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Anaerobe 10 (2004) 239–242

Anaerobe

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Veterinary anaerobes and diseases

Clostridium perfringens type E enteritis in calves: two cases and a brief review of the literature

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Received 5 April 2004; accepted 5 May 2004

Abstract

Toxigenic types of *Clostridium perfringens* are important causes of enteric disease in domestic animals, although type E is putatively rare, appearing as an uncommon cause of enterotoxemia of lambs, calves, and rabbits. We report here two geographically distinct cases of type E enterotoxemia in calves, and diagnostic findings which suggest that type E may play a significant role in enteritis of neonatal calves. The cases had many similarities, including a history of diarrhea and sudden death, abomasitis, and hemorrhagic enteritis. In both cases, anaerobic cultures of abomasum yielded heavy growth of *C. perfringens* genotype E. Four percent of > 1000 strains of *C. perfringens* from cases of enteritis in domestic animals were type E, and all ($n = 45$) were from neonatal calves with hemorrhagic enteritis. Furthermore, type E isolates represented nearly 50% of all isolates submitted from similar clinical cases in calves. Commercial toxoids available in North America have no label claims for efficacy against type E infections. Consideration should be given to type E-associated enteritis when planning for the health care of calves.

Keywords: *Clostridium perfringens*; Iota toxin; Bovine neonatal enteritis; Hemorrhagic enteritis

1. Introduction

Clostridium perfringens is an important cause of enteric disease in domestic animals [1–5]. Its virulence is based largely upon toxinogenesis [6,7], and production of four so-called major toxins is the basis for division of the species into types [6,8–11] (Table 1).

Type E is a putatively uncommon cause of enterotoxemia of lambs, calves, and rabbits [12] (Table 1). Iota enterotoxemia in calves and lambs was reported 50 years ago in Britain, and accounts published since that time have been of hemorrhagic, necrotic enteritis of calves [13] and of detection of type E organisms and iota toxin in ovine or bovine intestines at post mortem [14]. Suspected type E-induced disease in rabbits must be differentiated from that caused by *C. spiroforme* [3,15]. Strains of type E are distinguished from other toxinotypes by their production of iota toxin, which consists of two non-covalently associated components and ADP-ribosylates actin at Arg-177 [16–18]. Little is known of the pathogenesis of type E infections, although it is assumed that, in keeping with the pattern set by isolates of other toxin types, iota toxin plays an important role.

2. Case reports

Case one: Fixed and fresh tissues from a 2-week-old male Angus calf were submitted by a veterinarian in Wisconsin. The history included diarrhea and sudden death, and necropsy findings of hyperemia and edema involving the abomasum and small intestine. Microscopic examination of abomasum revealed mild multifocal mucosal hemorrhage and acute inflammation of the submucosal layer (Fig. 1). The submucosa was edematous and contained aggregates of neutrophils.

Fluorescent antibody examinations were negative for rotavirus, coronavirus, and BVD virus. Attempts at virus isolation yielded negative results, as did electron

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doi:10.1016/j.anaerobe.2004.05.001

Table 1
Diseases produced by toxigenic types of *C. perfringens*

Toxin type	Diseases	Major toxins
A	Myonecrosis, food poisoning, necrotic enteritis in fowl, enterotoxemia in cattle and lambs, necrotizing enterocolitis in piglets; possibly equine colitis, canine hemorrhagic gastroenteritis	Alpha
B	Dysentery in newborn lambs, chronic enteritis in older lambs (''pine''), hemorrhagic enteritis in neonatal calves and foals, hemorrhagic enterotoxemia in adult sheep	Alpha, beta, epsilon
C	Enteritis necroticans (pigbel) in humans, necrotic enteritis in fowl, hemorrhagic or necrotic enterotoxemia in neonatal pigs, lambs, calves, goats, foals, acute enterotoxemia (''struck'') in adult sheep	Alpha, beta
D	Enterotoxemia in lambs (''pulpy kidney'') and calves, enterocolitis in neonatal and adult goats, possibly enterotoxemia in adult cattle	Alpha, epsilon
E	Enterotoxemia likely in calves and lambs, enteritis in rabbits; host range and disease type unclear	Alpha, iota

