

# *Clostridium perfringens* Type A Infection in Cattle

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## Definition of *C. perfringens* Type A

*Clostridium perfringens* is divided into five types on the basis of its ability to produce one or more of the so-called major toxins (Table 1). Type A strains are those which produce alpha toxin (a characteristic of all, or nearly all, isolates) and which do not produce beta, epsilon, and iota toxins. They are widespread in the intestines of warm-blooded animals and in the environment,<sup>36</sup> and are well-known causes of wound contamination, anaerobic cellulitis, and gas gangrene in humans.<sup>15</sup> Strains of type A are associated with a wide variety of disease processes in many organ systems of myriad species of domestic animals.

## Type A Infections in Species other than Cattle

Perhaps the most common and widely-accepted enteric disease with a type A etiology is necrotic enteritis of domestic poultry.<sup>19,26</sup> The results of infection may be as mild as decreased weight gain, although in the more typical form, depression, inappetence, anorexia, and diarrhea occurs, with a brief clinical course. Necrosis of both jejunum and ileum can extend the entire width of the mucosa, while acute catarrh without necrosis is more common in the lower intestine. Gram-

positive bacilli are often detected on the lamina propria and attached to cellular debris. The disease can be reproduced by raising chicks on litter on premises with a history of the disease<sup>38</sup> or by administration of contaminated feed,<sup>19</sup> cultures of *C. perfringens*,<sup>2</sup> or culture supernatant fluids.<sup>3</sup> High-fiber litter can damage the intestinal mucosa, predisposing birds to development of necrotic enteritis,<sup>2</sup> as can concurrent infection with coccidia, which is often observed.<sup>34</sup>

Lamb enterotoxemia (yellow lamb disease) manifests itself clinically with signs of depression, anemia, icterus, and hemoglobinuria, and affected lambs die after a course of 6 to 12 h. The intestines often contain elevated numbers of *C. perfringens*. Goats<sup>33</sup> may be similarly affected.

Adult horses develop the so-called "intestinal clostridiosis," presenting with profuse watery diarrhea. Mortality is high, and large numbers of enteric type A organisms are demonstrated.<sup>39</sup> Hemorrhagic cecitis and peracute death without diarrhea may also be caused by *C. perfringens*. Neonatal foals can develop hemorrhagic diarrhea with extensive subserosal hemorrhage, marked, diffuse necrosis of the villous mucosa, and hyperemia and hemorrhage of lamina propria and submucosa.<sup>5</sup> Gram-positive bacilli are found in intimate association with necrotic villi.

**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

Clostridial enteritis in suckling and feeder pigs often takes the form of mild necrotizing enterocolitis and mild villous atrophy.<sup>6,29</sup> Lesions, which may be heavily colonized with *C. perfringens* are usually most severe in the jejunum and ileum.<sup>24</sup> The syndrome was reproduced, at least to a degree, by oral inoculation of gnotobiotic colostrum-deprived and conventional weaner pigs.<sup>18</sup> Alpha toxin alone, administered into ligated loops of small intestine, did not produce significant lesions or fluid loss.<sup>29</sup> It is important that these studies be repeated with better-defined preparations of alpha toxin, but it also seems likely that other virulence factors are involved and/or that virulence is multifactorial.

### Type A Infections in Cattle

The association of *C. perfringens* type A with lesions of the gastrointestinal tract of cattle is well-documented,<sup>1</sup> and many investigators have claimed an etiologic role for these organisms. In a study of more than 2500 isolates, type A was found to be the predominant type from cattle with enterotoxemia<sup>9</sup> and other studies<sup>41</sup> have had similar results.

Our experience, and that of others, has revealed lesion-associated type A strains in calves with tympany, abomasitis, and abomasal hemorrhage and ulceration. Calves may suffer from severe diarrhea, and enteric lesions can be obscured by rapid autolysis. Gram-positive bacilli are often found on the mucosa and in the submucosa.<sup>7,8,13,30,32</sup> Results of typing of isolates from enteric disease in domestic animals (Table 2) reveals the large proportion of infections which may be attributable to type A.

### Why has the Veterinary Community in the US Been Reluctant to Accept a Type A Etiology for Enteritis and Enterotoxemia?

