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Review paper

Fecal microbiome transplantation in the treatment of chronic enteropathies

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Abstract

The intestinal microbiome is essential for the proper functioning of the immune system and the course of metabolic processes in the living organism. Intestinal bacteria produce a variety of metabolites that affect the health of many organs, especially the intestines. Disturbances in the composition of the intestinal microflora are referred to as dysbiosis. Dysbiosis occurring in chronic enteropathies may exacerbate intestinal inflammation. Therefore, effective methods of treating enteropathy are still being sought, which involve restoring the proper composition of the intestinal microbiome. In recent years, many scientific centers have drawn attention to the possibility of treating enteropathy by transplanting intestinal contents from a healthy donor. This review presents the advantages and disadvantages of this therapeutic method, described in the latest available literature and the newest guidelines regarding the donor and transplant administration methods.

Keywords: intestinal microbiome, intestinal microbiome transplantation, dogs, cats

Introduction

Intestinal microbiome transplantation has been a well-known method for many years and is now attracting renewed interest from many scientists and clinicians. The first records of the use of intestinal flora in the treatment of various diseases in Chinese medicine date back to the 4th century AD (Shi and Yang 2018). Modern medicine became interested in the transfer of bacterial flora in the late 1950s. It began with the experiences of American surgeons who treated patients after colon cancer surgery. After surgical procedures, patients were experimentally administered capsules with powdered, dried or fresh stool derived from a healthy person. Patients were observed to recover much faster. In 1958, Dr Ben Eisenman from Colorado, USA, was the first to present attempts to treat inflammatory bowel disease using rectal infusions of feces collected from healthy individuals (Eisman et al. 1959). Despite good results, the procedure has not become widespread and has been performed sporadically in isolated centres around the world, as the administration of antibiotics was believed to be more effective in the treatment of *Clostridioides difficile* (CDI) infection. In

2000, there was renewed interest in intestinal microbiome ptantation when the Human Microbiome Project, aimed at sequencing the human microbiota, was launched in the United States (Cammarota et al. 2017, Borody et al. 2019). The project was a prelude to research into the development of the current transplantation procedure in humans and, subsequently, in animals. In 2006, Dr Max Nieuwdorp, an internal medicine specialist from Amsterdam, the Netherlands, published a paper presenting the bacterial microflora transplantation procedure (deClercq et al. 2019). In Poland, the first official transfer of bacterial flora in patients with advanced CDI was performed by Dr Paweł Grzesiowski, MD, PhD, and Dr Adam Herman, PhD, accompanied by the team of the Specialised Hospital in Wejherowo in 2012 (Jurkowska et al. 2014). Currently, the number of transplantations performed in humans has been steadily increasing all over the world, resulting from attempts to apply this method to a number of nosological units (Rosenbaum 2019).

In veterinary medicine, the transfer of gut microbiota is also not novel due to the phenomenon of coprophagia. In the animal world, there are several mammalian species in which this phenomenon occurs. Coprophagia in rodents and lagomorphs allows animals to absorb nutrients produced by their colon microbiota (Soave and Brand 1991). Coprophagia also occurs in approximately 16% of dogs and is considered a positive behavioural trait already present in the domestic dog's ancestors, which is aimed at reducing the risk of parasitic infection (Hart et al. 2018). Other authors also consider the transplantation of the rumen content as part of the therapeutic microbiome transfer. This procedure was documented in Europe as early as the 17th century (Klein and Müller 1941, dePeters 2014). In recent years, after the successful application of fecal microbiota transplant (FMT) in patients with CDI, this technique has also gained prominence in the gastroenterology of small animals. To date, however, most papers have focused on the clinical assessment of the application of transplantation in the recipient (Kelly 2015, Chaitman 2016, Burton et al. 2016, Chaitman et al. 2020).

