

## ***Clostridium perfringens* type A fatal acute hemorrhagic gastroenteritis in a dog**

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**Abstract** – The morning after participating in a dog show, a 2-year-old Pomeranian dog was found dead in a pool of bloody feces. Necropsy revealed hemorrhagic gastroenteritis of the entire gastrointestinal tract, with many Gram-positive bacilli on the surface and in the lumen and crypts of the intestine. Enterotoxin-positive type A *Clostridium perfringens* were isolated in large numbers. This dramatic case of fatal *C. perfringens* gastroenteritis highlights the need to better understand the role of this bacterium in enteric disease of dogs.

**Résumé** – Gastroentérite aiguë mortelle à *Clostridium perfringens* chez un chien. Le matin après avoir participé à une exposition canine, un chien Poméranien âgé de 2 ans a été trouvé mort dans une mare de fèces sanglantes. La nécropsie a révélé une gastroentérite hémorragique de l'ensemble du tube digestif, avec de nombreux bacilles à Gram positif sur la surface et dans la lumière et les glandes de l'intestin. Une quantité importante du bacille *Clostridium perfringens* de type A positif pour les entérotoxines a été isolée. Ce cas tragique de gastroentérite mortelle à *C. perfringens* souligne le besoin de mieux comprendre le rôle de cette bactérie dans la maladie entérique chez les chiens.

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**A** 2-year-old female Pomeranian dog was found dead in a pool of bloody feces the morning after it had been at a dog show. The previous day the dog was bright, alert, responsive, eating, and drinking. It had been fed a commercial diet. The dog was in good health; it had no history of vomiting or diarrhea. The dog had not been treated recently and was up-to-date on vaccinations. The owners were concerned about the possibility of poisoning so they submitted the body for necropsy to the Animal Health Laboratory (AHL) in Guelph, Ontario.

Necropsy revealed acute hemorrhagic gastroenteritis. Externally, the dog was in good condition except that the hair of the hind legs was matted in red fecal material. The serosal and mucosal surfaces of the entire gastrointestinal tract were diffusely hemorrhagic, with watery-red intestinal contents. Histopathology of the stomach, and small and large intestine revealed many Gram-positive clostridia-like bacilli on the surface of the mucosa and in the lumen and crypts of the small and large intestine (Figure 1). The mucosa of the villi in the small intestine appeared necrotic as did the superficial mucosal glands

in the stomach. The sub-mucosal vessels of the stomach and the colon were congested. The red pulp of the spleen was markedly congested. There was marked pulmonary congestion. Bacterial culture from the ileum isolated large numbers of *C. perfringens* on Shahidi-Ferguson Perfringens (SFP)-egg yolk agar plates (Becton Dickinson, Sparks, Maryland, USA) incubated overnight in anaerobic conditions at 37°C. Individual colonies were sub-cultured on blood agar plates (Becton Dickinson) incubated overnight in anaerobic conditions at 37°C. No *Campylobacter* spp. or *Yersinia* spp. were isolated from the ileum on selective media. No *Salmonella* spp. were isolated from the ileum on selective media after the use of an enrichment medium.

The *C. perfringens* isolates were type A, genotyped using a real-time polymerase chain reaction (PCR) assay based on the method of Albini et al (1) with the addition of primers and probes for the *netB*, *tpeL*, and atypical *cpb2* toxin genes. Five of six colonies from the same plate were positive for the atypical  $\beta_2$  (*cpb2*) toxin gene and the enterotoxin (*cpe*) gene, but were negative for the *cpb*, *etx*, *itx*, and consensus  $\beta_2$  (*cpb2*) toxin genes. The other isolate was Type A, positive for the alpha (*cpa*) toxin gene only. Immuno-histochemistry (IHC) using rabbit polyclonal antibody for CPB2 toxin on intestinal sections was negative (figure not shown). Based on these findings a diagnosis of acute hemorrhagic gastroenteritis due to *C. perfringens* type A infection was made.

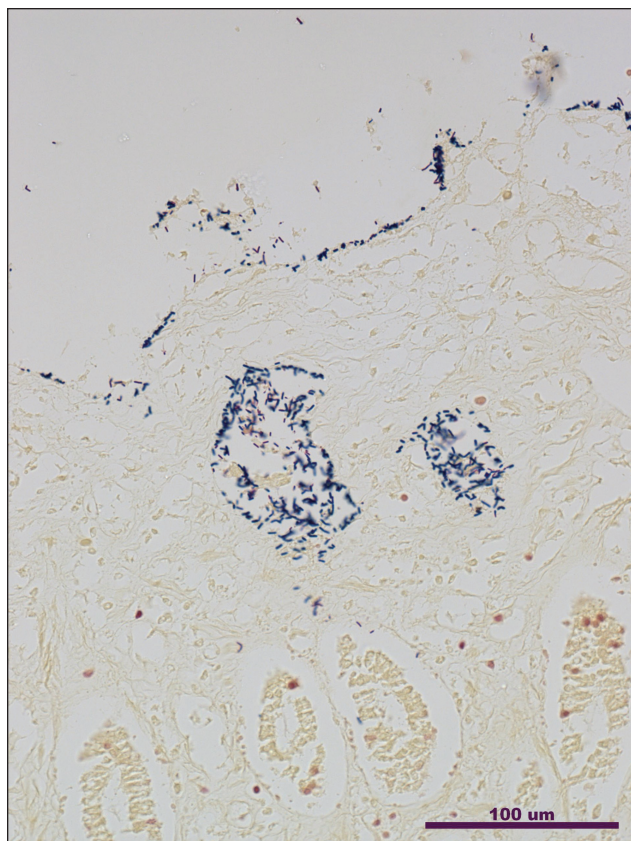
*Clostridium perfringens* is separated into 5 types (A through E) based on the production of alpha, beta, epsilon, and iota toxins;  $\beta_2$ , enterotoxin and several other important toxins have also been identified and may be produced by any type (2). Type A *C. perfringens* is a normal inhabitant of the environment and