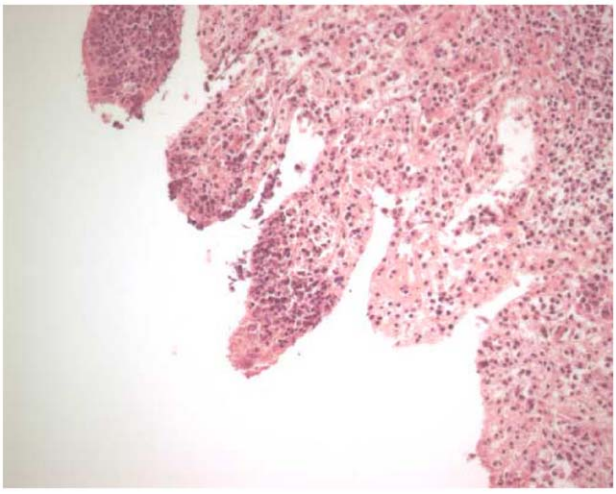

Fig. 1. Affected ileum from Case one. Tissue is autolysed, but necrotic leukocytes are evident in lamina propria.

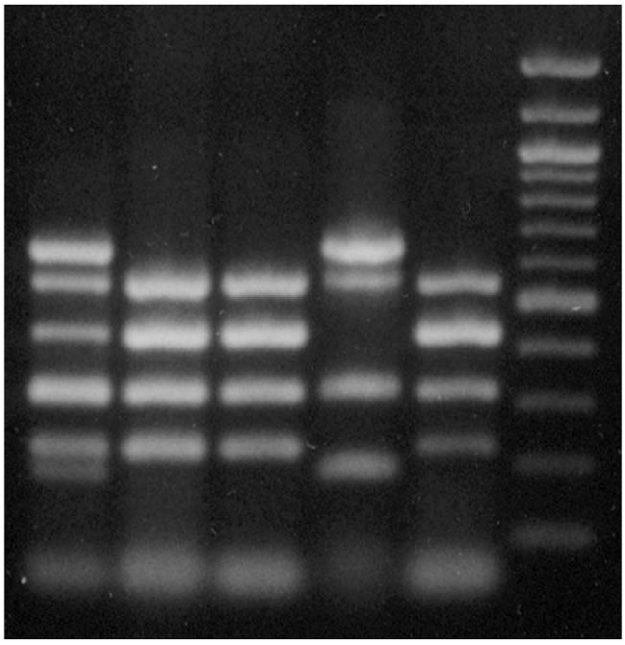

Fig. 2. PCR genotyping of type E isolates. Lane 1: standards (combined PCR amplification products from genotyping of types B and E control strains; from top down are bands indicating amplification of portions of genes for epsilon, beta2, iota A, and alpha toxins, enterotoxin, and beta toxin); Lane 2: Type E isolate from Case one; Lane 3: Type E isolate from Case two; Lane 4: Type B control; Lane 5: Type E control; Lane 6: standards (1 kbp ladder).

microscopic examination of intestinal contents. Direct fecal smears were negative for cryptosporidia. Anaerobic cultures of abomasum confirmed a heavy growth of *C. perfringens*, which was determined by PCR analysis to be genotype E; PCR results also revealed the presence of the genes for enterotoxin (*cpe*) and beta2 toxin (*cpb2*) [10,11,19] (Fig. 2). No significant organisms were found in small intestine, colon, or lymph node.

Case two: Fixed and fresh tissues from an 11-day-old crossbred female calf were submitted by a veterinarian in Nebraska. The calf was one of 70 in the herd, of which about 10% were affected and three had died. In the subject animal, death followed 3 days of scouring, and the veterinarian suspected a coronaviral infection.

