Clostridial Abomasitis and Enteritis in Ruminants

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KEYWORDS

- *Clostridium perfringens* • *Clostridium difficile* • Abomasitis • Enteritis
- Enterotoxemia • Ruminant

KEY POINTS

- Clostridial abomasitis and enteritis are characterized by necrosis of the abomasal or intestinal mucosa caused by exotoxins produced by *Clostridium perfringens* or *Clostridium difficile* in the lumen of the gastrointestinal tract.
- *C perfringens* types A, B, C, D, and E can cause enteric disease in all species of domestic ruminants. The 5 genotypes are identified by the presence of the genes for the lethal exotoxins alpha, beta, epsilon, and iota.
- Multiplex polymerase chain reaction is used diagnostically to identify *C perfringens* genotypes from anaerobic culture of samples.
- Proliferation of *C perfringens* in the ruminant gastrointestinal tract is associated with a combination of increased availability of carbohydrate or protein, and alteration in gastrointestinal motility.
- Treatment of abomasitis and enteritis caused by *C perfringens* should focus on 6 goals: relief of abdominal distention, systemic fluid support, prevention of *C perfringens* proliferation, decreasing or preventing exotoxin production, restoration of normal gastrointestinal flora, and providing pain management as needed.

INTRODUCTION

Clostridial diseases affecting the gastrointestinal tract are common in ruminant livestock; however, their classification can be confusing because of the varied nomenclature of the disease conditions.[1–4] Described diseases have included hemorrhagic enterocolitis, enterotoxemia, pulpy kidney disease, overeating disease, braxy, bradsot, struck, lamb dysentery, enterotoxemic jaundice, yellow lamb disease, clostridial

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abomasitis, and clostridial enteritis. Other than braxy (bradsot) of sheep, which is caused by *Clostridium septicum*, these diseases are all caused by different subtypes of *Clostridium perfringens*. *C perfringens* type A can also cause gangrenous mastitis and may be present in high numbers in spoiled milk.

Clinical disease is associated with rapid bacterial overgrowth within the gastrointestinal tract and subsequent exotoxin release. Although limited tissue invasion by *C perfringens* does occur, most local and systemic lesions result from the effects of potent exotoxins produced by certain genotypes of these bacteria. *C perfringens* is a large, gram-positive, anaerobic bacillus that exists ubiquitously in the environment and in the gastrointestinal tract of most mammals.[3,5–8] There are 5 defined types, or genotypes, of *C perfringens*: A, B, C, D, and E (**Table 1**). These genotypes are identified based on the lethal toxins that they produce: *C perfringens* alpha (CPA), *C perfringens* beta (CPB), epsilon (ETX), and *C perfringens* iota (CPI).[7–10] The alpha toxin gene (*plc*) is present on the chromosome of all *C perfringens* isolates.[7,8] All genotypes produce alpha toxin, although isolates differ significantly in the amount of alpha toxin produced.[11,12] The other lethal toxins, CPB (*cpb* gene), ETX (*etx* gene), and CPI (*iap/ibp* genes) are contained on transferrable plasmids.[7,8]

Two other toxins, enterotoxin (*C perfringens* enterotoxin [CPE], *cpe* gene) and the beta-2 toxin (CPB2, *cpb2* gene), are also carried on transferable plasmids in livestock isolates. Enterotoxin can be expressed by any of the subtypes if the plasmid containing this gene (*cpe*) is present, but it is not required for pathogenicity. Enterotoxin is not released by vegetative bacteria but only in sporulating *C perfringens* cells during lysis of the vegetative cell.[7] Thus, the toxin may not be present in intestinal contents of animals with *C perfringens* enteritis unless sporulation is occurring. The beta-2 toxin may be produced by type A as well as by some isolates of types B, C, and E.[13] Strains of *C perfringens* that carry the beta-2 toxin gene have been isolated from a variety of species of domestic animals, including horses, camelids, cattle, and swine.[13,14]

The genotype, and thus the specific subtype of *C perfringens*, can be determined by a multiplex polymerase chain reaction (mPCR) that detects the specific toxin genes carried by an individual isolate. Because the clinical and pathologic presentations of diseases caused by *C perfringens* types are not always distinct, anaerobic culture and polymerase chain reaction (PCR) genotyping of the isolates can be instrumental in determining the subtype involved and can help identify specific control measures. Each of the subtypes of *C perfringens* is associated with specific disease syndromes that are directly or indirectly related to the toxins they produce. The term enterotoxemia is often loosely used to describe enteric or systemic disease caused by any of the *C perfringens* toxinotypes. The term enterotoxemia refers to systemic disease caused by absorption of a toxin from the intestine. Clostridial abomasitis and enteritis does not require absorption of the toxins. The term enterotoxemia is best reserved for cases in

Table 1
Toxins, gene, genetic location, and toxin action expressed by different *Clostridium perfringens* toxinotypes (genotypes)

Toxin	*C perfringens* Type					Gene	Gene Location	Toxin Action
	A	B	C	D	E			
Alpha (CPA)	+	+	+	+	+	*plc*	Chromosome	Phospholipase
Beta (CPB)	—	+	+	—	—	*cpb*	Plasmid	Pore formation
Epsilon (ETX)	—	+	—	+	—	*etx*	Plasmid	Pore formation
Iota (CPI)	—	—	—	—	+	*Iap/ibp*	Plasmid	Cytoskeleton disruption

which systemic disease is associated with absorption of bacterial exotoxins, including hemolytic diseases in lambs with *C perfringens* type A, and systemic disorders caused by the epsilon toxin of *C perfringens* types B and D. Some of the disease conditions described for *C perfringens* are listed in **Table 2**.

Table 2		
Diseases caused by different subtypes of *Clostridium perfringens*		
Organism	**Disease**	**Notes**
C perfringens type A	• Enterotoxemic jaundice (lambs) • Yellow lamb disease • Rare in cattle • Abomasitis, enteritis (calves)[15] • Enteritis (swine)[16]	• Nutritional factors may predispose to bacterial overgrowth • Depression, anemia, icterus, hemoglobinuria • Hemolysis caused by action of alpha toxin • Lambs die within 6–12 h • Abdominal tympany • Abomasal ulceration • Mucosal necrosis and hemorrhage • Dietary factors, milk quality, and storage may predispose to disease • Found in both diseased and normal pigs
C perfringens type B	• Lamb dysentery • Enterotoxemia (calves, foals)	• Rare in North America; more common in United Kingdom and Europe • Lambs up to 3 wk of age • Calves up to 10 d of age • Hemorrhagic enterocolitis with bloody diarrhea
C perfringens type C	• Hemorrhagic enterocolitis (calves, lambs, foals, swine) • Struck (adult sheep)	• Calves up to 10 d of age • Trypsin inhibitors in colostrum/milk or other feeds (soy, sweet potatoes) may increase susceptibility by blocking degradation of beta toxin by trypsin • Hemorrhagic enterocolitis with bloody diarrhea • Immunize dam to provide lactogenic immunity to calf during critical first 10 d of life, when trypsin inhibitors are present in colostrum and milk • Acute fatal enterotoxemia of adult sheep • Organism in soil • Early spring • Colic, rigidity, sudden death • Rarely, diarrhea is observed • Abomasitis and enteritis
C perfringens type D	• Enterotoxemia • Pulpy kidney disease • Overeating disease	• Systemic distribution of epsilon toxin causes widespread endothelial damage and results in cerebral edema • Hyperglycemia and glucosuria occur caused by mobilization of hepatic glycogen • Primarily seen in lambs, less common in goat kids, and rare in calves • Lambs and goat kids 3–10 wk of age with heavy milking dams • Calves 1–4 mo of age • Predisposed by dietary conditions that promote high carbohydrate or protein bypass to the duodenum and secondary factors causing ileus • Vaccination of dams provides protective colostral antibodies in the neonates that last up to 8 mo
C perfringens type E	• Necrotic hemorrhagic enteritis (goats, calves)	• Outbreaks reported in adult goats • Otherwise rare in North America

Recent studies have suggested an association of hemorrhagic bowel syndrome (HBS) in cattle with *C perfringens* type A.[17–20] Although *C perfringens* is isolated from the intestinal tract and from lesions of animals with HBS, a causal link has not been established. It is possible that the heavy growth of *C perfringens* from animals with HBS is caused by an appropriate environment for growth that is associated with gastrointestinal (GI) hemorrhage and stasis. Studies trying to induce HBS by inoculation with *C perfringens* type A have not been able to reproduce the disease.[21]

