Review Article

Indications for canine fecal microbiota transplantation

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Abstract

Fecal microbiota transplantation (FMT) is a novel method to treat intestinal and extraintestinal diseases by transplanting the microbiota in the feces of healthy animals into the gastrointestinal tracts of patients and reconstructing intestinal flora in the patients' intestine. In this article, we reviewed the recent studies of canine FMT focusing on its indications. FMT was proved effective to treat canine *Clostridioides difficile* infection (CDI), inflammatory bowel disease (IBD), canine parvovirus (CPV) diarrhea and acute diarrhea; upregulate the intestinal microbiota in dogs with acute hemorrhagic diarrhea syndrome (AHDS); and may have benefit for preventing canine post-weaning diarrhea. However, the specific FMT protecting gut mechanism and the role of long-term colonized microbiota in dogs still need numerous basic researches to reveal in future.

Keywords: canine, fecal microbiota transplantation, indications

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Introduction

Fecal microbiota transplantation (FMT) is a current therapeutic method of intestinal diseases. The process of FMT includes extraction of intestinal microbes from healthy donors and transplanting it into the intestines of recipients. Symbiotic bacteria in the intestine are in a state of dynamic equilibrium, which play an important role in digesting complex carbohydrates, enhancing immunologic function, and preventing pathogenetic invasion (Wang *et al*.,2019). When animals suffering enteric diseases, the balance of microbial ecological environment in the animals' intestines will be destroyed. Due to the colonization of commensal microorganisms, FMT can repair damaged microbial flora and cure intestinal diseases (Wang *et al*.,2019). Currently, the studies and clinical applications of FMT in human medicine, especially in *Clostridioides difficile* infection (CDI), have attracted many scientists' attention (Ianiro *et al*.,2014). The study of FMT in dogs is becoming a tendency treatment in many gastrointestinal diseases.

Similar to human FMT, canine FMT procedure includes donor selection, fecal microbiota extraction and delivery (Chaitman *et al*.,2016). However, currently, there is not a standard comprehensive guideline for FMT treatment in small animal field formed. Only few cases and studies on canine FMT were reported. In this article, we tried to summarize the existing researches and clinical reports related to canine FMT, focused on presenting the indications of canine FMT, and discussed advantages and future potential challenges for canine FMT. After screening, 10 reports considered to be related to canine FMT were included, the general information of which were in Table 1. In each indication, the number of dogs who taken FMT both in successfully and unsuccessfully treated were counted from the reports for therapeutic effect assessment (Figure 1). We found that, of all indications, IBD was the most popular disease for FMT treatment. CPV had the lowest successful treatment rate (78.8%). CDI, AHDS (including *Clostridioides perfringens* infection), acute diarrhea and post-weaning diarrhea had the highest successful treatment rate (100%).

Clostridioides difficile infection

CDI is a severe diarrheal disease progressing from asymptomatic carrier status through mild diarrhea to colitis or pseudomembranous colitis. The appearance of CDI mostly associated with the use of antibiotics in animals. Antibiotics, especially broad-spectrum antibiotics, will kill several commensal bacteria in animal intestine, which may cause the reproduction and colonization of *Clostridioides difficile* (Czepiel *et al*.,2019). Under this circumstance, FMT becomes a better choice for treating CDI. For example, Sugita *et al*. (Sugita *et al*.,2019). reported a case that a bulldog suffered from intermittent diarrhea for 4 months and focal seizures for 7 days. The dog was diagnosed with CDI-associated colitis, as the antigen and toxins A&B genes and proteins of *Clostridioides difficile* bacteria in the canine fecal sample were detected by real-time PCR and immunochromatographic test. Instead of oral metronidazole, FMT therapy was performed in the dog due to the possibility of metronidazole-caused neuropathy. An excellent result was obtained: fecal color and consistency returned to normal 2 to 3 days after FMT and no *Clostridioides difficile* was detected 7 to 124 days after FMT. From this report, FMT can be a good treatment option for canine CDI and symbiotic bacteria transplanted from donor may colonize the recipient's intestines for a long time.

