vet Intern Med. 2012;26(6):1239-1250.

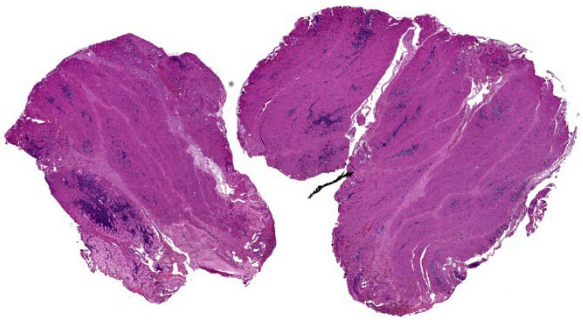
13. NVAP Reference Guide: Johne's Disease (Control and Eradication). NVAP Reference Guide. 2024:

14. Maxie G, editor. Jubb, Kennedy & Palmer's Pathology of Domestic Animals. Vol. 2. Elsevier 2015.

15. Tao R, Zheng W, Peng L, Lv S, Wang Q. Multistage pathogenesis and disease associations of *Mycobacterium avium* subsp. *paratuberculosis*. Front Immunol. 2026 Sep 15;17:1948490. doi: 10.3389/fimmu.2026.1948490. PMID: 42812195; PMCID: PMC13619385.

CASE IV:

Signalment:
A 7 year-old FS Viszla (*canis familiaris*)



4-1. Temporal muscle, dog: Two sections of skeletal muscle are submitted for examination. There are scattered areas of hypercellularity evident at subgross magnification. (HE, 27X)

History:
Fever, pain and swelling of the temporal muscle present for approx. 10 days. Unable to open mouth.

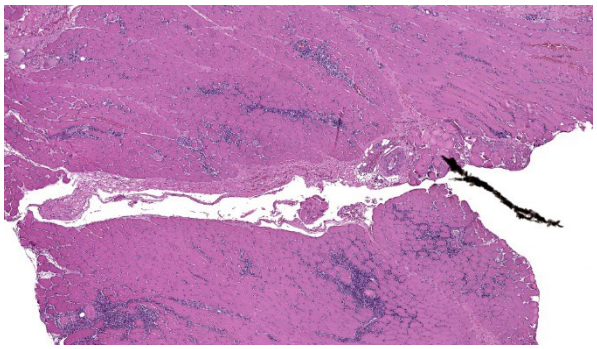
Gross Pathology:
None done

Laboratory Results:
Elisa test for 2M antibodies was positive at a dilution of 1:4000

Microscopic Description:
Temporal muscle: There is multifocal moderate to severe (depending on the sample) endomysial infiltration of lymphocytes, histiocytes, plasma cells, lesser eosinophils and neutrophils. In inflamed foci there is myofiber loss, necrosis, regeneration and in some areas a small amount of nuclear debris. Phagocytosis of necrotic myofibers is common. In the sample where inflammation occurs close to fascia, there is fibroblast hypertrophy and scattered microhemorrhages.

Contributor's Morphologic Diagnoses:
Multifocal moderate to severe subacute mononuclear and eosinophilic myositis