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**Figure 1.** Gram-stained section of the small intestine from the case of hemorrhagic gastroenteritis in a dog showing large numbers of clostridia-like bacilli on the surface of the mucosa and within the crypts (Gram stain, 20 $\times$ ).

gastrointestinal tract of many animals including dogs (2), since it can be cultured from more than 80% of both diarrheic and non-diarrheic dogs (3). *Clostridium perfringens* type A has been implicated in a wide range of enteric diseases including human food poisoning, abomasitis and enteritis in ruminants, and necrotic enteritis of broiler chickens (2). In recent years, additional toxins have been recognized in *C. perfringens* type A, including NetB (3) and TpeL (4), so that the full range of toxins associated with this type is probably under-recognized.

*Clostridium perfringens* type A-associated diarrhea and enteric disease in dogs is not well-characterized, but may range in severity from mild and self-limiting to the fatal acute hemorrhagic diarrhea seen in the current case (5). Acute hemorrhagic gastroenteritis associated with *C. perfringens* type A infection in dogs has been described previously, and is characterized by severe inflammation of the gastrointestinal tract, and hemorrhage and rapid death similar to the current case (6,7). The presence of large numbers of clostridia-like bacilli, identified as *C. perfringens*, adhering to mucosal surfaces (Figure 1) is a striking finding common to these cases (6,7). Isolates from previous case reports were not genotyped for the range of toxin genes tested for in the present study, and the current study reports for the first time the presence of the *cpe* and atypical *cpb2* genes in isolates from a case of fatal infection.

Understanding of the role of *C. perfringens* in diarrheal illness in dogs is incomplete, although less severe diarrheal

illness attributed to *C. perfringens* has been described (5). No gold standard for the diagnosis of diarrhea associated with *C. perfringens* exists, so veterinarians must carefully consider the clinical signs and molecular diagnostic evidence, and the absence of other pathogens, before making a diagnosis (5). In the present case, clinical signs, the presence of large numbers of clostridia-like bacilli on the surface of the mucosa in the small and large intestines, the necrotizing inflammation typical of severe *C. perfringens* enteritis seen in numerous other species (5), the heavy growth of *C. perfringens* from the ileum and the lack of isolation of other pathogens from the gastrointestinal tract all support a causative role for *C. perfringens*. The sudden death caused by this infection was so striking that the dog was initially assumed to have been poisoned at the dog show. What precipitated the infection is not clear. There was no history of unusual feeding. In other species, there is an association between colostrum- or food-associated trypsin-inhibition (which prevents trypsin breaking down secreted toxin) and severe *C. perfringens* enteritis (2), but such a possibility of food-associated infection is purely speculative.

Type A strains isolated in the present study were *cpe* and atypical *cpb2* toxin gene positive. *Clostridium perfringens* enterotoxin (CPE) production is associated with sporulation and is released upon lysis of vegetative cells, acting as a cytotoxic enterotoxin that causes tissue damage (8). Experimental canine models have shown CPE to induce fluid accumulation and diarrhea (9); however, the method by which CPE causes diarrhea is unclear (8). The presence of CPE in the feces of dogs is associated with diarrhea, but a small percentage of dogs without diarrhea also have CPE in their feces (8,10). The presence of the *cpe* gene has been associated with diarrhea in dogs, but the strongest association with diarrhea is shown when the toxin itself (and not just the *cpe* gene) is detected (10). The present study detected the gene for *cpe* but testing for CPE was not done. The established association of *cpe* with canine diarrhea and its presence in most isolates suggests that it may have played a role in the disease. This needs to be investigated further. Since both *cpe* and *cpb2* are commonly found on a plasmid (11) and since *C. perfringens* is known to carry multiple virulence-associated plasmids at a time, it is not possible to rule out the presence of other plasmid(s) carrying other as-yet-unknown virulence gene(s) contributing to the disease in this dog. The *cpb2* gene, in both the consensus and atypical variants, has been detected in *C. perfringens* type A isolates from healthy and diarrheic dogs in previous studies, and in combination with *cpe* (12–14). Jost et al (12) found a high rate of the atypical allele of *cpb2* in *cpb2*-positive *C. perfringens* type A canine isolates. Atypical *cpb2* may not be expressed *in vivo* (11), as was the case in this dog. Previous studies (13,14) examining the presence of *cpb2* in isolates of *C. perfringens* type A from diarrheic dogs have had small sample sizes, lacked control groups of healthy dog isolates (13) and have not differentiated between atypical and consensus alleles of *cpb2*. As a result there is no current evidence to support the role of *cpb2*-toxigenic *C. perfringens* type A strains in canine diarrhea (2).

Further research involving the characterization and genotyping of *C. perfringens* type A isolates from similar clinical cases

would be helpful in elucidating the role that *C. perfringens* type A virulence factors play in the pathogenesis of this apparently rare but serious disease.

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