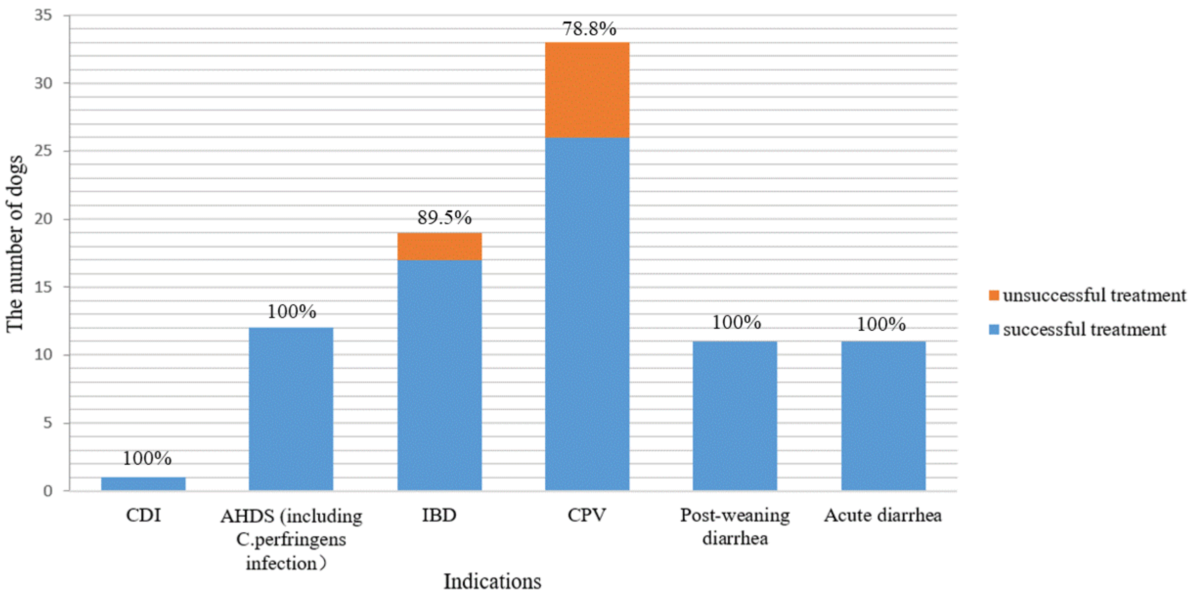

Figure 1 The number of dogs that were successfully treated and unsuccessfully treated in each indication. The successful treatment rates were on the top of columns.

Table 1 Indications of canine fecal microbiota transplantation

Diseases	Subjects	Dosage	Donors	Delivery methods	The results of treatment	Microbiota analysis	Observing time	Successful treated rate	Reference
CDI	Bulldog, 8-month-old, 11 kg, male	60 g feces/dog	Beagle, 9 years old, 11 kg	Oral	No Clostridioides difficile was detected after 7 days; CDI did not recur.	No	190 d	1/1, 100%	(Sugita *et al.*,2019)
AHDS	FMT treated: 4 dogs; sham-treated: 4 dogs.	2.5-3.8 g feces /kg	4 dogs	Endoscopy	FMT had no clinical benefit, however, increased abundance of SCFA-producing bacteria in intestine.	Yes	30 d	4/4, 100%	(Gal *et al.*,2021)
C.perfringens infection	8 dogs	-	-	Enema	Feces of 8 dogs became normal, and 6 of them did not detect *Clostridioides perfringens.*	Unavailable	-	8/8, 100%	(Murphy *et al.*,2014)
IBD	16 dogs	60-150 g feces/dog	-	Endoscopy, capsule	Clinical symptoms were improved in most dogs.	No	3 m	14/15, 93.3%	(Bottero *et al.*)
IBD	A 3 years old dog	-	-	Enema	Fecal consistency was improved within 24 hours, and other gastrointestinal symptoms were mostly reduced.	Unavailable	221 d	1/1, 100%	(Weese *et al.*,2013)
IBD	10 years old poodle	12 g feces/dog	Golden Retriever, 4 years old, 32.8kg, castrated male	Enema	The clinical symptoms were alleviated and fecal microbial diversity was restored.	Yes	-	1/1, 100%	(Niina *et al.*,2019)
IBD	2 dogs	-	-	-	1 dog was cured, the other was failed.	No	-	1/2, 50%	(Bryan *et al.*,2019)
CPV	Control group: 33 dogs received STD; Experimental group: 33 dogs received STD + FMT	10 g feces/dog	Pit Bull Terrier, 6 years old	Enema	The experimental group resolved diarrhea earlier than control group, the hospital stay was shorter, and mortality rate was smaller.	No	-	26/33, 78.8%	(Pereira *et al.*,2018)
Post-weaning diarrhea	23 dachshunds, 6-8 weeks old. FMT-treated: 11 dogs; sham-treated: 12 dogs	5 g feces/dog	Their dams	Oral gavage	The intestinal flora did not transfer to a state similar to that of the dams, but similar to that of sham-treated groups.	Yes	24 d	11/11, 100%	(Burton *et al.*,2016)
Acute diarrhea	Experimental group: FMT treatment of 11 dogs; Control group: oral metronidazole of 7 dogs.	2.5-5 g feces /kg	3 years old, male castrated dog	Enema	FMT group was more similar to healthy dogs in fecal consistency, fecal microbiota and metabolome profiles than oral metronidazole group.	Yes	28 d	11/11, 100%	(Chaitman *et al.*,2020)