The simple answer is the well-known occurrence of organisms of type A as part of the normal flora of the intestinal tract of virtually all warm-blooded animals. Studies comparing the rate of occurrence of *C. perfringens* type A in normal and diseased animals have consistently revealed the presence of this organism in both groups<sup>17</sup> and although higher numbers are sometimes found in animals with enteritis or enterotoxemia, even this has not been consistent.

However, it is probably useful to view this situation from a different perspective. Types B, C, D, and E are defined mainly by which toxins they **do** produce, and beta, epsilon, and iota toxins seem to be the most relevant factors in pathogenesis of disease caused by these types. It is fair to say that protection against the toxins (typically by an immune response to a toxoid antigen) is one and the same with protection against the

**Table 2.** Distribution of *C. perfringens* isolates by type

Type	Isolated from:	% of total
A	alpaca, birds, cats, cattle, deer, dogs, dolphins, feed, goats, horses, pigs, sheep	88.4
AE	cat, cattle, dog, horse, animal food, ostrich	5.5
B	goat, sheep	0.25
C	cattle, chickens, dogs, horses, pigs, sheep	3.4
CE	dog	0.05
D	cattle, sheep, wild turkey	1.39
DE	pig	0.05
EE	calves	0.82

disease. Type A, on the other hand, is defined by what it **does not** produce, namely beta, epsilon, and iota toxins. Thus, type A has become, literally and figuratively, a cesspool into which is thrown every isolate not making these three major toxins. Insufficient attention has been given to other potential virulence factors, production of which might set apart groups of isolates within what is now type A. Thus, if one takes the view that "type A is not always type A," the data presented by some [e.g., the isolation of *C. perfringens* type A from 78.6% of cases and 75% of controls]<sup>17</sup> could be viewed in manner which would be less likely to yield the conclusion that type A is not involved. Groups within type A might, in fact, be associated with specific syndromes (e.g., foal diarrhea or bovine enteritis) which are now considered by some to be idiopathic.

### Is *C. perfringens* Type A a Cause of Enteritis in Beef Calves?

A relatively common finding in beef calves is abomasal ulceration and tympany, and the association of this condition with *C. perfringens* infection has been noted by several investigators.<sup>20,22,30,31</sup> Histopathologic examination of abomasums frequently reveal abundant Gram-positive bacteria in close association with damaged mucosa, and *C. perfringens* type A is frequently isolated. In one specific study, a group of calves were submitted for diagnosis of suspected abomasal displacement or intestinal obstruction after acute onset of abdominal tympany, colic, depression, or death.<sup>31</sup> Upon necropsy, findings included abomasal distention and abomasitis, hemorrhage, and ulceration, but no displaced abomasum or obstructed intestine. Ruminant contents, collected either antemortem or at necropsy, yielded type A isolates in most cases. The conclusions of these

and other studies, plus the anecdotal findings of routine diagnostic work and the apparent efficacy of autogenous toxoids, suggest as a working hypothesis that *C. perfringens* type A may make a substantial contributory role in the pathogenesis of this acute abdominal syndrome in neonatal calves.

### Are Enterotoxigenic Strains of Type Involved in Enteric Disease of Cattle?

Certain isolates of *C. perfringens*, which may be of any toxigenic type, also produce enterotoxin (CPE). Toxin expression is during sporulation, rather than during vegetative growth. CPE has a demonstrated role in clostridial food poisoning in humans. The role of CPE in animal disease is much less clear, although there is a strong suggestion of its involvement in enteric disease in pigs. CPE-producing strains are not uncommon in cattle,<sup>23,37</sup> although some studies have found them distributed equally in affected and unaffected animals.<sup>11</sup> Enterotoxigenicity varies significantly from strain-to-strain, and may be greater in strains from enteritis cases. Production of large amounts of alpha toxin has been reported in enterotoxigenic strains from chickens with necrotic enteritis,<sup>26</sup> and one might hypothesize a synergistic effect between the two. In sum, no firm conclusions can be drawn, at this time, about a place for CPE in bovine enteric disease. It is unlikely, however, to play a major role, in that CPE-producing strains are not recovered from most cases of type A enteritis.

