Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2018-2019

C o n f e r e n c e 9

1 November 2018

Conference Moderator:

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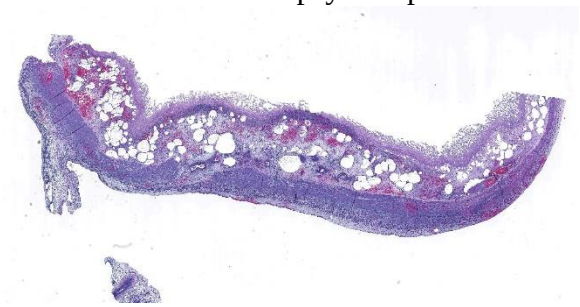
CASE I: 13-12484 (JPC 4050022-00).

Signalment: Two day old male warmblood horse, *Equus caballus*

History: This colt presented for bloody diarrhea, lethargy, and anorexia for ~1 day. His birth was not witnessed and he was noticed to be standing and nursing although the mare's udder was tight and he was not nursing well. He passed tan diarrhea at that time. Later that morning the diarrhea became bloody and he was increasingly lethargic. At presentation to Oregon State University Lois Bates Acheson Veterinary Teaching Hospital he was lateral, dull, ~ 5-10% dehydrated, and minimally responsive. Mucous membranes were pale, tacky, cold, and injected with a prolonged CRT. There were petechiae in the oral mucous membranes and in the pinna. Preliminary diagnosis was sepsis and despite supportive care there was little improvement. Due to the guarded prognosis the foal was euthanized and a necropsy was performed.



Intestine, foal: A section of intestine is submitted for examination. At subgross magnification, a linear band of cellular debris is present at the base of the mucosa, and the submucosa is markedly expanded by edema, hemorrhage, and multifocal emphysema. (HE, 6X)

Gross Pathology: Jejunal content was hemorrhagic, with multifocal zones of mucosal necrosis up to 50 x 15 cm throughout. There were multiple gastric ulcers with hemorrhagic gastric content and bloody liquid material throughout the small and large intestine. Petechiae and ecchymoses were present in the endocardium and kidneys.

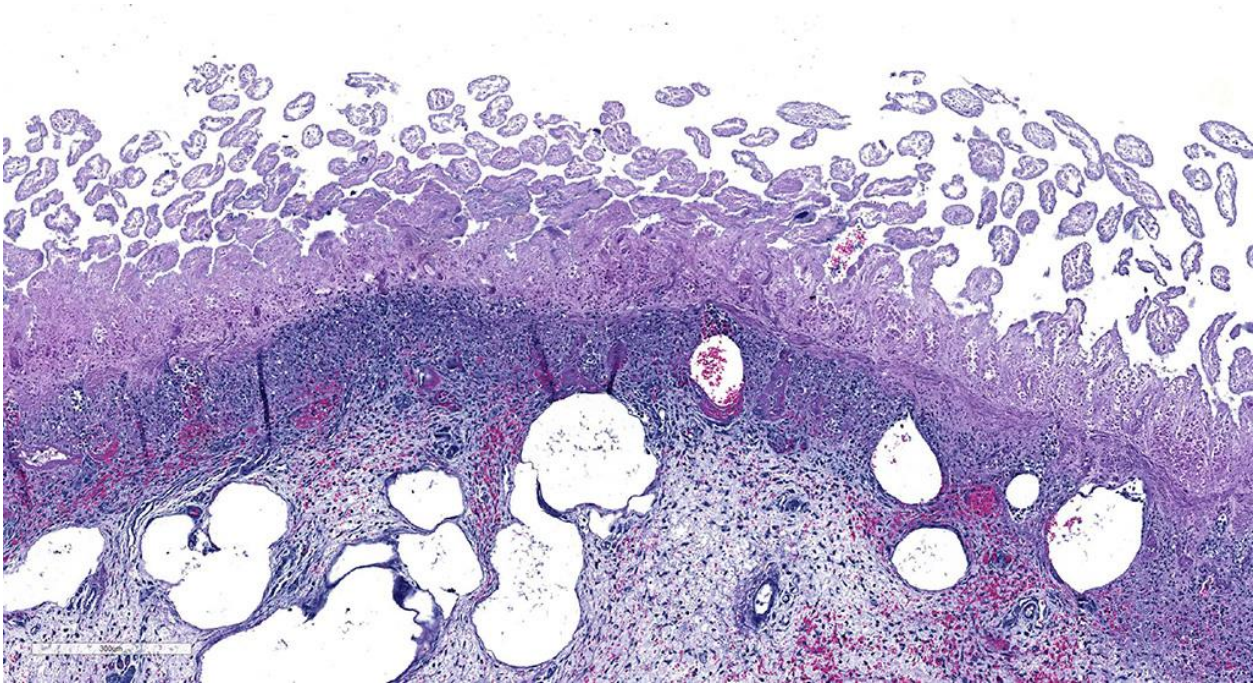
Laboratory results: CBC: Leukopenia (2810; RI: 6000-120000/ul), HCT 48.7% RI: 32-48%), hypoproteinemia (5.4; RI: 6.0-8.5 g/dL), hyperfibrinogenemia (400; RI: 100-400 mg/dl), lymphopenia (1208; RI: 1500-5000/ul), moderate neutropenia with a left shift (674; RI: 3000-6000/ul; bands 141 RI: 0-100ul).

Complete Chemistry: Mild azotemia (BUN 30; RI: 8-23 mg/dl, Crea 2.3; RI: 0.9-1.7 mg/dl), mild hypoglycemia (Glu 51; RI: 79-109), mild hyperbilirubinemia (3.4 RI: 0.8-2.6 mg/dl), moderate to marked hyperphosphatemia (10.1; RI: 1.9-4.1 mg/dl), increased SDH (12.9; RI: 2.4-7.2 U/L

IgG > 800 mg/dl
Blood culture: *Actinobacillus equuli* two colony types
Fecal *c. difficile* enterotoxin ELISA: positive
Fecal *c. perfringens* enterotoxin ELISA: negative

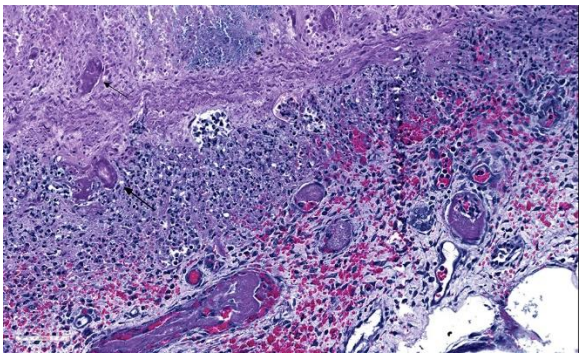
Microscopic Description: Small intestine: There are multifocal extensive areas of transmural hemorrhage. The submucosa is markedly edematous and there are multifocal fibrin thrombi with overlying mucosal necrosis. Within areas of necrosis there are numerous vessels occluded with amorphous, smudgy, eosinophilic deposition (fibrin thrombi) in the lamina propria and the tips of the villi are hypereosinophilic with necrosis and cellular debris. Also within affected areas there are multiple colonies of bacilli. The tissue is inflamed with mixed degenerate inflammatory cells and the base of the mucosa is expanded by plump fibroblasts (proliferative fibroplasia) and numerous small vessels lined by plump endothelium (reactive vasculature).