Acute hemorrhagic diarrhea syndrome

Canine acute hemorrhagic diarrhea syndrome (AHDS) is featured by sudden watery bloody diarrhea, vomiting, loss of body fluid and hemoconcentration (Unterer and Busch,2021). Till now, the cause of AHDS is not completely understood. *Clostridioides perfringens* has been found overgrowing in many AHDS cases, the toxin of which is considered to mediate necrosis of the intestinal mucosa (Leipig-Rudolph *et al*.,2018). Besides, the composition of other microbes also alters: *Fusobacteria* increases while *Actinobacteria* and *Ruminococcaceae* decrease (Ziese *et al*.,2018). Through active treatment, such as fluid correction, clinical symptoms of AHDS dogs with uncomplicated course can be improved within 24 to 48 hours (Unterer and Busch,2021). However, ~30% of the dogs who suffered from AHDS will develop chronic gastrointestinal diseases in later years (Unterer and Busch,2021). Therefore, instead of simple symptomatic treatment when acute diarrhea occurs, it is should be focus on preventing the appearance of resistant chronic diseases. Currently, the restoration of damaged barrier and the positive regulation of intestinal microflora are of great significance. Study has shown that after probiotic treatment the normalization of intestinal commensal bacteria was accelerated and the abundance of *Clostridioides perfringens* was significantly lower in dogs (Ziese *et al*.,2018).

FMT therapy may have similar effect on AHDS as probiotic treatment. Gal *et al*. (Gal *et al*.,2021) assessed the effect of FMT on clinical symptoms and intestinal microbiomes of 8 dogs with AHDS. FMT treated group (4 dogs) and sham treated group (4 dogs) were set in the study. The feces of FMT treated dogs, sham treated dogs and donors were collected at admission, discharge, and 30 days after discharge for 16S rRNA gene sequencing. α diversity (the Shannon diversity index, SDI) and β diversity (Principal coordinate analysis of the Bray Curtis dissimilarity matrix) were used for microbial analysis. From admission to discharge, the SDI in FMT treated group increased from a level that lower than healthy donors to a level that is not different from healthy donor, while in sham treated group did not change from admission to 30 days. Principal coordinate analysis (PCoA) of FMT treated dogs separated from healthy dogs at admission and clustered closer to donors at 30 days. Compared with sham treated group, the abundance of the SCFA-producing bacteria (*Eubacterium biforme* and *Faecalibacterium prausnitzii*) in FMT treated group was increased significantly to similar with donors at 30 days. SCFAs have been shown benefit for repairing intestinal mucosal barrier. However, 30 days after discharge, there were no significant difference in SDI between FMT treated group, sham treated group and donors. Through AHDS clinical scores, the two group are not significant different at admission or discharge. The authors explained that it might due to the small size of samples, mild clinical scores at admission and rapid correction of clinical symptoms. In another case, Murphy *et al*. (Murphy *et al*.,2014) reported the use of FMT in treating 8 dogs with recurrent diarrhea caused by *Clostridioides perfringens* infection. After the administration of fecal material, diarrheal problem got solved in all dogs. Among them, 6 dogs were *Clostridioides perfringens* α toxin negative by PCR. Therefore, in the long run, the microbial flora in the intestinal of AHDS dogs after FMT will alter towards a better condition.

