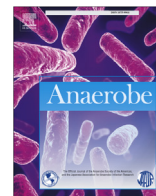




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Clostridium perfringens type A *netF* and *netE* positive and *Clostridium difficile* co-infection in two adult dogs



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ABSTRACT

The aim of this study was to report two cases of *Clostridium perfringens* type A and *Clostridium difficile* co-infection in adult dogs. Both animals were positive for A/B toxin. Toxigenic *C. difficile* and *C. perfringens* type A positive for NetE and NetF-encoding genes were isolated. This report reinforces the necessity of studying a possible synergism of *C. difficile* and *C. perfringens* in enteric disorders.

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Clostridium perfringens type A and *Clostridium difficile* are commonly described as enteropathogens in dogs. However, the role of both agents remains incompletely characterized, and diagnoses of these infections are still a challenge. Regarding *C. perfringens* type A, the high prevalence of enterotoxin-encoding gene *cpe* in diarrheic dogs led to a suspicion that this additional virulence factor was responsible for the pathogenesis of canine *C. perfringens*-associated diarrhea. However, several studies have failed to confirm this hypothesis [11]. Recently, three pore-forming toxins (NetE, NetF and NetG) that are always present in *C. perfringens* type A *cpe*⁺ strains were described in association with fatal canine hemorrhagic gastroenteritis [2]. Until now, the study by Gohari was the only study in the literature on this topic. Thus, the predisposing factors and occurrence of this disease remain unclear.

The role of *C. difficile* in canine diarrhea is also incompletely characterized. Nonetheless, there are reports of chronic and acute diarrhea caused by A/B toxins of *C. difficile*. However, it remains unclear whether *C. difficile* is the primary or secondary agent that causes diarrhea in dogs [7,17]. In addition, several studies showed a high similarity between *C. difficile* isolates from humans and animals, suggesting a possible zoonotic transmission [3].

Despite the known importance of both enteropathogens, there are no descriptions of co-infection of these two *Clostridium* species

in dogs. Thus, the aim of this study was to report two cases of *C. perfringens* and *C. difficile* co-infection in adult dogs.

An 18-month-old male German Shepherd (dog 1) was presented to the Veterinary Hospital of Federal University of Minas Gerais (Belo Horizonte city, Brazil) with a history of bloody diarrhea for two days. The dog was fed a regular commercial diet, had no history of other diseases in the past few months and was up-to-date on vaccinations and deworming. During the clinical examination, a stool sample was collected directly from the rectum for a differential diagnosis of the most common enteropathogens in dogs. The animal was treated with trimethoprim/sulfadiazine, omeprazole and fluid therapy. On the second day after admission, there was no blood in the feces, but the animal was still diarrheic. The consistency of the fecal material gradually returned to solid with a normal odor. The animal was discharged from the Veterinary Hospital five days after admission.

The second dog (dog 2) was a 12-year-old, mixed breed female. Similar to dog 1, this animal was also fed a regular commercial diet, had no history of other diseases and was up-to-date on vaccinations and deworming. The owner reported a history of bloody diarrhea for two days. During the clinical examination, the animal was apathetic and severely dehydrated. The dog died a few minutes after admission while receiving fluid therapy. During the *post mortem* examination, the intestinal contents from the small intestine were collected. Fragments of this organ were sampled in 10% neutral-buffered formalin. After fixation, specimens were routinely

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processed for histopathology, sectioned to 4 µm and stained with hematoxylin and eosin.

The stool sample of dog 1 and the intestinal contents of dog 2 were submitted to the following laboratory exams: rotavirus detection by polyacrylamide gel electrophoresis followed by silver staining [4]; parvovirus, rotavirus, coronavirus and *Giardia lamblia* detection by a chromatographic immunoassay (Ecodiagnostica, Brazil); isolation and genotyping of *C. difficile* [15] and *C. perfringens* [2,12,19]; *C. perfringens* enterotoxin detection by an enzyme-linked immunosorbent assay (EIA) kit (RIDASCREEN® *C. perfringens* Enterotoxin - R-Biopharm, Germany); *C. difficile* A/B toxin detection by ELISA (*C. difficile* Tox A/B II - Techlab Inc., USA); culture for *Salmonella* spp. in Hektoen Enteric Agar and XLT4 agar (BioBrás®, Prodimol Biotechnology); isolation of *Escherichia coli* in MacConkey agar (BioBrás®, Prodimol Biotechnology), followed by a previously described PCR method [8] to investigate the following virulence factor genes: *eae*, *stx1*, *stx2*, *ehxA*, *aggR*, *ipaH*, *eltA*, *etlB*, *est*, and *stap*.

Both dogs had positive *C. perfringens* and *C. difficile* isolations and A/B toxins detection. However, they were negative for *C. perfringens* enterotoxin by the EIA. *E. coli* was also isolated, but all strains were negative for virulence factors by PCR. All other exams were negative. Therefore, the involvement of *Giardia* spp., *Salmonella* spp., rotavirus, coronavirus and parvovirus was ruled out.