Contributor's Morphologic Diagnoses: Small intestine: Severe multifocal transmural necrohemorrhagic enteritis with fibrin thrombi and bacilli consistent with clostridia



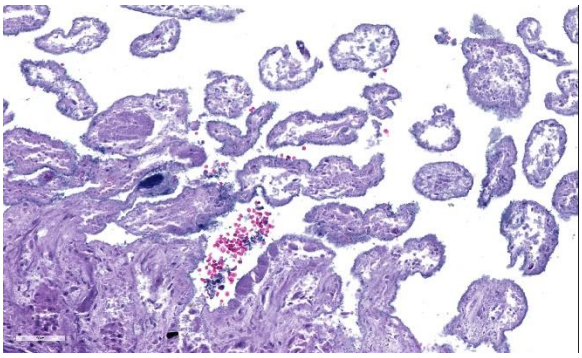
Intestine, foal: There is diffuse coagulative necrosis of the mucosa (top). There is a line of cellular debris immediately subjacent to the muscularis mucosae. The submucosa is multifocally diffusely expanded by edema, multifocal hemorrhage, and clear space (emphysema). (HE, 60X)

Contributor's Comment: *Clostridium difficile* is a gram-positive, anaerobic, spore-forming bacillus associated with diarrhea and (entero)colitis in humans and other mammals[3]. Colitis is most common in most species but the small intestine is affected in rabbits and foals[3]. In horses, *Clostridium difficile,* has been incriminated in hemorrhagic enteritis in foals and colitis in all ages and foals may be affected within the first few days of life[5]. The disease causes severe acute-to-peracute enterocolitis likely due to dysbacteriosis with clostridial overgrowth secondary to antibiotic therapy, stress, or dietary changes[3]. Although prior antibiotic treatment and/or hospitalization are significant predisposing factors in horses that develop *C.difficile*-associated disease (CDAD), disease may occur without prior treatment or hospitalization[1,2]. Foals < 7 days old without prior antibiotic therapy or hospitalization may frequently develop CDAD[2]. The primary virulence factors of *C. difficile* are toxin A (TcdA) and toxin B (TcdB) which act synergistically[3]. TcdA causes widespread damage to the mucosa (enterotoxic) allowing TcdB to affect epithelial cells (cytotoxic)[3]. TcdA enters the epithelial cell through endocytosis by coated pits and escapes into the cytoplasm by acidification of the endolysosome. In the cytoplasm, toxin inactivates Rho and other GTPase necessary for the regulation of cytosolic actin filaments leading to a loss of cell-to-cell contacts, increased paracellular permeability, and ultimately cell death[3]. TcdA and TcdB also initiate the inflammatory cascade including generation of reactive oxygen intermediates and elaboration of IL-8 and macrophage inhibitory protein 2 (MIP-2), both of which cause influx of neutrophils leading to host tissue damage[3]. TcdA also causes monocyte production of IL-1, IL-6, IL-8, and TNFα, and macrophage expression of COX-2 leading to release of prostaglandin E_2 which inhibits sodium chloride and water absorption in the intestine and enterocyte chloride secretion[3]. *Clostridium perfringens* is also an important cause of enteric disease but usually causes disease in more proximal segments of the gastrointestinal tract[3]. In foals, enteritis with extensive necrosis can occur in the small intestine due to both *C. difficile* and *C. perfringens*[3]. In this case the ELISA for *C. perfringens* toxin was negative.



Intestine, foal: Proprial vessels are often thrombosed; vessels in areas of necrosis have hyalinized walls, but this does not represent true vasculitis, but simply being in the wrong place at the wrong time. (HE, 275X)

This case demonstrates classic gross and histologic lesions of CDAD. Additional lesions, petechiation and ecchymosis, have been described in horses with CDAD and have been attributed to endotoxic shock and/or disseminated intravascular coagulation[1]. The most common histologic findings in foals with confirmed *C. difficile* infection are necrotizing or necro-hemorrhagic enteritis and submucosal thrombosis,[1] which were features of this case. Other infectious differentials for this type of enteritis in horses includes *C. perfringens* type C enterotoxemia, salmonellosis, or ehrlichial enteritis[1]. Culture and toxin assay should be interpreted together because culture sensitivity is below 100% and *C. difficile* can be isolated from a small number of healthy horses[1].



Intestine, foal: Close inspection of the HE section will disclose the presence of robust bacilli adherent to the necrotic remnants of the villi. (HE, 400X)

Actinobacillus equuli was isolated from the blood culture. *A. equuli* is a common cause of neonatal septicemia and death, with disease course ranging from abortion to early neonatal death to survival for several days with development of microabsesses in many organs[5]. *A. equuli* can occasionally be found as an opportunist in pathological tissue[5]. In this case there were fibrin thrombi identified in the kidney and edema of the choroid plexus that may have been caused by septicemia by *A. equuli*. The lesions in the intestine were most significant and the positive ELISA for *C. difficile* confirmed the diagnosis of CDAD. Mucosal damage due to *Clostridium* infection with subsequent *A. equuli* entry and sepsis is a likely sequence of events and has been suggested in a report of a foal with multiple concurrent diseases including CDAD and *A. equuli*[4].

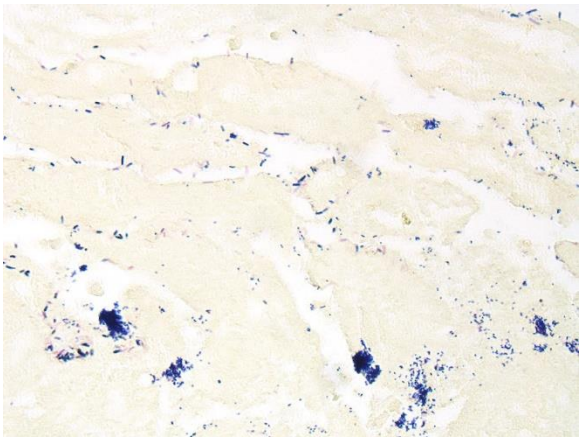
Contributing Institution:
Oregon State University College of Veterinary Medicine
Department of Biomedical Sciences and Veterinary Diagnostic Laboratory
http://vetmed.oregonstate.edu/diagnostic

JPC Diagnosis: Small intestine: Enteritis, necrotizing, diffuse, severe, with submucosal hemorrhage, edema and emphysema and numerous adherent robust bacilli.

Intestine, foal: Gram-positive bacilli are adherent to the necrotic remnants of the villi. (Brown-Hopps, 400X)

JPC Comment: The contributor provides an excellent review of the subcellular events associated with *C. difficile* infection in foals. In this species, both foals and horses are equally susceptible to infection, with antibiotic therapy and hospitalization being the main predisposing factors for the development of this disease in adult horses.[2] *C. difficile* has been the cause of disease in a wide variety of mammalian species. First identified from feces of clinically healthy human babies in the 1930s, the organism was originally named *Bacillus difficilis* because of the difficulties encountered in cultivating it. In humans, most infected people will remain symptomatic, with the remainder developing variable GI signs ranging from watery diarrhea to pseudomembranous colitis.[2] As in horses, hospitalization and antibiotic administration is a predisposing factor. Most *C. difficile*-positive infants, however, lack clinical signs, possibly due to lacking receptors for the toxin.

In humans, *C. difficile* associated disease (CDAD) was always assumed to affect individuals of any age, except during the neonatal period as it was thought that this specific group may lack specific *C. difficile* toxin receptors. Although between 25 and



70% of human neonates are colonized with *C. difficile*, these microorganisms have been largely considered part of the commensal microbiota. Recently, however, two 9 or 18 month-old children were diagnosed with CDAD, providing evidence that *C. difficile* is a potential cause of bloody diarrhea in neonates and young infants. In most animal species, CDAD is not age-dependent. The exception to this are pigs, which are almost exclusively affected during the neonatal period, up to approximately one week of age.[6]

C. difficile-associated disease (CDAD) affects a wide range of other mammals – while always resulting in enterocolitis, its manifestation varies widely with the affected species. Table 1 summarizes the severity of disease in various species by enteron segment.