CLOSTRIDIUM PERFRINGENS EXOTOXINS

The *C perfringens* exotoxins are all polypeptides that generally act by phospholipase damage to the plasma membrane or by forming pores or channels in the plasma membrane of host cells.[8] This process results in cellular membrane disruption and cell leakage of electrolytes or water. The alpha toxin gene is expressed from the chromosome of all *C perfringens* subtypes.[7,8] The genes encoding the other toxins are carried on large plasmids.[7,8] The enterotoxin gene can be either chromosomal or plasmid determined. In animal isolates, it is generally carried on a plasmid. However, it is chromosomally located on most (~70%) isolates from human cases of *C perfringens* food poisoning.[22] All of the toxins except CPE are secreted by vegetative bacteria. Enterotoxin is released after sporulation and bacterial cell lysis.[7,8,22]

Alpha Toxin

Alpha toxin is produced by all subtypes of *C perfringens*; however, the amount of toxin secreted varies by both isolate and type.[7] The alpha toxin gene (*plc*) is located on the bacterial chromosome. Alpha toxin is a phospholipase C sphingomyelinase (lecithinase) enzyme that binds to cell membranes and causes disruption of the lipid bilayer.[7,8,23] The toxin is hemolytic and dermonecrotic. Hydrolysis of lecithin by alpha-toxin produces diacylglycerol, which leads to stimulation of eukaryotic cell phospholipases and the arachidonic acid cascade.[8,23] This process can result in alterations in vascular permeability, platelet aggregation, and vasoconstriction. Alpha toxin has been shown to cause alterations in fluid transport within ileal and colonic loops in sheep.[24] Alpha toxin likely acts in concert with additional virulence factors, resulting in intestinal mucosal damage and, in particular, the *C perfringens* toxin perfringolysin (theta toxin) seems to work synergistically with alpha toxin in calves.[25,26]

Beta Toxin

Beta toxin is produced by type B and C strains of *C perfringens*. The beta toxin gene (*cpb*) is encoded on a large plasmid and codes for a small polypeptide protoxin. The protoxin is secreted from the vegetative bacteria and cleaved by proteases to produce the active toxin.[7] Beta toxin is classified as a pore-forming cytolysin, and is similar to other toxins, including *Staphylococcus aureus* alpha toxin. The channels formed by the beta toxin pores are selective for monovalent cations such as sodium and potassium.[27] Beta toxin is rapidly destroyed by trypsin.[3,7] Because of this, disease caused by types B and C tend to occur in young neonates less than 10 to 21 days of age because of the presence of trypsin inhibitors in colostrum and early milk. Disease may also occur later if bacterial or plant-derived trypsin inhibitors are present in the feed.

Epsilon Toxin

The epsilon toxin gene (*etx*) is carried by type B and D strains of *C perfringens*. The gene is encoded on a large plasmid.[7,8] The gene encodes a small polypeptide protoxin

that is cleaved to the active toxin by intestinal proteases, including trypsin and chymotrypsin.[28,29] *C perfringens* epsilon toxin is the third most potent bacterial toxin following botulinum toxin and tetanus toxins.[28] The toxin is lethal and dermonecrotic. Studies in lambs, goats, and calves show increased vascular permeability associated with exposure to epsilon toxin.[30–34] The toxin binds the plasma membrane of sensitive cells, including endothelial cells, and forms a complex with a particular membrane protein, resulting in altered membrane permeability by an unknown mechanism,[8,28,29] and this is the primary extent of disease observed in the intestine caused by epsilon toxin.

C perfringens epsilon toxin also results in systemic disorders in ruminant species (enterotoxemia). Toxin-induced damage to the intestinal mucosa allows absorption and systemic dissemination of the toxin. Disruption of endothelial cells causes vascular lesions and edema observed in the heart, lungs, and brain, resulting in pericardial effusion, pleural effusion, pulmonary edema, and perivascular cerebral edema.[28] Endothelial cell damage in the brain disrupts the blood-brain barrier, providing the toxin access to cerebral neurons and parenchymal cells. Epsilon toxin causes damage and necrosis to neurons, astrocytes, and oligodendrocytes, producing the characteristic lesion of focal symmetric encephalomalacia.[29] Pore formation disrupts transmembrane K^+, Cl^-, Na^+, and Ca^{++} transport in affected cells. There is also an overexpression of the membrane channel protein aquaporin-4, resulting in a disruption of intracellular water balance. Sublethal intracerebroventricular injections of epsilon toxin into mice and rats produced neuronal degeneration in the cerebral cortex, hippocampus, striatum, and hypothalamus, resulting in permanent behavioral changes.[35] Dexamethasone was able to reduce the effects of epsilon toxin in these studies.

Iota Toxin

The iota toxin is a binary cytotoxin consisting of 2 subunits: Ia and Ib.[7,8,36] The genes coding for the toxin (iap and iab) are located on an extrachromosomal plasmid. Both subunits are required for toxicity. The Ib subunit constitutes the binding component and the Ia subunit is the enzymatic component. The Ib precursor protein is cleaved by trypsin and binds to the cell membrane, forming a heptameric complex. Ia subunits bind to this complex and enter the cell by receptor-mediated endocytosis. The Ib heptamer forms a pore within the endosome, allowing translocation of the Ia subunits to the cytoplasm. The Ia subunit has ADP-ribosylation activity resulting in the depolymerization of actin and inhibition of cell functions that are dependent on actin.

Enterotoxin

C perfringens enterotoxin is a primary toxin associated with food poisoning in humans, but its relationship to enteric disease in animals is not fully established. The CPE gene (*cpe*) can be located either on the bacterial chromosome or on an extrachromosomal plasmid.[7–9,22] The *cpe* gene is chromosomally determined in *C perfringens* isolates that cause food poisoning in humans. However, the *cpe* gene is plasmid determined in isolates from animals. The *cpe* gene is carried by less than 6% of *C perfringens* isolates. The enterotoxin gene is most commonly found in type A isolates but is also present in some type C, D, and E isolates.[22] Although the gene is expressed in A, C, and D isolates, it is silent in the type E isolates that have been identified. Enterotoxin is only expressed during the process of bacterial sporulation and then released when the vegetative *C perfringens* cells undergo lysis.[7,22] Expression is regulated at the transcriptional level. The toxin binds claudins in the cell membrane of enterocytes forming a large transmembrane pore complex that allows for calcium influx and cell death by

either apoptosis or necrosis.[22] The CPE-claudin complex also causes destabilization of intercellular tight junctions and leakage between cells.

Beta-2 Toxin

The beta-2 toxin gene (cpb2) is located on an extrachromosomal plasmid and can be found in all subtypes of *C perfringens*. Although genetically distinct, the beta-2 toxin shows cytotoxic activity similar to that of *C perfringens* beta toxin (CPB; also designated beta-1 toxin).[8,37] The significance of the beta-2 toxin as related to disease caused by *C perfringens* has not been fully determined.[14,37–39] The toxin is likely a cofactor in the pathogenesis of abomasitis and enteritis when present but is not required for intestinal mucosal necrosis.

Novel Pore-forming Toxins

NetF toxin, a pore-forming toxin produced by a subset of *C perfringens* type A strains and related to the leukocidin/hemolysin superfamily, was recently associated with necrotizing enteritis in neonatal foals and with canine hemorrhagic gastroenteritis.[40,41] Similarly, NetB toxin (necrotic enteritis toxin B type) produced by some strains of *C perfringens* type A has been shown to be essential in causing necrotic enteritis in chickens.[42] These toxins form pores that result in disruption of the phospholipid membrane bilayer of cells and cause an ion influx that can lead to osmotic cell lysis.[43] Although it was previously thought that alpha toxin was the major toxin responsible for avian necrotic enteritis, Keyburn and colleagues[44] showed that alpha toxin null mutants were still virulent in an avian necrotic enteritis disease model. These novel toxins have not yet been shown to play a role in clostridial enteric diseases in ruminants.[45,46]

CLINICAL DISEASE

This article focuses on abomasitis and enteritis associated with *C perfringens* with clarification of the distinctions from true enterotoxemia. Enterotoxemia is a loosely used term for disease caused by *C perfringens* and is often used in reference to all clinical forms of *C perfringens*, including abomasitis and enteritis observed in cattle and other ruminants. This loose terminology can cause confusion when discussing the disease because it does not designate the specific genotype and clinical manifestations of *C perfringens* syndromes seen in cattle and other ruminants. The term enterotoxemia is best reserved for *C perfringens* diseases that include absorption and systemic spread of toxins with disorders in other organs beyond the intestinal tract. Clinical forms of the wide array of *C perfringens* disease syndromes are reviewed in **Table 2**.