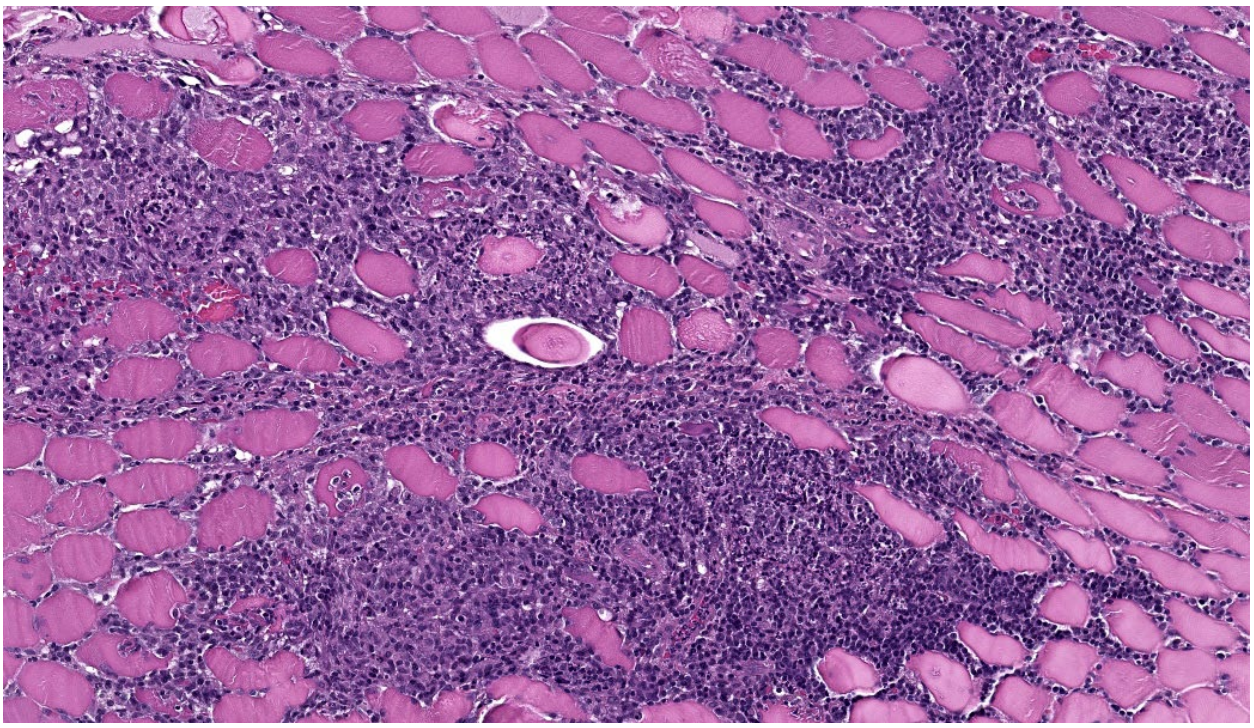
Contributor's Comment:
Masticatory muscle myositis (MMM) is an autoimmune myositis (or "inflammatory myopathy", according to others[2]) which affects the masticatory muscles (masseter, temporal, pterygoid and rostral digastricus) of dogs. This term has superseded two older designations - eosinophilic myositis and atrophic myositis - which were used when it was thought that each was a separate condition, rather than the acute and chronic phases of a single disease, the current understanding of this condition[1,2].



4-2. Temporal muscle, dog: There are multifocal areas of inflammation scattered throughout the sampled temporal muscle. (HE, 27X)

Clinical signs in the acute phase are muscle swelling which is most obvious in the temporal and masseter muscles, inability to fully open the mouth (trismus), jaw pain and in 44% of cases exophthalmos ascribed to retrobulbar swelling of the pterygoid muscle[2]. Pyrexia and mandibular and prescapular lymphadenopathy have also been reported during the initial 1 to 3 weeks of MMM[2]. The signs are usually bilateral but some cases may appear to be unilateral due to more pronounced involvement of one side. A classical clinical sign of MMM is inability to open the jaws under anesthesia [1,2,3]. Untreated attacks last up to 2-3 weeks with extended periods (weeks to 2-3 years) of quiescence between them[1]. If uncontrolled, the condition causes progressive and irreversible destruction of masticatory muscles. The end result is muscle atrophy which gives the skull a characteristic appearance[1] and may be accompanied by enophthalmos[2]. Unfortunately most cases are presented in the chronic phase of the disease[2]. Response to immunosuppressive doses of corticosteroids is usually rapid but cases may relapse if therapy is discontinued[1]. The condition affects many breeds but large breeds including GSD, Labrador retrievers, golden retrievers and Doberman pinschers appear to be predisposed[2]. The average age of onset is 3 years of age[2].