Table 1. Summary of lesion distribution by species.[3]

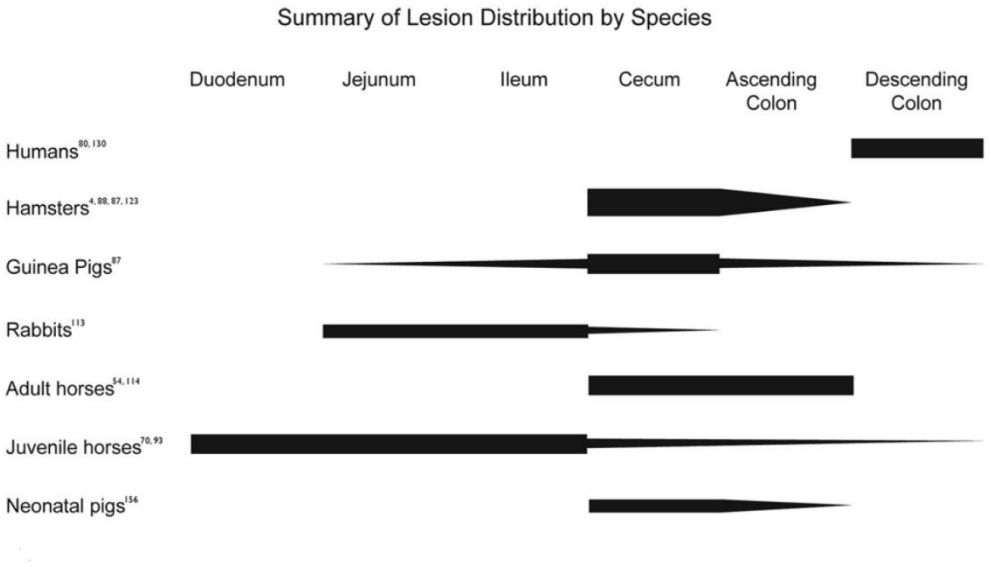


Fig. 1. Summary of the species-dependent distribution of intestinal lesions induced by *Clostridium difficile*. The thickness of the bars represents the severity of lesions typically seen in an infected individual of a given species at a particular site. The figure lists only those species for which the distribution of spontaneous lesions of *Clostridium difficile*–associated disease (CDAD) is well documented.

In rodents, CDAD is primarily cecal, resulting in ulcerative and rarely proliferative typhlitis and death. In pigs, the disease results in ulcerative typhitis or colitis with development of "volcano ulcers". An additional gross finding of mesocolonic edema (as well as diarrhea) makes CDAD a differential diagnosis for edema disease in swine.[2] The difference is that *C. difficile* infections occurs in young piglets (1-7 days of age), while edema disease is a disease of weanling age pigs. In rabbits, the lesion is primarily seen in the small intestine and concentrated in the ileum, often following antibiotic administration. It has also been reported sporadically in dogs, cats, ostriches, prairie dogs, and experimentally in non-human primates.[3]

Diagnosis of CDAD is usually based on a combination of clinical history, often with

previous antibiotic administration or hospitalization, characteristic microscopic lesions in appropriate segments of the enteron, and most importantly, ELISA testing of feces and/or intestinal for *C. difficile*-specific TcdA of TcdB toxins. Culture is useful because the carrier rate in horses is low, but care should be taken as: a) a few healthy horses (usually up to 5% although there is great variation in the literature) can be carriers of *C. difficile,* and b) occasionally non-toxigenic strains may be present in the intestine of horses, so any isolate should ideally be typed by PCR to confirm the presence of the genes encoding the major toxins of this microorganism. The latter is not routinely done in most veterinary diagnostic laboratories. Gross lesions, and to a lesser extent microscopic lesions are non-specific as to etiology, and in the horse, may resemble those of other diseases such as *C. perfringens* type C, salmonellosis, Potomac horse fever, and NSAID toxicity.[2]

In reviewing the slide, the moderator commented on the presence of numerous bacilli lining the necrotic villi as a relatively non-specific finding that should NOT be interpreted as evidence of clostridial infection, unless an appropriate immunohistochemical stain discloses that they are all of the same etiology. Many bacilli (and not just clostridia) will adhere to denuded mucosa. In addition, The moderator also pointed out that the presence of fibrin thrombi in the submucosa as evidence that the changes in the mucosa are truly antemortem and not simple autolysis.

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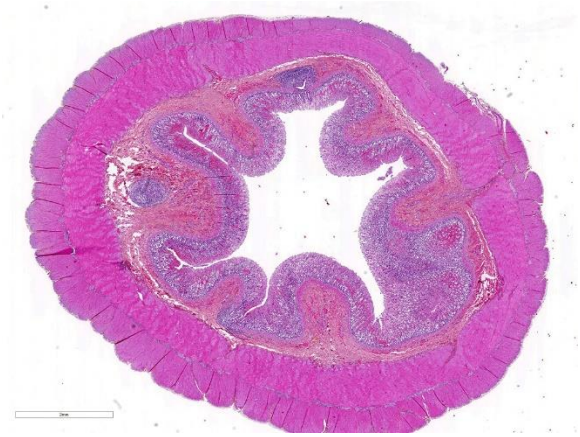
CASE II: H150787-83 (JPC 4085384-00).

Signalment: Dog (*Canis lupus familiaris*), intact female, 5-year-old, Chihuahua

History: The dog developed acute diarrhea and died suddenly. The dog was already dead at arrival to our teaching hospital and no further clinical investigations could be performed. The owner suspected intoxication and asked for a necropsy.



Colon, dog: A cross section of colon is submitted for evaluation. There is diffuse pallor (indicating necrosis) of the superficial mucosa and scattered hemorrhage. (H/E and Saffron, 6X).

Gross Pathology: This dog was in good body condition but showed marked dehydration. The stomach contained two plastic fragments and the mucosa was brown to green. A thick viscous content was in the small intestine associated with a diffuse and moderately congested mucosa.
The cecum, colon and rectum were moderately thickened with a severely and diffusely congested mucosa. The content was scant, hemorrhagic and thick.
A concentric hypertrophy of the left ventricle, a moderate distal tracheal collapse, a discrete mesenteric lymph node hyper-trophy and a mild meningeal congestion were also noted. All other organs and tissue were within normal gross limits.

Laboratory results: None given.

Microscopic Description: Colon: There is diffuse necrosis of the superficial mucosa, characterized by superficial mucosal hypereosinophilia with loss of cellular borders, pyknotic nuclei and loss of the mucosal epithelial lining. On the mucosal surface, admixed with necrotic debris, there are numerous 1-µm-long rod-shaped bacteria. The lamina propria and the submucosa present moderate to marked hyperemia and hemorrhage, and moderate infiltration by lymphocytes, plasma cells and degenerated neutrophils. There is multifocal thrombosis of small-sized blood vessels in the mucosa and submucosa.A Gram stain revealed numerous positive rods attached to the necrotic mucosal surface.

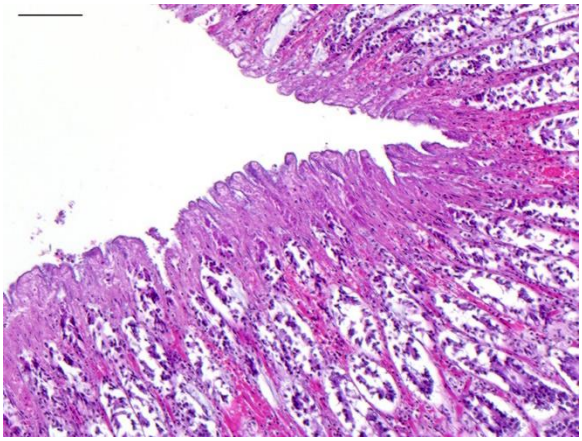
Contributor's Morphologic Diagnoses: Colon: Colitis, necrotizing and hemorrhagic, acute, diffuse, severe, with mucosal and submucosal vascular thrombosis and numerous gram-positive rod-shaped bacteria (consistent with *Clostridium sp.*) on to the mucosal surface

Name of the disease: Canine Hemorrhagic Gastroenteritis

Etiology: *Clostridium perfringens* type A (putative)

Contributor's Comment: Canine Hemorrhagic Gastroenteritis (CHG) is a sporadic, peracute, hemorrhagic gastro-enteritis of dogs. The etiology is not always identified, but *Clostridium perfringens* type A can been identified in some cases. A predilection for small breeds is described.[6]

Clostridium perfringens type A is responsible for mild and self-limiting diarrhea to fatal acute necrotizing and



Colon, dog. The mucosa shows superficial necrosis and hemorrhage. (H/E/Saffron, 200X) (Photo courtesy of: Unité d'Histologie, d'Embryologie et d'Anatomie pathologique, Département des Sciences Biologiques et Pharmaceutiques, Ecole Nationale Vétérinaire d'Alfort, FRANCE: www.vet-alfort.fr

