Fecal microbiota transplantation as a new treatment for canine inflammatory bowel disease

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In human medicine, fecal microbiota transplantation (FMT) is an effective treatment for recurrent Clostridioides difficile infection. It has also been tested as a treatment for multiple gastrointestinal diseases, including inflammatory bowel disease (IBD). However, only a few studies have focused on the changes in the microbiome following FMT for canine IBD. Here, we performed FMT in nine dogs with IBD using the fecal matter of healthy dogs and investigated the subsequent changes in the fecal microbiome and clinical signs. In three dogs, the fecal microbiome was examined by 16S rRNA sequencing. Fusobacteria were observed at a low proportion in dogs with IBD. However, the post-FMT microbiome became diverse and showed a significant increase in Fusobacteria proportion. Fusobacterium was detected in the nine dogs by quantitative polymerase chain reaction. The proportion of Fusobacterium in the post-FMT fecal microbiome was significantly increased (p<0.05). The changes in clinical signs (e.g., vomiting, diarrhea, and weight loss) were evaluated according to the canine inflammatory bowel disease activity index. The score of this index significantly decreased in all dogs (p<0.05) with improvements in clinical signs. These improvements were related to the changes in the proportion of microbes, particularly the increase in Fusobacterium. The dogs with IBD showed a lower proportion of Fusobacterium than healthy dogs. This suggests that a low proportion of Fusobacterium is a characteristic feature of canine IBD and that Fusobacterium is involved in this disease. The results of this study may help elucidate the pathogenesis of this disease and its association with Fusobacterium.

Key words: inflammatory bowel disease, inflammatory bowel disease activity index, canine, fecal microbiota transplantation, microbiome, Fusobacterium

INTRODUCTION

The gene composition and functional properties of the whole gut microbiome have been evaluated using a recently developed enteric bacterium analytical procedure [1]. Studies have revealed an association between abnormalities in the gut microbiome (dysbiosis) and various diseases (e.g., metabolic disorders, autoimmune disease, and mental disorders) [1]. Therefore, fecal microbiota transplantation (FMT) is performed to improve the enteral environment in patients with these diseases. In the FMT procedure, fecal matter is collected from a tested donor, mixed with saline or another appropriate solution, strained to exclude particles (mostly hair and other solid particles), and administered to a patient by colonoscopy, endoscopy, sigmoidoscopy, or enema [2–4]. The infusion site varies with the administration route; for example, the injection site is the colon or cecum with colonoscopy, the duodenum with endoscopy, and the colon or rectum with enema. Several studies have reported that FMT is an effective treatment for recurrent Clostridioides difficile (formerly Clostridium difficile) infections [5–9]. The potential of FMT as a treatment for various diseases, such as inflammatory bowel disease (IBD), including ulcerative colitis, Crohn’s disease, and irritable bowel syndrome, has been extensively investigated in recent years [6, 10–21].

Recently, FMT has been tested as a treatment for multiple gastrointestinal diseases in veterinary medicine [22]. Canine IBD is a common cause of idiopathic, chronic, and relapsing gastrointestinal (GI) diseases [23]. As a rule, dogs with IBD have been differentiatied clinically from dogs with other chronic intestinal diseases (e.g., food-responsive- and antibiotic-
responsive enteropathies) by performing a diagnostic treatment [23]. Endoscopy is a test for diagnosing IBD after excluding other chronic intestinal diseases [23]. The most common histological change associated with IBD is lymphocytic-plasmacytic inflammation; however, eosinophilic and neutrophilic inflammation can also occur [23]. The causes of IBD are unknown, but they are thought to be secondary to a complex interplay of genetics, immune dysregulation, and environmental factors, including the GI microbiome [24]. We previously reported the efficacy and safety of long-term FMT for canine IBD and demonstrated an association between improvements in clinical signs and changes in the fecal microbiome [23]. However, that study was conducted in just one dog; thus, the results needed to be confirmed in a larger number of cases. Here, we performed FMT in nine dogs with IBD to investigate the efficacy of this treatment for canine IBD.

