Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2025-2026

Conference #16 21 January 2026

CASE I:

Signalment:
15-yr-old pony mare (*Equus caballus*)

History:
Ten-day history of colic and diarrhea with suspected peritonitis. Tested positive by PCR for equine coronavirus (antemortem), and received aggressive supportive care (IV fluids, antibiotics, anti-inflammatories, etc.), but became persistently painful and was euthanized. The submitting veterinarian was concerned about intestinal infarction or displacement.

Gross Pathology:
The carcass was in good nutritional condition, with adequate amount of fat reserves, well fleshed, moderately dehydrated, and mild to moderate state of postmortem decomposition.

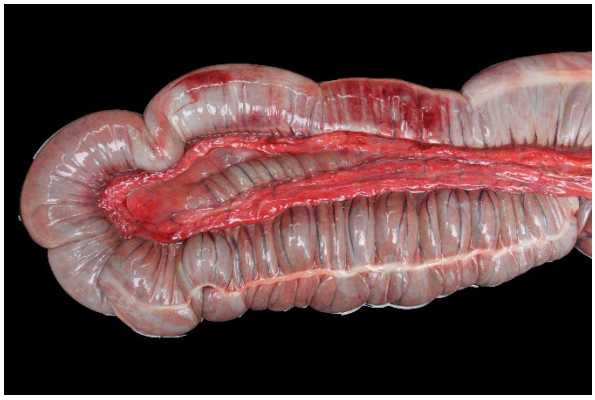

Figure 2-1. Colon, horse: The serosa of the colon was multifocally hemorrhagic. (Photo courtesy of: California Animal Health and Food Safety Laboratory, Sa Bernardino Laboratory. https://cahfs.vetmed.ucdavis.edu/)

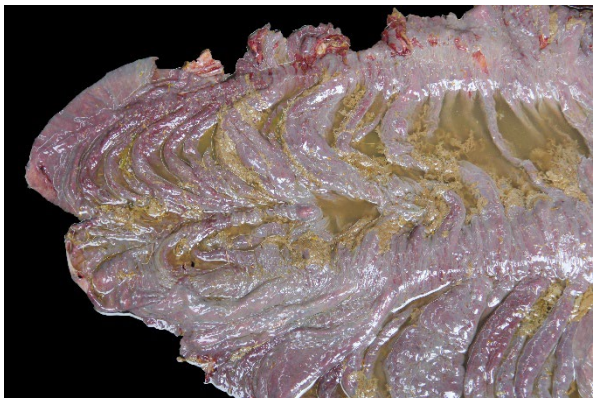

Figure 1-2. Colon, horse: There was clear liquid content throughout small and large intestine, and a pseudo membrane covering the mucosa of the right ventral colon and cecum. (Photo courtesy of: California Animal Health and Food Safety Laboratory, San Bernardino Laboratory. https://cahfs.vetmed.ucdavis.edu/)

About 10 liters of murky fluid were present in the abdomen. There were ~ 5 liters of clear thoracic fluid. The serosa of the colon was multifocally hemorrhagic. There was clear liquid content throughout small and large intestine, and a pseudo membrane covering the mucosa of the right ventral colon and cecum.

There was an~ 6 cm diameter jejunal diverticulum with very thick wall, that was adhered to the serosa of the cecum and the parietal peritoneum. The lungs were congested and edematous.

No other significant gross abnormalities were observed in the rest of the carcass.

Laboratory Results:
Clostridioides difficile and *Salmonella typhimurium* were isolated from small intestinal and colonic content. *S. typhimurium* was also isolated from the liver.

ELISA for toxins A and B of *C. difficile* was positive on small intestinal and colonic contents. ELISA for *Clostridium perfringens* alpha, beta and epsilon toxins was negative on the same specimens.

Mixed aerobic flora was isolated from small intestinal and colonic contents, liver and lung.

A heavy metal screen on liver revealed that copper and selenium were marginally below normal range. All other heavy metals were within normal range.

Microscopic Description:
Colon: There is severe, diffuse necrosis of the mucosa where the superficial epithelium and, less prominently, the crypt epithelium is lost and/or show hypereosinophilia, pyknosis, karyorrhexis and karyolysis. The lamina propria is diffusely eosinophilic and infiltrated by a large number of viable and degenerated neutrophils and fewer neutrophils, plasma cells and macrophages; this cellular infiltration extends to the submucosa. Multifocally, the mucosa is covered by a thick pseudo membrane composed of fibrin, cell debris, red blood cells, neutrophils and myriad mixed bacteria. Several blood vessels of the lamina propria show thrombosis. In areas where the superficial epithelium is still present, erosions are seen through which large number of neutrophils are seen exiting the lamina propria into the lumen (volcano lesions). The submucosa is severely dilated and edematous; there is vascular congestion and the lymphatic vessels are dilated. The serosa shows reactive mesothelial cells.

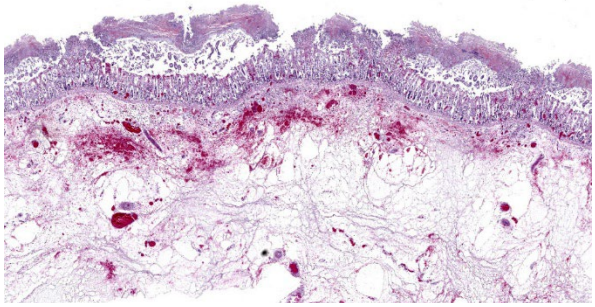

Figure 1-3. Colon, horse: The mucosa is covered by a serocellular crust and the submucosa is markedly expanded by edema and multifocal hemorrhage. (HE, 30X)

Contributor's Morphologic Diagnoses:
Colitis, fibrinonecrotizing, diffuse, with thrombosis, volcano lesions, submucosal edema and myriad intralesional mixed bacteria

Contributor's Comment:
This horse had severe colitis produced by a co-infection with *C. difficile* and *Salmonella* Typhimurium. Grossly and microscopically, there are several overlapping features in the lesions produced by these two microorganisms which make them impossible to differentiate based only on lesions.[3,7] An etiologic diagnosis cannot therefore be made without ancillary laboratory tests.[3,7]

In the case of *S. T*yphimurium, the infection was confirmed by PCR and culture followed by serotyping. Detection of this microorganism in intestinal content and liver suggests that the intestinal infection occurred before and there was systemic dissemination through the damaged intestinal mucosa, although intestinal mucosa damage is not a requirement for absorption and dissemination of this Salmonella spp.[5,7]

The diagnosis of *C. difficile* infection was confirmed by the detection of toxins A/B of this

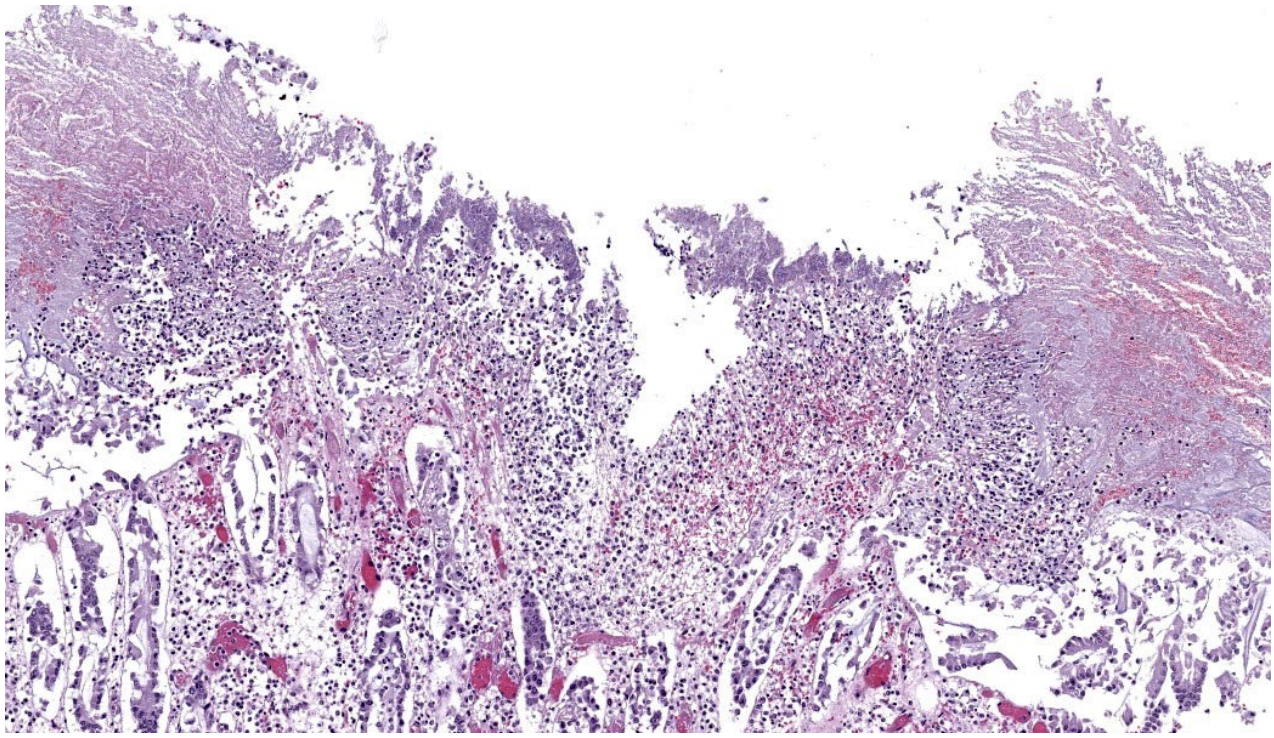

Figure 1-4. Colon, horse: Neutrophils extend vertically from the necrotic mucosa and peripherally within the overlying serocellular crust ("volcano ulcer") (HE, 180X)

microorganism in both small and large intestinal content. Because this ELISA test uses a cocktail of anti-toxin A and anti-toxin B antibodies, the positive result means that in the intestinal content there was one of the two toxins.[6] Isolation of *C. difficile* is suggestive of this microorganism involvement in the colitis, but not confirmatory as a number of clinically healthy horses can harbor *C. difficile* in the intestine.[4,5,6,7] Likewise, both gross and microscopic lesions are suggestive of, but not confirmatory, *C. difficile* infection. In particular, the volcano lesions are considered highly suggestive for *C. difficile* infection; this finding should, however, be interpreted with care, as other pathogens can also cause this lesion.[3,4,7]

The two main predisposing factors for *C. difficile* infection in horses are antibiotic therapy and hospitalization, both factors that were present in this case.[7] It is therefore speculated that salmonellosis occurred first and the attempts to control the disease with hospitalization and antibiotic treatment favored *C. difficile infection.*

No lesions suggestive of equine coronavirus were observed in the intestine of this horse; in particular, no intracytoplasmic inclusion bodies (the hallmark of such infection) were seen.[7] However, the necrosis of the mucosa was so extensive that if there were inclusion bodies, these may have been missed. Unfortunately, no post-mortem testing for equine coronavirus was performed.

