Enterotoxemia of Small Ruminants

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Abstract

Enterotoxemia is a common, frequently fatal disease of small ruminants caused by enteric superinfection with *Clostridium perfringens*. *C. perfringens* type C causes hemorrhagic enteritis of lambs. Type D causes colitis in goats and rapidly progressive, multi-organ failure in sheep, characterized by recumbency, convulsions, and opisthotonus. Prevention of enterotoxemia requires careful attention to feeding practices such that sudden ingestion of large quantities of concentrates or protein does not occur. Diligent vaccination is the cornerstone to enterotoxemia prevention; however, sheep and goats differ in their immune responses to conventional vaccines, and different vaccination regimens are necessary for these two species.

Résumé

L'entérotoxémie est une maladie courante et fréquemment mortelle des petits ruminants, qui résulte d'une superinfection par Clostridium perfringens. La bactérie C. perfringens de type C provoque une entérite hémorragique chez les agneaux. Celle de type D provoque la colite chez les chèvres et une défaillance multi-organes à progression rapide chez les moutons, caractérisée par le décubitus, des convulsions et l'opisthotonos. La prévention de l'entéro-toxémie exige d'apporter une attention minutieuse lors de l'alimentation, de façon par exemple à empêcher toute ingestion soudaine de grandes quantités de concentrés ou de protéines. Une vaccination soigneuse est la pierre angulaire de la prévention de l'entérotoxémie. Toutefois, les ovins et les caprins ont une réaction immunitaire distincte face aux vaccins conventionnels, d'où la nécessité d'un programme de vaccination différent pour ces deux espèces.

Introduction

Enterotoxemia, also known as overeating disease, is characterized by proliferation of and exotoxin production by *Clostridium perfringens* in the lumen of the gastrointestinal tract. Although limited tissue invasion by the causative organism does occur, most local and systemic lesions result from the effects of potent exotoxins produced by certain genotypes of this bacteria. *C. perfringens* is a large, gram-positive, anaerobic bacillus that exists ubiquitously in the environment and in the gastrointestinal tract of most mammals.[6,41] There are five defined types, or genotypes, of *C. perfringens*: A, B, C, D, and E. These genotypes are identified based on the lethal toxins that they produce: alpha, beta, iota, epsilon, and/or enterotoxin.[29] All genotypes produce alpha toxin, although isolates differ significantly in the amount of alpha toxin produced.[18,19] Additionally, the recently discovered beta2 toxin may be produced by type A, as well as by some isolates of types B, C, and E.[7] Strains of *C. perfringens* that carry the beta2 toxin gene have been isolated from a variety of species of domestic animals, including horses, camelids, cattle, and swine.[7,16]

Microbiology

In the past, typing of *C. perfringens* was conducted by mouse inoculation assay. At present, multiplex PCR is commonly used to classify the genotype, based on detection of gene sequences for alpha, beta, beta2, epsilon, and iota toxins and enterotoxin.[7,11,16,24] The presence of the gene for a particular toxin is currently considered to reflect the *potential* to produce that toxin. It is critical to note that major lethal toxin production is not consistent in quantity across clinical isolates within a particular genotype. Thus, the potential virulence of organisms within each genotype is quite 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, potentially crowding out other genotypes of the organism that might have played a role in the animal's death.[6,26] Thus, its isolation should be considered significant only from a fresh cadaver with compatible history, clinical signs, and lesions. The organism is relatively easily grown in vitro; in fact, *C. perfringens* type A grows relatively rapidly in anaerobic culture and may overgrow other potential pathogens that exist in the sample.[26]