Pathologic findings vary with the stage of the disease. In acute cases the muscles are swollen, dark red, may be doughy or hard and the



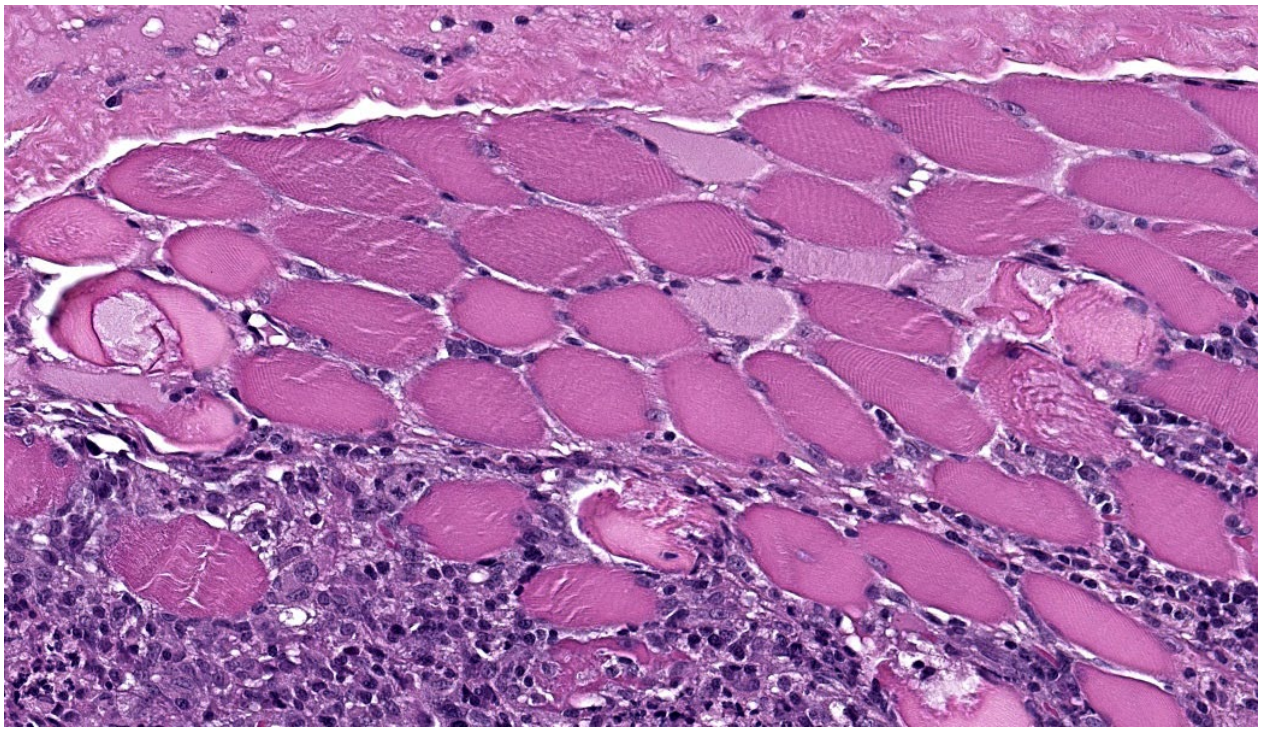
4-3. Temporal muscle, dog: Muscle fibers are separated, surrounded, and effaced by a dense inflammatory infiltrate of macrophages and lymphocytes. (HE, 270X)

cut surface reveals hemorrhagic streaks and irregular yellow white patches. In late stages there is advanced atrophy and fibrosis[1]. Microscopically, there is multifocal myonecrosis with perivascular to interstitial inflammatory infiltration comprising lymphocytes, plasma cells and in some cases eosinophils. Each of these 3 cell types may predominate in a given case. Cases with a strong eosinophilic component may be accompanied by eosinophilia. Macrophages are prominent in cases with marked myofiber necrosis[1].

Immunophenotyping of the lymphocytic infiltrates shows a mixture of T and B cells with more CD4+ cells than CD8+ cells[1]. The masticatory muscles are surrounded by a tough fascia and it is possible that some of the muscle damage in acute cases with significant swelling is a consequence of compartmental syndrome [1].