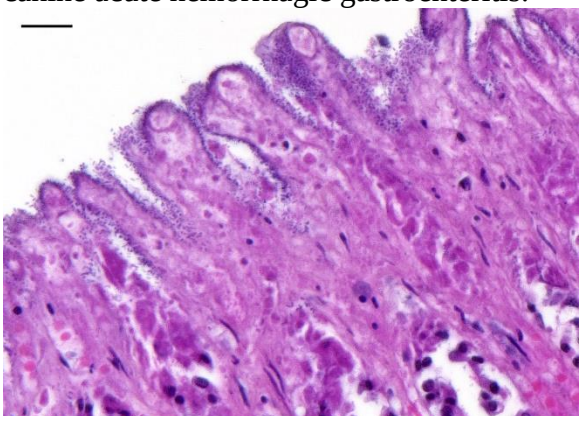
hemorrhagic enteritis in dogs and cats. It is also known to cause food poisoning in humans, necrotic enteritis in chickens, enterocolitis in horses and neonatal piglets, and enterotoxemia and hemorrhagic enteritis in lambs and calves.[9]

Clostridium perfringens is a gram-positive obligate anaerobic, spore-forming bacteria. It is part of the normal microbiota of human and animals, and a common enteric pathogen. It is separated into 5 types (A through E) based on the production of α, β, ε, and I toxins. Other important toxins, such as β2 and enterotoxin (CPE) may be produced by any of the five types, but the production of CPE is most commonly associated with type A strains.[11]

Clostridium perfringens type A major toxin is alpha toxin. Enterotoxin and β2 toxin may be produced by some strains. The bacteria require an anaerobic environment to sporulate and produce enterotoxin.[11]

CPE is a cytotoxic enterotoxin only elaborated during sporulation. It causes tissue damage and fluid secretion by binding to intestinal epithelial cells, forming pores in the plasma membrane that initiate cell death signaling pathway, and inducing structural damage to intercellular tight junctions resulting in increased epithelial permeability.[4] However, the pathogenesis remains poorly understood because CPE can be demonstrated in the feces without sporulation and in the feces of healthy dogs.[10]

In 2015, three novel putative toxin genes encoding proteins related to the pore-forming Leukocidin/Hemolysin Superfamily were identified, and named netE, netF, and netG. Only netF was associated with cytotoxicity. There was a highly significant association between the presence of netF with type A strains isolated from cases of canine acute hemorrhagic gastroenteritis.[4]

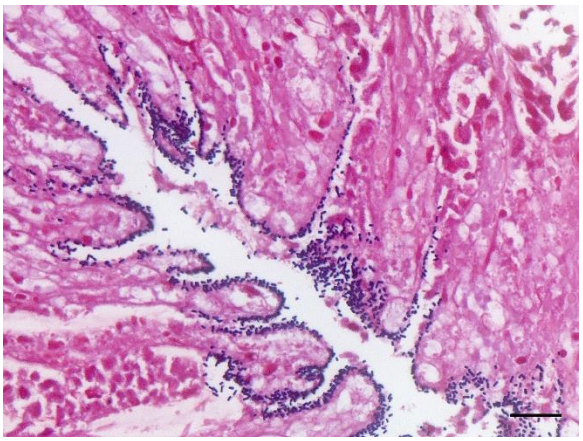


Colon, dog. Numerous rod-shaped bacteria are adherent to the mucosal surface. (H/E/Saffron, 1000X) (Photo courtesy of: Unité d'Histologie, d'Embryologie et d'Anatomie pathologique, Département des Sciences Biologiques et Pharmaceutiques, Ecole Nationale Vétérinaire d'Alfort, FRANCE: www.vet-alfort.fr

Three predisposing factors for CHG are described: enteric infection by another pathogen (such as canine parvovirus), antibiotic administration, and disruption of the normal microbiota (for example by a sudden change to a high protein diet). Dogs present with hemorrhagic diarrhea. In the acute form, dogs are often found dead lying in a pool of blood excreta. Gross lesions consist of necrotizing and hemorrhagic enterocolitis, and sometimes gastritis. Lesions in the colon tend to be more severe, as so well demonstrated in this case.

The histologic lesions consist of mural hemorrhagic necrosis of the gastrointestinal mucosa. Necrotic mucosal surface is lined by many gram-positive bacilli in association with fibrin and debris. The bacilli do not invade the lamina propria and can also be found within necrotic debris.[10]

Definitive diagnosis of CHG due to *Cl. perfringens* type A is not an easy task and cannot be based on macroscopic and microscopic lesions only. It requires combined testing for the CPE gene by PCR and for the *Cl. perfringens* enterotoxin by



Colon, dog. The bacteria are gram-positive. (Gram, 1000X)(Photo courtesy of: Unité d'Histologie, d'Embryologie et d'Anatomie pathologique, Département des Sciences Biologiques et Pharmaceutiques, Ecole Nationale Vétérinaire d'Alfort, FRANCE: www.vet-alfort.fr

fecal enzyme-linked immunosorbent assay, and exclusion of other potential enteropathogens.[11,13,16] In our case, such investigations could not be performed. However, the finding of an acute, fatal, necrotizing and hemorrhagic enterocolitis in a small-breed dog associated with numerous gram-positive bacilli is highly suggestive of CHG due to *Cl. perfringens* type A.

Contributing Institution:
Unité d'Histologie, d'Embryologie et d'Anatomie pathologique, Département des Sciences Biologiques et Pharmaceutiques, Ecole Nationale Vétérinaire d'Alfort, FRANCE : www.vet-alfort.fr

JPC Diagnosis: Colon: Colitis, necrohemorrhagic, diffuse, moderate, with thrombosis of submucosal vessels.

JPC Comment: The contributor provides an excellent review of this condition, which is well known to practitioners, but may be less well-known to veterinary pathologists. While the histologic lesion in this case is consistent with that seen in reported cases of acute canine hemorrhagic diarrhea syndrome (AHDS - a new and likely temporary name for this condition), the inability to either culture the pathogen or perform toxin identification is somewhat problematic in terms of a definitive identification of the etiologic agent. Mostly problematic is that the etiology remains unknown. *C. difficile* is identified in an equal or increased number of cases of hemorrhagic enteritis in dogs with similar predisposing factors to AHDS (previous antibiotic therapy, concurrent intestinal pathogens, and rapid dietary change.)[3] However, *C. difficile* is found in similar prevalence in healthy dogs and its role in enteric canine disease remains undetermined.[2]

Since the submission of this case to the WSC, a review of lesions in endoscopic biopsies obtained from dogs with acute hemorrhagic diarrhea syndrome (a name chosen to reflect the lack of gastric lesions in affected animals) has been published.[8] In endoscopic biopsies taken from 10 dogs with clinical AHDS, 9/10 demonstrate acute lesions of necrosis and neutrophilic infiltrate within the duodenum, 7/8 in the ileum, and 9/9 in the colon. Robust bacilli were identified in 5/6 duodenal samples, 5/8 ileal samples, and 7/9 colon samples.

C. *perfringens* type A was recently reported as a cause of necrotizing myositis in a German Shepherd Dog.[12] A large mass of crepitant necrotic muscle developed on the right hindlimb over a period of 72 hours which necessitated radical surgical excision, drainage, and prolonged antibiotics; the animal survived. Multiplex PCR identified the agent in initial aspirates as *Clostridium perfringens* type A with a cpA toxin.[12] *C. perfringens* type A is, beyond any doubt, responsible for human gas gangrene; alpha toxin is the main virulence factor of this type involved in gas gangrene.[1,14]

NetF is a novel toxin produced by some strains of *C. perfringens* type A. While preliminary evidence indicate that there seems to be a higher prevalence of NetF+ strains in dogs with AHDS than in healthy dogs[2,4], final proof of the role of this toxin in this or other enteric conditions is lacking.

