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MICROBIOLOGY

Fecal microbiota transplantation via colonoscopy in a dog with *Clostridioides* (*Clostridium*) *difficile* infection

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ABSTRACT: *In dogs, antimicrobial therapy for Clostridioides (Clostridium) difficile infection (CDI) is based solely on metronidazole, leaving limited treatment options in case of recurrent disease. Fecal microbiota transplantation (FMT) has been successfully used in humans with recurrent CDI, whereas the usefulness of this approach is largely unknown in dogs. In the present study, a dog with a chronic-recurring diarrhea was treated with FMT via colonoscopy. CDI was confirmed by A/B toxin detection and isolation of toxigenic C. difficile from ribotype 106, a strain also commonly associated with nosocomial infection in humans. The dog recovered well after the procedure and C. difficile was no longer isolated from its stool sample. The present research suggested that FMT could be a useful tool to treat recurrent CDI in dogs, corroborating the actual protocol in humans.*
Key words: *canine diarrhea, FMT, colitis.*

Transplante de microbiota fecal via colonoscopia em um cão com infecção por *Clostridioides* (*Clostridium*) *difficile*

RESUMO: *Em cães, a terapia antimicrobiana para infecções por Clostridioides (Clostridium) difficile é baseada apenas no uso de metronidazol, limitando as opções de tratamento nos casos de recorrência. O transplante de microbiota fecal (FMT) tem sido utilizado com sucesso em seres humanos com infecções recorrentes por C. difficile, porém a utilidade desse método é ainda amplamente desconhecida em cães. O presente trabalho relata a utilização de FMT para o tratamento de um cão com diarreia crônica-recorrente por C. difficile. A infecção foi confirmada por detecção das toxinas A/B e isolamento de uma estirpe toxigênica do ribotipo 106, linhagem comumente associada a infecção em seres humanos. Após o transplante via colonoscopia, o animal se recuperou do quadro e C. difficile não mais foi encontrado em novas amostras fecais. O presente trabalho sugere que o FMT possa ser útil para o tratamento de quadros de C. difficile em cães, corroborando protocolo atual de tratamento em seres humanos.*
Palavras-chave: *diarreia, FMT, colite.*

Clostridioides (Clostridium) difficile infection (CCD) is the main cause of nosocomial diarrhea in humans (DE ROO et al., 2020). In dogs, it has been identified as the etiological agent of 10–21% of cases of diarrhea worldwide with clinical signs varying from chronic-recurring to bloody life-threatening diarrhea (WETTERWIK et al., 2013; DINIZ et al. 2018). Studies have suggested that CDI can occur in dogs as a primary disease (DINIZ et al., 2018) or secondary due to allergic or inflammatory gut disease or even in association with other enteropathogens (SILVA et al., 2017; DINIZ et al., 2018; SILVA et al. 2018).

In addition to its possible role as an enteropathogen in dogs, *C. difficile* isolates from dogs have a high genetic similarity with those recovered from infected humans, raising the possibility of a zoonotic disease with animals playing a role in increasing cases of community acquired CDI in humans (RODRIGUEZ et al. 2016; WEESE et al. 2020).

The treatment of CDI in humans is mostly based on antimicrobial therapy with metronidazole, vancomycin or, less frequently, fidaxomicin (ASLAM et al., 2005). Regardless of which antimicrobial is used, 10 to 30% of the patients have a recurrence of the infection, which is partially due to the inability

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of their microbiota to quickly recover and prevent a new CDI episode. In these cases, fecal microbiota transplantation (FMT), or the "transfer of intestinal contents from a healthy donor to a diseased recipient", has been successfully used in humans (BORODY & KHORUTS, 2011; CHAITMAN et al. 2020). In dogs, antimicrobial therapy for CDI is based solely on metronidazole (MARKS et al., 2011). Interestingly, among the three main antimicrobials used for CDI in humans, metronidazole has the lowest cure rates (DI XIUZHEN et al., 2015). Despite the limited options for treatment of infected dogs, there are only two reports of oral FMT for treatment of CDI in animals: one in a dog (SUGITA et al., 2019) and another in a marmoset (YAMAZAKI et al., 2017). In the present research, we described, for the first time, a successful FMT via colonoscopy in a dog with chronic-recurring diarrhea associated with CDI.

In March 2018, a 4-year old female Golden Retriever was referred to the Veterinary Hospital of Federal University of Minas Gerais for consultation. The owner reported that the dog had been having chronic-recurring pasty large bowel diarrhea of two months duration. The dog had previously presented to two other clinics and the owners confirmed the historical use of metronidazole and amoxicillin with clavulanic acid. Deworming and vaccinations were up to date, the dog was fed with a homemade raw meat-based diet, and clinical examination revealed the animal was underweight but otherwise in good condition. Results of a complete blood count (CBC) and a serum biochemical analysis were normal.