This horse also had also a small intestine diverticulum, which was considered an incidental finding. Copper and selenium deficiency were also considered incidental findings, although a minor role in infection predisposition cannot be ruled out.

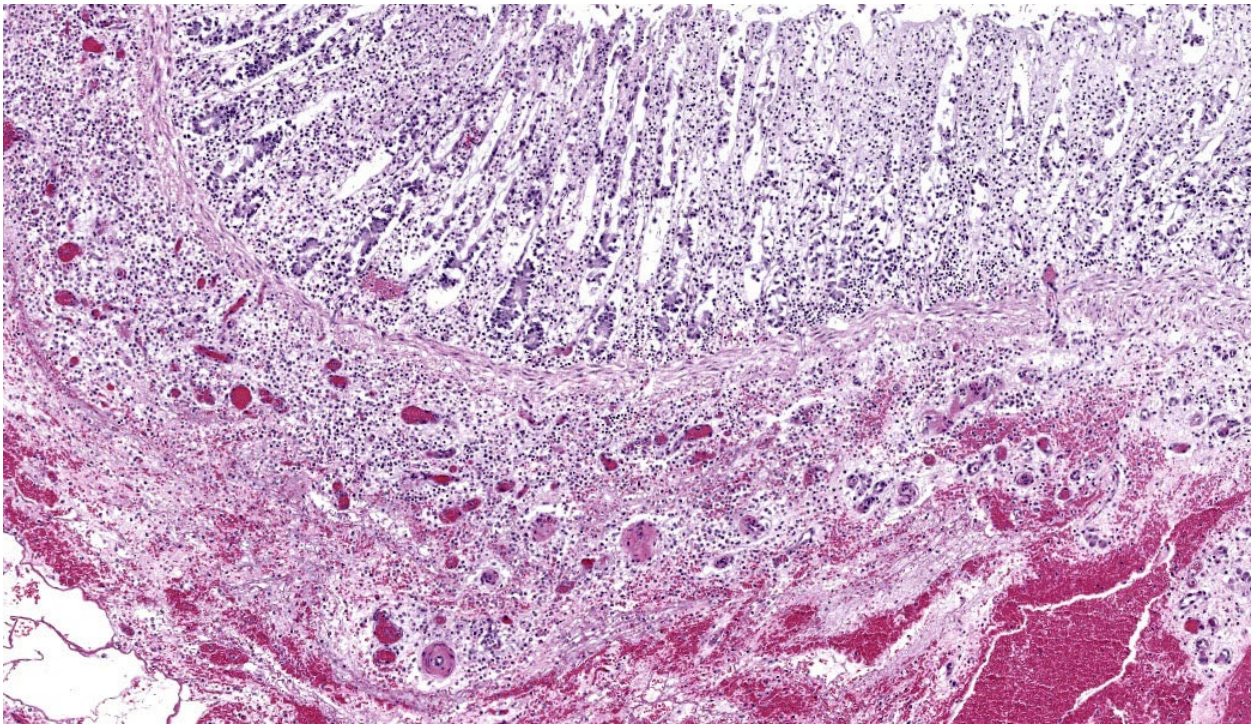

Figure 1-5. Colon, horse: The lamina propria and underlying submucosa are expanded by hemorrhage, edema, and numerous neutrophils. There are several thrombosed veins in the submucosa. (HE, 124X)

Contributing Institution:
California Animal Health and Food Safety Laboratory, Sa Bernardino Laboratory.
https://cahfs.vetmed.ucdavis.edu/

JPC Diagnoses:
Colon: Colitis, fibrinonecrotic, subacute, diffuse, marked, thrombosis, edema, and volcano lesions.

JPC Comment:
The JPC was thrilled to have the revered Dr. Paco Uzal, who needs no introduction, back again this year as a conference moderator. This first case provided a great example of an equine colitis case with multiple etiologies, something that is common in equine practice. The contributor provided an excellent write-up on this case and touched on many of the primary points of discussion during conference. Some participants struggled with determining if the tissue ID was small intestine or colon due to the degree of necrosis; this is reasonable and it can become incredibly difficult to differentiate the two when the architecture is lost. This horse also had a history of extensive NSAID administration and hospitalization, both of which are predisposing factors for both *C. difficile and Salmonella* infection. Grossly and histologically, *C. difficile*, *Salmonella* spp, and NSAID-induced ulceration can all look identical; culture and toxin typing are necessary for confirmatory diagnosis.[3] Participants agreed that the relevance of the previous equine coronavirus infection in this case could not be known.

Salmonella enterica var Typhimurium, thought to be the primary pathogen in this case by both the contributor and conference participants, is a non-host-adapted species of *Salmonella* that causes enteric disease in numerous species.[3] The overt lack of GI-associated lymphoid tissue (GALT) histologically should

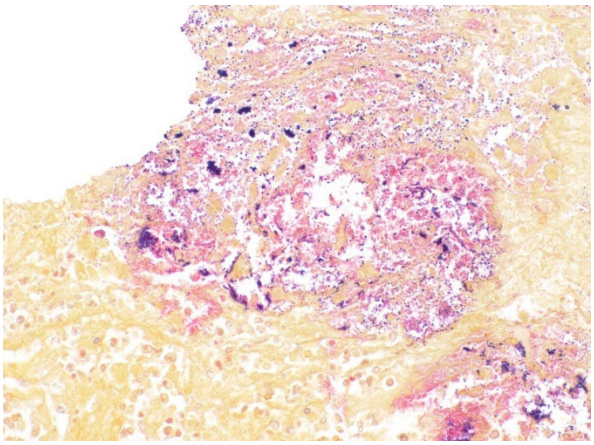

Figure 1-6. Colon, horse: There are colonies of mixed gram-positive and gram-negative bacilli within the necrotic mucosa. (Hucker Conn, 400X).

clue the pathologist in to the possibility for a *Salmonella* infection due to its tendency to infect the GALT first. *Salmonella* utilizes a Type 3 secretion system (T3SS) to stimulate phagocytosis and entry into M-cells and, subsequently, macrophages in the underlying lymphoid tissue. *Salmonella* inhibits fusion of the phagosome and lysosome to ensure its survivability within the macrophage and begins replicating safely within a Salmonella-containing vacuole (SCV). From there, it can be trafficked throughout the body hidden within macrophages. Later, using a Type 1 secretion system (T1SS), *Salmonella* stimulates apoptosis of the macrophage via activation of caspase 1, enabling each *Salmonella* to escape the host cell and infect additional cells. In this case, *Salmonella* Typhimurium was cultured from the small intestine, colon, and liver, indicating a septic process.

Clostridioides difficile (renamed in 2016 from *Clostridium difficile*) affects many species of all ages.[1,3] The primary clinical sign is profuse diarrhea. Histologically, "volcano lesions", as seen in this case, are a hallmark of *C. difficile* in the acute phase of infection.[3,4] Although other organisms (including *Salmonella*) can cause these lesions, they are considered classic for *C. difficile*. These lesions are characterized by micro-ulcerations of the mucosa with necrotic debris and neutrophils being "spat out" into the lumen, giving it the appearance of lava spewing from an erupting volcano. Following acute infection, volcano lesions become difficult to see histologically due to the progression to a full-blown necrotizing colitis, the lesions of which are non-specific and can be caused by a number of enteric pathogens (i.e. *Salmonella spp, Brachyspira spp*, etc.). As mentioned by the contributor, culture is a more suggestive method for confirming a diagnosis of *C. difficile*, but the results must be interpreted with caution as, according to Dr. Uzal, 3% of clinically normal horses will grow *C. difficile* on culture of enteric content.[4]

ELISA testing for *C. difficile* A/B toxin from the intestinal content/feces of affected animals, which was performed in this case, is considered confirmatory for a diagnosis of *C. difficile*. These toxins are the primary drivers of disease in *C. difficile* infection. TcdA (enterotoxin) binds to intestinal brush border receptors, while TcdB (cytotoxin) binds to receptors like LRP1. Both are internalized via receptor-mediated endocytosis, where they inactivate small GTP-binding proteins of the Rho/Ras family via mono-O-glucosylation.[2] These proteins play key roles in regulating the cytoskeletal dynamics of actin. Their inactivation leads to changes in the shape of the cell, retraction of cell processes, detachment from neighboring cells, and cell rounding.[1,2] This causes the cell to undergo apoptosis.[1,2] In the case of detachment-mediated stress, this form of apoptosis may be similar to anoikis, a form of apoptosis that occurs in "homeless", anchor-dependent cells that have become detached from the extracellular matrix and/or surrounding cells.

