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Short communication

# *Clostridioides difficile* infection in dogs with chronic-recurring diarrhea responsive to dietary changes

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ABSTRACT

Five dogs with chronic-recurring diarrhea were positive for *Clostridioides difficile* infection (CDI), but were unresponsive to treatment with metronidazole. One of these animals was subjected to a colonoscopy, which revealed eosinophilic infiltration of the colon. All five animals completely recovered after dietary changes. The present work suggests that CDI might occur in dogs with other intestinal alterations. In addition, this report suggests that dysbiosis should be considered in animals that have chronic-recurring diarrhea and test positive for *C. difficile*.



*Clostridioides* (previously *Clostridium*) *difficile* is recognized as the main infectious cause of antibiotic-associated diarrhea in humans worldwide [1]. In dogs, the role of this agent in diarrhea is incompletely characterized, and it remains controversial whether *C. difficile* is a primary or secondary agent [2,3]. It is commonly described as a community-acquired enteropathogen responsible for acute canine hemorrhagic diarrhea, but it is also reported by some authors as a cause of chronic disorders in dogs [4–7]. The predisposing factors associated with *C. difficile* infection (CDI) in dogs remain largely unknown. Antibiotic therapy is, so far, the only known risk factor for *C. difficile* colonization in dogs [8,9]. In the present work, five cases of chronic-recurring diarrhea in dogs with CDI have been reported. These animals did not respond to metronidazole therapy, but recovered after dietary changes.

The first animal (dog 1), an 18-month-old female Rottweiler, was brought to a veterinary clinic with a history of chronic diarrhea, including three or four episodes of hematochezia per week in the previous ten months. This dog was fed a regular commercial diet; the vaccination and deworming statuses were up-to-date. The owner reported that the diarrhea started around one month after starting cyclosporine treatment (2.0 mg/kg/q.d.) for atopic dermatitis (AD). Treatment was then changed to oclacitinib maleate (0.4 mg/kg/day (Zoetis, USA), and oral antibiotic therapy was prescribed (Table 1); however, the dog continued to have diarrhea.

During the clinical examination, a stool sample was collected directly from the rectum and screened for common canine enteropathogens. The following laboratory examinations were performed: chromatographic immunoassays to detect rotavirus, parvovirus, coronavirus, and *Giardia* sp. (Ecodiagnostica, Brazil); culture and genotyping of *C. difficile* [10] and *C. perfringens* [11–13]; enzyme-linked immunosorbent assay (EIA) (Ridascreen® *Clostridium perfringens* Enterotoxin - R-Biopharm, Germany) to detect *C. perfringens* enterotoxin; and EIA (*C. difficile* Tox A/B II - Techlab Inc., USA) to detect *C. difficile* A/B toxin. For the isolation of

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**Table 1**
Details of five adult dogs with chronic-recurring diarrhea and diagnosed with *Clostridium difficile*-infection (CDI).

| Dog | | | | Diarrhea | | Comorbidities | *C. difficile* isolates | | |
|---|---|---|---|---|---|---|---|---|---|
| Number | Breed | Age (months) | Antibiotic therapy[a] | Stool type | Frequency | | Toxin types | Ribotype | MLST |
| 1 | Poodle | 18 | AMC and STP | Bloody | 3-4 episodes per week | Atopic allergy | $A^+B^+CDT^-$ | 014/020 | ST2 |
| 2 | Mixed breed | 48 | STP | Bloody | Daily | – | $A^+B^+CDT^-$ | 012 | ST54 |
| 3 | Bichon Frise | 25 | STP and MET | Mushy | Daily | Atopic-dermatitis-like lesions | $A^+B^+CDT^-$ | 106 | ST42 |
| 4 | Mixed breed | 24 | STP | Mushy | 3-4 episodes per week | – | $A^+B^+CDT^-$ | 106 | ST42 |
| 5 | Mixed breed | 38 | AMC and STP | Mushy | Daily | – | $A^+B^+CDT^-$ | 014/020 | ST2 |

Legend: AMC - Amoxicillin/clavulanic acid; STP - Trimethoprim/Sulfamethoxazole; MET - Metronidazole.
[a] Antibiotic therapy before diagnosis of *C. difficile* infection.