To investigate an infectious cause of diarrhea, a stool sample was collected and submitted for evaluation of several enteropathogens. Commercial immunochromatographic tests were used to test for parvovirus (Alere, USA), rotavirus and coronavirus (Ecodiagnostica, Brazil) similar to previous studies (DINIZ et al., 2018). The presence of *Giardia* sp. was evaluated using a commercial enzyme immunoassay (RIDASCREEN® Giardia - R-Biopharm AG). *Clostridium perfringens* isolation was conducted as previously described (MEER & SONGER, 1997; SILVA et al., 2015) followed by the detection of the NetB-, NetE-, NetF and NetG-encoding genes (KEYBURN et al., 2008; GOHARI et al., 2015). For diagnosis of CDI, the stool sample was first subjected to immunochromatographic tests for detection of the *C. difficile* glutamate dehydrogenase (GDH). Samples with a positive result were then subjected to a previous described isolation protocol used to evaluate for the presence of *C. difficile* (SILVA et al., 2013). Colonies underwent PCR for a housekeeping gene (*tpi*), toxins A (*tcdA*) and B (*tcdB*), and a binary toxin gene (*cdtB*) (SILVA et al., 2011). The stool sample was also subjected to A/B toxin detection (*C. difficile* Tox A/B II - Techlab Inc., Blacksburg, VA, USA).

For *E. coli* isolation, stool samples were plated on MacConkey agar (Difco, USA). Up to three lactose-fermenting colonies were identified by PCR (MCDANIELS et al., 1996) and virulence genes associated with diarrhea were investigated as previously described (FRANCK et al., 1998; YAMAMOtO et al., 1997; GUNZBURG et al.,1995; TOKUDA et al., 2010). For the isolation of *Salmonella* spp., the stool sample was inoculated in Rappaport-Vassiliadis Broth (Oxoid, USA) followed by plating in Hektoen Agar (Oxoid, USA). Suggestive colonies (sulfite-reducing colonies) were submitted to PCR analysis (KWANG et al., 1996). The fecal flotation method was used for detection of nematode and cestode eggs (DE SANTIS et al., 2006).

The stool sample was positive for A/B toxins, and a toxigenic *C. difficile* strain was isolated confirming CDI in the patient. No other enteropathogens were detected. A recent study in our institution had already diagnosed CDI in dogs with chronic recurring diarrhea (SILVA et al., 2018). Interestingly, those animals were later diagnosed with alimentary allergies, suggesting that CDI was secondary to the dysbiosis caused by the associated inflammation of the intestines. Considering this possibility, the owner agreed to present the dog from the present study for a colonoscopy. The macroscopic findings suggested mild colitis—characterized by hyperemic areas in the ileocolic and cecocolic sphincters in the descending colon and in the rectum. In this last intestinal segment, a moderate amount of whitish fibrin filaments was observed. Samples of mucosa biopsies from the ascending colon, transverse colon, descending colon, and rectum were collected for later histopathological examination. Due to the macroscopic alterations seen in the colonoscopy and the confirmed laboratory diagnosis of CDI, the clinician proceeded with the FMT.

The donor fecal solution used in the FMT was previously prepared. First, a healthy donor was selected based on previous publications (CHAITMAN et al., 2020; CHAITMAN et al., 2016; REDFERN et al., 2017; PEREIRA et al., 2018). Briefly, a seven-year old dachshund dog without history of gastrointestinal disorder in the last six months and fed commercial dry food was invited to donate. The stool sample of the donor tested negative for all enteropathogens previously described in the present study. Approximately 65 g of donor´s

feces were diluted in 250 ml of sterilized phosphate buffered saline (REDFERN et al., 2017; PEREIRA et al., 2018) and, after filtration through a sterilized medical gauze pad (SUGITA et al., 2019), were stored in 60 mL syringes at -80 °C until the procedure, which occurred 14 days later. For the FMT, a total of 60 ml of the donor fecal solution was warmed to 36 °C and administered directly into the ascending colon using the working channel of the endoscope. The animal's pelvis was kept elevated with an orthopedic pillow for 20 minutes to prevent the fluid from escaping. All animal procedures in this study were approved by the Animal Experimentation Ethics Committee of the Universidade Federal de Minas Gerais (protocol number 109/2017).