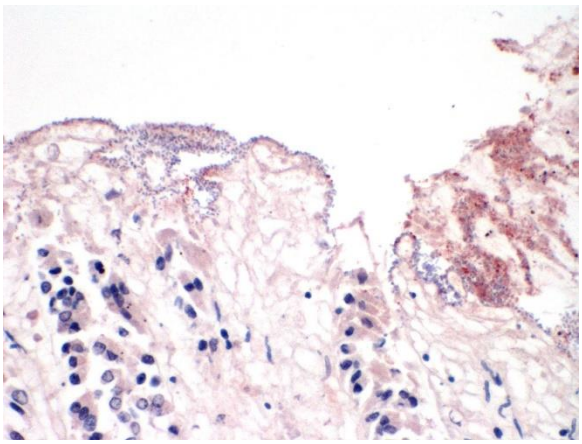
Upon review of the slide, the moderator discussed parameters which might be used in determining the presence of inflammation within the lamina propria versus a normal inflammatory population. While various criteria exist, but the separation of crypts and or lifting of crypts off of the muscularis mucosa by more than 3 layers of inflammatory cells suggests the presence of a true inflammatory infiltrate within the lamina propria.

Attendees discussed the possibility of parvoviral in this case based on morphologic finding, but the group consensus is that this differential diagnosis is not morphologically supported by the lack of profound crypt necrosis, necrosis of superficial mucosa and sparing of lymphoid tissue.

An immunohistochemical stain was performed for *Clostridium perfringens* type A at the San Bernardino lab of the California Animal Health and Food Safety system, and only a small percentage of the mucosal-adherent bacilli stained positively. The role of *C. perfringens* type A in enterocolitis and/or enterotoxemia of mammalian species is controversial and poorly supported by scientific evidence.[15] The fact that *C. perfringens* type A has been identified in some cases (most cases actually) does not support a causal role in the disease, as this microorganism may be isolated from a large percentage of normal animals as well. One possible exception to the relationship between *C. perfringens* type A is perhaps yellow lamb disease.[15] The contributor's assertion that combined testing for the CPE gene by PCR and for the *Cl. perfringens* enterotoxin by fecal enzyme-linked immunosorbent assay would be helpful in this diagnosis is problematic as well in light of recent developments in the field as the production of *C. perfringens* enterotoxin is now restricted only to *C. perfringens* type F in humans, a common cause of food poisoning which has not be identified in animals.[10]

The consensus diagnosis of the attendees in this particular case is that, without any other submitted diagnostics, the etiology of this particular case remains in question.

Colon, dog: In areas of necrosis, numerous robust bacilli are adherent to the devitalized mucosa. Numerous (but certainly not all) of these bacilli stain immunopositively for a stain for C. perfringens (which does not differentiate for toxinotype). (pan-C. perfringens, 400X)(Photo courtesy of: California Animal Health and Food Safety Laboratory, San Bernardino Branch.)



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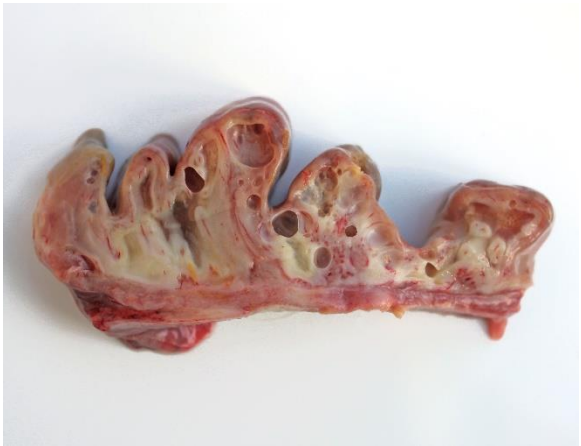
CASE III: H17-1982 (JPC 4105936-00).

Signalment: 16 day-old Holstein-Friesian male (*Bos taurus*).

History: The calf became acutely dull and lethargic, with subnormal body temperature, despite being placed under a heating lamp. The animal did not feed and died overnight.

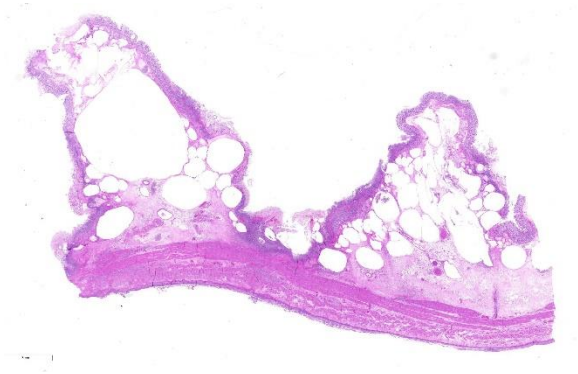
Gross Pathology: The calf was in reasonable body condition. Bilaterally, sunken eyeballs within sockets. Abundant serofibrinous fluid in abdominal cavity with fibrin adherent to a large (4 cm diameter) non-penetrating serosal tear along the greater curvature of the abomasum. Within the abomasum, the mucosa was diffusely congested, with two large (approximately 5 cm diameter) areas of ulceration. Diffusely, there was marked thickening, edema and emphysema of the abomasal folds.



Abomasum, calf. The submucosa is markedly expanded by edema, hemorrhage, and emphysema with multiple areas of mucosal ulceration. (Photo courtesy of: UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland, http://www.ucd.ie/vetmed/)

Laboratory results:
Abomasal tissue ***Clostridium sordellii*** FAT – negative
Abomasal tissue ***Clostridium septicum*** FAT – negative

Microscopic Description: Abomasum: There are marked multifocal areas of degeneration, necrosis and loss of the glandular mucosa and gastric pits. Necrotic areas contain amorphous eosinophilic cellular debris, basophilic karyorrhectic debris and are infiltrated by large numbers of viable and degenerate neutrophils and some macrophages. Occasionally both within and overlying necrotic areas there are



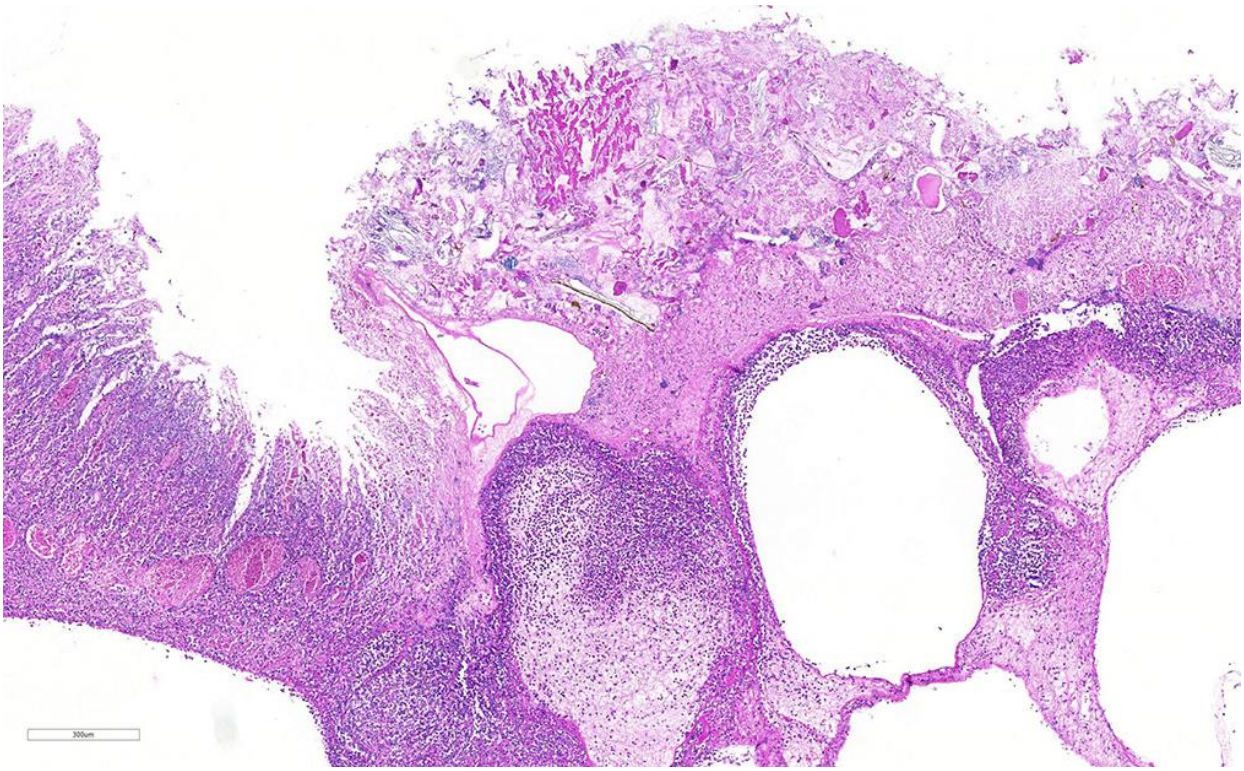
Abomasum, calf. Subgross examination details the extent of the emphysema within the submucosa and areas of mucosal necrosis. (HE, 5X)