Inflammatory bowel disease

Inflammatory bowel disease (IBD) in dogs is a chronic gastroenteritic disease characterized by excessive inflammation, including ulcerative colitis, crohn's disease and undetermined colitis. Clinically, IBD manifests as recurrent symptoms, covering diarrhea, colorectal bleeding, and abdominal pain (Jergens and Simpson,2012). Lymphocytic-plasmacytic enteritis is the main form of canine IBD. The appearance of canine IBD is related to multiple factors like mucosal immune system, genetic susceptibility, and enteric microenvironment (Peiravan *et al*.,2018). The intestinal microbial diversity of IBD patients is lower than healthy animals: anti-inflammatory bacteria reduce and pro-inflammatory bacteria increase in the intestine of IBD patients (Nishida *et al*.,2018). *Enterobacteriaceae* and *Clostridiaceae* families are rich in the intestine of IBD dogs, and the components of *Paraprevotellaceae* family and *Porphyromonas* genus in IBD dogs are higher than healthy dogs (Omori *et al*.,2017). Primary task of treating IBD is anti-inflammatory including dietary therapy and pharmacologic interventions, regulation of microbiota by antibiotics, probiotics and prebiotics (Jergens and Simpson,2012). Until 2021, four studies have reported that FMT is effective on IBD dogs that is unresponsive to common medical treatments.

The first report registered 16 dogs with idiopathic IBD that did not respond to traditional treatment (Bottero *et al*.). The dogs were treated with FMT in different ways: 9 dogs used fresh feces via endoscopy (5 of them combined with oral procedure) and 7 dogs used frozen fecal bacteria capsule orally. The results showed that 14 dogs' clinical symptoms were improved, one dog became more serious, and one dog was excluded since other reason. However, in this study most of the dogs were using drugs during the FMT process, which might have effects on the results. In the second report, Niina *et al*. (Niina *et al*.,2019) described an IBD dog that was administrated FMT 9 times in 6 months. The dog was observed in the following 221 days after FMT. Microbial diversity analysis indicated that, 42 days after FMT, the proportion of *Proteobacteria* reduced from 52.2% to 1.5% while *Fusobacteria* increased from 0 to 35%, *Firmicutes* and *Bacteroidetes* also increased. In healthy dogs, the proportion of *Proteobacteria* and *Fusobacteria* were 5-7% and 23-40% respectively. The case suggested that FMT can restore the recipient flora to a similar state of the donors. Similar to the second case, the third report discovered that an IBD dog could be treated with FMT successfully (Weese *et al*.,2013). In this report, fecal samples of the patient dogs were collected before and after FMT for 16s rRNA sequence analysis. The result showed that the microbiome of recipient clustered with donor phylogenetically on day 2 after FMT. The fourth study (Bryan *et al*.,2019) reported two of five IBD dogs failed to be treated by antibiotics, prednisolone, probiotic and dietary modification, then additionally used FMT treatment. Eventually, one dog was cured by FMT while the other one was eventually euthanized since other diseases.

From the above four reports, a total of 19 IBD dogs failing to respond to conventional antibiotics were treated with FMT, only 2 of them were not cured, which may be affected by other conditions. Therefore, FMT may have a good function for canine IBD.

Canine parvovirus diarrhea

Canine parvovirus (CPV) diarrhea and enteritis is the reason for highly infectious acute hemorrhagic enteritis, which is usually accompanied by high morbidity and mortality. The mortality rate of untreated puppies with parvovirus infection is over 90% (Parker *et al*.,2017). CPV invades germinal epithelium of the intestinal crypt, leading to shortening or obliteration of villi (Goddard and Leisewitz,2010). On the one hand, intestinal injury increases the risk of bacteria transferring from intestinal track to blood circulatory system thus raising the possibility of canine death (Goddard and Leisewitz,2010). On the other hand, the breakdown of intestinal epithelium causes the intestinal microbiota disorder (Wang and Wang,2019). FMT as a microflora-improving treatment has a promoting effect on parvovirus diarrhea in dogs. Pereira *et al*. (Pereira *et al*.,2018) divided 66 parvovirus-infected dogs into two groups: the control group (33 dogs) receiving standard therapy (STD), and the experimental group (33 dogs) receiving STD+FMT. Their result showed that the treatment of STD+FMT had a shortened therapeutic period: 61.5% of the dogs treated with STD+FMT were discharged within 48 hours, while only 4.8% of the dogs treated with STD were discharged in the same time. The mortality rate of STD+FMT treatment was 21.2% less than the STD group rate (36.4%). However, microbial variation in the gut of parvovirus-infected dogs is unknown in this study since microbial evaluation is absent unfortunately. From the study, FMT is a good choice for CPV as adjuvant therapy.