### Is *C. perfringens* Associated with Enterotoxemia and Sudden Death in Dairy and Feedlot Cattle?

There is an increasing number of reports of type A infection as a cause of enterotoxemia and sudden death in dairy and feedlot cattle. The clinical picture is not well-defined, but many animals display depression and inappetence. When present, diarrhea varies in characteristics from watery to bloody. Some animals may exhibit signs of shock, but in most, the progression of the disease is very rapid, and sudden death is not uncommon. At post mortem, the most impressive lesion is typically "redgut," in which large segments of the small intestine are a deep reddish-purple color and are often filled with blood. Microscopic examination of tissues reveals mild to moderate necrosis, and there may be large numbers of Gram-positive rods on the intestinal surface. Recovery of large numbers of *C. perfringens* type A is routine.

A clostridial etiology for such problems is not as yet universally accepted, with good reason. A recent study<sup>10</sup> measured the effect of booster vaccination with a clostridial bacterin-toxoid on feedlot sudden death syndrome (SDS) mortality. Nearly 90,000 cattle (suffi-

cient numbers to detect a treatment effect if the mortality rate was reduced at least 40%) were immunized against *Clostridium chauvoei*, *C. speticum*, *C. novyi*, *C. sordellii*, and *C. perfringens* types C and D. Ninety days pre-slaughter, cattle in the principal group received a booster injection, but the SDS mortality rate remained the same (0.24%) in both principal and control groups. Thus, if an efficacious immune response is elicited against the subject clostridia by the bacterin:toxoid used in this study, a clostridial etiology for SDS seems unlikely. Furthermore, there is good evidence that at least some of the lesions attributed to *C. perfringens* type A are in fact caused by the terminal effects of bloat.

On the other hand, routine immunization with *C. perfringens* types C and D toxoids does not give rise to alpha-toxin neutralizing antibodies (our unpublished data). Thus, if type A and alpha toxin are involved in the pathogenesis of SDS, the immunization protocol would not have been predicted to prevent disease. Lending further support to a type A etiology is the fact that equine origin hyperimmune serum against types C and D **does** contain alpha-toxin neutralizing antibodies, and there is anecdotal evidence that such serum plays a positive role in therapy of putative cases of type A enteritis.

### What is Known about the Pathogenesis of Type A Enteritis in Cattle?

There is very little concrete information pertaining to pathogenesis of type A enteric infections in cattle. Disease in lambs and calves is sometimes compatible with the action of a hemolytic toxin in the circulation, causing intravascular hemolysis and capillary damage, inflammation, platelet aggregation, shock, and sometimes-fatal cardiac effects.<sup>35,36</sup> Large amounts of alpha toxin can be found in feces in natural cases of disease in cattle, and in feces and intestinal contents of birds with necrotic enteritis; alpha toxin may be responsible for intestinal mucosal necrosis in the latter condition. More alpha toxin is produced by many isolates of *C. perfringens* from birds with necrotic enteritis than by isolates from normal birds.<sup>16</sup> Lesions can be reproduced with crude preparations of alpha toxin,<sup>2,3,26</sup> and this effect is neutralized by antiserum. IV injection of lambs<sup>28</sup> or calves<sup>25</sup> with alpha toxin produced transitory diarrhea and intestinal mucosal hyperemia, and one might expect similar effects if toxin diffused from a site of production in the intestine. Alpha toxin is a major element in pathogenesis of muscle disease,<sup>4</sup> and alpha antitoxin protects against myonecrosis.<sup>40</sup>

A lingering question relates to the fact that **all** isolates of *C. perfringens* produce alpha toxin, and could, thus, be expected to participate to an equal degree in the etiology of enteritis. However, the variation in quantity of alpha toxin production by various strains is well-known,



and it is possible that this alone is enough to differentiate virulent and avirulent isolates. In addition, the structure of alpha toxin is not uniform across all strains. Changes in amino acid sequence (Ala174 to Asp174; Thr177 to Ala177; Ser335 to Pro335) affected the sensitivity of alpha toxin to chymotrypsin,<sup>13</sup> and it is tempting to speculate that this might allow accumulation of the toxin in the gut, with subsequent systemic effects.

If type A is a factor in enteritis in cattle, it is still possible that the pathogenesis is mediated by some element other than alpha toxin. The recent description of beta2 toxin, and its common production by strains from cattle and pigs with enteritis, is an example.

### What are Important Areas of Work for the Immediate Future?

Clearly, the most important area for investigation is the unequivocal testing of hypotheses regarding etiology. Reproduction of disease with pure cultures of type A is important, and should be accompanied by identification of possible virulence attributes and studies *in vivo* with specific mutants. Companion work might include the experimental or field studies of the efficacy of immunization against various products of type A cultures.

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