Intestinal microbiome

Interest in intestinal microbiome transplantation is linked to studies of the bacterial distribution in the gastrointestinal tract. These studies showed that in both humans and animals, there is considerable variation in the microbiome due to different environments and nutrient availability. This means that different sections of the digestive tract are populated by different bacterial flora. Each individual's microbiome, similar to fingerprints, is unique. In other words, there are no two individuals with an identical microbiome among a human or animal population (Schmitz and Suchodolski 2016). These differences are mediated by factors such as age, sex, race/breed as well as diet, medical history, and the treatments applied (Chun et al. 2020, Ganz et al. 2022, Rojas et al. 2023). An imbalance of the gut microflora is referred to as dysbiosis, which is a result of a deficiency of beneficial bacteria and an excess of pathogens (yeasts, parasites and harmful bacteria). The balance of the microbiota in the gastrointestinal tract can be disturbed for a variety of reasons: as a result of antibiotic therapy, inflammatory conditions of various etiologies (allergy, idiosyncrasy, parasites, infection), periodic or persistent medication intake (non-steroid anti-inflammatory drugs, proton pump inhibitors), the consumption of highly processed feeds, a single-food diet, malnutrition, and the physiological condition of the body (joy, stress, fatigue, excessive physical activity) (Honneffer 2014, Pilla R. et al. 2020, Tuniyazi et al. 2022). Dysbiosis is most commonly diagnosed in patients with gastrointestinal diseases, as it leads to chronic inflammation of the intestinal mucosa with impaired Paneth cell function (Chaubeck et al. 2016). In clinical practice, the most suitable test in the diagnostic process to identify the presence of dysbiosis is the determination of the Dysbiosis Index (DI). The Dysbiosis Index is a study based on the PCR technique, which determines the counts of seven bacterial groups (plus the total bacterial count) and summarises them into a single number. Numerical values above 2 in dogs and 1 in cats are indicative of the presence of dysbiosis (Sung et al. 2022). In addition, by assessing the count of *Peptacetobacter hiranonis* (*C. hiranonis*), it is possible to demonstrate normal or abnormal bile acid conversion, which may facilitate the diagnosis of chologenic diarrhoea (Torenssen 2021). A more sensitive test of the intestinal microflora is the analysis of the complete DNA of the bacteria found in a fecal sample based on the whole genome sequencing method. Microorganisms are identified by molecular methods, mainly involving the analysis of 16S rRNA genes, 18S rRNA genes or other marker genes and genomic regions, amplified and sequenced from microbiomes (Xenoulis et al. 2008, Suchodolski et al. 2010, Gliński and Żmuda A. 2024). It is also possible to assess bacterial cultures in the feces (culture on suitable media). However, this method only determines a small percentage of the bacteria that can be identified by routine methods or tests (Werner et al. 2021) (this particularly applies to anaerobes). The authors of this publication suggest that when analysing a rectal swab, one should remember that only the colon microbiome is assessed and not the small bowel microbiome.

Indications for FMT

Restoration of the proper intestinal microbiome can be achieved through a change in diet, administration of probiotics, prebiotics or synbiotics, and fecal transplantation, i.e. the transplantation of intestinal microbiome. Intestinal microbiome transplantation, otherwise known as fecal microbiota transplant (FMT), is the transfer of fecal or intestinal contents from a healthy donor to an ill patient. The aim of FMT is to treat and prevent viral, bacterial and parasitic diseases by improving the gut microbiome and raising the patient's immune status through the stimulation of the immune system and administration of antibodies, especially to puppies. Although the exact mechanism of FMT efficacy is still poorly understood, it is believed that in the context of treating patients with dysbiosis, the mode of action is mainly determined by the increasing diversity of bacteria (Dwyer 2019, Chaitman 2020, Quraishi 2020, Gal et al. 2021).