C. perfringens strains were genotyped as type A and positive for *cpe*, *netE* and *netF* in both cases. Interestingly, the *C. perfringens* isolate from dog 1 was also positive for *netG*. Both *C. difficile* isolates were positive for toxin A- and toxin B-encoding genes (*tcdA* and *tcdB*) but negative for binary toxin gene (*cdtB*). PCR ribotyping was conducted as previously described [5]. Both strains were identified as O14/O20. Table 1 summarizes the laboratory findings from both dogs.

During the *post mortem* examination of dog 2, the small intestine was distended by a hemorrhagic fluid and gas content, with markedly reddish serosa (congestion). The mucosal surface was diffusely red and covered with reddish and viscous material. The microscopic evaluation was limited by autolysis of the organ. However, evidence of necrosis of the villi and hemorrhage in the lamina propria and intestinal lumen were observed. Numerous Gram positive rods were attached to the luminal surface of the denuded villi (Fig. 1). The lamina propria and submucosa were mildly expanded by edema, and the blood vessels of the submucosa were markedly congested.

To clarify whether dog 1 was permanently colonized by a *C. perfringens* *cpe*⁺ *netE*⁺ *netF*⁺ *netG*⁺ strain, a new stool sample was collected approximately eight months after the diarrhea episode. This second stool sample was positive for *C. perfringens* type A, but the isolated strain was negative for *cpe*, *netE*, *netF* and *netG*.

Gohari et al. [2] described an association of these three new *C. perfringens* pore forming toxins (NetE, NetF and NetG) in fatal cases of canine hemorrhagic diarrhea. Both dogs in the present report presented at a Veterinary Hospital with bloody diarrhea, but dog 1 recovered completely after a 3-day treatment. Despite the bloody diarrhea, this animal was clinically healthy and not severely dehydrated during the clinical examination. In contrast to the previous report by [2]; our findings suggest that a *C. perfringens*

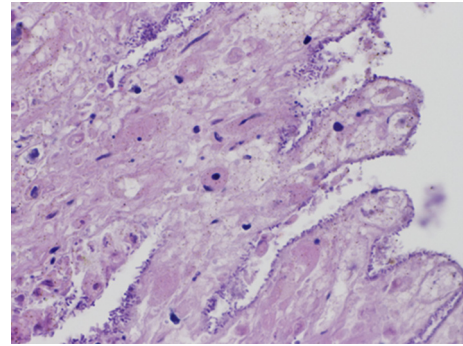


Fig. 1. Dog 2 – Duodenum. Large numbers of clostridia-like bacilli attached to the necrotic villi.

infection might be involved in less dramatic cases of hemorrhagic enteritis.

In contrast, the acute clinical evolution experienced by dog 2 and the *post mortem* findings corroborate the observations described in acute canine *C. perfringens* type A-associated diarrhea [7,9,17]. Regardless, comparisons of clinical evolution are still limited. This is only the second study on *C. perfringens* *netE* positivity in dogs. In the first description of this condition, the authors did not provide clinical details of the affected animals [2]. Some studies suggest that changes in feed is an important predisposing factor to trigger a *C. perfringens* type A infection in dogs and other species [7,11]. According to the owners, both dogs in the present case report were only fed a commercial diet. The absence of a known diet alteration or any other relevant predisposing factor corroborates the only study available thus far on *C. perfringens* *netE* positivity in dogs [2] and highlights the need for additional studies on this matter.

Both dogs were also positive for A/B toxins, and toxigenic strains were isolated, which suggested the involvement of *C. difficile*. Both strains were identified with PCR as ribotype O14/O20, which is recognized as one of the most common causes of *C. difficile* infection (CDI) in the European community. It also has been previously described in dogs in several countries [6,18]. In Brazil, ribotype O14/O20 was previously reported in several animal species, including domestic and wild animals, and also in humans with confirmed CDI [1,10,13,14].

C. perfringens infection in adult dogs is commonly linked to food or environmental changes, but no marked changes were reported by the owners in both cases described here. Specifically for *C. difficile*, antibiotic therapy is well known as the most common predisposing factor for CDI. However, in the present report, neither dog received any antibiotics before being sampled [16], described five cases of *C. perfringens* type C and *C. difficile* co-infection in foals. Similar to the two cases described here, none of the affected foals were on antibiotic therapy. In light of this, the authors suggested that the *C. perfringens* infection may have acted as a predisposing factor for *C. difficile* or vice versa. Anyway, Together, these two reports reinforce the need to study a possible synergism of

Table 1
Details of the two adult dogs with *Clostridium perfringens* type A and *Clostridium difficile* co-infection.

Dog	Age	<i>Clostridium perfringens</i>		<i>Clostridium difficile</i>			Outcome
		Genotyping ^a	EIA (CPE)	Isolation	Ribotyping	EIA (A/B)	
1	1.5 years	<i>cpe</i> ⁺ <i>netE</i> ⁺ <i>netF</i> ⁺ <i>netG</i> ⁺	–	A ⁺ B ⁺ CDT ⁻	O14/O20	+	Recovered
2	12 years	<i>cpe</i> ⁺ <i>netE</i> ⁺ <i>netF</i> ⁺	–	A ⁺ B ⁺ CDT ⁻	O14/O20	+	Death

^a Both *C. perfringens* isolates were also negative for *netB*, *tpel* and *cpb2*.

C. perfringens and *C. difficile* in enteric disorders.

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