**MATERIALS AND METHODS**

**Dogs with IBD and sample collection**

This study was conducted in nine dogs with clinical signs of chronic GI disease (e.g., vomiting, diarrhea, weight loss, hypoalbuminemia, and ascites); they were subjected to endoscopic examination in the medical center of Nippon Veterinary and Life Science University between 2016 and 2019. The profiles of these dogs are shown in Table 1. Inflammatory bowel disease was diagnosed based on histopathological evidence of lymphocytic–plasmacytic enteritis after exclusion of food- and antibiotic-responsive enteropathies [26]. Medication (e.g., antibiotics, antidiarrheal compounds, antiflatulents, corticosteroids, and cyclosporine) was discontinued 1 week before FMT. Feces samples collected from the dogs with IBD 6 hr before FMT were used as the pre-FMT samples.

**Donor dog characteristics**

We collected fresh feces from five donor dogs. Physical and clinical examinations, complete blood count measurement, serum biochemical analysis, radiography, abdominal ultrasound, and fecal examination revealed that the donor dogs were in good health.

**Fecal microbiota transplant protocol**

The optimum dose and treatment interval for FMT procedures have not been established. We determined the optimum dose for FMT on the basis of similar ratios that proved to be successful in previous reports [2, 27].

Immediately after collection, approximately 3 g/kg feces was dissolved in Ringer’s solution. The slurry was then passed through sterilized gauze to filter out particulate matter. We administered 10 mL/kg slurry to the dogs with IBD during each FMT procedure. Generally, FMT is performed either orally (e.g., nasoduodenal intubation and enteroscopy) or rectally (i.e., rectal enema and colonoscopy). We chose rectal enema as the route of administration for all dogs because of its efficacy and safety, as observed in our previous study [27]. In this study, we performed FMT one time after collection of the pre-FMT feces samples (on the same day). The symptoms improved and remained stable in all cases for 2 weeks. Feces samples collected by the dog owners 2 weeks after FMT were used as post-FMT samples. These were stored at −80°C until investigation.

![Image](308x434 to 549x590)

**Table 1. Profiles of dogs with IBD used in this study**

| Age (years) | Sex  | Breed                   |
|------------|------|-------------------------|
| 10         | F, spayed | Miniature Dachshund     |
| 12         | M, neutered | Toy Poodle              |
| 12         | M, neutered  | Cavalier King Charles Spaniel |
| 12         | M, neutered  | Toy Poodle              |
| 10         | M, neutered  | Mix                     |
| 7          | F, spayed    | Border Collie           |
| 7          | M, neutered  | Beagle                  |
| 8          | F, spayed    | Beagle                  |

IBD: inflammatory bowel disease.

**Fig. 1.** Clinical observation according to the canine inflammatory bowel disease index (CIBDAI). The normal range is 3 or less. The post-fecal microbiota transplantation (FMT) CIBDAI score is significantly lower than the pre-FMT score \((p<0.05)\). The data were analyzed using a t-test with R (version 2.8.1).

**Quantitative polymerase chain reaction (qPCR) analysis**

To exclude the occurrence of pathogenic microbe-related digestive system disease, a qPCR analysis (IDEXX Laboratories, Inc., Tokyo, Japan) of fecal samples from all dogs was performed. The dogs were found to be negative for *Cryptosporidium* spp., *Giardia* spp., *Clostridium perfringens* a toxin, *C. difficile* toxins A and B, *Campylobacter jejuni*, *Campylobacter coli*, *Salmonella* spp., canine parvovirus type 2, canine distemper virus, and canine enteric coronavirus.

**Evaluation of clinical signs**

We evaluated the pre- and post-FMT clinical signs of IBD according to the canine inflammatory bowel disease activity index (CIBDAI) (Fig. 1). The CIBDAI is based on six criteria, each scored on a scale of 0–3: attitude/activity, appetite, vomiting, stool consistency, stool frequency, and weight loss. The total composite scores are evaluated as follows: 0–3, clinically insignificant; 4–5, mild; 6–8, moderate; 9 or higher, severe (Fig. 2) [27, 28]. After FMT, we requested that the owners of the dogs check the dogs’ GI health.