Clostridial Abomasitis

All genotypes of *C perfringens* can cause abomasitis or enteritis in some or all domestic ruminant species. *C perfringens* type A is an increasingly common isolate associated with clostridial abomasitis in ruminants.[4,15,30,45,47–49] Type E has also been isolated in cases of abomasitis but seems to be rare.[47,49,50] Clostridial abomasitis has been reproduced experimentally by intraruminal inoculation of *C perfringens* type A in calves.[51] Clostridial abomasitis may be present by itself or combined with enteritis.

Type A abomasitis is most commonly observed in neonatal and juvenile animals but can also be observed in adult ruminants. *C perfringens* abomasitis is a sporadic disorder of neonatal to weanling calves, lambs, and kids.[15,45,47,49,52] The condition is common in both dairy and beef calves and seems to be less common in lambs and

kid goats. Clinical signs include decreased nursing, lethargy, fluid distension of the abomasum, abdominal tympany, colic, and bruxism.[49] Animals generally have a normal temperature unless complications of systemic sepsis or peritonitis have developed, in which case their temperature can be decreased or increased. Mucous membranes may be pale with a prolonged capillary refill time indicating systemic shock. Hydration status is often normal in acute cases but dehydration can develop in animals that are not treated soon. Abdominal distension with a succussion splash is a predominant and consistent clinical sign (**Fig. 1**). The ability of *C perfringens* to produce gas contributes to gastric dilatation and intramural emphysema evident in affected animals. Abomasal tympany and the associated abdominal distension also causes respiratory difficulty because of pressure placed on the diaphragm. Abomasal distension can also result in hemodynamic effects that can lead to shock. The case fatality rate of clostridial abomasitis seems to be high when rapid treatment is not instituted (75%–100%).[15,45,47–49,51]

At necropsy, the abomasum is grossly distended and filled with hemorrhagic fluid and gas (**Fig. 2**A). Gross pathology is characterized by diffuse, hemorrhagic to necrotizing inflammation of the abomasal mucosa, frequently involving the deeper layers of the abomasal wall in severe or chronic cases (**Fig.** 2B). Abomasal ulceration (**Fig.** 2C) and perforation with peritonitis (**Fig.** 2D) may occur in a subset of affected animals. Intramural emphysema and edema of the abomasal wall may be present.

Other differential causes of abomasitis and abomasal tympany in ruminants include *Sarcina* spp,[47,53–55] *Salmonella*,[56–58] coccidiosis,[59] fungal infection,[60] immunosuppression, pica, trauma from coarse feed, trichobezoars, gastric ulcers, and

Fig. 1. A 3-week-old calf with abdominal distension caused by *C perfringens* type A abomasitis and enteritis.
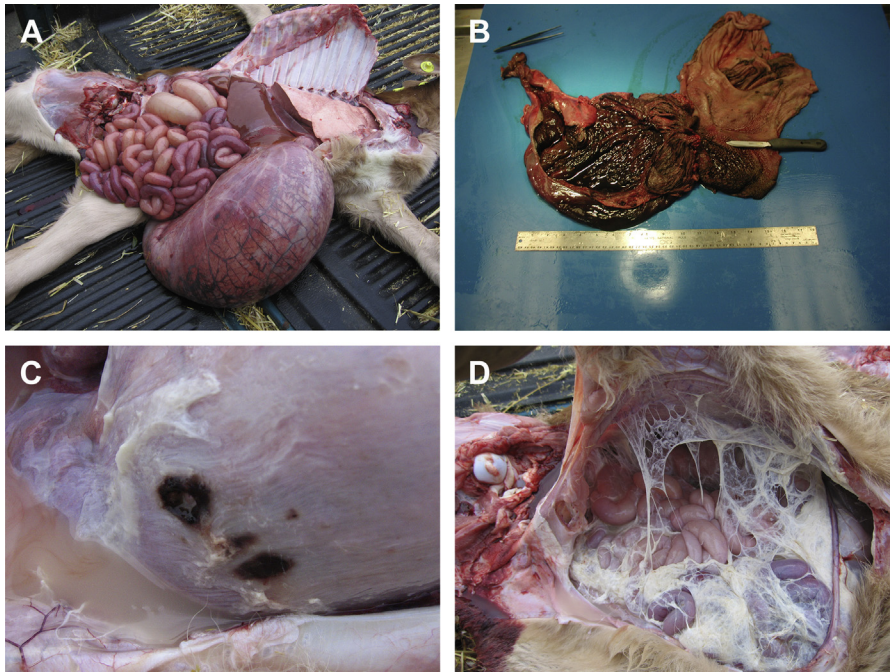
Fig. 2. Necropsy findings that may be observed in neonates with clostridial abomasitis. Common findings include grossly distended abomasum containing fluid and gas (*A*); diffuse, hemorrhagic to necrotizing inflammation of the abomasal mucosa (*B*); abomasal ulceration (*C*) with or without perforation resulting in fibrinous peritonitis (*D*).

vitamin or mineral deficiencies.[61] *Sarcina* spp are implicated as a cause of abomasitis and abomasal tympany in calves, sheep, and goats.[47,53–55] Abomasal coccidiosis is described in an 11-month-old female sheep with proliferative abomasitis.[59] *Salmonella typhimurium* DT104 was isolated from the abomasal wall of Midwestern veal calves with abomasitis.[56,57] *S typhimurium* was also isolated from an outbreak in ewes and lambs in which the primary clinical finding was abomasitis.[58] Copper deficiency has been associated with abomasitis and abomasal ulcers in beef calves.[61–63] However, Roeder and colleagues[51] showed that abomasitis can occur spontaneously and be induced experimentally in the absence of copper deficiency. Thus, although copper deficiency may act as a contributory factor for abomasitis and enteric disease of calves, it does not seem to be a requisite factor for either condition.

Clostridial Enteritis

Clostridial enteritis (inflammation of the small intestine) may be seen in concert with abomasitis or on its own. The condition is most commonly observed in young animals but may also occur in adult ruminants. Clostridial enteritis can be caused by all toxinotypes of *C perfringens*, although there are important differences. *C perfringens* type A is the most common cause of clostridial enteritis currently observed in ruminants in the United States, which may in part be caused by the routine use of clostridial C and D toxoids in ruminants in the United States resulting in antibodies to

beta and epsilon toxins and providing effective protection against enteritis caused by *C perfringens* types B, C, and D. It may also be caused by geographic differences in the environmental and host distribution of toxinotypes B, C, D, and E.

Type A

Enteritis caused by *C perfringens* type A is observed in calves, lambs, and kid goats.[1,3,10,25,30,47,50,64,65] Enteritis is generally acute and develops over a period of hours. Appetite is significantly decreased. In most cases, diarrhea is not present in the acute stages. The animals generally have a normal temperature and may not show physical or clinicopathologic signs of sepsis or systemic inflammation unless the disease has progressed and generalized sepsis is present. At necropsy, the small intestine is hemorrhagic and distended with hemorrhagic contents (**Fig. 3**). The intestinal wall may be thickened with edema or, if intestinal distension is severe, may appear thin. Ultrasonography examination of affected patients often shows local or generalized areas of distended small intestine. The motility pattern of the affected segments is decreased, or seems to have a lack of coordinated progressive peristalsis. Gross pathology is characterized by segmental to diffuse, hemorrhagic or necrotizing inflammation of small intestinal mucosa. In some cases, mucosal damage can also affect the cecum or spiral colon.

