

Enteric infection of swine with *Clostridium perfringens* types A and C

J. Glenn Songer, PhD; Robert D. Glock, DVM, PhD

Summary

Hemorrhagic necrotic enteritis in piglets, induced by *Clostridium perfringens* type C, is a well-known disease syndrome that remains a problem in spite of long-term availability of inexpensive, generally-effective toxoids. Type A infections are now recognized with increasing frequency in neonatal and weaned pigs, and approaches to diagnosis and prophylaxis are both different and more complex than those for type C infections. Careful documentation of the clostridial etiology of these cases, including exclusion of other enteric pathogens, can provide the basis for effective strategies for prevention and control.

C*lostridium perfringens* is the etiologic agent of multiple syndromes in domestic animals, which represent some of the most important conditions confronting producers and veterinary practitioners. The pathogenesis of *C. perfringens* infections is mediated by one or more of nearly 20 potent exotoxins. Indeed, the species is divided into types on the basis of production of α , β , ϵ , and ι toxins, the so-called major toxins (Table 1). A fifth toxin, enterotoxin (CPE), is important in disease in humans and probably in domestic animals.

The four major toxins are elaborated during growth of vegetative cells, but CPE production is co-regulated with sporulation, and the toxin is released upon lysis of vegetative cells. It can be produced by strains of any of the toxigenic phenotypes (Table 1).

Widespread, long-term availability and use of toxoids has not eliminated clostridial enteric disease in pigs. Improper administration of immunoprophylactic products and nonresponding sows may be a small part of the problem, but evidence increasingly suggests that strains of *C. perfringens* type A may be common causes of enteric disease, in pigs and other animals.

Practitioners and diagnosticians have traditionally been reluctant to accept *C. perfringens* type A as a cause of enteric disease, probably because strains of type A are part of the normal intestinal flora of virtually all warm-blooded animals. However, consideration of the information in Table 1 reveals that the only criteria for membership in type A

Table 1

Diseases caused by the five toxigenic types of *Clostridium perfringens*

Type	Diseases in domestic animals	Major toxins
A	Myonecrosis, fowl necrotic enteritis, bovine and ovine enterotoxemia, porcine necrotic enterocolitis, equine colitis, canin hemorrhagic gastroenteritis	α
B	Dysentery, chronic enteritis in lambs, ovine hemorrhagic enterotoxemia, equine and bovine hemorrhagic enteritis	α, β, ϵ
C	Fowl necrotic enteritis, neonatal hemorrhagic or necrotic enterotoxemia (ovine, porcine, bovine, caprine, equine), ovine enterotoxemia	α, β
D	Enterotoxemia (lambs, calves), caprine enterocolitis, bovine enterotoxemia	α, ϵ
E	Bovine, ovine enterotoxemia, rabbit enteritis	α, ι

are that the strain:

- produce α toxin, and
- fail to produce β , ϵ , and ι toxins.

Thus, it seems reasonable (even likely) that there exist groups of strains, within what we now call type A, that produce heretofore-undescribed virulence attributes (such as toxins) and that cause specific disease syndromes in domestic animals.

We were recently asked to examine a large group of isolates of *C. perfringens* obtained from piglets with classical hemorrhagic, necrotic enteritis to confirm that they were type C. In fact, 95% were type A, and considering that true type-C cases yield pure cultures of type C, it seems unlikely that these were “normal flora” type-A strains picked inadvertently from a background of disease-causing type-C strains. Elucidation of the role of type-A strains will require amassing epidemiologic and microbiologic data, as well as genotyping and phenotyping the isolates. For the present, the differential diagnosis for swine enteric disease should include both type A and type C.

JGS, RDG: Department of Veterinary Science and Microbiology, University of Arizona, Tucson, Arizona 85721; Email gsonger@u.arizona.edu

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Clinical manifestations and pathologic lesions

Type-C infections are common in newborn animals of many species, perhaps because this organism is able to colonize rapidly in the absence of well-established normal intestinal flora. Damage to microvilli and terminal capillaries occurs prior to adhesion to the jejunal mucosa and progressive mucosal necrosis. Bacterial invasion, and further multiplication and toxin production, follows desquamation of epithelial cells. Vegetative cells and spores can be observed on the mucosa.