Rations rich in concentrates have been shown to increase the rate of isolation of *C. perfringens* from the rumen and cecum of healthy ruminants.[2] Proliferation of *C. perfringens* in the ruminant gastrointestinal tract, resulting from concentrate feeding or overeating, is considered the pivotal event in the onset of enterotoxemia. Unlike type A, *C. perfringens* types C and D are not found as frequently in the gastrointestinal tracts of healthy ruminants.[26] Proliferation of type C in the

gut appears to occur in close temporal association with clinical disease.[26] Therefore, determination of the genotype of *C. perfringens* isolated from cases of ruminant gastroenteritis is a critical step in diagnostic testing. The diagnostic significance of isolation of each genotype of *C. perfringens* from ruminants with enteric disease is increased if the corresponding major lethal toxins can be demonstrated in gut contents and/or blood.[6,35] However, major lethal toxins may not be detected in cases of enteric disease because these toxins are labile, particularly in the protease-rich environment of the gut lumen.[35] Preservation of toxin in samples requires the use of special buffers to optimize preservation of the toxin. Thus, compilation of the "optimal" microbiologic data for *C. perfringens* in enteric disease cases can be problematic.

Type A Enterotoxemia and Abomasitis

Enterotoxemia associated with *C. perfringens* type A has been reported in goats but appears to be relatively rare.[34] Experimental intraduodenal administration of *C. perfringens* type A to goat kids led to transient diarrhea but no fatalities.[30] Type A enterotoxemia of sheep (yellow lamb disease) is an apparently rare but highly fatal disorder that manifests as an acute hemolytic disease. The major lethal toxin produced by *C. perfringens* type A is alpha toxin, which hydrolyzes phospholipids in cell membranes.[6] As it apparently translocates from the gut lumen to the bloodstream of affected lambs, alpha toxin causes rapid hemolysis and subsequent icterus.[6,23,35]

Abomasitis is a sporadic disorder of neonatal to weanling calves, lambs, and kids. This disease 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. Abomasal ulceration and perforation may occur in a subset of affected animals. Intramural emphysema and edema of the abomasal wall may be present. Clinical signs include lethargy, abdominal tympany, colic, bruxism, fluid distension of the stomach, diarrhea, and death.[32] Although the number of case studies on abomasitis is few, upon review of the available literature, the case fatality rate appears to be high (75-100%).[32,33]

A number of putative etiologies for this disease exist, including primary bacterial or fungal infection, immunosuppression, pica, trauma from coarse feed or trichobezoars, and vitamin / mineral deficiencies. In 1987, investigators at Kansas State University detected *C. perfringens* types A and E in stomach contents of affected calves,[32] and the following year reproduced the disease experimentally by intraruminal inoculation of *C. perfringens* type A in calves.[33] Belgian investigators have also detected *C. perfringens* in the abomasums of affected calves.[22] The ability of this organism to produce gas is considered to contribute to gastric dilation and intramural emphysema evident in affected animals. More recently, *Salmonella typhimurium* DT104 was isolated from the abomasal wall of midwestern veal calves with abomasitis.[9,10] Although authors of earlier case reports associated copper deficiency with abomasitis and abomasal ulcers in beef calves,[21] Roeder and colleagues demonstrated that abomasitis could occur spontaneously and be induced experimentally in the absence of copper deficiency.[33] Thus, although copper deficiency may act as a contributory factor for abomasitis and enteric disease of calves, it does not appear to be a requisite factor for either condition. Cases of this disease in neonatal calves have been associated temporally with management practices that cause delays in regular nursing patterns (e.g. calf separation at branding) or changes in environment that interrupt normal nursing patterns (e.g. winter storms).[21] In dairy calves and hand-raised goat kids, poor milk hygiene, intermittent feeding of large volumes of milk, and altered milk temperature have been empirically incriminated as potential contributory factors for abomasal tympany, ulceration, and abomasitis. *C. perfringens* type A and type A+beta2 have been isolated from adult dairy cows and beef cattle affected by jejunal hemorrhage syndrome.[1,13]

Type C Enterotoxemia

Enterotoxemia 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.[6,17,26] 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.[6,26] Neonates nursing heavily lactating dams appear 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.