References:

1. Di Bella S, Ascenzi P, Siarakas S, Petrosillo N, di Masi A. *Clostridium difficile* Toxins A and B: Insights into Pathogenic Properties and Extraintestinal Effects. *Toxins (Basel)*. 2016;8(5):134.
2. Liu Z, et al. Structural basis for selective modification of Rho and Ras GTPases by *Clostridioides difficile* toxin B. *Sci Adv*. 2021;7:eabi4582.
3. Mendonça FS, Navarro MA, Uzal FA. The comparative pathology of enterocolitis caused by *Clostridium perfringens type C, Clostridioides difficile, Paeniclostridium sordellii, Salmonella enterica* subspecies *enterica* serovar Typhimurium, and nonsteroidal anti-inflammatory drugs in horses. *J Vet Diagn Invest.* 2021;34:412-420.
4. Uzal FA, Diab SS, Blanchard P, Moore J, Anthenill L, Shahriar F, Garcia JP, Songer JG.. *Clostridium perfringens* type C and *Clostridium difficile* co-infection in foals. *Vet Microbiol.* 2012;156:395-340.
5. Uzal FA, Diab SS. Gastritis, enteritis, and colitis in horses. *Vet Clin North Am Equine Pract*. 2015;31:337-358.
6. Uzal FA, Navarro MA, Asin J, Henderson EE. Clostridial Diseases of Horses: A Review. Vaccines (Basel). 2022;10:318.
7. Uzal FA, Arroyo LG, Navarro MA, Gomez DE, Asín J, Henderson E. Bacterial and viral enterocolitis in horses: a review. *J Vet Diagn Invest.* 2022;34:354-375.

CASE II:

Signalment:
8-month-old, castrated male domestic shorthair cat (*Felis catus)*

History:
The cat was neutered and was recovering well until the evening following surgery. After then, the cat was inappetent and lethargic. He developed diarrhea the second day after surgery. The cat was found dead the third day after surgery.

Gross Pathology:
At postmortem examination, the cat was in good body condition with minimal postmortem decomposition. The skin of the scrotum, prepuce and perineum was swollen with large amounts of subcutaneous edema and emphysema. The right and left inguinal areas and medial aspect of the thighs were swollen by subcutaneous edema and emphysema, but the right rear leg was particularly swollen. The skin of the medial aspect of the thigh of the right rear leg was mottled red and green. The semitendinosus and semimembranosus muscles of the right and left rear legs were dark red to black, edematous and emphysematous with the most severe lesions in the right rear leg. There was marked subcutaneous edema consisting of light red fluid cloudy fluid in the skin of the dorsal lumbar area extending ventrally into the right and left flanks.

Figure 2-1. Skeletal muscle, cat: (Photo courtesy of: New Mexico Department of Agriculture Veterinary Diagnostic Services http://www.nmda.nmsu.edu/home/divisions/vds/)

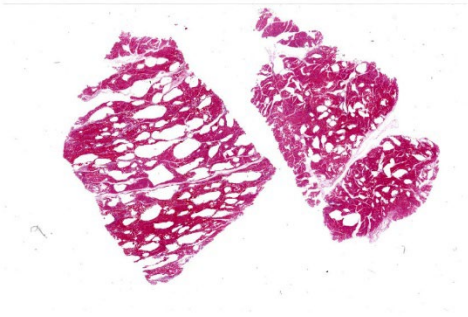

Figure 2-2. Skeletal muscle, cat: At subgross magnification, there is marked emphysema within the sections of skeletal muscle. (HE, 10X)

Laboratory Results:
Fluorescent antibody testing of the affected skeletal muscle was positive for *Clostridium novyi* and negative for *Clostridium chauvoei*, *Clostridium septicum* and *Clostridium sordellii*.

Moderate numbers of *Clostridium novyi* and rare *Clostridium perfringens* were isolated on anaerobic culture. There were no bacteria isolated on aerobic culture.

Microscopic Description:
The grossly affected skeletal muscle is necrotic with swollen, hypereosinophilic to pale staining myofibers that have lost their cross striations. The sarcoplasm of small numbers of myofibers is fragmented and replaced by eosinophilic flocculent material. The interstitium is emphysematous and contains small amounts of hemorrhage and edema that separate individual myofibers and the endomysium. There are also numerous large bacilli and a few perivascular to random multifocal infiltrates of small numbers of degenerate neutrophils in the interstitium. There are small numbers of arterioles and venules with necrosis of the vascular wall.

Contributor's Morphologic Diagnoses:
Skeletal muscle: Acute necrosis with emphysema, hemorrhage, mild suppurative myositis and numerous intralesional bacilli; etiology, *Clostridium novyi.*

Contributor's Comment:
Clostridium species are large, gram-positive, rod-shaped bacteria that produce endospores.[4] They are obligate anaerobes with some species being difficult to grow (*Clostridium novyi* and *C. haemolyticum*) because they can rapidly die (within fifteen minutes) after being exposed to oxygen. The pathogenic *Clostridium* can be divided into three general groups based on the disease they cause. The neurotoxic clostridia include *C. botulinum* and *C. tetani*. The histotoxic clostridia include *C. chauvoei*, *C. haemolyticum*, *C. novyi* types A and B, *C. perfringens* type A, *C. septicum* and *C. sordelli*. The enteric clostridium include *C. perfringens* types A-E, *C. difficile*, *C. spiroforme* and *C. colinum*. Some authors will placed *C. piliforme* in a group by itself and classify it as an atypial clostridium. The histotoxic clostridia produce toxins, are invasive, and are responsible for the various forms of clostridial myonecrosis (clostridial myositis) such as malignant edema, gas gangrene, blackleg, and pseudoblackleg.[2,5,6] Other than blackleg in ruminants being specifically caused by *C. chauvoei*, the other forms of clostridial myonecrosis are not specifically caused by any species of *Clostridium*. The clostridia causing myonecrosis are typically found in the soil or are part of the flora of the gastrointestinal tract. Clostridial myonecrosis can be the result of endogenous latent clostridial spores that reside in the skeletal muscle or exogenous bacteria that are introduced as wound contaminants. In both cases, the pathogenesis of myonecrosis is the same. Trauma to the skeletal muscle results in localized tissue hypoxia and anaerobic conditions allowing the clostridia to proliferate. As the pH and oxygen content of the traumatized skeletal

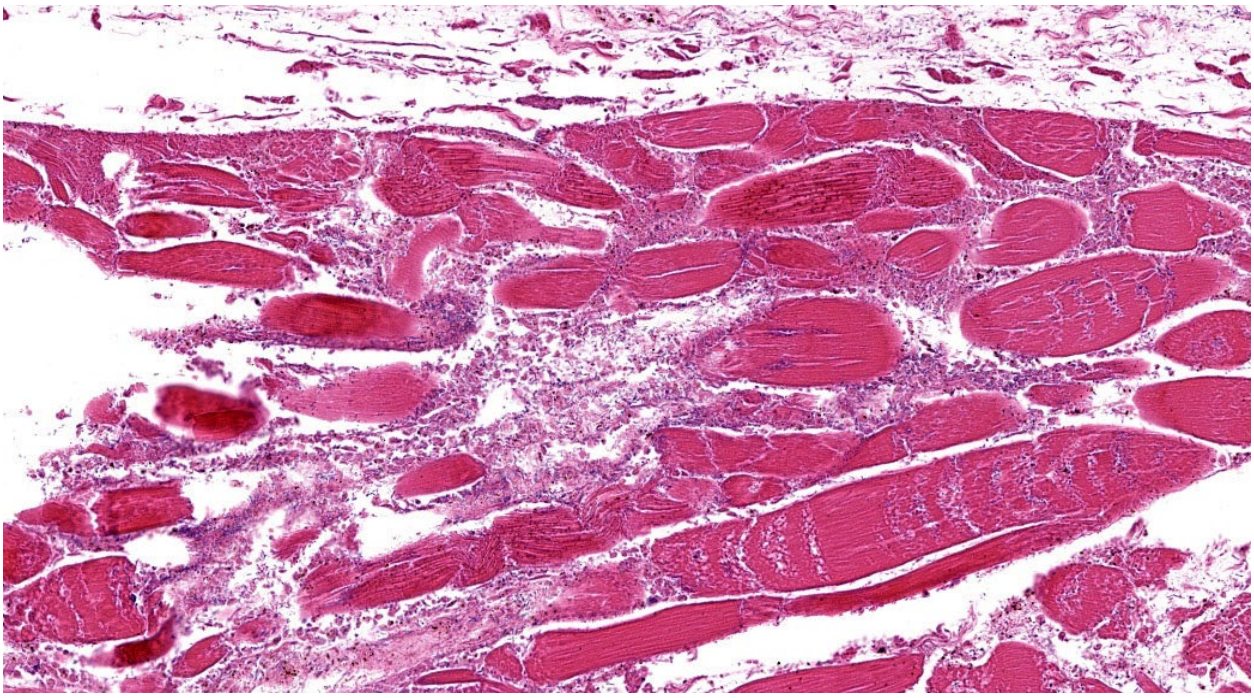

Figure 2-3. Skeletal muscle, cat: Necrotic fibers demonstrate contraction band formation, myofibrillolysis, and fragmentation. There are numerous robust bacilli within the necrotic myofibers and adjacent endo- and perimysium. (HE, 394X)

muscle decreases, the conditions for clostridial growth and toxin production become ideal. The clostridial toxins inhibit neutrophil infiltration into the lesion as well as weakening endothelial cell-to-cell contact. The clostridial toxin's effects on endothelial cells can result in vascular thrombosis causing further tissue hypoxia and necrosis. The clostridial toxins are eventually absorbed systemically and can result in hypovolemia, cavitary effusion, low cardiac output, low cardiac contractility, shock and death.

In the submitted case, the histotoxic clostridium *C. novyi* was detected in the skeletal muscle by fluorescent antibody testing as well as being isolated from the affected skeletal muscle. *Clostridium novyi* has three types.[2,4] *Clostridium novyi* type A produces alpha toxin (a cytotoxin) and causes clostridial myonecrosis. *Clostridium novyi* type B produces both the cytotoxic alpha toxin and a beta toxin (a phospholipase). *Clostridium novyi* type B causes infectious necrotizing hepatitis (black disease) following liver fluke infections in ruminants. *Clostridium novyi* type C does not produce toxins and is not pathogenic. The *C. novyi* alpha toxin as well as four other large clostridial cytotoxins function by glucosylating cellular GTPase proteins.[1,2] The clostridial cytotoxins have a particular affinity for the GTPase proteins that are involved in maintaining the cytoskeletal function. Inhibition of the cytoskeletal proteins results in loss of endothelial cell integrity that results in edema and thrombosis locally as well as hypovolemia, hypotension, organ failure and death systemically.