Neonatal pancreatic secretion deficiencies and ingestion of protease inhibitors in colostrum favor the action of β toxin. Intestinal lesions are usually extensive and severe, but death is probably due ultimately to toxemia. Peracute disease in piglets 1–2 days of age is characterized by diarrhea and dysentery, with blood and necrotic debris in feces. Hemorrhagic necrosis of the mucosa, submucosa, and muscularis mucosa is extensive, with gas accumulation in tissue and hemorrhagic exudate in the lumen. Morbidity is 30%–50% and the case fatality rate is 50%–100%. Older piglets often develop nonbloody, yellowish diarrhea, with jejunal mucosal necrosis. Sows may be the major source of infection for newborn pigs.

Strains of type A are prevalent in the intestines of warm-blooded animals, and in the environment. However, isolates of this phenotype are also consistently associated with enteric disease (Table 1). α toxin probably does not cause lesions or fluid loss, so it is likely that yet-undescribed virulence attributes are involved in the pathogenesis of swine enteric disease. However, intravascular hemolysis, capillary damage, inflammation, platelet aggregation, shock, and fatal cardiac effects in lambs and calves with enterotoxemia are consistent with the action of a hemolytic toxin, such as α toxin, in circulation, and these possible systemic effects in any domestic species should not be ignored.

Type A enteritis in neonatal pigs is most often characterized by mild necrotizing enterocolitis and damage to the tips of the villi, affecting primarily the jejunum and ileum. A form of the disease has been reproduced by inoculation of conventional and gnotobiotic, colostrum-deprived pigs, in which enteropathy followed substantial adherence and multiplication of an α -toxigenic *C. perfringens* in the gut. α toxin administered alone to neonatal piglets caused mild enteritis and villous edema, with epithelial and vascular damage.

There is an increasing awareness of the possible role of enterotoxin-producing strains of *C. perfringens* (mainly of type A) in swine enteric disease. Clinical materials are not routinely examined for CPE, nor are isolates of *C. perfringens* from animals, but the CPE gene has been commonly found in isolates from horses, cattle, sheep, swine, poultry, and other species. CPE may be found in stools of diarrheic pigs with superficial mucosal necrosis and villous atrophy, although not consistently. Spore counts are often two logs higher in pigs with diarrhea than in normal pigs. Inoculation of hysterectomy-derived, colostrum-deprived (HDCD) piglets with enterotoxigenic strains of type A resulted in disease ranging from profuse, bloody diarrhea, enteritis, and death,

to nonbloody diarrhea and intestinal gas accumulation, with low mortality. The latter syndrome resembles the experimental infection in conventionally weaned pigs. CPE caused fluid accumulation in ileal loops, and, when administered intragastrically to HDCD piglets, led to transient diarrhea. Diarrhea and death in HDCD pigs were prevented by giving serum, milk, or colostrum from sows immunized against enterotoxin, and conventional pigs born to the same sows were protected against oral challenge with an enterotoxigenic strain. Parenteral inoculation with CPE does not stimulate protective antibodies, but pigs naturally infected with enterotoxigenic strains produce anti-CPE antibodies. Furthermore, protection of neonatal pigs by colostrum from immunized dams suggests that commercial products for immunization of pigs against clostridial enteric disease might benefit from inclusion of some form of CPE toxoid.

Diagnosis

Diagnosis should begin with a thorough evaluation of herd history and current clinical signs. Typically affected, untreated pigs should be submitted for postmortem examination. Clinical signs and gross lesions allow a tentative diagnosis in type-C infections, but diagnosing type-A infections is more complex (see below). Appropriate specimens (Table 2) should be fixed in formalin for histopathologic examination or submitted fresh for microbiologic examination.

Bacteriologic culture for *C. perfringens* is straightforward. Scrapings obtained from the mucosa with a sterile microscope slide are first examined by Gram staining. It is frequently possible to see large numbers of Gram-positive or Gram-variable rods, and it is not uncommon to observe spores. *Clostridium perfringens* organism is perhaps the most oxygen-tolerant of the pathogenic clostridia, and growth can occasionally be observed on aerobically-incubated plates in the presence of extensive growth of facultative organisms. Thus, it is not absolutely essential that solid media be freshly prepared. Typically, cultures would be made on an agar medium containing 5% bovine or ovine blood, incubated in an oxygen-free atmosphere in a jar or anaerobic chamber. The atmosphere may be generated by commercially-available reagent envelopes or by evacuation and refilling with oxygen-free gases. Identification of *C. perfringens* is facilitated by the nearly invariable production of a double zone of hemolysis. About 2% of strains do not produce θ toxin, and, thus, have no clear inner zone of

Table 2

Specimen selection for diagnosis of clostridial enteritis in nursing and weanling pigs

Activity	Specimen
Bacteriologic culture	Scrapings of affected tissue or loops of fresh gut on ice
Histopathology	Formalinized small intestine
Genotyping	Isolate(s) from affected tissue (not feces)
Preparation of autogenous toxoids	Genotyped isolate; if type A, there should be isolate present in high numbers

hemolysis. Lack of the outer zone of hemolysis, which is produced by α toxin, is extraordinarily rare. Gram stains of colonies should reveal Gram-positive or Gram-variable rods (depending upon the age of the culture), rarely with spores.