The available literature provides several indications for the application of this method. The primary indications for FMT include animals with chronic enteropathy. This term encompasses a variety of gastrointestinal conditions that may have different causes, such as food allergies and intolerances, autoimmune diseases, and infections (Malewska et al 2011, Allenspach and Mochel 2021, Rychlik and Kaczmar 2020, Jergens and Heilman 2022). Before making the decision to apply FMT, it is advisable to carry out appropriate diagnostic tests in order to identify the cause of the enteropathy, as the diarrhoea is likely to recur if the underlying causes are not addressed (Malewska et al. 2011, Rychlik and Kaczmar 2020, Allenspach and Mochel 2022, Jergens and Heilmann 2022). Furthermore, it is not recommended to administer FMT to patients who are concurrently receiving antibiotics, as antibiotics will have a negative effect on the microbiome and reduce the beneficial effects of FMT, as demonstrated in patients with CDI (Tariq et al. 2021). Microbiome transplantation can be applied in the treatment of inflammatory bowel disease in dogs and cats. The author's experience and data from other publications suggest that FMT can be safely administered in patients with IBD, treated with immunosuppressive preparations, glucocorticoids, chlorambucil and/or cyclosporine (Dai et al. 2014, Sugita et al. 2021, Huang et al. 2022).

Donor selection rules

The key to achieving successful treatment outcomes with FMT is appropriate donor selection. In human medicine, fecal donors are subjected to very strict screening procedures aimed at preventing the transmission of infections or other diseases (Cammarota et al. 2017, Silva 2023). Potential donors are screened for dietary habits, the use of antibiotics, immunosuppressants, chemotherapeutic drugs and proton pump inhibitors, and a history of chronic diseases, including gastrointestinal diseases, diabetes, obesity and mental disorders. The tests are also aimed at excluding parasitic diseases as well as the presence of intestinal pathogens and MDR (multiple drug resistance) bacteria in the feces (Zhang et al. 2019, Vendrik et al. 2020). In small animal veterinary medicine, there are many screening protocols that vary depending on published studies (Kao et al. 2017, Chaitman and Gaschen 2021). The aim of these proposed protocols is to ensure that the feces used for FMT do not infect the recipient with enteropathogens and that the transplanted microbiome is of good quality. Most authors recommend that the donor selection procedure should include a detailed history, clinical examination and additional tests, with particular emphasis on the Dysbiosis Index. According to a recent guideline study by Winston et al. 2024, a good donor should exhibit the following characteristics:

- recommended age of over 12 months, not exceeding 75% of the animal's life expectancy,
- donor should not travel longer distances (especially abroad),
- the absence of health problems over the past 4-6 months; in particular, the absence of episodes of chronic gastrointestinal diseases, allergies and immune-mediated diseases. As emphasised by the study authors, acute gastrointestinal symptoms (lasting for up to 2 weeks) do not exclude the transplant donor but require re-assessment after a minimum of 3 months after the symptoms have subsided. Such an interpretation is in line with studies which indicate that in such cases, in contrast to the chronic process, the return to a normal microbiome occurs within several weeks (Suchodolski et al. 2012, Ziese et al. 2018, Werner et al. 2020),
- it is very important that the donor should not have received any antibiotics over the last six months, and should not have received proton pump or H2-receptor blockers for a minimum of two weeks. The author of this publication believes that this period, in case of long-term administration, should be extended to a minimum of four weeks, as proton pump blockers are a known factor in inducing dysbiosis (Naito et al. 2018, Kiecka and Szczepanik 2023),
- the donor should be vaccinated regularly in accordance with current guidelines,

- the donor should be fed a balanced diet. The authors recommend that animals that have been fed raw meat in the last 30 days should be excluded as donors, justifying this approach on the grounds of the increased number of pathogens in the composition of the intestinal microbiome,
- the donor should not be overweight or underweight (from 4 to 6 points on a 9-point body condition scale),
- the donor's stool consistency should be normal (2 or 3 points on a 7-point consistency scale),
- a normal result of a clinical (physical) examination.

Laboratory tests performed on the donor should show:

- normal blood chemistry parameters, and normal complete blood count results. The authors recommend the determination of cobalamin, folic acid, TLI, specPL, cortisol, and thyroxine,
- negative results of parasitological tests (deworming, if applicable),
- correct assessment of the fecal microbiome (Dysbiosis Index value below 0). The authors do not recommend fecal cultures for the identification of bacteria. Instead, they recommend PCR or immunohistochemistry tests for the presence of pathogens, such as *Salmonella spp., Campylobacter spp.* or *C. perfringens, Giardia spp*.