  C perfringens type A is also associated with nonobstructive hemorrhagic enteritis in adult ruminants, particularly cattle. This condition in adult ruminants is poorly described in the literature. Type A hemorrhagic enteritis is less common in adults than in young animals. Clinical signs include acute onset of decreased appetite with right side or bilateral ventral abdominal distension (**Fig. 4**A). The animals may have a fever or be afebrile. Inflammatory changes to the complete blood count (CBC) are often absent but may be present because of translocation of enteric Gram-negative bacteria and secondary septicemia. Hemorrhagic diarrhea may not be apparent at the time of presentation (**Fig. 4**B) but may be apparent later in the course of the disease (**Fig. 4**D). Abdominal ultrasonography often reveals multiple loops of fluid-distended small intestine up to 5 cm in diameter (**Fig. 4**C). Intestinal motility is often decreased or lacks coordinated peristalsis. Scant feces may be noted upon rectal examination in early cases. The clinical signs are often difficult to discriminate from intestinal obstruction caused by intussusception, entrapment, or obstructing HBS. *C perfringens* type A can be cultured from affected small intestine and these
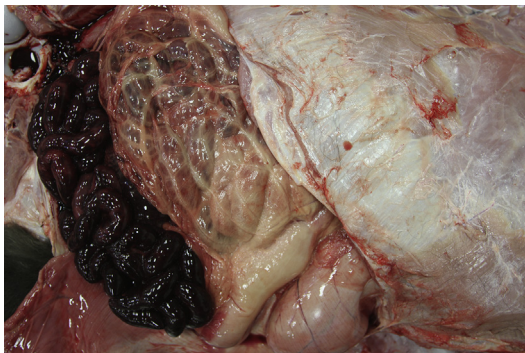
Fig. 3. Necropsy of a 3-day-old beef calf with *C perfringens* type A hemorrhagic enteritis. The cause was confirmed with anaerobic culture and mPCR genotyping.
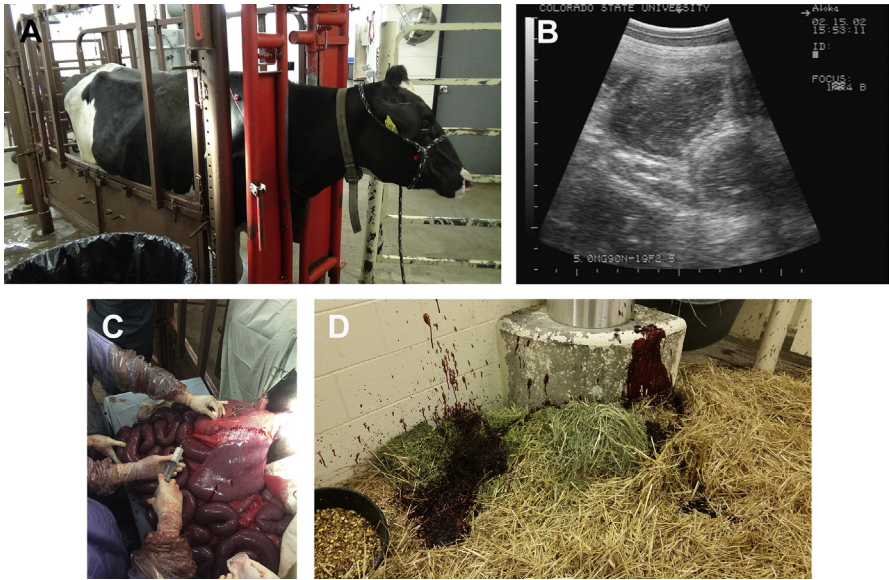
Fig. 4. Hemorrhagic *C perfringens* enteritis in an adult dairy cow. Note the right ventral abdominal distension caused by distension of the small intestine (*A*). Transabdominal ultrasonography shows dilated loops of small intestine up to 5 cm in diameter (*B*). A right flank exploratory laparotomy was performed and confirmed diffuse hemorrhagic enteritis (*C*). The cow received intraluminal procaine penicillin G in surgery. The following morning the cow had profuse hemorrhagic diarrhea (*D*), returned to eating, and recovered.

animals often respond to treatment directed toward intraluminal clostridial infection. At this time, it is unclear whether this is an enteritis condition distinct from HBS or part of a continuum of enteric disease caused by *C perfringens* type A that includes HBS.

Type A enterotoxemia of sheep (yellow lamb disease) is an apparently rare but highly fatal disorder that manifests as an acute hemolytic disease. Hemolysis is thought to be caused by either alpha toxin or perfringolysin (theta toxin) following absorption into the circulation secondary to damaged intestinal mucosa.

Type B
C perfringens type B causes acute hemorrhagic enterocolitis in neonatal ruminants. Type B enteritis is uncommon in North America but more common in the United Kingdom and Europe. In lambs, the condition is called lamb dysentery. Type B enteritis is also observed in calves and foals. The main contributing toxin is the beta toxin. The disease is restricted to young animals; generally, lambs less than 21 days of age and calves less than 10 days. The main factor responsible for this age restriction is the susceptibility of the beta toxin to destruction by trypsin (discussed further for *C perfringens* type C). Infection results in a severe acute hemorrhagic enterocolitis with high mortality similar to type C. Type C isolates also express epsilon toxin and can cause clinical signs of enterotoxemia because of systemic absorption and the action of the epsilon toxin.

Type C
Hemorrhagic enterocolitis caused by *C perfringens* type C is a commonly fatal disease that occurs in calves and lambs and is suspected to occur on rare occasions in

goats.[5,65,66] The hallmark of this disease is acute profuse hemorrhagic diarrhea. Neonates are most commonly affected, although disease losses in older calves and lambs can be significant. Intake of large quantities of soluble carbohydrate and/or protein is considered a risk factor for the development of type C enterotoxemia.[5,65] Neonates nursing heavily lactating dams seem to be at higher risk. Heavy grain feeding, foraging on grain crops, sudden access to high-quality forage, or overfeeding following a period of hunger are also considered risks.

The beta toxin is thought to be responsible for the acute necrohemorrhagic enterocolitis. The disease is generally restricted to neonatal ruminants because of the susceptibility of the beta toxin to proteolytic destruction by trypsin.[3,7] Beta toxin is active in young neonates because of the presence of trypsin inhibitors in colostrum and early postpartum milk. Trypsin inhibitors are present in milk for up to the first 10 to 21 days after parturition and place neonates that nurse postpartum dams at risk. The risk is less for neonates fed pooled milk, including milk from later lactation animals or milk replacer. Disease can occur in older ruminants if bacterial or plant-derived trypsin inhibitors are present in the feed.

Affected animals are acutely listless and reluctant to nurse. Ataxia, colic, bloody diarrhea, depression, and recumbency soon follow. Extensor rigidity and opisthotonus may be seen terminally, and death usually occurs within hours of the onset of signs. Severe hemorrhagic enterocolitis is the primary gross lesion at necropsy. This lesion tends to be most pronounced and is consistently found in the distal jejunum and ileum, although occasionally the entire small intestine or colon is involved.[5,67] Fibrin clots, casts of necrotic mucosa, and red-brown blood may be present within the intestinal lumen. An increased amount of clear, straw-colored, or serosanguinous fluid may be found within the peritoneal, pleural, and pericardial cavities.

Type D

Enteritis is not the predominant clinical feature of enteric infection with *C perfringens* type D. Clinical enteritis is rare in calves and lambs. Goats more consistently show clinical signs of GI dysfunction with type D infection, including gross and histologic lesions of enteritis.[33,68–71] In the peracute form of this disease, affected goats may be found dead or may have colic. Abdominal distension, vocalizing, dyspnea, tachypnea, and watery diarrhea containing fibrin, mucus, or strands of blood may occur. Recumbency, respiratory distress, and convulsions associated with true enterotoxemia usually follow, and death typically occurs within hours of the onset of signs.

C perfringens type D is responsible for true enterotoxemia in small ruminants of all ages.[5,65] Type D enterotoxemia in calves is rare.[72] Enteritis is not a consistent clinical finding with enterotoxemia except in goats. *C perfringens* type D is not considered to be a common inhabitant of the GI tract of normal ruminants, although it can be carried sporadically by healthy animals.[65] As with type C enterotoxemia, passage of soluble carbohydrates or protein into the small intestine is thought to induce rapid replication and elaboration of epsilon toxin from this organism.[66] Unlike beta toxin, however, epsilon toxin is activated by intestinal and pancreatic proteases.[5,7,8] Once absorbed into the bloodstream, epsilon toxin causes loss of endothelial integrity, increased capillary permeability, and edema formation in multiple tissues.[73] Epsilon toxin also causes damage and necrosis to neurons, astrocytes, and oligodendrocytes, producing the characteristic lesion of focal symmetric encephalomalacia and is responsible for the neurologic signs observed with this disease.[29]

Type D enterotoxemia in sheep is typically a peracute illness, with many cases simply being found dead. If a live ovine case is detected, neurologic signs predominate.