Because of the multifocal and polyphasic nature of the condition, biopsies from suspected cases may show scant or no inflammation. In later stages, the only finding may be generalized myofiber atrophy. Hence, it is thought that with the right clinical history, any evidence of fiber degeneration or regeneration even without_obvious inflammation, suggests MMM[1]. Biopsies are usually collected from the temporalis muscle but care must be taken to avoid sampling the frontalis muscle which overlies the temporal muscle and is unaffected in MMM. This is reportedly a common mistake [2].

Comparison of fiber type composition between limb and masticatory muscles demonstrated that the latter are rich in a unique fiber type called type 2M fiber. This fiber type is only found in muscles innervated by the mandibular branch of the trigeminal nerve and contains a unique myosin isoform known as type 2M myosin. Antibodies against type 2M myosin are present in dogs with MMM and are



4-4. Temporal muscle, dog: Myocytes demonstrate a range of degenerative and necrotic changes including pallor, loss of cross striations, contraction bands, myofibrillolysis and inflammatory cells within large cytoplasmic vacuoles. (HE, 425X)

highly specific for this condition (100% specific and 85-90% sensitive, [2]). A positive serologic result establishes the diagnosis but biopsy is recommended because it provides information important for prognosis[3]. Early cases with significant inflammation and minimal fibrosis are more likely to recover following therapy than cases with minimal inflammation and significant fibrosis[1].

It is unknown why autoantibodies against type 2M myosin are produced. One possible mechanism is molecular mimicry i.e. antibodies or T cells generated in the course of infections may cross react with type 2M myosin[1,2]. False negative serologic results may occur in cases that have received immunosuppressive dosage of corticosteroids for longer than 7-10 days or in end stage MMM [1,2,3]. Serum levels of CK and AST may be normal or only moderately elevated – this is attributable to the focal nature of the lesions and / or the stage of the disease[2].

The masticatory muscles of dogs, especially the temporal muscle, are prone to a variety of generalized myopathic and systemic disorders[1]. The differential diagnosis for trismus, jaw pain, swelling or atrophy of the masticatory muscles includes the following conditions:

1. Some cases of polymyositis present with subclinical involvement of limb muscles and appear primarily as a problem of masticatory muscles [1, 3]. Without the benefit of clinical data indicating involvement of other muscles, serologic studies (presence of absence of type 2M antibodies) and IHC (in polymyositis infiltrating lymphocytes are T cells with more CD8+ than CD4+ cells), MMM may be diffi-

cult if not impossible to distinguish from polymyositis in routine HE-stained sections. The infiltrate is of similar composition in both conditions and can be variable in individual cases[1].

2. Atrophy of the masticatory muscles may occur as a result of chronic corticosteroid therapy. Prior to assuming a diagnosis of MMM, it should be determined if atrophy occurred after the initiation of corticosteroid therapy for another problem[3].

3. Dogs with any generalized illness often develop rapid atrophy of the temporal muscles that resolves with treatment of the primary problem[1].

4. Idiopathic cases of persistent, bilaterally symmetric atrophy of masticatory muscles not associated with pain, immobility of the jaw or generalized disease are reported in dogs. In these cases, atrophy does not appear to follow inflammation[1].

5. Atrophy of the masticatory muscles and muscle pain may occur in endocrine disorders including hypothyroidism and Cushing's syndrome[3].

6. Idiopathic inflammatory polymyopathy is reported in the Viszla (the same breed from which the submitted biopsy was obtained). Atrophy of masticatory muscle was a common presenting sign of this condition, usually with dysphagia. Serology for 2M antibodies was negative in all cases that were tested[4].