According to the author of this article and a large group of the most prominent gastroenterologists, the balanced behaviour of the donor is also important as highlighted in the publication "Clinical Guidelines for Fecal Microbiota Transplantation in Companion Animals" (Winston et al. 2024). This is the most detailed compendium with guidelines on the principles of donor selection to date.

Rules for performing the procedure

In human and veterinary medicine, it is recommended that fresh feces from a tested donor be used for transplantation within six hours after defecation (during this time, storage at room temperature is sufficient). It is recommended that storage and preparation be as short-term as possible in order to protect anaerobic bacteria. Longer storage requires the addition of glycerol to represent approximately 10% of the transplant material. Material prepared in this way can be stored for up to 6 months at a minimum temperature of -20 degrees Celsius. One study showed that transplants made of fresh and frozen feces were equally effective (Brandt and Aronidas 2013, Lee et al. 2016). A majority of authors describing the procedure for administering faecal content emphasise that the process is not complicated. The process of preparing the material for transplantation begins with the addition of 0.9% NaCl solution in a ratio of 1 volume of feces to 4 volumes of 0.9% NaCl. The amount of feces administered varies considerably, ranging from 1 to 5 g of feces per kg of body weight (Chaitman J et al. 2020, Kao D et al. 2017). The material is then blended to obtain a homogenate. The next step is to filter the resulting homogenate through gauze or a fine sieve to avoid clogging the administration equipment. The final stage is to collect the filtered homogenate into a syringe (5-10 ml of homogenate per kg of recipient body weight) and, depending on the method of application, administer it to the recipient (Fig. 1). In the available literature, authors describe the administration of the transplant in several ways (Table1). Most authors, including the author of the current study, recommend the administration of the material into the rectum or the colon via a rubber catheter (Chaitman et al. 2016, Pereira et al. 2018, Alves et al. 2023). Another way is to administer the transfer during endoscopy to the duodenum or the colon or *per os* in special capsules dissolving in the small intestine (Bottero et al. 2017, Dwyer 2019, Ramai et al. 2021). However, the administration of the transplant during an endoscopic procedure in companion animals requires general anaesthesia, which limits its widespread application. In the case of the application of fecal contents using a catheter, the author of this publication recommends that, before the administration, the recipient should take a 30-minute walk and should not be fed 6 to 8 hours before the administration. When dealing with restless animals, some authors recommend the administration of acepromazine or other drug with a similar effect. The author of this publication does not use such medications, as the procedure is simple and short-term (administration of the contents with a catheter) that there has been no need for the administration of sedatives to date. The homogenate dose, as recommended by most authors, is 5-10 mL per kg of body weight. A larger amount of the transplant administered can accelerate intestinal peristalsis and the expulsion of the material (Chaitman et al. 2020, Tuniyazi 2022). As emphasised by most clinicians performing this procedure, after the administration, it is important to handle the post-transplant recipient appropriately. It is advisable to keep the patient's hindquarters lifted for several minutes so that the homogenate administered can reach the farthest sections of the colon. At the author's clinic, the owner is advised to return home by car if possible. It is not advisable to walk or feed the patient for several hours in order not to stimulate peristalsis. However, research on the optimal preparation and administration of FMT in human and veterinary medicine is still ongoing.