Clostridial myonecrosis can develop in multiple species, but is most common in ruminants, horses, and swine.[3] In all species, skeletal muscle necrosis in the primary lesion. The muscle necrosis is accompanied by edema, hemorrhage, and often emphysema. The hemorrhage and lysis of erythrocytes cause the affected skeletal muscle to be dark red to black.

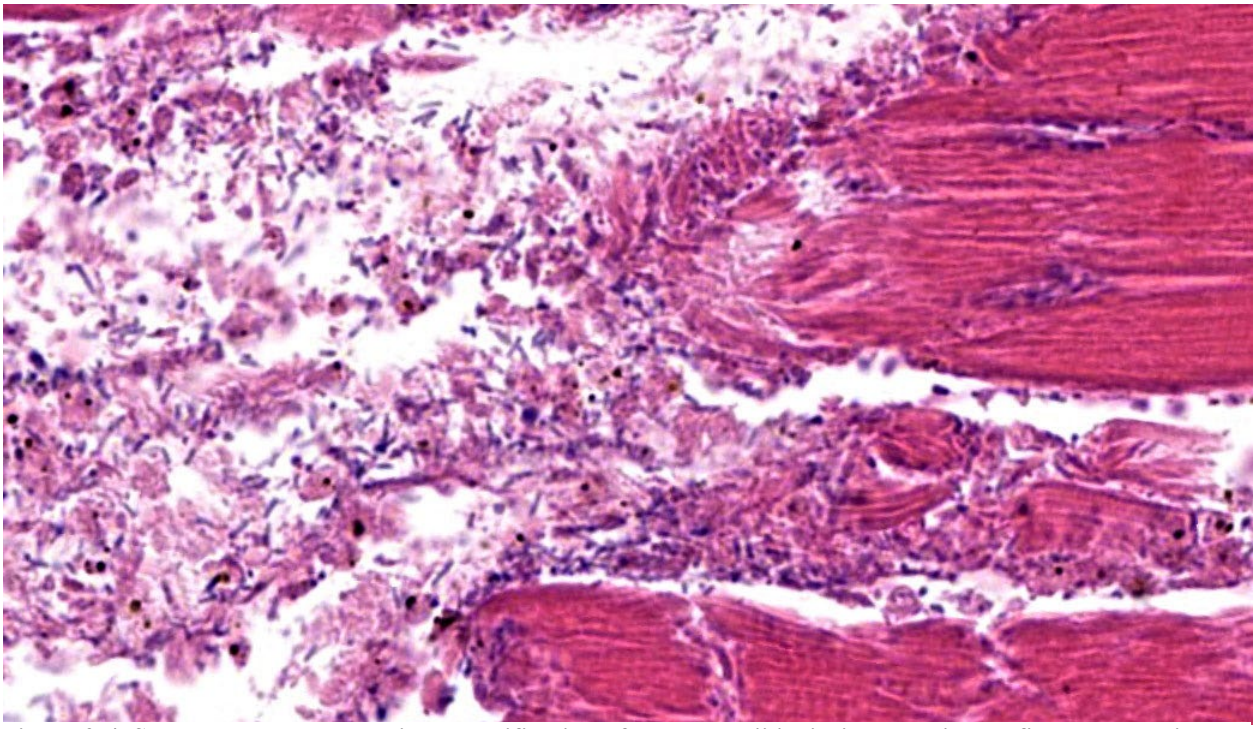

Figure 2-4. Skeletal muscle, cat: High magnification of robust baciili within necrotic myofibers and their cellular debris. (HE, 1800X)

The necrotic skeletal muscle often has a rancid odor. The microscopic lesions mirror the gross lesions. The primary microscopic lesion is necrotic myofibers that are separated by edema, hemorrhage and lysed erythrocytes. The skeletal muscle may be emphysematous. There typically are very few neutrophils within the lesion. The number of bacteria in the lesion varies widely with the lesions in some animals containing large numbers of bacteria with the lesions in other animals containing small numbers of bacteria. Most lesions have small numbers of bacteria. Fragmentation of the necrotic myofibers in the deeper aspects of the lesions may be present. The affected animals die quickly usually within twenty-four hours if not treated. The histotoxic clostridia spread quickly throughout the carcass postmortem resulting in rapid decomposition. Because these bacteria spread quickly throughout the carcass postmortem, one has to be careful in interpreting positive identification of histotoxic clostridia within muscle lesions as the *Clostridium* species identified may be a postmortem contaminant rather than the cause of the muscle necrosis.

Contributing Institution:
New Mexico Department of Agriculture
Veterinary Diagnostic Services
http://www.nmda.nmsu.edu/home/divisions/vds/

JPC Diagnoses:
Skeletal muscle: Myositis, necrotizing, acute, diffuse, severe, with emphysema and large, occasionally sporulating bacilli.

JPC Comment:
This contributor's outstanding comment pretty much covers it all! The few points from the conference discussion not covered in the write-up above focused on a review of some of the terminology utilized to describe degenerative and necrotic lesions in skeletal muscle ("loss of cross striations," "vacuolation,"

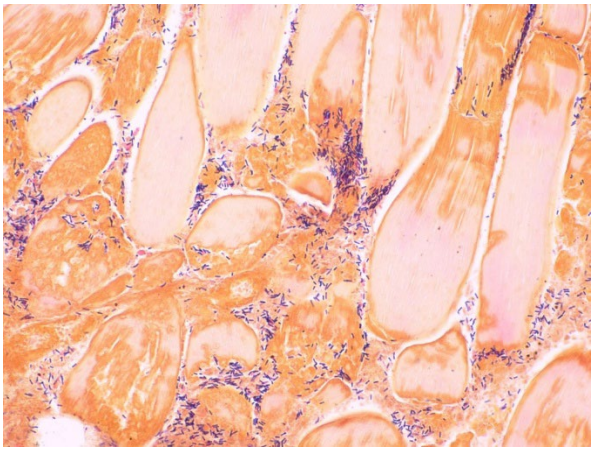

Figure 2-5. Skeletal muscle, cat: High magnification of robust gram-positive bacilli within areas of necrotic skeletal muscle. (Hucker Conn, 400X)

"fragmentation," "myofibrillolysis," "contraction bands", etc.). Contraction bands, in particular, take some practice to see on an H&E and are characterized by hypercontracted, intensely eosinophilic transverse bands within the sarcoplasm of myocytes. These represent focal myofibrillar disruption and are often seen in rhabdomyolysis, trauma, or ischemia.[7] They correspond to extremely shortened sarcomeres with obliterated I-bands and H-zones and are an indication of myocyte necrosis.[7] The contraction bands in these cases can be beautifully revealed with a PTAH stain. For both written and oral descriptions of slides in which a clostridial organism is suspected, Dr. Uzal additionally made a point to mention that there should be mention of whether the bacteria are sporulating or non-sporulating. In this case, there were a few sporulating bacteria present on the H&E; this is reflected in the JPC's morphologic diagnosis.

Dr. Uzal ran immunohistochemical stains on this case for multiple gas gangrene-causing clostridial species in his lab, as gas gangrene cases are frequently infected with multiple organisms. In this case, there was immunoreactivity for both *Clostridium novyi* and *Paraclostridium sordellii* (previously *C. sordellii*). Of the types of *C. novyi* listed by the contributor, type A was most likely the offender in this case. The Type A toxin of *C. novyi* behaves similarly to the A-B toxin of *C. difficile* discussed above in Case 1. Participants also took note that C. perfringens had been cultured by the contributor and speculated that the *C. perfringens* were present as bystanders and were not actively contributing to the infection in this cat.

References:

1. Aronoff DM. Clostridium novyi, sordellii, and tetani: mechanisms of disease. *Anaerobe*. 2013;24:98-101.
2. Aronoff DM and Kazanjian PH. Historical and contemporary features of infections due to Clostridium novyi. *Anaerobe*. 2018;50:80-84.
3. Cooper BJ and Valentine BA. Muscle and tendon. In: Maxie MG, ed. *Pathology of Domestic Animals*. 6th ed. vol. 1. St. Louis, MO: Elsevier; 2016:230-233.
4. Markey BK, Leonard FC, Archambault M, Cullinane A, Maguire D. Clostridium species. In: Markey BK, Leonard FC, Archambault M, Cullinane A, Maguire D, eds. *Clinical Veterinary Microbiology*. 2nd ed. St. Louis, MO: Mosby Elsevier; 2013:215-229.
5. Nagahama M, Takehara M, Rood JL. Histotoxic clostridial infections. *Microbiol Spectrum*. 2019;6(4):1-17.
6. Stevens DL, Aldape MJ, Bryant AE. Life-threatening clostridial infections. *Anaerobe*. 2012;18:254-259.
7. Venance SL, Burns KL, Veinot JP, Walley VM. Contraction bands in visceral and vascular smooth muscle. *Hum Pathol*. 1996;27(10):1035-1041.

CASE III:

Signalment:
9-month-old, female, spayed Bernese Mountain dog (*Canis lupus familiaris)*

History:
The dog presented for a 1-month history of severe ileus that was unresponsive to medical management. Abdominal radiographs showed a diffusely fluid and gas distended small intestine, with an intestinal diameter reaching 3.3 cm. Laparotomy to obtain biopsies of the gastrointestinal tract was performed. Due to poor prognosis and progressive clinical deterioration the patient was euthanized. A post-mortem examination was declined by the owners.

Gross Pathology:
During surgery, the gastrointestinal tract was reported to be diffusely distended and friable without any appreciable peristaltic movement.