There is little evidence that quantitation of *C. perfringens* is useful as a diagnostic aid. However, culture of dilutions of mucosal scrapings will allow isolation of the strain of *C. perfringens* that is present in highest numbers. In disease caused by type A, this will increase the chances of isolating the etiologic strain, rather than a "normal flora" strain. Given the current state of knowledge of pertinent virulence attributes within type A, this may be most important if the producer or practitioner wishes to prepare and use an autogenous toxoid.

Demonstration of toxins by in vivo assays is no longer a common practice. Culture supernatant fluids or eluates from gut contents (trypsin-treated or untreated, neat or mixed with antiserum) are examined in mice (injected intravenously) for lethality or guinea pigs (injected intradermally) for dermonecrosis. Detecting toxins in clinical specimens does not necessarily confirm the existence of disease, and false negatives may occur due to the lability of these proteins, especially β toxin. CPE can be detected by cytotoxicity assays and immunoassays, and the latter are now commercially available. The commercial reverse-passive latex agglutination test is apparently subject to false positives, but the enzyme immunoassay is significantly more accurate. The missing element in the array of diagnostic tools is a simple in vitro method by which to detect α , β , and ϵ toxins.

Use of polymerase chain reaction (PCR) to genotype field isolates has provided new opportunities to understand the natural history of porcine clostridial enteritis, and provides a powerful tool to generate information on which to base prevention and control strategies. Template DNA is obtained from isolates of *C. perfringens*, and segments of the genes for the major toxins, plus CPE, are amplified. Detection of specific products allows placement of an isolate into one of the five genotypes. Silent toxin genes are apparently rare in isolates from domestic animals, and the correlation of genotype (presence of toxin genes) with phenotype (production of toxins) is nearly 100%. An exception is type-E strains, which are occasionally isolated from calves with hemorrhagic enteritis, and which carry a silent copy of CPE. The availability of PCR genotyping is increasing.

If isolates of genotype C are obtained from pigs with typical lesions in nonvaccinated herds, diagnosis is unequivocal. Current evidence suggests that commercial type-C toxoids are effective in preventing enteritis and enterotoxemia associated with this toxigenic type, but the possibility of intragenotype variability in virulence attributes makes it wise to

retain isolates against the possible need to produce an autogenous toxoid.

If isolates of genotype A are obtained, if clinical signs and lesions are compatible, and if other etiologies (viruses, parasites, such as *Isospora suis*, and other bacteria, such as *Escherichia coli*) are ruled out by concurrent microbiologic examination, a diagnosis of type-A enteritis should be considered. A positive enzyme immunoassay for CPE in gut contents is supportive, but most type-A isolates causing enteritis do not produce CPE. Unfortunately, limitations in current knowledge and available diagnostic technology do not allow positive confirmation of the type-A etiology; thus one must take a Sherlock Holmes-like approach to finding an answer by eliminating the alternate possibilities. Perhaps the most meaningful confirmation of the occurrence of type-A enteritis is decreased incidence in the face of vaccination with a carefully-prepared autogenous toxoid. However, the overall utility of autogenous toxoids can be difficult to evaluate, since other management changes are often made concurrently.

Prophylaxis and therapy

Type-C infections can usually be prevented by vaccinating sows with a commercial toxoid, and there is anecdotal evidence that autogenous toxoids prepared against type-A strains are useful. Immunization of sows must be linked to efforts to maximize the quality and quantity of colostrum uptake. *Clostridium perfringens* is susceptible to many antimicrobials, and prophylaxis of type-C disease in swine has been achieved by use of feed-grade bacitracin (at 250 g per ton of feed), lincomycin, and others, from 2 weeks pre-farrowing through the course of lactation. One would predict a similar positive effect against type-A infections. Treating individual piglets with oral or injectable antimicrobials has often been successful, but the high rate of recovery in untreated pigs makes it difficult to interpret anecdotal reports.

Beyond this, heightened attention to sanitation is important, including cleaning the sow prior to farrowing, and routinely disinfecting farrowing houses and crates.

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