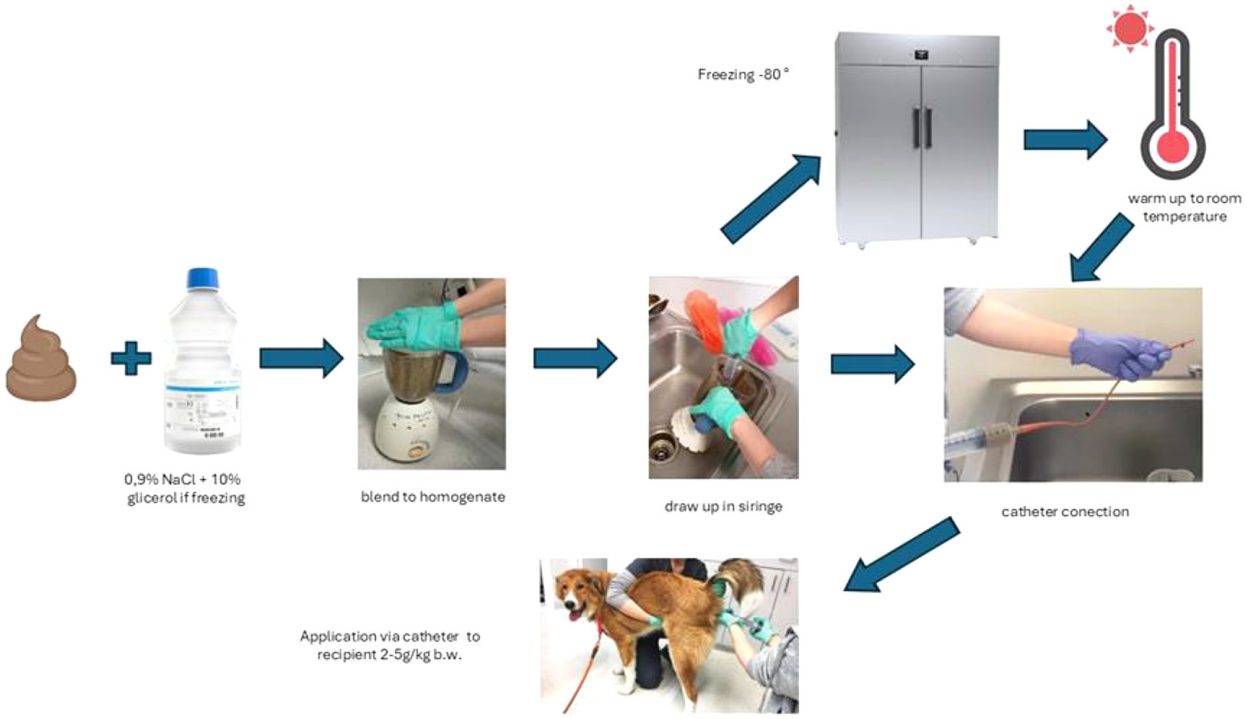

Fig. 1. Fecal Microbiota Transplantation preparation and administration diagram.

Advantages and disadvantages of FMT

The effectiveness of fecal transplantation in the treatment of enteropathy is assessed positively in the vast majority of studies (Weese 2013, Gerbec 2016, Chaitman et al. 2020; Table 1). It should be added that it should be used only as a complementary method for treating enteropathy (Bryan CE, Jergens and Heilmann 2022, Toresson et al. 2023). Fecal transplantation is one of the components of dysbiosis treatment in chronic enteropathies in companion animals. In such patients, dietary changes, the adminitration of probiotics and prebiotics (symbiotics), and, in advanced clinical cases, reducing inflammation by administering low-dose glucocorticoids for several days should also be considered. This is confirmed by research comparing the administration of FMT with supplementation with psyllium alone. In this study, the authors did not note any differences in the effectiveness of both monotherapies (Alvesz et al. 2023). Since the observations of the author of this article indicate that, in patients with IBD, a single transplantation may not show satisfactory clinical results, several administrations of the fecal content every three weeks or so are required. Patients in the author's clinic were administered the transplant several times every 48 hours until clinical improvement was achieved. Puppies of small breeds were administered 10 mL of the transplant, whereas those of larger breeds were given 20 mL. Larger amounts of the material being transplanted often caused rapid expulsion of the content. According to the author, better efficacy of FMT was observed if the recipient's abnormal ID values were lower, which is in line with the information provided by other clinicians (Toresson et al. 2021).

In humans, studies of complications following FMT include both short-term and long-term side effects. Table 2 presents the most commonly reported side effects in humans (Park and Seo 2021). The method of administration may also influence the occurrence of side effects. Studies in humans have shown that treatment of the anterior gastrointestinal tract with FMT was associated with a higher incidence of adverse events than FMT treatment of posterior gastrointestinal tract (Bonovas et al. 2017). This was due to aspiration pneumonia, associated with the administration of FMT through the nasoduodenal tube (Baxter et al. 2015).