Post-weaning diarrhea

Weaning usually leads to reduction of weight, nutrient absorption, and immune homeostasis, thus causing impaired barrier function, susceptibility to pathogens, and diarrhea (Yi *et al*.,2016). The intestinal flora of early weaning animals will undergo a substantial change to establish a characteristic and dynamic state of microbes (Gresse *et al*.,2017). Microflora disorders are recognized as one of the main factors leading to post-weaning diarrhea (Gresse *et al*.,2017). FMT by decreasing microflora disorders may prevent dogs from post-weaning diarrhea. Burton *et al*. (Burton *et al*.,2016) used FMT for 23 weaning puppies. 11 dogs were treated with FMT alone and the remaining 12 dogs received the same dose of 2% reduced-fat milk as sham-treated controls. Healthy fecal materials were transplanted from dams to these puppies for consecutive 5 days, beginning at 3 days before weaning. After administration, feces of puppies and dams were collected for 16S rRNA amplification sequencing at 3 days before weaning and 3, 10, and 24 days after weaning. The result indicated that diarrhea got improved after FMT, while α diversity (Chao1 indices) and β diversity (PCA) showed that the microbiota in recipient puppies did not shift to a similar state to that of donor dogs. Additionally, the healthy score of FMT-treated group was not different from that of sham-treated group. Although FMT was not proved an enhacing effect on post-weaning diarrhea in this study, FMT was proved that could ameliorate diarrhea, limit colon inflammatory and strengthen intestinal barrier function in piglets with weaning from a recent study (Tang *et al*.,2020). Therefore, FMT could be a potential treatment approach for post-weaning diarrhea. More studies may be needed for determining the influence of FMT on puppies with post-weaning diarrhea.

Acute diarrhea

Acute diarrhea means that the water content of feces raises in a short term, resulting in an increase in fecal fluidity, volume, and frequency of defecation. Fecal microbiome with acute diarrhea will be changed. Therefore, a regulatory restoration of the microflora may be considered when treating acute diarrhea (Suchodolski *et al*.,2012). Probiotics and prebiotics can inhibit the growth of gastrointestinal pathogens and shorten the duration of clinical symptoms of acute diarrhea in dogs (Nixon *et al*.,2019). FMT, similar to probiotics and prebiotics, may become an effective method for treating acute diarrhea in dogs. Chaitman *et al*. (Chaitman *et al*.,2020) compared the fecal consistency, fecal microbiota and metabolome profiles between FMT-treated group and metronidazole group at 0, 7, and 28 days after treatment. The techniques they used to analysis were Fecal Dysbiosis Index (DI), α diversity (Shannon Index), and β diversity (PCoA) for fecal microbiota; Targeted Metabolomic and Untargeted Metabolomic ((PCA) for metabolome analysis. On day 7, the percentage of primary bile acid of feces decreased in FMT group but increased in metronidazole-treated group. Till 28 days, the fecal consistency of the FMT-treated group was better than that of the oral metronidazole group; DI decreased and Shannon Index increased significantly; the microbial communities and metabolite composition of the FMT-treated group clustered closer to healthy dogs. After FMT, *Escherichia. coli* deceased while *Faecalibacterium* and *Clostridioides hiranonis* increased. These results showed that FMT rebuilt the gastrointestinal environment of the diseased animals to a condition close to healthy dogs.