*Salmonella* spp., stool samples were enriched in Tetrathionate Broth (Oxoid, USA) and plated on Hektoen Enteric Agar (BD, Germany) and XLT4 agar (Prodimol Biotechnology, Brazil) [7]. Samples were inoculated on MacConkey agar (Prodimol Biotechnology, Brazil), and lactose-fermenting colonies were subjected to PCR to detect the common virulence genes of diarrheagenic *E. coli* for pathotyping [14].

The stool sample from dog 1 tested positive for A/B toxins, and a toxigenic *C. difficile* strain (A + B + CDT-) was isolated (Table 1), confirming CDI. In addition, testing of all virulence factors for *E. coli* revealed negative results. *C. perfringens* type A enterotoxin-negative and NetF-encoding genes were isolated; they can be considered as part of the normal microbiota. The stool sample tested negative for all other enteropathogens.

Considering the laboratory results, this animal was treated with metronidazole (20 mg/kg – b.i.d.) for 14 days. According to the owner, the episodes of diarrhea were less frequent, (one per week) and no blood was visible in the feces during this time. One diarrheic stool sample was collected in the second week of treatment and tested negative for both A/B toxins and *C. difficile*. Despite the clinical improvements, the dog was returned to the clinic with hematochezia a few days after completion of the antibiotic therapy. Another stool sample was collected and tested positive for A/B toxins and toxigenic *C. difficile*. Since the owner did not agree to a colonoscopy examination of the animal, treatment consisting of a second round of metronidazole and a probiotic (*Saccharomyces boulardii*; Floratil, Merck, Germany) was prescribed.

The dog again experienced diarrhea, although less frequently and without visible signs of blood in the feces, during the treatment regimen. However, the clinical signs intensified a few days after the end of the treatment. The owner finally agreed to submit the dog to a colonoscopy examination. No macroscopic alterations were seen, but a biopsy revealed eosinophilic inflammation of the colon. The dog's regular diet was immediately changed to a commercial feed commonly used for the treatment of food allergy in dogs (Hypoallergenic Canine, Royal Canin, France), and the feces became normal within a few days. A stool sample collected one month later tested negative for A/B toxins and *C. difficile*. After one year of follow-up, dog 1 remained healthy and there were no clinical signs of relapse.

Following this first case, four other animals (dogs 2 to 5) that had a history of chronic-recurring diarrhea unresponsive to previous antibiotic therapy (Table 1) were presented at three veterinary clinics. In all dogs, the vaccination and deworming statuses were up-to-date. Similar to dog 1, dog 3 also developed recurrent diarrhea five months after unsuccessful treatment with glucocorticoids for AD-like lesions, while the remaining animals had no history of other diseases. It is important to note that dog 2, which also presented with hematochezia, was fed a raw chicken-based diet. The other animals (dogs 1 and 3 to 5) were fed a regular commercial diet largely based on meat and bone meal, whole-wheat flour, ground yellow corn, and a chicken by-product meal.

As in the case of dog 1, stool samples from these four animals were submitted for differential diagnoses of the most common enteropathogens in dogs, and CDI was confirmed in all cases (Table 1). Initial treatments based on metronidazole were commenced in all cases. Dogs 2 and 3 were partially responsive to treatment, while no improvements of the clinical signs were observed in dogs 4 and 5. The owners did not agree to their dogs undergoing colonoscopy examinations; thus, the commercial diet adopted for dog 1 (Hypoallergenic Canine, Royal Canin, France) was prescribed for dogs 2, 3, and 4 as well. In the case of dog 5, for financial reasons, the owner adopted a diet based on cooked rice and chicken meat. All animals recovered in less than one week after starting the new diets. After around 10 days, stool samples were again collected; *C. difficile* was not isolated from any of the samples. The owners reported no relapses among the animals six months after the new diets were adopted.

It is important to note that three owners (of dogs 1 to 3) reported that their animals experienced a reduction in the clinical signs during treatment with metronidazole, including decreased evacuation frequency and absence of hematochezia. However, in all cases, the clinical signs returned—including hematochezia in dogs 1 and 2—a few days after completion of antibiotic therapy. In addition, at the end of metronidazole treatment, A/B toxin was detected in stool samples from all five animals. These observations suggest that *C. difficile* was not the primary cause of diarrhea, but was likely responsible for the worsening of diarrheic symptoms.