Laboratory Results: N/A

Microscopic Description:
Duodenum, jejunum and ileum: Diffusely throughout the sections, there is partial to complete loss of the leiomyocytes of the outer and inner muscularis, which are multifocally replaced by loose fibrous connective tissue (fibrosis). The remaining smooth muscle is infiltrated by large numbers small and intermediate lymphocytes, few macrophages and neutrophils. The same inflammatory infiltrate is present throughout the muscularis mucosae. Remaining leiomyocytes have pale, frequently vacuolated cytoplasm. In the submucosa, muscularis and serosa there are small to moderate numbers of perivascular lymphocytes. Small lymphocytes, and infrequently neutrophils, surround or infiltrate the myenteric and submucosal ganglia, though neurons do not exhibit degenerative changes. The lamina propria contains a markedly increased number of lymphocytes, plasma cells and a mildly increased number of eosinophils. The epithelium is diffusely overlain by myriad bacterial rods. The serosa is mildly expanded by increased clear space (edema) and is lined multifocally by markedly hypertophied mesothelium. In the stomach a similar process is observed but restricted to the outer aspect of the muscularis with a patchy/multifocal distribution and sparing of the muscularis mucosae. (section not submitted)

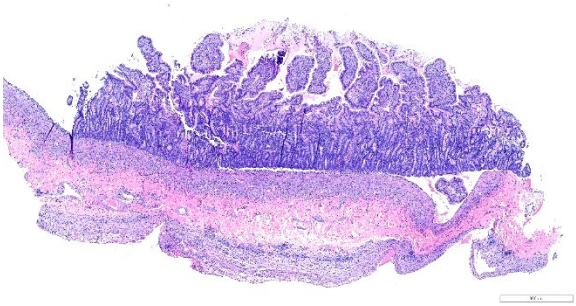

Figure 3-1. Jejunum, dog. The jejunal wall is markedly thinned and the muscularis propria and muscularis mucosae have a marked inflammatory infiltrate. (HE, 10X) (Photo courtesy of: Cummings School of Veterinary Medicine at Tufts University, Department of Biomedical Sciences, Section of Pathology, http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/)

Contributor's Morphologic Diagnoses:
Duodenum, jejunum and ileum: Severe, diffuse, chronic lymphocytic leiomyositis with severe muscularis atrophy; marked, diffuse, chronic lymphoplasmacytic enteritis.

Contributor's Comment:
Chronic intestinal pseudo-obstruction (CIPO) is a well-documented, rare gastrointestinal syndrome and represents a disorder leading to impaired intestinal motility that results in signs compatible with intestinal obstruction without occlusion of the intestinal lumen.[4,6,8,15]

Etiology of CIPO can be broadly considered primary (idiopathic), secondary or familial

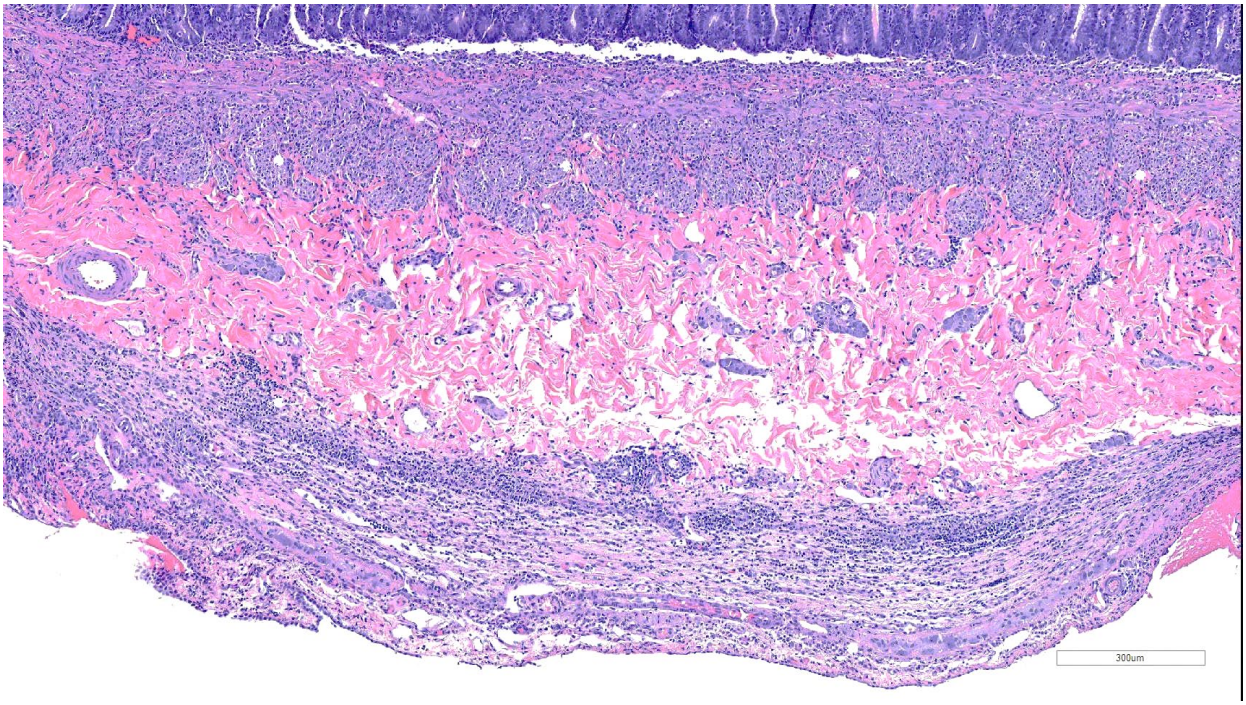

Figure 3-2. Jejunum, dog. Higher magnification showing the thinning of the muscularis with a marked lymphocytic inflammatory infiltrate that spares the submucosa. (HE, 100X) (Photo courtesy of: Cummings School of Veterinary Medicine at Tufts University, , Department of Biomedical Sciences, Section of Pathology, http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/)

where primary CIPO has no demonstrable etiopathologic cause, and secondary CIPO may be due to systemic pathology and thus, is more commonly seen in older human patients.[4] Regardless of the cause, CIPO is classified histologically in humans as myopathic, mesenchymopathic, and neuropathic groups, based on the involvement of the nerve pathways supplying the gastrointestinal tract, the interstitial cells of Cajal, and smooth muscle cells, respectively.[1,4,15]

The classic clinical presentation of affected dogs is marked gastric and small intestinal dilation with severe hypomotility and clinical signs of abdominal discomfort, vomiting, regurgitation, small bowel diarrhea, anorexia, and weight loss. Clinicopathologic changes are variable and nonspecific. Radiographic and ultrasonographic imaging of the abdomen shows variable involvement of the stomach, small intestine and colon in all dogs with diffuse dilatation of the small intestine, variable dilation of the stomach and large intestine.[15]

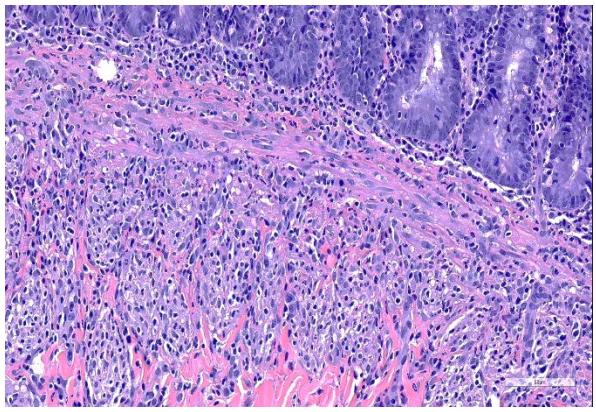

Figure 3-3. Jejunum, dog. Higher magnification showing the marked lymphocytic infiltrate affecting the muscularis mucosae and the mixed inflammatory infiltrate in the lamina propria. (HE, 400X) (Photo courtesy of: Cummings School of Veterinary Medicine at Tufts, , Department of Biomedical Sciences, Section of Pathology, http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/)

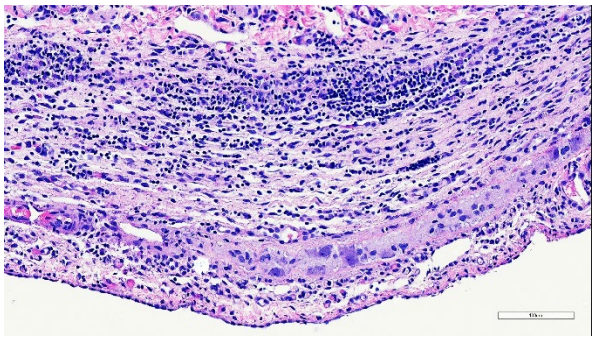

Figure 3-4. Jejunum, dog. There is marked loss of the smooth muscle of the muscularis with a marked lymphocytic infiltrate that spares the myenteric ganglia. (HE, 400X) (Photo courtesy of: Cummings School of Veterinary Medicine at Tufts University, Department of Biomedical Sciences, Section of Pathology, http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/)

CIPO in dogs is a rare disorder that is increasingly being diagnosed, and is attributed to intestinal leiomyositis.[10,15] Prior reports of leiomyositis have been described in large breed dogs and similar to this case, Bernese Mountain dogs are featured in several of these reports.[3,6,15] Other breeds reported in the literature include Portuguese Water dogs, Yorkshire Terrier, Border Collie, American Staffordshire Terrier, English Springer Spaniel, German Shepherd Dog, and mixed breed dogs.[6,8,15] The ages of affected dogs range from 6 months to 10 years old, which emphasizes that this disorder should be a differential in both young and older dogs.[15] In human pediatric patients, a similar idiopathic/autoimmune myositis restricted to the muscularis propria has been described as an isolated condition or arise as part of more generalized connective tissue disease.[9,13] This pattern differs from other congenital conditions in humans where smooth muscle from the urinary tract, particularly the urinary bladder, is also affected.[14]