Discussion

Microbial and metabolome analysis were usually used for assaying FMT for intestinal diseases. For microbiome analysis, α diversity (including Chao1 and Shannon Index) and β diversity (including PCA and PCoA) were used mostly, both of which can reflect the diversity of fecal microbial community. Fecal Dysbiosis Index (DI) calculated from Quantitative PCR (qPCR) assays summarizes the abundances of total bacteria and 7 bacterial taxa in one single number showing the degree of microbial community disorder. When Chaitman *et al*. (Chaitman *et al*.,2020) analyzing FMT on acute diarrhea, they performed qPCR assays of total bacteria, *Faecalibacterium*, *Turicibacter*, *Escherichia coli*, *Streptococcus*, *Blautia*, *Fusobacterium*, and *Clostridium hiranonis*, and get their DIs for microbial analysis. The value of DI less than 0 is normal; between 0 and 2 indicates the intestinal microbiota has a slight fluctuation; greater than 2 shows the microbiota has a large change (AlShawaqfeh *et al*.,2017). Targeted Metabolomic and Untargeted Metabolomic were used for metabolome analysis based on Gas chromatography-mass spectrometry (GC-MS). Targeted Metabolomic is for identifying and quantifying of known metabolites, like fecal bile acid concentrations Chaitman *et al*. (Chaitman *et al*.,2020) detected. Untargeted approach is aimed at obtaining a large amount of metabolite data, annotating metabolites, and finding the changes of known and unknown metabolites. Analysis methods of Untargeted Metabolomic data are commonly principal component analysis (PCA), partial least squares projection to latent structures discriminant analysis (PLS-DA), orthogonal projections to latent structures discriminant analysis (OPLS-DA), etc (Schrimpe-Rutledge *et al*.,2016).

Thanks to these analysis techniques, scientist could make the intestinal microbiota and metabolites shift visualized, which is important to explain that the dogs could not be treated with antibiotics or probiotics but responded to FMT treatment. Antibiotics may inhibit intestinal microbiota indiscriminately, resulting in reduced microbial diversity and beneficial bacterial groups (Suchodolski *et al*.,2009). The mechanism of FMT restoring intestinal microbial structure mainly due to fecal commensal bacteria: 1) competing with pathogens for nutrition and planting resources, 2) directly killing pathogens by producing bacteriocin, 3) preventing mucus layer from disruption by stimulating intestinal cells to secrete mucin, antimicrobial peptides and IgA, 4) maintaining the intestinal epithelial cell barrier and promoting tight connections between intestinal epithelial cells, 5) suppressing excessive inflammatory reaction in the gut through regulatory T cells (Treg), 6) adjusting SCFAs, secondary bile acids and other metabolites to normal concentrations (SCFAs provide main energy for colonic epithelial and secondary bile acids are proved to inhibit the reproduction of *Clostridioides difficile*) (Hudson *et al*.,2017; Khoruts and Sadowsky,2016). Therefore, FMT has great potential in treating antibiotic complications and diseases that not respond to antibiotic.

The mechanism of FMT altering intestinal microbial structure brings some advantages to FMT compared with animal microecological preparations. Animal microecological preparations, such as probiotics, prebiotics and synbiotics, may improve the health of animals by modifying intestinal microbiota like FMT (Chaitman *et al*.,2016). However, current research showed that the impact on mucosal bacteria of probiotic therapy is similar to that of standard therapy in IBD dogs, and both of them can't improve intestinal inflammation in histopathologic (White *et al*.,2017). On contrast, in a study we described above, the diarrheal symptom of one IBD dog can be improved by FMT instead of probiotic (Bryan *et al*.,2019). The reason that FMT has a better effect on IBD and other diseases compared with microecological preparations may be that healthy gut microbes in feces have better adaptation in the intestinal environment of recipients after transplantation than routinized probiotics. The nutrients in the feces could enable intestinal microorganisms to have a better living environment when they are transplanted together. On the other hand, microorganisms in the gut depend on each other as a global functional organization. Probiotics as single species may not replace the full function of microflora in the intestine (Chaitman *et al*.,2016; Fuentes *et al*.,2017). What is more, compared with animal microecological agents, FMT may introduce host intestinal cells, immune factors, SCFAs and secondary bile acids from donors to recipients during the transplantation (Bojanova and Bordenstein,2016). Intestinal cells can participate in the repair and reconstruction of the intestinal environment and act as a physical barrier between bacteria and host intestinal tissues (Maynard *et al*.,2012). Intestinal immune molecules protect host cells against pathogens and toxins in the gut (Arpaia *et al*.,2013).