tetrad packets of basophilic cuboidal 2-3 um bacteria (*Sarcina spp*.). Diffusely, the abomasal leaves are thickened with severe submucosal expansion by large (up to 6mm) multifocal-coalescing clear spaces (emphysema), abundant finely fibrillar eosinophilic mesh (fibrinous exudate) and dilated lymphatics. There is marked submucosal infiltration of numerous neutrophils. The neutrophilic infiltrate and fibrinous oedema obscures the muscularis mucosa and extends through the submucosa, muscularis externa and serosa. The external serosa is covered by a thick mat of fibrin and neutrophils.

Contributor's Morphologic Diagnoses: Abomasum: Severe, acute, diffuse, necrosuppurative and emphysematous abomasitis with *Sarcina* spp.

Etiology: *Sarcina spp.* (putative)

Contributor's Comment: The association of *Sarcina spp.* with fatal emphysematous abomasitis, abomasal bloat and abomasal ulceration in both young calves and lambs has been reported.[2,3] The current case demonstrates a number of typical features; submucosal emphysematous bullae, fibrinous exudate, neutrophilic infiltrates, foci of mucosal necrosis and the characteristic colonies of *Sarcina spp*. bacteria[2,5]. *Sarcina spp*. are gram-positive anaerobic cocci, are carbohydrate fermenters and can grow in an acid environment (as low as pH 1.0). They have a characteristic microscopic morphology: basophilic, cuboidal bacteria with flattened walls occurring in tetrad packets. They are not invasive and are typically found along the mucosal surface.[1] The main products of *Sarcina* carbohydrate fermentation are ethanol, acetaldehyde, carbon dioxide and hydrogen. In human gastritis associated with *Sarcina ventriculi*, it is postulated that the mucosal damage is the result of alcohol production, as the lesions are reminiscent of acute alcohol poisoning[1]. In animals it is postulated that production of large volumes of carbon dioxide induce abomasal bloat and emphysema[2] with the distension in turn inducing ulceration, edema and haemorrhage[5]. *Sarcina spp*. are present in the soil and environment. When ingested by



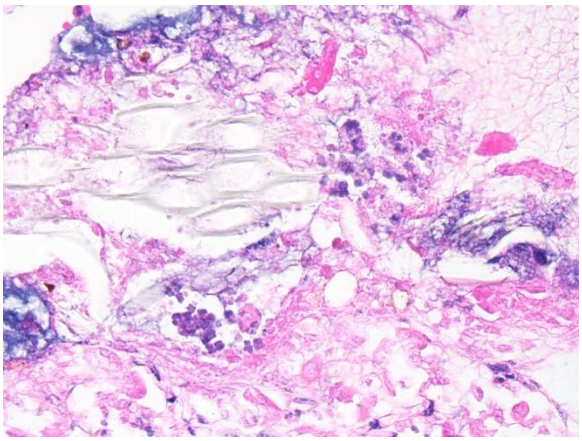
Abomasum, calf. Area of full-thickness mucosal necrosis overlying an emphysematous submucosa infiltrated by large numbers of neutrophils enmeshed in edema and polymerized fibrin. (HE, 69X)

pre-ruminant calves the milk diet provides a rich fermentative substrate, and the low pH environment favoured by the bacteria, enabling them to outgrow the commensal abomasal flora and cause gas accumulation. Although *Sarcina spp.* are difficult to culture under routine conditions, their histological features are quite characteristic/pathognomonic.

Contributing Institution:
UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
http://www.ucd.ie/vetmed/

JPC Diagnosis: Abomasum: Abomasitis, necrotizing and neutrophilic, transmural, diffuse, severe, with marked submucosal emphysema and edema, and numerous bacterial tetrads and colonies of bacilli.

JPC Comment: *Sarcina ventriculi* is an interesting presumptive pathogen of young ruminants, and occasionally in humans. Since its original association with abomasal bloat in goat kids and lambs in the 1990s, it has been incriminated as a cause of various syndromes of abomasal bloat, tympany, and necrotizing abomasitis. It is a relatively fastidious anaerobe and notoriously difficult to culture, rendering inoculation studies difficult at best, and the additional observation that it may be found in the stomachs of healthy as well as diseased animals has led to great consternation for pathologist trying to add it to the established pathogens *Clostridium septicum* and *Clostridum perfringens type A* in the development of braxy or braxy-like syndromes in young ruminants. The concurrent identification of various clostridial species, notably *C. sordelli*, in affected animals has further clouded the diagnostic picture[5]; hence, the role of this microorganism in abomasitis of calves has,



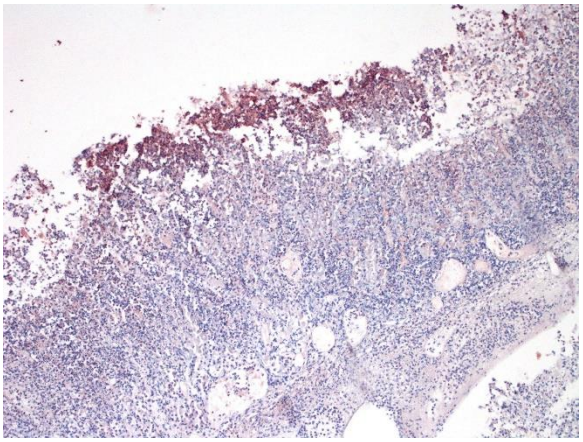
Abomasum, calf: In areas of necrosis, bacterial tetrads consistent with S. ventriculi are present among cellular debris and colonies of bacilli. (HE, 200X).

however, not been definitely demonstrated and it is possible that *Sarcina* is part of a multifactorial etiology.[3,4]

To complicate the etiology of the lesion in this individual even more, several rods identified in the lumen of this abomasum were immunopositive for *C. perfringens* IHC at the San Bernardino laboratory of the California Animal Health and Food Safety System.

In humans, *S.* ventriculi was first identified as a gastric pathogen by Dr. John Goodsir in 1842, while only 19 cases of gastritis due to *S. ventriculi* infection have been confirmed since, with more than half of these cases occurring in patients with a history of previous gastric surgery resulting in outflow impairment. The term "sarcinous vomiting" referring to a characteristic "obstinate, frothy" vomit has been described in these patients as well as others with chronic gastritis.[1]

Sarcina is a genus of three species of gram-positive coccobacteria in the family Clostridiaceae which are known commensals of human skin and GI tract, and synthesize microbial cellulose. It derives its



Abomasum, calf: In areas of necrosis, numerous robust bacilli are adherent to the devitalized mucosa. Numerous (but certainly not all) of these bacilli stain immunopositively for a stain for C. perfingens (which does not differentiate for toxinotype). (pan-C. perfringens, 400X)(Photo courtesy of: California Animal Health and Food Safety Laboratory, San Bernardino Branch.)

name from "sarcina" from the Latin meaning a pack or bundle (similar to that worn by Roman legionaries). The bacteria grow in two planes, resulting in characteristic tetrads, with cell wall compression against adjacent bacilli giving them a cuboidal appearance. (A similar tetrad appearance may also be seen with the much smaller *Micrococcus*.) Interestingly, they are purportedly refractile (not demonstrated in this slide), mimicking plant material. [1]