Microscopic findings reported for this condition consist of mild to marked mononuclear infiltrate, myofiber degeneration and fibroplasia or fibrosis centered within the muscularis propria of the stomach, small and large intestinal wall with the jejunum being the most severely and chronically affected segment.[7,15] In early lesions the inflammation and smooth muscle degeneration are segmental, random between the outer and inner muscularis layers and sparing of the muscularis mucosae.[15] Concurrent cecal involvement leading to impaction has also been described.[5] Consistent histologic findings included T-lymphocyte inflammation within the muscularis propria with relative sparing of the mucosa, submucosa and neural plexuses.[14] In one case report the inflammatory infiltrate was predominantly of B-lymphocytes and featured extensive angiogenesis.[6] Leiomyocytes appear to be the target of the inflammation given the presence of various stages of degeneration leading to complete myofiber loss in chronic stages of the disease. Superficial inflammation is a frequent secondary finding in CIPO and has been associated with bacterial overgrowth, which were changes observed in this case.[6] Neuropathy has not been a feature in most reports of canine CIPO. Inflammatory cells obscuring the

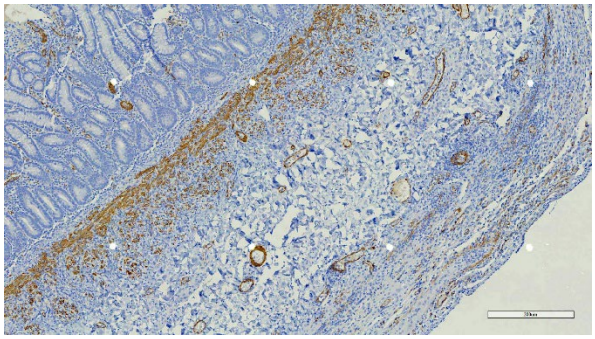

Figure 3-5. Jejunum, dog. Loss of smooth muscle is stressed by loss of smooth muscle actin immunoreactivity of the muscularis with relative sparing of the muscularis mucosae. (Anti-SMA, 400X) (Photo courtesy of: Cummings School of Veterinary Medicine at Tufts University, Department of Biomedical Sciences, Section of Pathology, http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/)

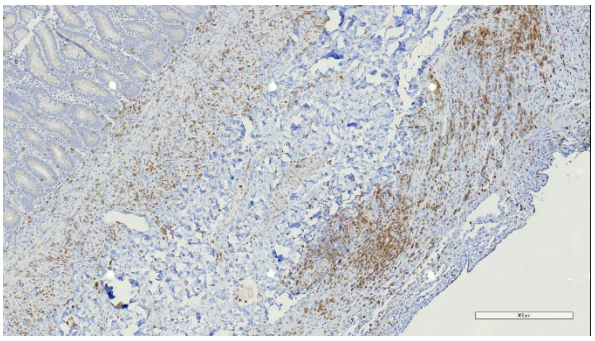

Figure 3-6. Jejunum, dog. The lymphocytic infiltrate in the muscularis is composed predominantly of lymphocytes showing strong cytoplasmic and membranous immunoreactivity for CD3, proving T-cell origin. Anti-CD3. (HE, 200X) (Photo courtesy of: Cummings School of Veterinary Medicine at Tufts University, Department of Biomedical Sciences, Section of Pathology, http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/)

myenteric and submucosal plexuses in this case were considered an extension from the neighboring inflammation as no degenerative changes were observed in the neurons. Myenteric ganglionitis has, however, been reported occurring concurrently with leiomyositis.[10]

The pathogenesis for this condition remains unclear, though the targeting of smooth muscle by T-lymphocytes is supportive of a cell-mediated inflammatory process suggestive of an immune-mediated process against intestinal smooth muscle.[11,14,15] In humans with intestinal leiomyositis, the inflammation is specific for the intestinal musculature as leiomyocytes of vessels or other tissues are unaffected.[15] Humans with intestinal leiomyositis can have autoimmune disease as a preexisting condition, including systemic lupus erythematosus, rheumatoid arthritis, scleroderma, and autoimmune hepatitis.[7] Neither the dog in this case nor those in the largest case series in dogs with leiomyositis had concurrent evidence of systemic autoimmune disease.[14] In few human cases, acute episodes of infectious gastroenteritis or diarrhea preceded the development of gastrointestinal distention.[7] Molecular mimicry has been proposed as a mechanism for the development of leiomyositis, although an explanation of restriction of the inflammation to the gastrointestinal tract was not elucidated.[7]

An important diagnostic consideration for cases of leiomyositis is mechanical obstruction secondary to foreign body. The combination of complementary imaging methods such as abdominal radiographs with ultrasonography is the most effective method to rule out the presence of a mechanical obstruction, though exploratory laparotomy is required for sampling when leiomyositis is suspected.[15]
The prognosis of dogs with intestinal leiomyositis is generally poor with survival times ranging from 10 days to 5 weeks after diagnosis.[14] Management of CIPO in humans is challenging and aimed at controlling symptoms and minimizing complications and typically involves immunomodulatory therapy.[12,15] Early diagnosis and treatment, results in a better long-term prognosis in humans. One case report in a dog described a favorable response to therapy after early diagnosis and treatment, suggesting better prognosis if the condition is identified before the onset of muscle atrophy.[11]

Contributing Institution:
Cummings School of Veterinary Medicine at Tufts University
Department of Biomedical Sciences, Section of Pathology
200 Westboro Rd.
North Grafton, MA. 01536
http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/

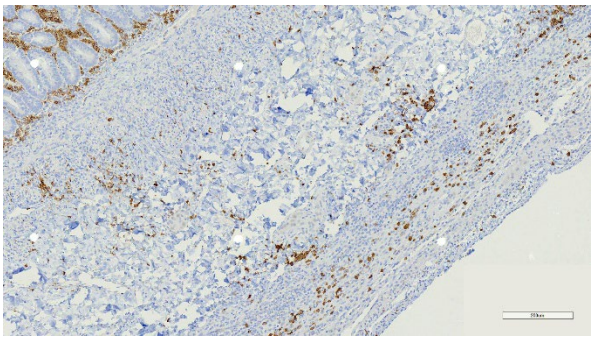

Figure 3-7. Jejunum, dog. A small percentage of the inflammatory infiltrate shows strong membranous and variable cytoplasmic immunoreactivity for CD20, consistent with B-cells. Anti-CD20. (HE, 400X) (Photo courtesy of: Cummings School of Veterinary Medicine at Tufts University, Department of Biomedical Sciences, Section of Pathology, http://vet.tufts.edu/department-of-biomedical-sciences/research/pathology/)

JPC Diagnoses:

Small intestine: Leiomyositis, lymphocytic, chronic, diffuse, severe, with marked smooth muscle loss and fibrosis.

JPC Comment:

Yet again, a fantastically written comment by the contributor that hits on the majority of conference discussion points! Chronic intestinal pseudo-obstruction (CIPO) has also been known by many names throughout its history, including fibrosing gastrointestinal leiomyositis, intestinal leiomyositis, idiopathic intestinal pseudo-obstruction, chronic intestinal dysmotility, and enteric myopathy. Many of these names are no longer in use, but intestinal leiomyositis and CIPO are most commonly utilized in the current literature. A good way to think about CIPO is that it is the clinical disease that can result from intestinal leiomyositis. This entity was last seen in the WSC in 2017 during Conference 4, Case 2. Histologically, the focus of lymphocytic inflammation on the smooth muscle cells of the intestine with replacement fibrosis and an absence of infectious organisms should make one think about this clinical syndrome, especially if the history fits.

The contributor thoroughly describes the syndrome in dogs, and differential diagnoses were mentioned in conference. These should include lymphocytic polymyositis, as some cases of this condition can have similar lesions in the esophagus and other areas of smooth muscle, and Chagas disease, caused by *Trypanosoma cruzi* (last seen in the WSC in 2024 during Conference 3, Case 3). T. cruzi can cause similar symptoms by causing chronic inflammation that leads to the destruction of the enteric nervous system (ENS), particularly the myenteric plexus.[13] This destruction results in severe neuromuscular dysfunction, leading to conditions like megaesophagus and megacolon.[13]

One participant brought up the possibility of a dysautonomia in this case, which is a great thought since CIPO may result from segmental or diffuse neuromuscular dysfunction in the gut. There are multiple forms of dysautonomia, such as specific autoimmune forms like autoimmune autonomic ganglionopathy (AAG) that involves autoantibodies attacking nerve cells.[2] Other forms arise secondary to other autoimmune disorders (i.e., Sjögren's, systemic lupus erythematosus).[2] However, in this case, the ganglia in the intestine looked great! This steered attendees away from considering a neuromuscular autoimmune condition. That being said, the pathogenesis of this condition has yet to be elucidated, and there is evidence that an autoimmune attack against smooth muscle cells could be at play.[12]

References:

1. Antonucci A, Fronzoni L, Cogliandro L, et al. Chronic intestinal pseudo-obstruction. *World J Gastroenterol.* 2008;14:2953–2961.
2. Blitshteyn S. Dysautonomia: a common comorbidity of systemic disease. *Immunol Res*. 2025;73(1):105.
3. Couraud L, Jermyn K, Yam PS, Ramsey IK, Philbey AW. Intestinal pseudo-obstruction, lymphocytic leiomyositis and atrophy of the muscularis externa in a dog. *Veterinary Record.* 2006;159:86–87.

