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Toxigenic *Clostridium perfringens* from a Parvovirus-Infected Dog

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A strain of *Clostridium perfringens*, type A, has been isolated from the intestine of a dog which died from parvovirus infection. This *Clostridium* strain produces a toxin which can be detected by counterimmunoelectrophoresis, using *C. difficile* antitoxin, and produces cytotoxicity in WI-38 cell culture. Cytopathology can be blocked by *C. difficile* antitoxin. Its role in canine parvovirus infection is unknown.

A recent report from our laboratory (4) demonstrated the utility of counterimmunoelectrophoresis (CIE) for the rapid detection of *Clostridium difficile* toxin in human feces. The test is sensitive and allegedly specific, there being no reports of bacterial antigens which cross-react with the rabbit antitoxin to *C. difficile* (2). The U.S. standard *Clostridium sordellii* antitoxin, however, has been shown to cross-react with the toxin of *C. difficile* (1). Welch et al. (5) used *C. sordellii* antitoxin to identify *C. difficile* isolates by CIE and reported immunological cross-reactions with *C. sordellii* and *Clostridium bifermentans* but no cytoxicity caused by the clostridia in WI-38 cell cultures. The one strain of *C. perfringens* tested (5) reacted in neither CIE nor the cell culture.

Five dogs with fatal canine parvovirus infection were examined at necropsy. The pathological results and clinical signs were consistent with acute parvovirus infection of the gastrointestinal system. Intestinal contents and a portion of the small intestine were collected and brought immediately to the microbiology laboratory. The contents of the small intestine were suspended in an equal volume of phosphate-buffered saline, pH 7.2, and the tissue was minced in the same buffer. Both the intestinal contents and the tissue were cultured on 7% sheep blood agar and cefoxitin-cycloserine agar incubated in an anaerobic atmosphere (GasPak; BBL Microbiology Systems, Cockeysville, Md.). Although no *C. difficile* cells were recovered from any of the dogs, large numbers of *C. perfringens* cells were observed on BAP from two of five dogs. In five of five dogs, a precipitin line was observed when both intestinal contents and tissue were tested with *C. difficile* antitoxin (T. Wilkins, Virginia Polytechnic Institute, Blacksburg, Va.). In all cases, these reactions could be blocked with prior treatment of the specimens with *C. difficile* antitoxin. None of the initial specimens from the five dogs was tested in cell culture. Similarly, no other fecal isolates were tested for toxin production by either CIE or cell culture.

The two isolates of *C. perfringens* were repurified and grown in chopped meat glucose broth for 48 h under anaerobic conditions. The culture supernatants were centrifuged at 10,000 × *g* for 30 min and tested for *C. difficile* toxin by both CIE and cell culture techniques. Toxin was detected in one of the isolates by both methods.

The activity of this toxin from the *C. perfringens* isolate was blocked in WI-38 cells by preincubation with *C. difficile* antitoxin. Similarly, formation of an immune precipitate was blocked in CIE by preincubation with antitoxin. Substitution of the *C. difficile* antitoxin by pentavalent gas gangrene antitoxin (Lederle Laboratories, Pearl River, N.Y.) resulted in similar protection.

The toxigenic isolate of *C. perfringens* was type A and toxigenic for mice. The type A toxin was neutralized by *C. perfringens* type A antitoxin at the Centers for Disease Control, Atlanta, Ga. Whether there is a single cross-reacting toxin or multiple toxins present is unknown. Poxton and Byrne (3) have recently shown that *C. difficile*, *C. sordellii*, and *C. bifermentans*, but no other clostridial species tested, possess some common surface carbohydrate antigens. Although it is possible that the CIE positive reactions with *C. perfringens* may be due to these cross-reacting surface carbohydrates, it is unlikely that these carbohydrates would be cytotoxic.

It is premature to speculate on the role played by clostridia in canine parvovirus infection. However, a toxin synthesized by *C. perfringens*, type A, biologically and immunologically similar

to *C. difficile* toxin, has been isolated from the intestine of a dog with clinical and pathological evidence of parvovirus infection. Whether such clostridia produce gastrointestinal disease in humans is unknown.

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