Lethargy and ataxia are evident early, with collapse, hyperesthesia, lateral recumbency, convulsive paddling, and opisthotonus following within hours. Diarrhea is inconsistently seen. Glucosuria is frequently present in sheep.[74]

At necropsy examination, the effusion is typically observed in the peritoneal, pleural, or pericardial spaces with variable volumes of straw-colored or red fluid that may contain fibrin clots. Petechial hemorrhages are often visible on the visceral surfaces. Pulmonary and mesenteric edema may be evident. Gross lesions of the intestinal tract are frequently absent in affected sheep. Dipstick analysis of urine collected from the bladder frequently reveals the presence of glucose in sheep. The renal cortex may be softened (hence the term pulpy kidney), although this is a nonspecific autolytic change seen on occasion in small ruminant cadavers. The classic pathologic lesion is focal symmetric encephalomalacia. Occasionally, no gross lesions are seen in ovine cases of type D enterotoxemia.[66]

Unlike sheep, goats affected by type D enterotoxemia more consistently show signs of GI dysfunction, and gross and histologic lesions are more consistently found in the GI tract.[33,68–71] In the peracute form of this disease, affected goats may be found dead or may have colic. Abdominal distension, vocalizing, dyspnea, tachypnea, and watery diarrhea containing fibrin, mucus, or strands of blood may occur. Recumbency, respiratory distress, and convulsions usually follow, and death typically occurs within hours of the onset of signs. Glucosuria is not consistently detected in goats with enterotoxemia.[69]

The clinical signs of the acute form of type D enterotoxemia in goats are similar to those of the peracute form, but the progression of the disease occurs over 2 to 4 days. Intermittent or protracted diarrhea, weight loss, and reduced milk production are evident in the chronic form of enterotoxemia in goats. In this form of the disease, clinical signs may persist for several days or occur intermittently over weeks or months. Chronic enterotoxemia may be difficult to diagnose unless prior peracute or acute cases are known to have occurred in the herd. GI parasitism, salmonellosis, Johne disease, and rumen acidosis are important differential diagnoses.

The most prominent gross postmortem lesion in goats with peracute or acute type D enterotoxemia is fibrinohemorrhagic colitis, which is usually most severe in the spiral colon.[33] Luminal casts of fibrin, blood, and mucus may be present, and a pseudomembrane may form in affected colonic segments. The colonic serosa may be hyperemic or edematous, with edema evident in the colonic mesentery and mesenteric lymph nodes. Pulmonary edema, fluid, and fibrin in the thoracic and abdominal cavities and pericardium, and scattered ecchymotic hemorrhages on serosal surfaces may be present. Occasionally caprine cases of peracute type D enterotoxemia show no gross lesions. Chronic cases may show scant body fat reserves and ulcerated colonic mucosa. Glucosuria is inconsistently found.[69]

Type E

Enteritis caused by *C perfringens* type E is rare but has been reported in goats, calves, and adult cattle.[50,75,76] Clinical signs range from mild diarrhea to severe hemorrhagic enteritis. Type E isolates are not considered to be a common cause of abomasitis or enteritis in cattle. However, their presence makes appropriate diagnostic differentiation important when evaluating cases of clostridial abomasitis or enteritis in ruminants.

PREDISPOSING FACTORS

The underlying factors that allow overgrowth and toxin expression of *C perfringens* in ruminants are considered multifactorial. Three key components seem to be involved in clostridial abomasitis and enteritis:

1. The presence of *C perfringens* within the GI tract
2. Sufficient carbohydrate or protein nutrients to support bacterial growth
3. Decreased GI motility that allows segmental overgrowth of the bacteria within the GI tract

C perfringens type A is ubiquitous in the livestock environment and is considered normal flora at low levels in the healthy ruminant.[7,8,45,47,77] Fecal shedding maintains persistence in the environment. Other toxinotypes are not found as consistently in the environment or from fecal samples of ruminants.

All forms of enteric disease caused by *C perfringens* seem to be associated with increased intake of feeds rich in soluble carbohydrate, protein, or both. Heavy grain feeding, foraging on grain crops, sudden access to high-quality forage, or overfeeding following a period of hunger are also considered risks. Consumption of large volumes of milk at individual feedings also seems to increase risk in nursing ruminants.

Clostridial abomasitis and enteritis in neonatal beef calves is associated with management practices that cause delays in regular nursing patterns (eg, calf separation at branding) or changes in environment that interrupt normal nursing patterns (eg, winter storms).[62] In dairy calves, poor milk hygiene, intermittent feeding of large volumes of milk, and milk replacers with higher carbohydrate or protein concentrations are thought to be contributory factors.

There are many factors that can decrease intestinal motility. Abomasal emptying in nursing calves can be delayed by feeding large volumes in single feedings, feeding with an esophageal tube, high caloric content, and high osmolality of the milk.[78] Enteric pathogens can alter intestinal motility, including rotavirus, coronavirus, cryptosporidia, and coccidia. Cases of *C perfringens* enteritis have been associated with concurrent coccidiosis in cattle and New World camelids.[79–81] Other factors, such as abomasal ulcers, coarse feed, foreign bodies, hairballs, and mineral deficiencies, may be associated with abomasal tympany and increase the risk of *C perfringens* overgrowth in the abomasum.[61]

CLINICAL PATHOLOGY

Because the pathogenesis of the disease is primarily an enterotoxemia, signs of systemic inflammation are often not observed. A CBC may show a neutrophilia without a left shift and normal fibrinogen, suggestive of a physiologic response or a stress response. An inflammatory leukogram is rarely seen unless there is sufficient mucosal compromise to allow for Gram-negative bacterial translocation and/or systemic endotoxin absorption. Most parameters on the serum chemistry are normal. The creatinine and/or blood urea nitrogen level may be increased if systemic shock has resulted in decreased renal perfusion. Sheep and sometimes goats with type D enterotoxemia may be hyperglycemic and glucosuric. Total protein level is usually not increased because systemic dehydration is often not present in peracute or acute cases. The serum chloride may be decreased because of intestinal ileus and delayed abomasal emptying, and metabolic alkalosis may be present in early cases. Metabolic acidosis and increased lactate levels are common in later stages of the disease.

DIAGNOSIS

Definitive diagnosis of *C perfringens* abomasitis or enteritis has challenges and must rely on clinical signs, gross and microscopic pathology, and appropriate microbiological tests to show the presence of the organism.[10] mPCR is currently used to categorize this diverse species into distinct types, or genotypes. Genotyping is

based on detection of gene sequences for alpha, beta, beta-2, epsilon, and iota toxins and enterotoxin.[13,14,82,83] The presence of the gene for a particular toxin reflects the potential to produce that toxin. It is critical to note that expression of major lethal toxins is not consistent across clinical isolates within a particular genotype. Thus, the potential pathogenicity of isolates within each genotype is suspected to be variable.

Interpretation of positive culture results for *C perfringens* from the intestinal lumen of a ruminant is a complicated matter. *C perfringens* type A inhabits the intestine of normal animals and can overgrow in the gut lumen postmortem.[5,65] Thus, its isolation should be considered significant only from a fresh cadaver with compatible history, clinical signs, and lesions. The organism is easily grown in vitro; *C perfringens* type A grows rapidly in anaerobic culture and may overgrow other potential pathogens.[65] Although quantitative culture is considered helpful in discriminating normal flora from overgrowth, a recent study in veal calves showed no difference in bacterial counts between normal veal calves and veal calves with clostridial enteritis.[84] Diagnosis of clostridial abomasitis or enteritis therefore requires a combination of observations that may include:

- Observation of abdominal distension with colic.
- The presence of hemorrhagic abomasitis or enteritis (see **Figs. 2**B and **3**). Abomasitis or enteritis may additionally be suggested by ultrasonographic findings.
- Cytologic evidence of high numbers of gram-positive rods in abomasal or intestinal contents, feces, or on the mucosal surface of tissues (**Fig. 5**).
- Culture of *C perfringens* in high numbers from abomasal or intestinal contents, feces, or tissue samples.
- Demonstration of the presence of alpha, beta, or epsilon toxin by mouse neutralization test or enzyme-linked immunosorbent assay (ELISA). However, toxin assays for the lethal toxins are not readily available from commercial diagnostic laboratories in the United States. Toxin detection test kits are available from BioX in Belgium (http://www.biox.com).
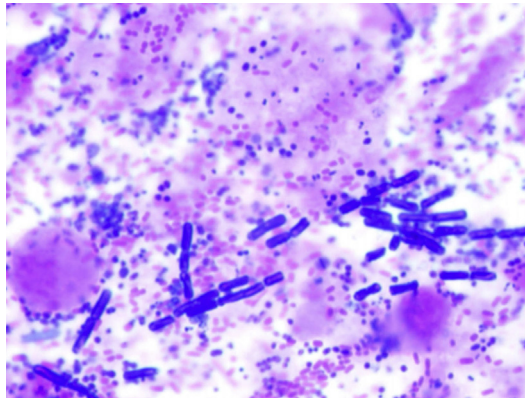
Fig. 5. Gram stain of feces from a calf with clostridial enteritis (original magnification, ×1000). Note the group of large Gram positive rods within the background of other bacterial flora. Cytologic evidence of high numbers of large Gram-positive rods in ingesta or feces is suggestive of, but not definitive for, clostridial abomasitis and/or enteritis. The presence of white blood cells, red blood cells, and sloughed mucosal epithelium indicates inflammation with mucosal necrosis and hemorrhage. In early cases of abomasitis or enteritis, changes in fecal flora may not initially be observed.