4. Downes TJ, Cheruvu MS, Karunaratne TB, De Giorgio R, Farmer AD. Pathophysiology, Diagnosis, and Management of Chronic Intestinal Pseudo-Obstruction. *Journal of Clinical Gastroenterology.* 2018;52:477–489.
5. Eastwood JM, McInnes EF, White RN, Elwood CM, Stock G. Caecal Impaction and Chronic Intestinal Pseudo-Obstruction in a Dog. *Journal of Veterinary Medicine Series A*. 2005;52:43–44.
6. Gianella P, Tecilla M, Bellino C, et al. An unusual case of intestinal leiomyositis in a Bernese mountain dog. *Schweiz Arch Tierheilkd*. 2015;157:563–567.
7. Haas S, Bindl L, Fischer H-P. Autoimmune enteric leiomyositis: A rare cause of chronic intestinal pseudo-obstruction with specific morphological features. *Human Pathology*. 2005;36:576–580.
8. Johnson CS, Fales-Williams AJ, Reimer SB, Lotsikas PJ, Haynes JS. Fibrosing Gastrointestinal Leiomyositis as a Cause of Chronic Intestinal Pseudo-Obstruction in an 8-Month-Old Dog. *Veterinary Pathology.* 2007;44:106–109.
9. Kapur RP. Intestinal Motor Disorders. In: Pathology of Pediatric Gastrointestinal and liver disease. New York: Springer; 2014:249–316.
10. Kopke MA, Ruaux CG, Gal A. Myenteric ganglionitis and intestinal leiomyositis in a Jack Russell terrier: Myenteric ganglionitis and intestinal leiomyositis in a dog. Journal of Small Animal Practice. 2018: 1-4.
11. Murtagh K, Oldroyd L, Ressel L, Batchelor D. Successful management of intestinal pseudo-obstruction in a dog. *Veterinary Record Case Reports*. 2013: e000025
12. Oton E, Moreira V, Redondo C, et al. Chronic intestinal pseudo-obstruction due to lymphocytic leiomyositis: is there a place for immunomodulatory therapy?. *Gut*. 2005;54(9):1343–1344.
13. Pérez-Molina JA, Crespillo-Andújar C, Trigo E, et al. Chagas disease is related to structural changes of the gut microbiota in adults with chronic infection (TRIPOBIOME Study). *PLoS Negl Trop Dis*. 2023;17(7):e0011490.
14. Ruuska T, Karikoski R, Smith VV, Milla PJ. Acquired myopathic intestinal pseudo-obstruction may be due to autoimmune enteric leiomyositis. Gastroenterology. 2002;122:1133–1139.
15. Zacuto AC, Pesavento PA, Hill S, et al. Intestinal Leiomyositis: A Cause of Chronic Intestinal Pseudo-Obstruction in 6 Dogs. *Journal of Veterinary Internal Medicine.* 2016;30:132–140.

CASE IV:

Signalment:
6-week-old, female, New Zealand white rabbits (*Oryctolagus cuniculus*)

History:
A total of 15 rabbits arrived from the vendor and appeared in good health. Six days post-arrival, animal A was found dead in the cage, and B was found moribund. Animal B died before treatments could be commenced.

Gross Pathology:
The only obvious gross lesions in both animals were severely edematous large intestine which contained scant liquid feces..

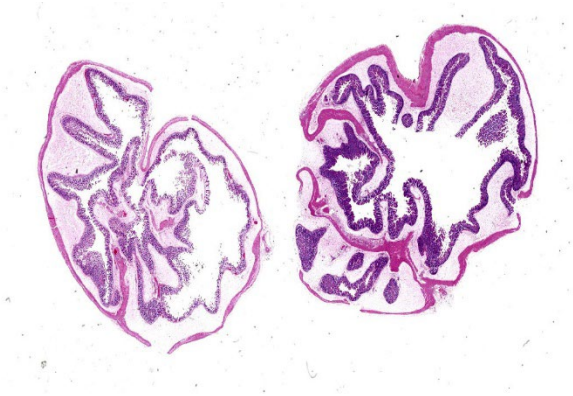

Figure 4-1. Colon, rabbit: Two sections of colon are submitted for examination. At subgross magnification, there is profound submucosal edema. (HE, 12X)

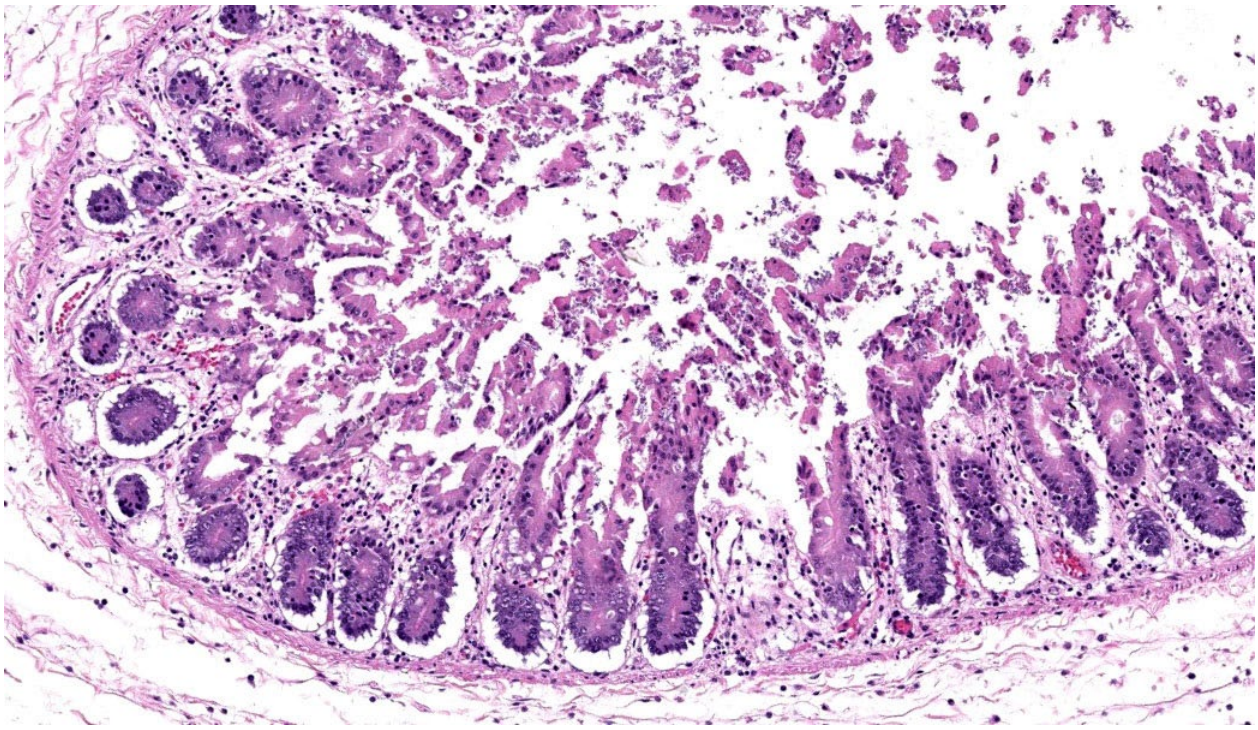

Figure 4-2. Colon, rabbit: There is ulceration of the colonic mucosa. (HE, 100X)

Laboratory Results:
Feces submitted to IDEXX yielded positive results for *Clostridium difficile* toxins A&B via ELISA.

Microscopic Description:
Cecum at ileocecocolic junction: One full-thickness cross sections of cecum at the ileocecocolic junction are examined from each animal (2 total). Diffusely, the submucosa is expanded by clear space (edema) and admixed inflammatory cells (neutrophils, macrophages, lymphocytes, and fewer plasma cells)(Fig 1). The lamina propria is multifocally expanded by edema, hemorrhage, the previously mentioned admixed inflammatory cells, and karyorrhectic debris (Fig 2). The overlying epithelium has multifocal erosions and ulcerations with surface necrotic debris and mixed bacterial colonies.

Contributor's Morphologic Diagnoses:
Typhlocolitis, acute, ulcerative, with edema and hemorrhage.

Contributor's Comment:
Clostridioides difficile (renamed from *Clostridium difficile* in 2016) is a toxin-producing, gram-positive, spore-forming anaerobe that affects humans and a variety of animal species.[4,9] When it was first identified from the feces of clinically normal babies in the 1930's it was named "the difficult clostridium" due to the fact that it was difficult to isolate and slow to grow in culture.[4,8] Sometimes called "pseudomembranous colitis", this entity causes classic histopathology lesion in humans and non-human primates including pseudomembrane formation on the intestinal surface.[2,8,13] In humans and animals, the pathogenesis involves disruption of commensal bacteria flora, colonization by C. difficile, and the release of toxins that cause significant mucosal damage.[4,8,13]

Rabbits are subject to a number of different clostridial diseases including enterotoxemia (*C. difficile, D. perfringens, C. spiroforme*), Tyzzer's disease (*C. piliforme*), dysautonomia

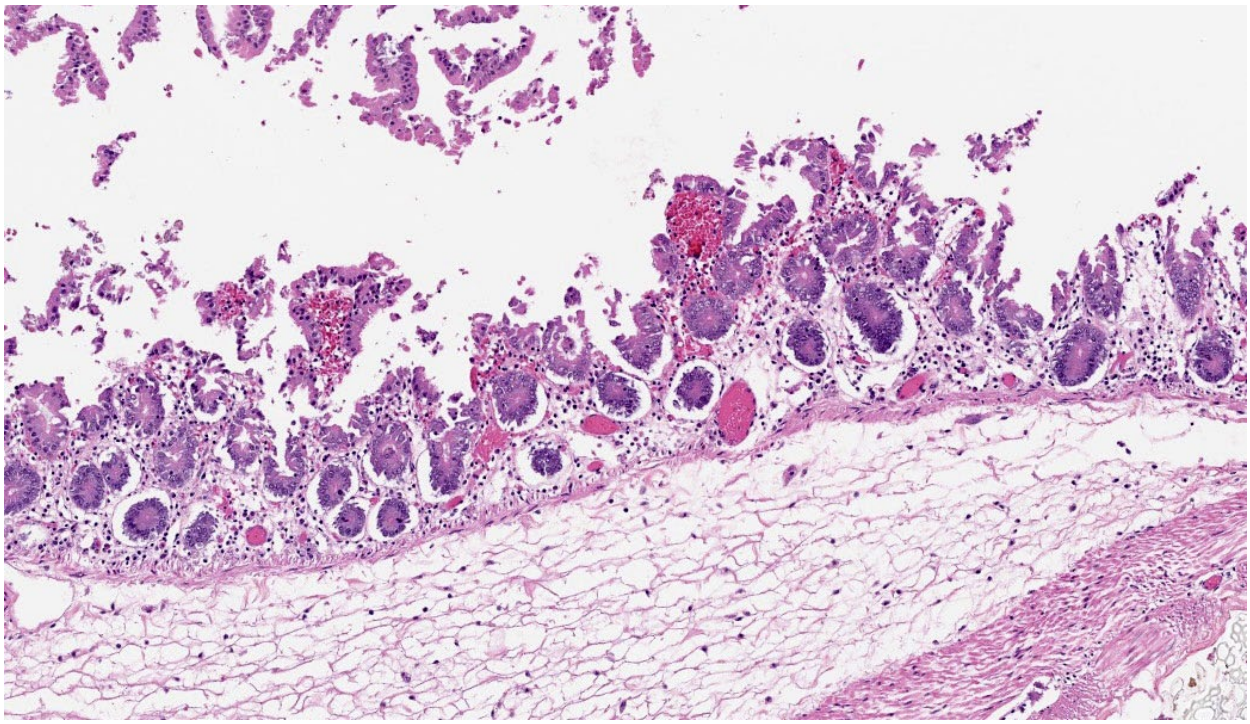

Figure 4-3. Colon, rabbit: There is multifocal hemorrhage of the colonic mucosa and marked submucosal edema. (HE, 200X)

(*C. botulinum*), and epizootic rabbit enteropathy (*C. perfringens* alpha toxin). Clostridial enterotoxemia has similar features in rabbits regardless of the species.[12] Chronicity can be acute or peracute.[6] In these cases, a peracute infection is likely since diarrhea, dehydration, or weight loss were not noted prior to death.