Sarcina sp. are naturally present in the soil and is likely ingested with feedstuffs. *Sarcina* sp. possess an exclusive carbohydrate fermentative metabolism, producing ethanol, acetaldehyde, carbon dioxide and hydrogen. Theories of pathogenesis include the production of large amounts of carbon dioxide resulting in bloat and emphysema, with production of ethanol and acetaldehyde resulting in mucosal necrosis. Abomasal tympany may additionally result in secondary damage to the overstretched mucosa.[2]

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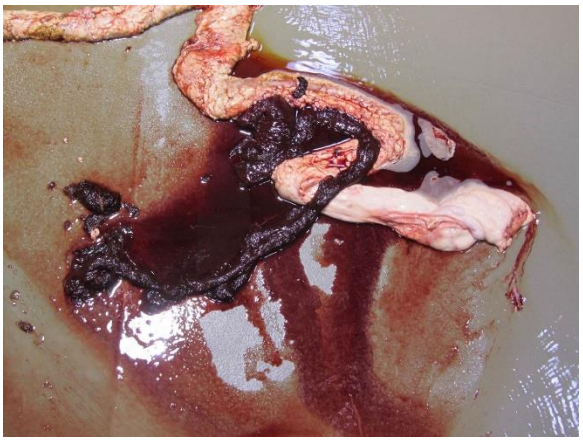
CASE IV: 13101599 (JPC 4048573-00).

Signalment: 13-month-old, intact male, Angus, *Bos taurus*, bovine

History: Two, 13-month-old, Angus bulls from the same farm presented for acute, voluminous, red, watery diarrhea. The diarrhea was noticed by the owner while animals were being gathered into the barn for a routine testing procedure. Both bulls



Colon, ox. There was extensive blood and a large clot within the lumen. (Photo courtesy of: Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, www.cvm.okstate.edu)

were in excellent body condition (body condition score of 5/9) and one of the two was said to be the owners fastest grower. Both bulls were routinely vaccinated and dewormed. One of the bulls died while in transit to Oklahoma State University and a necropsy was performed at the Oklahoma Animal Disease Diagnostic Laboratory. Multiple diagnostic tests were performed on the other bull and the only significant finding on rectal examination was frank blood within the colon and a CBC measuring a PCV of 19%. This bull fully recovered with symptomatic care.

Gross Pathology: On gross examination, the bull was in excellent body condition. Approximately 7cm proximal to the spiral colon, there was an abrupt change in the intestinal content from normal to watery dark red fluid containing blood clots. This continued throughout the remainder of the spiral and descending colon. Segmentally (30 cm), the distal colonic mucosa had a mild cobblestone appearance with innumerable pinpoint to coalescing petechiae and ecchymosis and approximately four 0.5 x 0.2 cm, elliptical, shallow mucosal ulcers. Otherwise, the colonic mucosa outside of this segment was within normal limits.

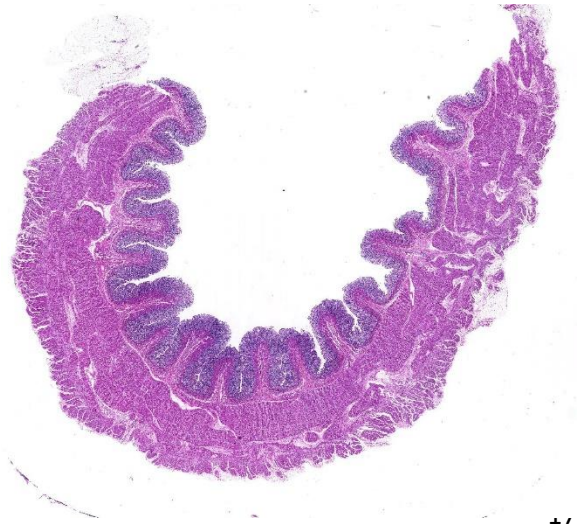
Colon, ox. A section of colon is presented for examination – there are no obvious lesions at subgross examination. (HE, 5X)

Laboratory results:
Fluorescent antibody (FA) staining for coronaviral antigens was conspicuously positive in the colonic mucosal sections. Immunostains (IHC) were also strongly positive (13101599 BCoV IHC). Positive and negative controls for both FA and IHC stains behaved as expected.

Bacterial culture of distal colon, spiral colon, and feces yielded large numbers of growth of *E. coli* and *Streptococcus bovis*. A *Clostridium perfringens* Type A was also isolated, but was determined opportunistic with little contributory role to death.

Fecal floatation was negative for parasites, including coccidia.

Microscopic Description: Histologically, colonic sections exhibited multifocal loss of colonic glands with scattered remnant glands present in an uneven distribution. The remnant glandular epithelium was either



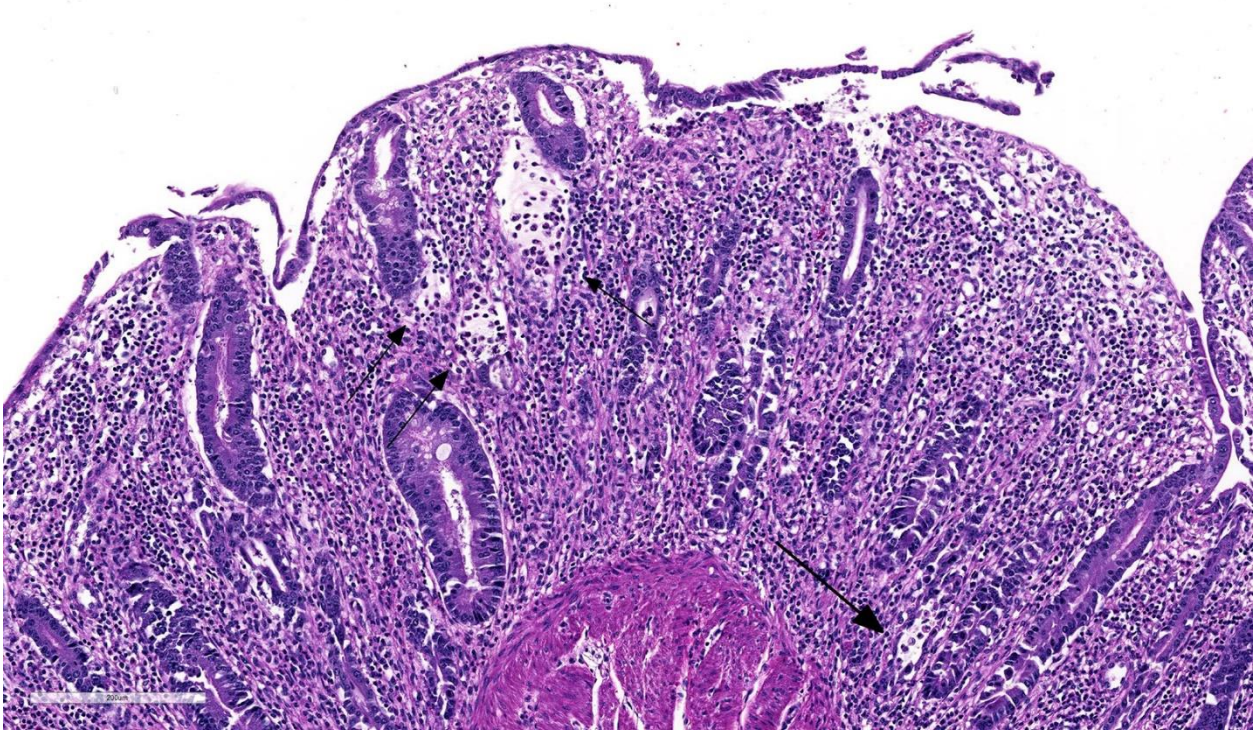
hyperplastic or was exhibiting features of degeneration and necrosis with occasional sloughing of necrotic epithelial cells into the glandular lumen. The hyperplastic epithelium exhibited re-epithelialization of expansive sections of the surface mucosa. In most regions, goblet cells were absent. The lamina propria had a moderate infiltrate of lymphocytes and plasma cells. Multifocally, hemorrhage was present within the lamina propria.