Later, histopathological analysis of the samples collected via colonoscopy revealed a mild lymphoplasmacytic and neutrophilic colitis, corroborating the macroscopic findings and the laboratorial diagnosis of CDI. As previously planned, the dog returned 15 days after the procedure. The animal was in good condition and had had no diarrheic episodes since the FMT. A new fecal sample was collected and was negative for A/B toxins and for *C. difficile* isolation. Six months after the transplantation, the animal was still healthy and without relapse. This result is similar to that previously described by Sugita et al. (SUGITA et al., 2019) which reported the absence A/B toxins and *C. difficile* strains in stool samples collected 7, 36 and 124 days after oral FMT in a dog with CDI. Together, these results suggested that the FMT not only successfully treated the diarrhea associated with CDI these two dogs, but it also eliminated *C. difficile* colonization—thus preventing the animal from becoming an asymptomatic carrier of this possible zoonotic pathogen.

In order to better characterize this case, the isolate was submitted to multi-locus sequence typing (MLST) and ribotyping (GRIFFITHS et al., 2010; JANEZIC & RUPNIK, 2010). The isolate was from ribotype 106 and sequence type 42, which is frequently reported in dogs and is also one of the most prevalent ribotypes implicated in human CDI worldwide (JANEZIC et al., 2014; SILVA et al., 2015; CARLSON et al., 2019). This finding is accordance with other studies, highlighting the possibility of dogs being reservoirs of toxigenic *C. difficile* strains for humans (RODRIGUEZ et al., 2016; STONE et al., 2016).

Due to the possibility of zoonotic disease, the antimicrobial resistance of *C. difficile* isolated from dogs is also a matter of concern. In the last years, strains from dogs resistant to several antimicrobials, including metronidazole, have been reported (ORDEN et al., 2017; ANDRÉS-LASHERAS et al., 2018). The clinical inefficacy of the previous treatments with metronidazole in this dog also raises the possibility of colonization by a resistant strain. Thus, the minimal inhibitory concentrations (MIC) of metronidazole, vancomycin, clindamycin, moxifloxacin, rifampicin, tetracycline, and ciprofloxacin were determined by gradient test with M.I.C. Evaluator™ strips (M.I.C.E.™, Oxoid, USA). Interestingly, the isolate was susceptible to metronidazole and vancomycin—drugs of choice for treating CDI in humans— and to moxifloxacin, rifampicin, tetracycline. Conversely, the strain was resistant to clindamycin and ciprofloxacin—antimicrobials that are known to increase the risk factors for CDI development (PIRŠ et al., 2013; BANDELJ et al., 2017). This result rules out, at least partially, the hypothesis of recurrence due to infection by a metronidazole resistant strain. Nevertheless, it is important to remember that up to 30% of human patients with CDI have a recurrence after antimicrobial treatment despite being infected with metronidazole-susceptible strains, showing that the recurrence is more strongly linked to inadequate restoration of the patients' microbiota than the antimicrobial susceptibility of the strain (ASLAM et al., 2005; BORODY et al.,2011).

Previous studies have shown that FMT is effective for the treatment of recurrent CDI in humans in more than 90% of cases (CAMMAROTA et al., 2014). Although, not fully understood, it is believed that the rapid reestablishment of the microbiota is the main mechanism underlying the effectiveness of FMT in these cases (BORODY et al., 2011; KELLY et al., 2015). In fact, human patients with CDI are known to have a lower bacterial diversity (VAN NOOD et al., 2013). There are few studies on intestinal microbial composition of diarrheic dogs, and none were specifically done on animals with confirmed CDI (NIINA et al., 2019; NIEDERWERDER et al., 2018). Nevertheless, previous studies showed that dogs with chronic diarrhea, which was the case of the dog in the present study, have lower bacterial diversity in their fecal samples compared to healthy dogs (SUCHODOLSKI et al., 2012; MINAMOTO et al., 2014). FMT for treatment of infectious diarrhea deserves more investigation in animals. So far, there is one study involving dogs with parvovirus infection (PEREIRA et al., 2018) and two reports of oral FMT for treatment of CDI—in a dog and in a marmoset (YAMAZAKI et al., 2017; SUGITA et al., 2019), which all showing promising results. Together, these previous studies and the present research suggested that FMT might be a safe and useful tool for the treatment of CDI diarrhea in dogs. Further studies are

still needed to clarify the benefits of this procedure as well as determine indications and selection criteria for donors and recipients.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

All animal procedures in this study were approved by the institutional ethics committee of Universidade Federal de Minas Gerais (CEUA-UFMG), protocol 109/2017.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception. And read and approved the final manuscript.

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