Recommended samples for diagnostic testing include[2,10]:

- Abomasal or intestinal contents or feces for cytologic evaluation.
- Impression smear of the mucosal surface of the affected GI tract for cytology including Gram stain.
- Intestinal contents, tissues, or feces for anaerobic culture and PCR identification of genotype. Samples should be collected antemortem and/or immediately post-mortem to avoid normal postmortem clostridial overgrowth.
- Abomasum, small intestine, and colon in 10% buffered formalin for histopathology.
- Brain in 10% formalin for histopathology if neurologic signs are observed and *C perfringens* type D (or B) is suspected.
- Intestinal contents, refrigerated or frozen for toxin detection.

TREATMENT

There is very limited information on the efficacy of treatment methods for abomasitis and enteritis caused by *C perfringens*. The following information is based on treatment protocols at the contributing authors' institution that seem to be effective in treating animals with clinical signs consistent with clostridial abomasitis or enteritis. Treatment of abomasal or enteric clostridial disease should focus on 6 goals:

1. Relief of abdominal distension, particularly if respiration is compromised because of abdominal pressure on the diaphragm.
2. Systemic and nutritional support with intravenous (IV) fluids if indicated.
3. Preventing ongoing bacterial proliferation.
4. Decreasing or preventing production of and neutralizing clostridial exotoxins.
5. Restore normal GI flora.
6. Pain management as needed.

The abdominal distension in cases of clostridial abomasitis and enteritis is caused by gas and fluid distension of the abomasum and/or small intestine. The gaseous distension is a result of a combination of ileus and bacterial gas production. In most cases, passage of an orogastric tube does not provide significant relief. If the distension is severe enough that it is compromising respiration, then percutaneous decompression of the abomasum can be performed. This decompression is best done with the animal in dorsal or left lateral recumbency (**Fig. 6**).[85] The area of tympany is localized and quickly clipped and prepared for an aseptic procedure. A percutaneous abomasocentesis can then be performed using a 16-gauge to 20-gauge, 38-mm (1.5-inch) needle inserted directly through the skin into the abomasum. An extension set can be attached to the needle. Allow the gas to freely escape while placing slight pressure on the abdomen. Fluid that drains from the needle can be collected for anaerobic culture, PCR typing, or toxin assays. Before removing the needle, antimicrobials and *C perfringens* type C and D antitoxin can be administered directly into the abomasum (these syringes should be prepared and ready before starting the abomasocentesis).

Systemic support generally involves administration of IV fluids. Because the animals typically have ileus and abdominal distension, oral fluids, including milk, are not indicated until the distension is resolved (generally 12–24 hours). IV fluids provide systemic support in treatment of the hypovolemic or maldistributive shock that is often observed in these patients. Some animals with clostridial enteritis do not show significant clinical dehydration, most likely because of the rapid effects of the toxins. Shock fluid rates are 80 mL/kg, and can be divided into one-quarter doses

Fig. 6. Abomasocentesis performed in a 3-week-old Holstein calf in order to decompress a tightly distended abomasum. This procedure is best done with the calf in dorsal or left lateral recumbency. Following decompression, fluid samples can be obtained for culture and genotyping as well as cytology. Antibiotics and antitoxin can also be administered directly into the abomasum.

given to effect at intervals of 15 to 30 minutes. Once the shock has been addressed, the fluid rate can be decreased to a maintenance rate of 3 mL/kg/h. Dextrose (5%) can also be provided in the maintenance fluids, because milk is likely to be withheld for 12 to 24 hours. Many animals with clostridial enteritis show mild to moderate hypochloremic metabolic alkalosis caused by the intestinal ileus and delayed abomasal emptying. Thus, isotonic sodium chloride is a suitable initial fluid type to resolve the hypochloremic alkalosis if present. Animals in later stages of the disease may show metabolic acidosis and require the addition of sodium bicarbonate to fluids. IV fluids can be changed to a balanced electrolyte solution after resolution of the shock, or within the first 12 hours.

Antimicrobials are used to slow or stop clostridial proliferation in the GI tract. The challenge is that the bacteria reside within the lumen so antimicrobial treatment must be selected such that an appropriate antimicrobial is delivered to the lumen of the abomasum and small intestine. Many antimicrobials are effective against *C perfringens*. Penicillin is one of the most effective antibiotics at killing *C perfringens*; however, penicillin does not immediately kill the bacteria. During the initial phase, the bacteria may continue to produce and release lethal exotoxins.[86] Further, systemically administered penicillin is excreted via the renal system and is minimally excreted or secreted into the GI tract. Thus, oral procaine penicillin G (22,000 IU/kg, by mouth, every 24 hours for 3–5 days) may be recommended for treatment and has been used by

the authors. Most *C perfringens* isolates are susceptible to oxytetracycline. Oxytetracycline is a bacteriostatic agent that inhibits protein synthesis. Oxytetracycline reduces *C perfringens* toxin production in vitro more rapidly than penicillin.[86] Systemically administered oxytetracycline is partially excreted through the liver and biliary system and enters the lumen of the GI tract through the common bile duct. Both oral penicillin and systemic or oral oxytetracycline, alone or in combination, have been found to be clinically effective in treating acute cases of clostridial enteritis at Colorado State University (authors' personal experience). Metronidazole, although effective against *C perfringens*, is a substituted benzimidazole and is illegal in all food animals including cattle, sheep, goats, and swine.

One study with a food safety focus has suggested that use of egg yolk antibody from hens immunized against *C perfringens* could significantly inhibit the growth and sporulation of *C perfringens* in vitro.[87] An oral product intended for calves that contains egg-derived antibodies from hens immunized against *C perfringens* types A, C, and D is commercially available (EPIC Calf Scour Formula, Tomlyn), but its use in treatment or prevention of clinical cases of *C perfringens* has not been described.

In addition to administering an appropriate antimicrobial, bacterial proliferation may be decreased by withholding milk feedings for a period of 12 to 24 hours. In cases in which medical treatment is successful, the calves generally want to nurse within 12 to 24 hours. It is critical that, after this time, milk feeding is reinstituted slowly in small volumes (500 mL [1 pint]) at more frequent intervals (every 4 to 6 hours). Overfeeding can result in a relapse of *C perfringens* overgrowth.

Attempts to restore normal GI flora may also be beneficial once oral intake resumes. Oral administration of fresh rumen fluid may help restore normal GI flora in affected animals. Alternatively, commercially available probiotics can be administered. Placement of an indwelling nasogastric tube greatly facilitates frequent administration of oral medications and transfaunation in patients that do not quickly regain nursing activity.

There is limited information on effective treatments for neutralizing *C perfringens* toxins. In the case of clostridial toxins that work directly on the GI mucosa, neutralization must occur within the GI lumen to be most effective. *C perfringens* type C and D antitoxin contains antibodies against the beta and epsilon toxins and may provide efficacy against enteritis caused by *C perfringens* types B, C, and D. Systemically administered antitoxin may not reach effective concentrations within the GI tract. However, it is highly effective at neutralizing systemically absorbed epsilon toxin (*C perfringens* types D and B) associated with neurologic signs of enterotoxemia. When treating enteric clostridiosis, the authors recommend a combination of both systemic and oral C and D antitoxin at the labeled amounts.

Di-tri-octahedral smectite (Bio-Sponge, Platinum Performance) has been shown to bind *C perfringens* alpha, beta, and beta-2 toxins, and enterotoxin.[88,89] Kaolin-pectin and bismuth subsalicylate have also been used to bind *C perfringens* toxins; however, there are limited data showing efficacy. Both kaolin-pectin and bismuth subsalicylate could have clinical benefit in treating the damaged mucosal epithelium. Kaolin in Kaopectate is a potent activator of the extrinsic clotting cascade and may help decrease intestinal blood loss in cases with hemorrhagic gastroenteritis. Use of proton pump inhibitors in an attempt to limit mucosal ulceration in clostridial abomasitis cases should likely be discouraged, because there is evidence that these are a risk factor for the development of some types of clostridial GI infections in humans.[90]

Pain management should be used on an as-needed basis in these cases. Nonsteroidal antiinflammatories should be used judiciously, because severe shock and

decreased renal perfusion may be present. In addition, GI ulceration can occur from the primary disease process, and this could be exacerbated by use or overuse of nonsteroidal antiinflammatory drugs such as flunixin meglumine or meloxicam. A low dose of flunixin meglumine, as is used to treat the effects of Gram-negative sepsis (0.25 mg/kg IV every 6 hours or 0.3 mg/kg IV every 8 hours) may be preferable to using the labeled cattle dose (up to 2.2 mg/kg IV every 24 hours). Opioids are an alternative selection for pain control but can decrease GI motility and should therefore be used cautiously in cases of suspected clostridial gastroenteritis. However, use of butorphanol intramuscularly (0.02–0.1 mg/kg every 6–8 hours) or as a constant rate infusion (CRI) (13 μg/kg/h) in horses with anterior enteritis is reported to have minimal effects on GI motility.[91] As with all drugs used extralabel in food-producing species, the Food Animal Residue Avoidance Databank (www.farad.org) should be consulted for a meat and/or milk withdrawal time before the administration of any of these agents.