**Fecal microbiome analysis**

A rarefaction analysis of the 16S rRNA sequence was performed at Anicom, Inc. (Tokyo, Japan) using the MiSeq Reporter software.
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Raw sequence data were screened, trimmed, and filtered with default settings using the QIIME 2 View tool. The analysis was performed on a randomly selected subset of 30,213 ± 4,721 sequences from three dogs with IBD and three donor dogs. The V3–V4 16S rRNA sequence was analyzed to identify the bacterial groups Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria, Tenericutes, and others (Fig. 3A–C).

Because a considerable number of sequence results were obtained for Fusobacteria, we performed the qPCR analysis on all dogs to determine the number of *Fusobacterium* (Fig. 4).

The oligonucleotide sequences of the primers and the respective annealing temperatures are summarized in Table 2 [29, 30].

Fecal bacterial DNA extraction for qPCR

DNA was extracted from each fecal sample (100 mg) using a genomic DNA isolation kit for stool samples (Macherey-Nagel GmbH & Co. KG, Düren, Germany) according to the manufacturer’s instructions. The qPCR assay was performed as reported previously [29, 30]. The total extracted DNA was mixed with 100 µL of TE buffer. The final reaction mix consisted of 10 µL of Promega GoTaq® qPCR Master Mix (Promega, Madison, WI, USA), 0.4 µL each of forward and reverse primers (final concentration: 4 pmol), 7.2 µL of double-distilled water, and 2.0 µL of normalized DNA (final concentration: 50 ng/µL).

Statistical analysis

All statistical analyses were conducted using R (version 2.8.1). Clinical signs evaluated according to the CIBDAI were statistically analyzed using the t-test (all p-values <0.05). The Wilcoxon rank-sum test was used to examine the post-FMT changes in the number of *Fusobacterium* (all p-values <0.05).

### Ethics approval and informed consent

This study was approved by the Ethical Committee of Nippon Veterinary and Life Science University (Permission number: 29-5).

### Consent for publication

Written informed consent was obtained from the owners of the patient dogs for publication of this report.

### RESULTS

#### Clinical signs

Improvements in the clinical signs were observed in all dogs at 3 days after FMT. The most common clinical sign was chronic diarrhea, followed by chronic vomiting. Some dogs with IBD that presented with chronic diarrhea and vomiting also showed weight loss. The post-FMT CIBDAI score was significantly lower in the dogs than the pre-FMT score (p<0.05) (Fig. 1). Additionally, no adverse effects were observed during FMT treatment in the dogs.

#### Fecal microbiome analysis

The rarefaction analysis of the V3–V4 16S rRNA sequence revealed changes between the proportions of the different bacteria in the pre-FMT feces compared with those in the post-FMT feces and in the donor fecal samples (Fig. 3A–C). The major bacterial phyla in the pre-FMT feces of the dogs with IBD were Firmicutes (51.7%; Fig. 3A) and Proteobacteria (80.3%, 52.2%; Fig. 3B and C). The proportions of Actinobacteria, Tenericutes, and Proteobacteria in the microbiome of the donor dogs were low, and the major bacterial phyla were Bacteroidetes, Firmicutes, and Fusobacteria. Generally, the proportion of Fusobacteria in the pre-FMT microbiome of the dogs with IBD was lower than that in the microbiome of the donor dogs and that in the post-FMT microbiome of the dogs with IBD.

The results of the qPCR analysis are shown in Fig. 4. The post-FMT number of *Fusobacterium* was significantly higher than the
Fig. 3. Rarefaction analysis of the V3–V4 16S rRNA sequence to determine the changes in the proportions of bacteria in the fecal samples of the dogs with inflammatory bowel disease (IBD) between before and after fecal microbiota transplantation (FMT) and to determine the proportions of bacteria in the fecal samples of the donor dogs. The phyla are shown in order from the top of the bar graph: other, Tenericutes, Proteobacteria, Fusobacteria, Firmicutes, Bacteroidetes, and Actinobacteria (Fig. 3A–C). The major bacterial phyla in the dogs with IBD were Firmicutes (51.7%), as shown in Fig. 3A, and Proteobacteria (80.3%, 52.2%), as shown in Fig. 3B and C. The proportions of Actinobacteria, Tenericutes, and Proteobacteria in the microbiome of donor dogs were low, and the major bacterial phyla were Bacteroidetes, Firmicutes, and Fusobacteria. Generally, the proportion of Fusobacteria decreased in the microbiome of dogs with IBD.
DISCUSSION

In this study, we performed FMT in nine dogs with IBD and then investigated the changes in clinical signs and fecal microbiome. The CIBDAI score significantly decreased in all dogs, indicating improvements in clinical signs. Additionally, a lack of adverse effects during FMT demonstrated its safety. Thus, FMT could be an effective and safe treatment for canine IBD.