7. Disorders of the temporomandibular joint [3] or trauma to the jaws[2]

8. Disorders affecting peripheral nerves e.g. trigeminal neuritis and peripheral nerve sheath tumors [2]

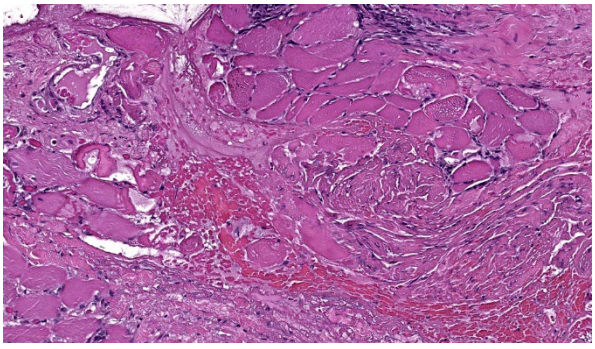
Additional DD may be found in references 2 and 3.

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JPC Diagnoses:
Skeletal muscle: Rhabdomyositis, lymphohistiocytic, polyphasic, chronic, multifocal, marked, with myofiber degeneration, necrosis, and regeneration.

JPC Comment:
The contributor has provided an outstanding review of this uncommon entity in the dog. This disease appears unique to the animal kingdom, as no similar entity exists in humans. While numerous inflammatory myopathies in humans (as addressed in Case 2 above) may involve the muscles of mastication, no myopathy targets these muscles and generates specific autoantibodies against their components.

This entity has also been identified in gray wolf (not terribly surprising, as it is also a canid), and more recently, a cat.[5] In this case, a 1-year-old mixed breed cat presented with trismus and difficulty eating hard food. An ELISA test against canine type 2M myosin fibers was positive. A somewhat uncooperative



4-5. Temporal muscle, dog: There are focal areas of hemorrhage within areas of myofiber degeneration(HE, 389X)

owner intermittently administered corticosteroids, and a year later, marked atrophy of the muscles of mastication was identified. Biopsies of these muscles showed marked lymphoplasmacytic inflammation and fibrosis.[5]

A unique syndrome affecting the muscles of mastication in farmed mink was reported by Needle *et al*. in 2014.[6] Affected animals demonstrated necrotizing myositis of muscles containing 2M myosin, including the masticatory muscles; however, the inflammation in these muscles was primarily eosinophilic in character. Some animals had myodegeneration and lymphohistiocytic inflammation in other groups, including the diaphragm, epaxial muscles, and quadriceps femoris.[6]

References:

1. Cooper BJ, Valentine BA. Muscle and tendon. In: Jubb, Kennedy and Palmer's *Pathology of Domestic Animals*. Ed: M Grant Maxie. 6th ed. vol. 1, Elsevier, 2016:226-228.

2. Melmed C, Shelton GD, Bergman R, Barton C. Masticatory Muscle Myositis: Pathogenesis, Diagnosis, and Treatment, *Compendium*, 2004; 26(8):590-6

3. http://vetneuromuscular.ucsd.edu/cases/2010/Jan10.html; http://vetneuromuscular.ucsd.edu/cases/2007/Dec07.html

4. Tauro A, Addicott D, Foale RD et al. Clinical features of idiopathic inflammatory polymyopathy in the Hungarian Vizsla. *BMC Veterinary Research*, 2015; 11:97

5. Kent M, Glass EN, Castro FA, Miller AD, de Lahunta A. Masticatory muscle myositis in a gray wolf (Canis lupus). J Zoo Wildl Med. 2017 Mar;48(1):245-249.

6. Blazejewski SW, Shelton GD. Trismus, masticatory myositis and antibodies against type 2M fibers in a mixed breed cat. JFMS Open Rep. 2018 Apr 26;4(1):2055116918764993.

7. Needle DB, Hollinger C, Shelton GD, Fitzgerald SD. Necrotizing and eosinophilic masticatory myositis in farmed mink: a preliminary description. J Comp Pathol. 2014 Aug-Oct;151(2-3):217-27.

8. Fisher R, Goldschmidt S, Knipe MF, Soltero-Rivera M, Vapniarsky N, Arzi B. Differential causes of masticatory muscle disorders in dogs: a review of diagnosis, treatment and long-term management. Front Vet Sci. 2026 Feb 10;13:1750095.