Contributor's Morphologic Diagnoses: Large intestine, distal colon: Ulcerative colitis, lymphoplasmacytic and hemorrhagic with multifocal colonic epithelial necrosis and colonic gland collapse, severe

Contributor's Comment: Coronavirus has been implemented in causing three diseases in cattle: neonatal diarrhea in calves (between 1 day and 30 months old, with diarrhea most common at 1 to 2 weeks of age), respiratory disease in a wide age range of calves (between 2 and 19 weeks of age), and winter dysentery, which affects adult dairy and beef cattle.[1]

Genus *Coronavirus* is found in the family Coronaviridae within the genus *Torovirus*. They have a single-stranded, positive sense, RNA genome, are pleomorphic and vary in size from 70 to 200 nm in diameter.[2] They contain four major structural proteins: the nucleocapsid protein N, the integral membrane glycoprotein M, the spike glycoprotein S, and the hemagglutinin-esterase glycoprotein HE. N protein is associated with the genome to form the nucleocapsid. M glycoproteins are found within the virus envelope. S and HE glycoprotein receptors are used in attachment of the virus to the target cell membrane.[1]

The exact cause of winter dysentery has not yet been determined; however mounting scientific evidence strongly suggests the etiologic agent to be coronavirus. Winter dysentery has been described in cattle worldwide, and in the United States, is more common in the northern states.[1,3] Typically, this disease occurs in cattle during the cooler winter months and is associated with multiple environmental risk factors, including low drop in atmospheric temperature, close confinement, poor ventilation, and using manure handling



Colon, ox. There is marked necrosis and loss of glands with numerous crypt abscesses (arrows).

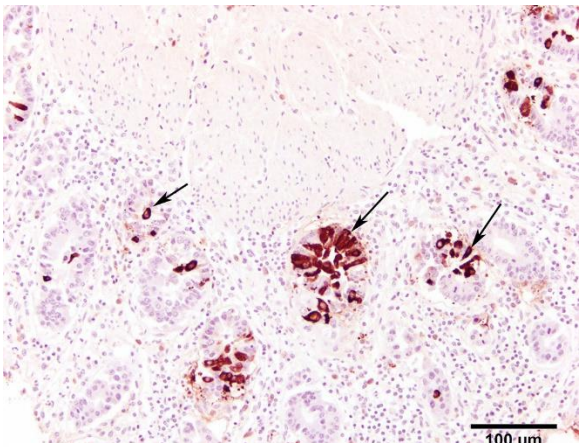
equipment to handle feed.[3] Viral transmission is by respiratory or oral routes.

Winter dysentery is a sporadic, acute, contagious, hemorrhagic enterocolitis that predominately affects adult dairy cows, and infrequently adult beef cattle. Clinical signs include dark brown, hemorrhagic, watery, and commonly profuse diarrhea, often with concurrent anorexia, depression, and dehydration. The disease typically has a high morbidity (50-100%) and low mortality (1-2%). Significant economic losses are associated with this disease, especially regarding milk drops in lactating dairy cows.[3] The length of acute disease is brief, often with spontaneous recovery within a few days. Persistent diarrhea can lead to dehydration, with secondary polydipsia and reduced ruminal motility. Rarely, hemorrhage from the large colon leads to acute, severe anemia and death[4], as seen in this case.

Differential diagnoses for adult cattle with acute, profuse, watery, diarrhea include BVDV, coccidiosis, and salmonellosis. Diagnosis of winter dysentery requires physical examination and exclusion of the above causes of acute and contagious diarrhea in cattle. Diagnostic tests include PCR, virus isolation, or IHC on ear notch to rule out BVDV, fecal floatation to rule out coccidiosis, and fecal bacterial culture to rule out salmonellosis. At necropsy, spiral colon is the ideal sample for virus detection because the virus persists for the longest time here after oral infection.[3] Immunohistochemistry was used in this case to detect BCoV antigen in positively staining crypts within the colonic mucosal epithelium.
Coronavirus has a tropism for respiratory and intestinal epithelium.[4] Coronavirus implicated in winter dysentery causes lesions comparable to neonatal calves with coronaviral diarrhea. In winter dysentery, epithelial cells of colonic crypts are destroyed by virus, leading to degeneration and necrosis of crypt epithelium. Sloughing of damaged and necrotic epithelium can be seen grossly as massive quantities of frank, often clotted, blood within the spiral and distal colon. Viral particles leave the cell by exocytosis at the apical or lateral cell surface, or by lysis of the cell.[2,3] As in this case, marked mucosal atrophy and re-epithelialization can also be seen. In addition, fine streaks of hemorrhage were noted along the edges of the mucosal folds in the distal colon. [2,3]

The diarrhea that occurs in winter dysentery is a malabsorptive diarrhea and death in this case was most likely due to severe, acute anemia from profound colonic hemorrhages.

Contributing Institution:
Department of Veterinary Pathobiology
Center for Veterinary Health Sciences



Colon, ox. Immunohistochemistry for bovine coronavirus showing variably intense positive staining for epithelial intracytoplasmic viral antigen (arrows). BCV, HRP method, counterstained with hematoxylin. Bar = 100µm. (Photo courtesy of: Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, www.cvm.okstate.edu)

Oklahoma State University
www.cvm.okstate.edu

JPC Diagnosis: Colon: Colitis, necroulcerative and hemorrhagic, diffuse, severe with marked loss of glands and multifocal crypt abscessation.

JPC Comment: The contributors provide an excellent review of this case, and subsequent to its submission for the Wednesday Slide Conference, published this is a case report including three additional cases, from which further details are now publicly available. In 2012-2013, four calves from two herds died of acute necro-hemorrhagic colitis. Colonic tissue from all calves were positive by fluorescent antibody and IHC for BoCV. The virus was isolated, and the genomic information from a variable region of the Spike gene revealed BoCV clade 2 in all cases, which had been previously seen as a respiratory infection in postweaned beef calves. (To date, 4 separate clades of BoCV have been isolated.)

Coronaviruses are well known as pathogens of both the digestive system (dog, swine, mice, rats) as well as the respiratory system (humans, cattle, rats). In the early 2000s, SARS-CoVs was discovered in civet cats, raccoon dogs, and bats) that were associated with both respiratory and enteric infections, and were subsequently identified in humans, camelids, and a wide variety of species. BoCV in itself is a pneumoenteric virus infecting both the upper and lower respiratory tract in respiratory infections.

BoCV is ubiquitous worldwide based on antibody seroprevalence data In a study of 2311 calves with diarrhea in Tulare, CA, 30.5% were positive for BCV; the age range of affected animals was 1-30 days with an average of 10.4 days.[1] BoCV is also commonly incriminated as one of the inciting agents in the bovine respiratory disease complex in feedlot cattle with some studies identifying the virus through the use of RT-PCR testing in up to 96% of feedlot cattle. A number of wild species may serve as wildlife reservoirs for BoCV, including white-tailed deer, Sambar deer, elk, waterbuck, and giraffes; isolates from each of these species share amino acid sequences with over 99% homology to BoCV. Dogs have also been implicated as a potential reservoir for a coronavirus which may potentially infect ruminants.

In 2-6 month old calves, respiratory infections are associated with coughing, rhinitis, and pneumonia, which may or may not be accompanied by enteric signs; fecal shedding of the virus is commonly documented. It has been proposed by researchers following the time course of pneumoenteric infections that following clinical upper respiratory infection, large amounts of the virus, coated in mucus, may be swallowed, facilitating infection of the intestinal epithelium. A number of intra-nasal modified live vaccines designed primarily to protect against coronavirus-associated "calf scours" have been released on the market in the last five years.

The moderator gave the following differentials – BoCV, BRotaV, Coccidiosis (gross appearance, but too old an animal), and salmonellosis. The moderator commented on the age of the affected (13 months) in this case, which is consistent with "winter dysentery" (which is another misnomer as it may occur in hot months or in tropical climates which do not see a cold season as well.)[7]

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