Affected animals are acutely listless and reluctant to nurse. Ataxia, colic, bloody diarrhea, depression, and recumbency soon follow. Extensor rigidity and opisthotonus are brief events that immediately precede death, and death usually occurs within hours of the onset of signs. Severe hemorrhagic enteritis is the primary gross lesion at necropsy. This lesion tends to be most pronounced and consistently found in the distal jejunum and ileum, although occasionally the entire small intestine is involved.[6,28] 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 serosanguineous fluid may be found within the peritoneal and pleural cavities and the pericardial sac. Petechiae or ecchymoses may be evident on the surface of multiple organs.

Beta toxin is the principal major lethal toxin of type C, although variable amounts of alpha toxin are produced by this organism.[6,26,28] This toxin induces necrosis of enterocytes in the small intestine, thereby allowing toxin access to the deeper layers of the gut wall, which creates extensive submucosal necrosis and intraluminal hemorrhage. Terminally, multisystemic signs of disease can result from absorption of the major lethal toxins as well as other gut-origin toxins and/or organisms from the damaged gut into the bloodstream.

Beta toxin is inactivated by trypsin.[6,26,28] Thus, the lethal effects of beta toxin may be exacerbated in neonates due to either low pancreatic trypsin production or the presence of trypsin inhibitors in colostrum. In older lambs, experimental reproduction of fatal type C enterotoxemia required concurrent administration of *C. perfringens* type C and protease inhibitors in the form of soybean flour.[27]

Type D Enterotoxemia (Overeating Disease, Pulpy Kidney Disease)

Clostridium perfringens type D causes enterotoxemia in small ruminants of all ages;[6,26] disease in cattle appears to be very rare.[36] *C. perfringens* type D is not considered to be a common inhabitant of the gastrointestinal tract of normal ruminants, although it can be carried sporadically by healthy animals.[26] As for 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 type D.[17] Unlike beta toxin, however, epsilon toxin is activated by intestinal and pancreatic proteases.[6] Once absorbed into the bloodstream, epsilon toxin causes loss of endothelial integrity, increased capillary permeability, and edema formation in multiple tissues.[15]

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 on, with collapse, hyperesthesia, lateral recumbency, convulsive paddling, and opisthotonus following within hours. Diarrhea is inconsistently seen. Glucosuria is frequently present, so the bladder urine should be analyzed during necropsy.[8]

At necropsy examination, the peritoneal, pleural, and/or pericardial spaces are filled with variable volumes of straw- or red-colored 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. 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 thalamus and cerebellum may be appreciably soft, with scattered hemorrhages therein. Occasionally, no gross lesions are seen in ovine cases of type D enterotoxemia.[17]

Unlike sheep, goats affected by type D enterotoxemia more consistently show signs of gastrointestinal dysfunction, and gross and histological lesions are more consistently found in the gastrointestinal tract.[3-5,39] In the peracute form of this disease, affected goats may be found dead or may display 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 usually occurs within hours of the onset of signs. Glucosuria is not consistently detected.

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 two to four 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. The most prominent gross postmortem lesion in goats with peracute or acute type D enterotoxemia is fibrinohemorrhagic colitis, usually most severe in the spiral colon.[39] 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 body cavities and heart sac, and scattered ecchymotic hemorrhages on serosal surfaces may be present. Occasional 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 in type D enterotoxemia of goats.[4,38] Chronic enterotoxemia may be difficult to diagnose unless prior peracute or acute cases are known to have occurred in the herd. Gastrointestinal parasitism, salmonellosis, Johne's disease, and rumen acidosis are important differential diagnoses.

Prevention

Presentation of excessive amounts of starch, sugar, or soluble protein into the stomach and/or intestine is considered pivotal in the development of these diseases; thus, all potential influences on this pivotal 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 slug feeding. As a conservative rule of thumb outside of feedlots, modest (10-15%) increases in carbohydrate or protein in rations for small ruminants should be gradually implemented by small increments, such that five to seven days are required to complete the transition. Along those lines, for pasture-fed animals, turnout onto a new pasture should be very gradual (e.g. day 1 - 15 minutes of grazing; day 2 - 30 minutes; day 3 - 1 hour; day 4 - 2 hours, etc.).