Early medical treatment of *C perfringens* type A abomasitis and enteritis cases can often have dramatic success (**Fig. 7**). Calves are often standing and eager to nurse within 12 to 24 hours when treatment is initiated early. It is important to initially limit milk intake to smaller frequent quantities and gradually return to a normal feeding over 5 to 10 days. Differentiation of *C perfringens* type A hemorrhagic enteritis in adult cattle may be difficult to distinguish from intestinal obstruction. In these cases, an exploratory laparotomy may be performed. Distended hemorrhagic small intestine without intraluminal obstruction is observed (see **Fig. 4**C). During surgery, intestinal samples can be obtained at surgery for bacterial culture, genotyping, and cytology if indicated. Treatment can be initiated intraoperatively with intraluminal injections of penicillin. In our experience, exploratory laparotomy in these cases has not seemed to be detrimental and greatly aids confirmation of a diagnosis and initial treatment.

PREVENTION
Vaccination

Vaccination is considered to be the cornerstone of preventive programs for clostridial diseases in livestock.[72,92] Conventional commercial *C perfringens* C and D or 7-way or
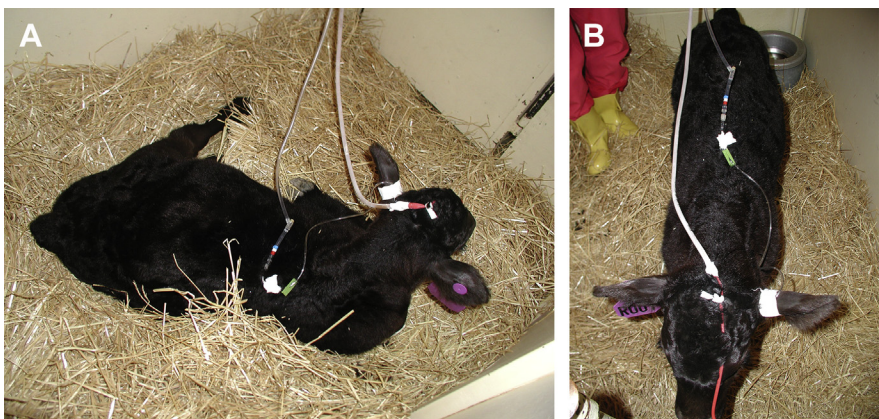
Fig. 7. Beef calf with suspected *C perfringens* abomasitis. The calf showed marked abdominal distension and abomasal tympany at presentation (*A*). The calf was treated medically and the abdominal distension resolved within 24 hours (*B*).

8-way toxoid vaccines are very effective at preventing disease caused by *C perfringens* types B, C, and D in young animals. Type B disease, however, is very rare in the United States. In the following review of the literature, summaries to facilitate evidence-based decisions are presented. It is critical to understand that the conclusions reached in these studies should be related to the specific vaccine products tested in each trial.

In sheep in North America, immunization against the major toxins of *C perfringens* types C and D is warranted. Tetanus is also considered an essential component of a flock immunization program.[93,94] In a 1962 study in sheep, Sterne and colleagues[95] showed that a multivalent, alum-adjuvanted, formalin-inactivated clostridial bacterin-toxoid administered to sheep in 2 doses induced titers deemed protective against the beta and epsilon toxins of *C perfringens*. In another study, antibody titers to epsilon toxin of *C perfringens* type D were induced in sheep immunized with a 2-dose series of a multivalent (8-way) clostridial vaccine.[96] Immunization of ewes 3 weeks before lambing has been shown to induce colostral antibody titers against epsilon toxin that were adequate to impart protection of lambs for up to 12 weeks of age.[97] In that study, adding a 2-dose immunization of the lambs at either days 1 and 21 of age or days 21 and 42 of age did not significantly change the titer of passively protected lambs. Immunization of lambs from vaccinated ewes (that do not have failure or suspected failure of passive transfer) with a *C perfringens* C and D and tetanus toxoid is recommended at 8 to 12 weeks of age and again 3 to 4 weeks later.[94] Feeder lambs (and ideally replacement ewe lambs) can also receive a booster before entering the feedlot or being placed on a high-grain ration.[94]

In goats, most enterotoxemia cases in North America seem to be caused by *C perfringens* type D.[66] Administration of multivalent ovine enterotoxemia vaccines twice annually to goats has been shown to be ineffective in protecting goats against fatal type D enterotoxemia.[70] Goats do respond, albeit variably, to the epsilon toxin component of *C perfringens* type D vaccines labeled for sheep.[68] The disparity in protection among the two species may reflect disparate mechanisms of disease. In sheep, most pathologic lesions seem to be the result of translocation of epsilon toxin from the gut to remote organs; for example, the brain. Circulating antitoxin antibodies against epsilon toxin seem to be critical in protecting sheep. In goats, however, the more localized disease process (enterocolitis) does not seem to be effectively or consistently curtailed by anti–epsilon toxin antibodies in the bloodstream.[33,68,69] However, in a 1998 study, immunization with an epsilon toxoid combined with Freund incomplete adjuvant did protect goats against intraduodenal challenge with purified epsilon toxin, whereas a commercial, aluminum hydroxide–adjuvanted product did not.[33] Existing *C perfringens* C and D toxoids may need to be administered to goats more than twice per year to confer adequate (albeit partial) protection.[70]

Administration of *C perfringens* type C and D to cattle has been shown to induce protective titers against both the beta toxin[98] and epsilon toxin[99] in recipients. Neonates and young, growing animals in stocker operations and feedlots are considered to be at higher risk for clostridial diseases relative to adults, but the risk seems to vary among herds.[72,92] For the former group, immunization of pregnant cows and heifers has been shown to produce antitoxin titers considered adequate for protection against type C and D infections in colostrum-fed calves.[99] Many ranchers immunize calves with multivalent clostridial vaccines before weaning, but, in many cases, repeat immunization of calves to provoke an anamnestic titer is not consistently performed during the preweaning period. Troxel and colleagues[99] determined that vaccination

of colostrum-fed calves (from immunized dams) with a multivalent clostridial vaccine at 50 to 53 days of age and again at weaning at ~170 days produced titers to beta and epsilon toxin that were not considered protective for calves during the preweaning period. The investigators concluded that this 4-month gap between the first and second immunizations may not be optimal for herds in which clostridial diseases occur in preweaned calves, and alterations in management and/or vaccination schedule would be warranted in such cases. In another study, immunization of colostrum-fed calves (from immunized dams) at 3 weeks of age with a single-dose clostridial bacterin-toxoid did not significantly affect type C or D antitoxin titers over the first 4 months of life; however, significant differences in antitoxin titers among different vaccines were apparent.[100]

In a 2000 survey of feedlot operators, more than 90% of operators overseeing lots with a capacity more than 8000 head used at least 1 type of clostridial vaccine in their cattle.[101] In a prospective feedlot study involving nearly 19,000 animals, death losses were compared among calves immunized against clostridial diseases and those that were not.[102] Treated calves were administered a 7-way clostridial vaccine at arrival and 30 days later. Reduction in death loss in the vaccinated calves, weighed against purchase price and vaccine cost, provided an additional net profit of more than $10 per vaccinate.[102] More recently, DeGroot and colleagues[103] found no detectable effect of booster vaccination with a multivalent clostridial vaccine on sudden death syndrome mortality in feedlot cattle.