Necropsy revealed jejunal hemorrhage, which was especially notable from the serosal surface. Streaks of inflammation were seen on the mucosal surface of the spiral colon, and mesenteric lymph nodes were edematous and inflamed.

Microscopic examination of tissues revealed mild autolytic change in segments of jejunum, ileum, and colon. Some areas of ileum appeared necrotic, with inflammation of some villous tips, and lymphadenitis was evident.

Fluorescent antibody examinations of intestine were negative for rotavirus and coronavirus, as was electron microscopic examination. Direct fecal smears were negative for cryptosporidia. No significant bacteria were found in lymph nodes or colon, but heavy growth of *C. perfringens* was obtained upon anaerobic culture of intestine. The isolate was determined by PCR analysis to

be genotype E; PCR results were also positive for *cpe* and *cpb2* [10,11,19] (Fig. 2).

In both cases, findings were consistent with *C. perfringens* type E enteritis.

3. Discussion

As noted, infection by *C. perfringens* type E has been a rare diagnosis since its first description more than 50 years ago. Diagnoses in rabbits may, in fact, have been infections by *C. spiroforme*, an organism which is very different from *C. perfringens* but which produces a toxin quite similar to iota toxin [3]. An important point illustrated by the cases presented here is that, without bacteriologic culture and genotyping, these infections, if attributed to *C. perfringens*, would likely have been assumed to be caused by organisms of type A or C.

In fact, strains of type E are not uncommon in certain niches. Examination of 1113 strains of *C. perfringens* from cases of enteritis in domestic animals revealed 45 type E strains (4%), all from different herds. The origin of these isolates was uniformly from neonatal calves with hemorrhagic enteritis (and most experiencing sudden death), which is consistent with the findings of others [15,19]. Samples submitted to us for diagnostic screening are not necessarily random or representative; nevertheless, the fact that all 45 type E isolates identified in this study originated from a single host type and condition is notable, since these type E isolates represented 46.9% of all isolates submitted from similar clinical cases in calves. More rigorous epidemiologic and diagnostic pursuit of similar cases is perhaps warranted.

It is also important to note that currently available commercial toxoids will likely offer little or no protection against type E infections. Thus, disease could occur even in the face of faithful use of an immunoprophylactic product directed against other genotypes. It is tempting to speculate on a role for type E strains in so-called vaccine breaks.

The finding of *cpe* in isolates from these cases is consistent with previous work by ourselves and others [19–22], in which the gene could be amplified from strains of type E, but enterotoxin (CPE) was not expressed. Silent *cpe* sequences, found near the iota toxin genes on episomal DNA, were highly conserved among type E isolates, but contained nine nonsense and two frameshift mutations and lacked the initiation codon, promoters, and ribosome binding site. This is remarkable, given that sequencing of *cpe* from eight different isolates revealed 100% sequence homology [19,23–25]. These strains were apparently not clonal; location of *cpe* sequences, with *iap* and *ibp*, on episomal DNA and lack of isolate-specific mutations suggests recent wide distribution among *C. perfringens* isolates.

Detection of *cpb2* sequences by PCR analysis is of uncertain importance. CPB2 has been associated with enteric disease in pigs [26], horses [27], and feedlot cattle [28]. We have found *cpb2* in 12.9% of bovine isolates across-the-board, and in 30.1% of isolates from cattle with enteritis (unpublished data). However, there is no direct experimental evidence for a role in pathogenesis, in cattle or any other species. Furthermore, we have found that *cpb2* is expressed by only $\sim$50% of all bovine isolates; *cpb2* is silent in all type E isolates we have examined.

In sum, consideration should be given to type E-associated enteritis when planning for the health care of calves. Greater attention to bacteriologic culture and genotyping as part of diagnostic approaches will provide more information on the true importance of this problem. Development of immunoprophylactic products is desirable, but may not be considered financially viable.

Acknowledgements

The authors gratefully acknowledge Connie Gates, Jane Christopher-Hennings, and Dawn M. Bueschel for technical assistance. Supported in part by funds from Boehringer Ingelheim Vetmedica and USDA-Hatch.

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