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 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, 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."

For hand-fed lambs and kids, prevention requires division of the daily allotment of milk or milk replacer into as many milk feedings as is practical for that operation. In problem herds and flocks, reducing of the volume of milk fed per feeding and increasing the number of milk meals provided is often helpful; the added expense for labor is often offset by reduction in morbidity and mortality. Equipment used to prepare and store milk or milk replacer should be cleaned as diligently as feeding equipment. Milk replacer preparation should be reviewed, and the water temperature at which milk replacer is prepared should be measured and compared to manufacturer's recommendations. Anecdotally, milk and milk replacers that are allowed to cool to below body temperature are considered to be risk factors for abomasal bloat and enterotoxemia.

Clean water and small quantities of good-quality hay should be provided within the first seven to 10 days of life. A small allocation of starter pellets can be provided; however, soiled or wet pellets and hay should be removed and fed as waste to older animals. Soiled water should be changed. Feeding off of the ground or floor should be limited whenever possible.

Vaccination is considered to be the cornerstone of preventive programs for clostridial diseases in livestock.[31,36] In the following review of the literature, summaries to facilitate evidence-based decisions will be presented. It is critical to understand that the conclusions reached in these studies should be related to the specific vaccine product(s) 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.[14,25,31] In a 1962 study in sheep, Sterne and colleagues demonstrated that a multivalent, alum adjuvanted, formalin-inactivated clostridial bacterin-toxoid administered to sheep in two doses induced titers deemed protective against the beta and epsilon toxins of *C. perfringens*.[37] In another study, antibody titers to epsilon toxin of *C. perfringens* type D were induced in sheep immunized with a two-dose series of a multivalent (8-way) clostridial vaccine.[20] Immunization of ewes three 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.[12] In that study, adding a 2-dose immunization of the lambs at either day 1 and 21 of age or day 21 and 42 of age did not significantly change the titer of passively protected lambs. Repeat immunization of lambs with a *C. perfringens* C and D and tetanus toxoid *or* a multivalent 8-way clostridial vaccine is recommended at six to 10 weeks of age and again four weeks later.[14] Feeder lambs and replacement ewe lambs should receive a booster immunization following weaning.[35]

In North American goats, the majority of enterotoxemia cases appear to be caused by type D.[17] Administration of multivalent ovine enterotoxemia vaccines twice annually to goats has been demonstrated to be potentially ineffective in protecting goats against fatal type D enterotoxemia.[4,5] Goats do respond, albeit variably, to the epsilon toxin component of *C. perfringens* type D vaccines labeled for sheep.[38] The disparity in protection among the two species may reflect disparate mechanisms of disease. In sheep, the majority of pathologic lesions appear to be the result of translocation of epsilon toxin from the gut to remote organs, e.g. the brain. Therefore, circulating antitoxin antibodies against epsilon toxin appear to be critical in protecting organs against the vascular damage characteristic of this disease. In goats, however, the more localized disease process (enterocolitis) does not appear to be effectively or consistently curtailed by anti-epsilon toxin antibodies in the bloodstream.[4,38,39] However, in a 1998 study, immunization with an epsilon toxoid combined with Freund's incomplete adjuvant did protect goats against intraduodenal challenge with purified epsilon toxin, while a commercial, aluminum hydroxide-adjuvanted product did not.[40] Existing *C. perfringens* C and D toxoids may need to be administered more than twice per year to confer adequate (albeit potentially partial) protection to goats.[5] Based on this literature review,

it is evident that fatal enterotoxemia can occur in vaccinated goats, particularly if feeding practices allow ingestion of large meals of soluble carbohydrates and protein. Immunization of pregnant does at roughly one month prepartum is recommended to impart passive protection to kids.[14]

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