Although the former study has sometimes been used as justification for use of clostridial vaccines in feedlot calves,[92] additional research has investigated the potential detrimental effects of this use. Because ownership is not always retained for groups of cattle moving from ranch to feedlot, the immunization history of incoming cattle at a feedlot is not always known. As a result, some animals may receive multiple (predicted as many as 6) clostridial vaccinations before marketing, potentially compounding problems with injection site reactions in carcasses.[104] When administered subcutaneously twice to feedlot calves, a 7-way clostridial vaccine induced significant reduction in feed intake after the second immunization and larger injection site lesions than did a *C perfringens* type C and D toxoid.[104]

C perfringens type C and D toxoid vaccines are not designed to specifically stimulate an immune response against alpha toxin. Thus, these vaccines are generally not considered to be effective at preventing abomasitis or enteritis caused by type A strains. One commercial type A toxoid vaccine is available (Clostridium Perfringens Type A Toxoid, Elanco) and has been shown to be safe and stimulate an antibody response against alpha toxin. However, because of the difficulty in developing a satisfactory experimental challenge model, efficacy has not yet been shown in order to have a preventive label. Autogenous *C perfringens* type A vaccines can also be used. The efficacy of these vaccines for *C perfringens* is still not established. A recent study has shown that although formaldehyde inactivated alpha toxin vaccines stimulated antibody production similar to native alpha toxin vaccines, only antibodies produced from the native toxin vaccines provided protection in an intestinal loop assay.[105] Recombinant vaccines may offer advantages compared with conventional toxoid vaccines in the future.[106]

C perfringens vaccination strategies based on species, age, and animal use are provided in **Table 3**.

Feeding and Environmental Management

Presentation of excessive amounts of starch, sugar, or soluble protein into the stomach and/or intestines is considered pivotal in the development of these diseases; thus,

all potential influences on this crucial event must be considered when formulating a preventive plan. Evaluation of ration net energy, fiber content and forage length, bunk space, animal hierarchy within a pen, feeding frequency, the rate and magnitude of changes in ration between successive production groups, and feed mixing practices is essential to identify and correct problems with carbohydrate overload and/or so-called slug feeding. For pasture-fed animals, turnout onto a new, particularly lush pasture should be very gradual (eg, day 1, 15 minutes of grazing; day 2, 30 minutes; day 3, 1 hour; day 4, 2 hours; and so forth).

Prevention of enterotoxemia in nursing animals requires consideration of environmental or management factors that may trigger changes in milk composition or volume for lactating dams. Intermittent provision of high-energy supplements to range animals may trigger changes in milk production. Similarly, management practices that cause prolonged interruption of suckling (eg, cow processing for pregnancy examination) must be made time-efficient in order to limit engorgement of the udder and subsequent ingestion by the neonate of a larger-than-normal milk meal. Sudden and severe changes in weather may cause dams and their offspring to seek shelter or remain recumbent for prolonged periods of time; provision of multiple locales for shelter and bedding, or simply encouraging dams to eat by providing hay (weather permitting) may encourage more frequent nursing than if the animals were left to "sit the storm out."

In dairy calves, variable or improper mixing of milk replacers may increase the incidence of clostridial GI disease. Milk replacers should be mixed at the appropriate concentration and temperature. Some milk replacers are prone to settling out and can result in variable concentrations between what is fed from the start to the finish of a single batch. Milk replacers high in protein and carbohydrate may also promote clostridial overgrowth in the GI tract.

Clostridium difficile

Clostridium difficile is another potential cause of enteritis, but the relationship between *C difficile* infection (CDI) and GI disease in ruminants is unclear because the bacterium may be present in the feces of clinically normal animals. A high prevalence of *C difficile* shedding has been described in otherwise healthy young calves; in one study, 14.9% of calves without clinical disease were shedding the organism.[108] In otherwise healthy veal calves, CDI has been reported to be as high as 51%.[109] *C difficile* shedding has also been detected in dairy cows,[110] feedlot cattle,[111] sheep,[112] and goats.[113]

Hammitt and colleagues[114] found *C difficile* and its toxins (toxin A and toxin B) in 25.3% and 22.9%, respectively, of calves with diarrhea. Diarrheic calves were twice as likely to be culture positive compared with those without diarrhea.[114] Nondiarrheic calves, however, more commonly had toxin-positive stools.[114] This team of researchers concluded that these findings lent credence to the idea that CDI may manifest as diarrhea in young cattle.[114]

C difficile–associated diarrhea (CDAD) was also suspected in a neonatal elk calf, based on finding a toxigenic *C difficile* fecal isolate in the absence of other more common enteropathogens.[115] Similarly, the authors have tentatively diagnosed 3 alpaca crias with CDAD based on fecal ELISAs positive for *C difficile* toxins A and/or B, in combination with exclusion of other common infectious causes of camelid diarrhea and a history of prior antimicrobial administration (authors' personal experience). It is possible that CDAD in livestock occurs more frequently than reported, but it remains underdiagnosed because attempted identification of *C difficile* and its toxins is not routinely performed in most diarrheic ruminants.

Table 3
Summary of current best-practice *Clostridium perfringens* toxoid vaccine recommendations for disease prevention, based on review of the literature

Species and Age Group	Type of Vaccine	Timing and Frequency of Vaccination	Reasoning and Type of Immunity Induced
Bred heifers and adult cows, both beef and dairy	• *C perfringens* type C and D, or 7-way or 8-way vaccines	• Initial immunization followed by booster 3–4 wk later • Second dose (and additional boosters) 2–3 wk before calving[107]	• Provides immunity against the beta and epsilon toxins in calves via colostral transfer • Enteric immunity from anti–beta toxin colostral antibody during the first 2 wk of life is critical in preventing disease from types B and C, because trypsin inhibitors in colostrum prevent denaturation of beta toxin • Systemic immunity from anti–epsilon toxin antibodies are effective at neutralizing systemically absorbed epsilon toxin and preventing neurologic signs from type D ± type B infections
	• *C perfringens* type A	• Initial immunization followed by booster in 2–4 wk, then: • Yearly 2–4 wk before calving	• *C perfringens* C and D toxoid vaccines are not designed to specifically stimulate an immune response against alpha toxin and usually are not considered effective at preventing abomasitis or enteritis caused by type A • May be particularly important in farms with a history of type A outbreaks in calves • Might also be useful in adult cows on dairies with a history of HBS
Feedlot cattle	• *C perfringens* types C and D, 7-way or 8-way vaccines	• At arrival and 30 d later, or: • Initial immunization and booster 3–4 wk apart before arrival	• Potential reduction in death loss • 7-way vaccines may induce decreased feed intake and larger injection-site lesions after booster compared with C and D vaccines
Beef and dairy calves (particularly from herds with previous *C perfringens* type C or D outbreaks)	• *C perfringens* types C and D, or 7-way or 8-way vaccines	• 3–6 mo of age • Initial immunization followed by booster 3–4 wk later	• In cattle, classic type D disease most commonly observed in older, weaned animals on high-concentrate feed • Maternal antibodies may have decreased to nonprotective levels by this age

Adult sheep	• *C perfringens* types C and D, or 7-way or 8-way vaccines	• Initial series of 2 doses 3–4 wk apart, then yearly boosters[94]	• Provides immunity against beta and epsilon toxins
Bred ewes	• *C perfringens* types C and D, or 7-way or 8-way vaccines	• Yearly booster 3–4 wk before lambing	• Provides immunity in lambs against epsilon toxin for up to 12 wk
Lambs from vaccinated dams	• *C perfringens* types C and D	• 8–12 wk of age and booster 3–4 wk later	• Feeder lambs and replacement ewe lambs should receive boosters again before feedlot entry or being placed on high-grain diet
Lambs from unvaccinated dams or with suspected FPT	• *C perfringens* types C and D	• 1–3 wk of age • Booster twice: 3–4 wk and 6–8 wk later[94]	• Feeder lambs and replacement ewe lambs should receive boosters again before feedlot entry or being placed on high-grain diet
Adult goats and kids	• *C perfringens* types C and D, or 7-way or 8-way vaccines	• >2 immunizations per year • 3 immunizations per year, with initial booster dose 3–4 wk later	• May still only provide partial protection • Type D disease most common in goats, and is not prevented by 2 doses of multivalent ovine enterotoxemia vaccines • This may in part be caused by minimal antibody excretion into GI tract in localized enterocolitis in goats vs systemic antibody protection needed in sheep because of effects of absorbed epsilon toxin
Bred does	• *C perfringens* types C and D, or 7-way or 8-way vaccines	• One of the yearly boosters given 3–4 wk before kidding	—

Abbreviation: FPT, failure of passive transfer.

SUMMARY

Clostridial abomasitis and enteritis remain common clinical diseases in livestock, particularly in young ruminants. *C perfringens* types A, B, C, and D are the most common causes, although enteritis caused by *C difficile* is also reported. Commercial *C perfringens* C and D vaccines are effective at preventing disease caused by type B, C, and D strains but do not provide adequate protection for type A strains. When clinical cases are identified early, treatment can be successful if provided promptly.

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