Potential causes of chronic diarrhea in dogs include food allergy, bacterial or parasitic infections, and inflammatory or neoplastic conditions [6,15]. *C. difficile* has been reported in some studies to be one of the possible bacterial agents [2,4–6], although no studies to date have confirmed this hypothesis [3]. It is recognized that disruption of the gut microbiota is the main predisposing factor for colonization by *C. difficile* [8], and recent studies have shown dysbiosis in dogs with many gastrointestinal diseases including chronic diarrhea [15]. One known consequence of gut dysbiosis is alteration in bile acids, which can facilitate the germination of *C. difficile* spores [16,17]. In the present study, five dogs with chronic-recurring diarrhea were diagnosed with CDI. In one animal (dog 1), eosinophilic inflammation of the colon was confirmed via biopsy. This condition is commonly reported in dogs with food allergies and inflammatory bowel disease (IBD) [18,19]. Thus, it seems reasonable to hypothesize that dysbiosis associated with eosinophilic inflammation might have led to *C. difficile* colonization and toxin production in this case. Curiously, human patients with IBD and other inflammatory conditions, including celiac disease, are known to have a higher incidence of CD [20–22].

In the present study, two animals were previously diagnosed with AD (dog 1) or had atopic-dermatitis-like lesions (dog 3). Interestingly, AD was recently implicated in dysbiosis and intestinal inflammation in dogs [23]. Unfortunately, these diagnoses were determined at other institutions, and there is limited information on the protocols used. Food allergy can cause skin lesions that are

identical to those of AD, and thus, it is possible that the chronic-recurring diarrhea and skin lesions observed in these animals were associated only with food allergy. It is also possible that AD and food-responsive diarrhea occurred concomitantly, as previously described in other studies on dogs [24,25]. In addition, it is important to note that dog 1 developed CDI around one month after starting treatment with cyclosporine. Diarrhea is commonly reported after treatment with cyclosporine in human patients, and some cases are associated with CDI. Nonetheless, this symptom usually disappears when the use of cyclosporine is interrupted or after antibiotic treatment for CDI [26], which is in contrast to our results with dog 1.

It is also important to note that none of the five dogs received a laboratorial diagnosis of CDI during the first episode of diarrhea, and antibiotic therapy was prescribed before any laboratorial diagnosis of enteropathogens was attempted. Thus, association of intestinal inflammation and antibiotic therapy, which is a known risk factor for CDI [8], is also possible in these cases.

Several authors include both clostridial infections and diet-responsive colitis in the list of differential diagnoses for chronic-recurring canine diarrhea, but an association between these two etiologies has never been reported [5,6]. Moreover, a diagnosis of food-responsive diarrhea is usually determined by response to treatment after the exclusion of other causes, including CDI [27]. In this situation, it is possible that diarrhea occurring after treatment in dogs diagnosed with CDI could be wrongly considered as refractory or recurring CDI; however, the present work suggested that *C. difficile* colonization was secondary to other causes. In addition, although it is not always possible to diagnose CDI in clinics, the protocols for the treatment of chronic-recurring canine diarrhea commonly include dietary modifications in combination with antibacterial therapy [28,29]. Thus, it is possible that cases of food-responsive CDI are underdiagnosed.

To obtain information on *C. difficile* isolates, one strain from each dog was submitted to PCR ribotyping as previously described [30] and Multilocus Sequencing Typing (MLST) as proposed by Griffiths et al. [31]. Analyses of sequences were made in Unipro UGENE 1.28 (UniPro, Russia), and MLST profiles were obtained from the public database (https://pubmlst.org/cdifficile/). Three ribotypes (RTs) and strain types (STs) (Table 1), which have all been described in human patients with CDI around the world, were obtained [32,33]. Our results corroborate the findings of recent studies, which have shown substantial similarities between human and dog *C. difficile* isolates, suggesting possible zoonotic transmission [34,35].

The present study indicated a possible association between CDI and food-responsive diarrhea in dogs. In addition, this report suggests that dysbiosis should be considered in animals that have chronic-recurring diarrhea and test positive for *C. difficile*.

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