Although FMT has many advantages when treating intestinal diseases, it may have multiple defects. Presented by a few studies currently, some operations and theories of FMT for dog are still controversial. For example, there is a debt on if FMT can thoroughly improve the intestinal microecology, or only inhibit certain pathogens and pathological indicators below the detection limit. In addition, there is not an uniform standard for the amount of stool required for transplantation. In the studies summarized above, fecal usage and fecal material delivery routes are different. The fecal delivery routes can be roughly divided into upper gastrointestinal and lower gastrointestinal channel. The upper gastrointestinal includes oral administration, esophagogastroduodenoscopy, nasogastric tube, duodenal infusion, and fecal bacteria capsule; the lower gastrointestinal includes colonoscopy and rectal enema (Wang *et al*.,2019). For different routes of FMT delivery, they have different efficacy rates, as discussed in a human metanalysis, multiple colonoscopy delivery had the highest efficacy rates (98%), single enema infusion had the lowest efficacy rates (56%) (Ianiro *et al*.,2018). For human, colonoscopy can observe the intestinal conditions, and allow a large amount of fecal fluid to reach the intestine. Enema might be more convenient than colonoscopy, while the retention time of the injected bacterial fluid in the intestine is shorter (Wang *et al*.,2016). Oral administration is simple to operate, but the success rate is lower than that of the lower gastrointestinal tract (Kassam *et al*.,2013). Studies shown that the upper gastrointestinal tract was more prone to adverse reactions than the lower gastrointestinal tract (Wang *et al*.,2016). For dogs, Bottero *et al*. (Bottero *et al*.) claimed that the combination of endoscopic and oral transplantation seems to have a better effect than using the endoscope or oral alone. Enema was used mostly in the above studies, probably on account of convenient. The standing posture of dogs is different from that of humans, so the effects of different delivery routes on dogs are probably different from humans. However in the current circumstance, it is hard to define which is the best method for performing FMT, since different indications give various difficulty to treatments, thus bring different therapeutic effects. For example, the lower successful treatment rate of FMT on CPV (Figure 1) is probably caused by the refractory of CPV. Even under the same indication the number of studies may not enough to evaluate.

The risks of co-transfer of pathogenic microorganisms and the transmission of drug-resistant genes in FMT were considered as the other limits for canine FMT. The contamination of transferred feces by environmental microbes when donor feces are collected, stored and processed in the early stages of sample preparation may make recipient intestinal diseases worse. Recipient dogs have low intestinal immunity during disorder and are susceptible to infection. All donors in every indication we mentioned had gone through a rigorous screening process to confirm that the fecal donors not contain pathogens of intestinal diseases, not take antibiotics within 3 months prior to the delivery of feces, and not suffer infectious diseases, nutritional metabolic diseases, immune diseases, tumors, obesity, chronic pain, etc (Bhutiani *et al*.,2018; Chaitman *et al*.,2016). Additionally, the dogs chosen to be donors should be vaccinated yearly and dewormed twice a year (Gal *et al*.,2021). Before collecting stool, donors should be fed with commercial diet. The results of clinical and laboratory examinations, including complete blood count, serum biochemical analysis, radiography, abdominal ultrasound and fecal pathogenetic examinations (canine parvovirus, canine distemper virus, canine adenovirus II, canine parainfluenza and other pathogens), should be normal (Niina *et al*.,2019; Pereira *et al*.,2018; Sugita *et al*.,2019). Except for the longitudinal screening of a large number of fecal donors, functional metagenomic studies on fecal probiotics, drug resistance genes, transposons and other aspects are also required. After collecting stools, fresh feces need to go through a series of treatments and finally turn into fecal concentrate that with better color, taste and viscosity. The concentrate can be used immediately for transplantation or stored at -80℃ for 1 to 8 weeks (Hamilton *et al*.,2012). During this process, a sterile environment is required.

In conclusion, FMT has advantages in treating canine CDI, IBD, canine parvovirus diarrhea and acute diarrhea, and can upregulate the intestinal microbiota in AHDS dogs. It was also able to be a useful method in preventing post-weaning diarrhea, antibiotic complications and diseases not responding to antibiotic in dogs, from current limited reports. However, the specific FMT protecting gut mechanism and the role of long-term colonized microbiota in dogs still need numerous studies to reveal in future.

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