The fecal microbiome was investigated in three dogs by 16S rRNA sequencing. Notably, the pre-FMT proportion of Fusobacteria was lower in the dogs with IBD than in the donor dogs, whereas the post-FMT proportion in dogs with IBD was significantly higher. Fusobacterium was detected by qPCR in all nine dogs. The post-FMT number of Fusobacterium was significantly increased (p<0.05), which was consistent with the results of 16S rRNA sequence analysis. This suggests that a low proportion of Fusobacterium is a characteristic feature of canine IBD and that Fusobacterium is involved in this disease.

Fusobacterium is a butyric acid-producing bacterium. Butyric acid is used as a major energy source by epithelial cells in the mucous membrane of the large intestine; it inhibits the growth of colorectal cancer cells and induces differentiation and apoptosis of them [31–35]. Butyric acid promotes the maturation of acquired immune system cells that play a central role in suppressing inflammation and allergic reactions [36, 37]. It also inhibits the production of inflammatory cytokines [38]. Butyrate suppressed the onset of colorectal cancer in a model animal [39, 40]. Additionally, some studies have reported that butyric acid improves the symptoms of bowel-related diseases and that the butyric acid concentration is lower in the feces of patients with ulcerative colitis [41–43]. Therefore, butyric acid is considered important for maintaining large intestine function and for preventing and improving large intestine-related diseases.

However, several studies have reported that Fusobacterium is a pro-inflammatory pathogen [25, 44–46], with a high abundance in patients with IBD and mouse models of IBD [25, 46]. Other studies have concluded that Fusobacterium nucleatum may promote colonic neoplasia development by downregulating antitumor T-cell-mediated adaptive immunity [47]. Although Fusobacterium may be a risk factor for colorectal carcinoma in mice and humans [45–47], a low proportion of Fusobacterium may be specific to canine IBD.

In this study, FMT was used to effectively treat canine IBD. The proportion of Fusobacterium is higher in the gut microbiome of the canine or has been reported to be higher in the gut microbiome of the canine than in the microbiome of other animals, including mouse models and humans [25, 44–47]. Species differences may exist in the gut microbiome, which is affected by various factors, including diet, habitat, and gastrointestinal anatomical differences.

Here, the proportion of Fusobacterium tended to be low in dogs with IBD, although two dogs (22%) showed normal proportions of Fusobacterium. However, Fusobacterium may be associated with canine IBD. Further studies are needed to investigate the effect of Fusobacterium on FMT for canine IBD, because the proportion of Fusobacterium was increased by FMT, even in the two dogs that showed normal proportions.

Future studies should examine the differences in the proportion of Fusobacterium in dogs with IBD. It should also be noted that we did not perform endoscopy on the dogs after FMT due to a lack of consent from the owners. Therefore, we were unable to confirm any changes in the intestinal mucosa resulting from FMT. Therefore, it is necessary to identify a marker indicating pre-FMT number (p<0.05). However, in two dogs with IBD, the pre-FMT number of Fusobacterium was similar to that in donor dogs.

Table 2. Oligonucleotide primers/probes used in this study

| Target     | Primer | Annealing | Reference |
|------------|--------|-----------|-----------|
| Fusobacterium | Fuso-F | KGG GCT CAA CMC MGT ATT GCGT | 51℃ 30 sec [29, 30] |
|            | Fuso-R | TCG CGT TAG CTT GGG CGC TG    |           |
Fecal microbiota transplantation (FMT) is an effective treatment for patients with functional gastrointestinal disorders (FGID) and inflammatory bowel disease (IBD). We would like to thank Editage (www.editage.com) for English language editing.

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