The primary virulence factors of *C. difficile* are two major exotoxins, toxin A and toxin B.[1-14] Both are enterotoxins, while Toxin B is both an endotoxin and cytotoxin. These toxins have the ability to glycosylate and inactivate Ras GTPases, disabling key cell signaling pathways, and glycosylate Rho and interfere with its regulation of cytoskeletal actin leading to apoptotic cell death of colonic epithelium.[1,13] Fecal ELISA is commonly used to identify these toxins and was used as a confirmation method in these rabbits to confirm the presence of *Clostridium difficile* A and B toxins in these rabbits. Fecal culture is the most sensitive method, but it is not very specific due to the possibility of isolating non-toxigenic isolates.[6] PCR detection of *C. difficile* is also highly sensitive and can discriminate between toxigenic and nontoxigenic strains of the organism by detecting its toxin-producing genes.[6]

As in humans, *C difficile* enterotoxemia in rabbits is associated with anything that disrupts the normal intestinal flora including antibiotic treatment.[1-14] These animals had not been treated with antibiotics, and it is suspected that stress from shipping was the primary factor leading to Clostridial disease in this case.

Although antibiotic treatment with metronidazole is considered the first choice of treatment in both humans and companion animals, treatment of *C. difficile*-associated disease has increasingly been associated with failure and recurrence.[3,11,14] In humans and marmosets, fecal transplantation shows promising results as a treatment for recurrent CDAD.[11,14] Additionally, a 2024 paper shows promising results

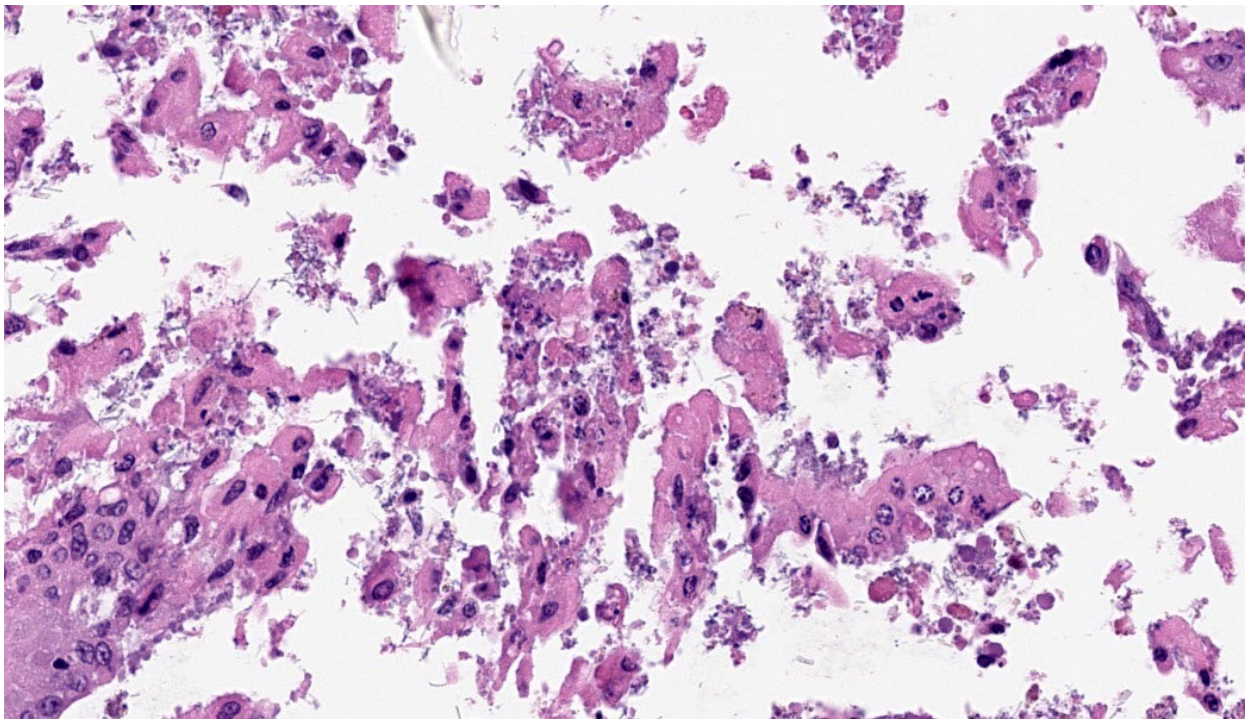

Figure 4-4. Colon, rabbit: Sloughed and necrotic enterocytes are admixed with numerous robust bacterial rods within the lumen (HE, 800X)

in the development of a *C. difficile* vaccine that uses metal-catalyzed oxidation (MCO) to inactivate toxins A & B after 2-3 intramuscular injections in rabbits.[1]

Contributing Institution:
Division of Laboratory Animal Resources
University of Pittsburgh
S1040 Thomas E. Starzl Biomedical Science Tower
200 Lothrop Street
Pittsburgh, PA 15261
http://www.dlar.pitt.edu/

JPC Diagnoses:
Colon: Colitis, necrotizing, subacute, multifocal, moderate, with volcano lesions, hemorrhage, and severe submucosal edema.

JPC Comment:
Wrapping up this conference is a classic example of another *C. difficile* infection, this time in a rabbit. Rabbits, along with hamsters and mice, are utilized in laboratory medicine in *C. difficile* studies. Rabbits specifically have been utilized because their gut tends to be colonized by heterogeneous *C. difficile* ribotypes, many of which are commonly isolated in humans.[6] As mentioned by the contributor, despite the lack of aggressive antibiotic therapy in the history, this animal had been recently transported and participants agreed that the stress of travel could have served as the predisposing factor for *C. difficile* overgrowth.

Similar to Case 1, due to the presence of volcano lesions coupled with mucosal ulceration/necrosis and heterophilic inflammation, Dr. Uzal says this is *C. difficile* until proven otherwise! This case had some solid examples of volcano lesions in which heterophils could be seen exploding out of tiny ulcers in the mucosa.

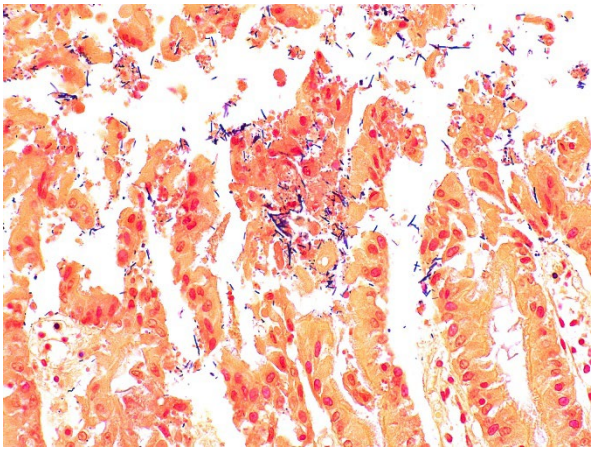

Figure 4-5. Colon, rabbit: Numerous robust gram-positive bacilli are present in the necrotic superficial mucosa. (Hucke , 400X)

Histologically, the colon was severely edematous and had gram-positive bacilli present within areas of mucosal necrosis that showed up beautifully on a Gram stain performed by Dr. Uzal's lab. A secondary bacterial infection was considered by conference participants in the markedly dilated sections of gut in this case, especially since rabbits frequently have multispecies enteric infections similar to cattle (i.e. coronavirus, rotavirus, *C. difficile, E. coli,* etc.), but there was no laboratory testing available to confirm this. *C. difficile*, however, was confirmed by the contributor via ELISA for A/B toxin.

References:

1. Aminzadeh A, et al. Immunogenicity and safety in rabbits of a *Clostridioides difficile* vaccine combining novel toxoids and a novel adjuvant. *Vaccine*. 2024;42(7):1582-1592.
2. Armstrong AR, Wünschmann A, Rigatti LH, Klein EC. *Clostridium difficile* enterocolitis in a captive Geoffroy's spider monkey (*Ateles geoffroyi*) and common marmosets (*Callithrix jacchus*). *Veterinary Pathology*. 2019;56(6):959-963.
3. Aslam S, Hamill RJ, Musher DM. Treatment of *Clostridium difficile*-associated disease: old therapies and new strategies. *Lancet Infect Dis*. 2005;5(9):549-57.
4. Czaepiel J, et al. *Clostridium difficile* infection: a review. *Europ J Clin Microbiol Inf Dis.* 2019;38:1211-1221.
5. Delmée M. Laboratory diagnosis of *Clostridium difficile* disease. *Clin Microbiol Infect*. 2001;7(8):411-6.
6. Drigo I, Mazzolini E, Bacchin C, et al. Molecular characterization and antimicrobial susceptibility of Clostridium difficile isolated from rabbits raised for meat production. *Vet Microbiol*. 2015;181(3-4):303-307.
7. Keel MK, Songer JG. The comparative pathology of *Clostridium difficile*-associated disease. *Vet Pathol.* 2006;43(3):225-40.