In veterinary medicine, studies on FMT administration for the treatment of enteropathy have most often shown only short-term side effects, mostly gastrointestinal, or no side effects at all. Most authors concluded that it is a safe therapeutic method (Table 1). Lee et al. 2024 also studied the effects of FMT administration on healthy dogs. They observed no negative effects on blood counts, selected biochemical and immunological parameters, or the Dysbiosis Index. In animals receiving fecal transplantation, only short-term clinical symptoms were observed, including vomiting, diarrhea, and a slight decrease in activity and appetite. These symptoms resolved without treatment.

Like most gastrointestinal disease treatment

Table 1. Clinical cases describing fecal transplantation in dogs.

Authors	Indication	Number of animals studied	Administration method	Effect	Comments
Chaitman et al. 2021	Acute diarrhea without complications	11 dogs	Enema, single dose	Clinical improvement after 7 days	Improving the Dysbiosis Index
Berlanda et al. 2021	Chronic enteropathy, relapses for 3 years, no therapeutic effects of current treatment	1 dog	Orally, capsules two cycles with a break of 8 months, one cycle involved administration of one capsule for 30 days	Improvement, gastric symptoms disappeared, general condition improved	Improving the Dysbiosis Index, no side effects
Niina et al. 2021	IBD	9 dogs	Enema, single dose	Improvement, clinical symptoms disappeared after 3 days	No side effects, reduced CIBDAI index
Collier et al. 2022	Assessment of the inclusion of FMT in standard therapy (corticosteroids and hypoallergenic diet) in dogs with IBD	13 dogs with IBD randomly divided into two groups: one with FMT treatment and one with placebo	Enema, a total infusion volume of 10 ml/kg b.w. FMT or sterile saline solution	A decrease in the CCECAI index was observed, but there were no significant differences between the index in the placebo and FMT groups.	The CCECAI clinical index and microbiota sequencing (16S rRNA) were assessed.
Alves et al. 2023	Chronic large intestine enteropathy	30 dogs, comparison of the effectiveness of therapy for dogs receiving FMT and psyllium	Enema, single dose	After 30 days, the study showed greater improvements in body weight and BCS in dogs with FMT, but no differences were observed in fecal examination results, defecation frequency, or duration of diarrhea episodes.	Assessment based on the CIBDAI index and body condition score (BCS) in both groups showed similar effects in terms of improving clinical symptoms.
Toresson et al. 2023	Chronic enteropathy, no effects after diet treatment	41 dogs	Enema 5-7g/kg b.w., 3 doses with 10-20 day intervals	Improvement was observed in 31 dogs (26 without relapse after 6 months), 10 showed no improvement.	Minor side effects in 7 dogs: diarrhea, which resolved after 2-3 days
Lee et al. 2024	Assessment of the occurrence of side effects in healthy dogs	10 dogs	Enema, given in a dose of 5g /kg b.w.	Short-term clinical signs included vomiting (3 dogs), diarrhea (4 dogs), decreased activity (2 dogs), and loss of appetite (1 dog). Symptoms resolved without treatment.	No changes in morphology and biochemical parameters
Min-OK et al. 2024	Chronic enteropathy, no improvement with diet, antibiotics and steroids	8 animals (5 dogs and 3 cats)	Oral capsules, The duration of treatment from 14 to 100 days in dogs, 30 days in cats	Improvement in clinical signs in all eight animals, normalization of appetite and activity levels.	No side effects significant reduction in CIBDAI, CCECAI, and FCEAI
Hanifeh M et al. 2024	Antibiotic (tylosin) dependent enteropathy	14 dogs with enteropathy (7 with FMT, 7 placebo)	Enema, FMT administered for 4 weeks.	Effectiveness of FMT treatment (71.4%) was slightly higher than placebo (50%), but this difference was not statistically significant	Assessment of CCECAI, fecal consistency, and microbiome in dogs with tylosin-sensitive enteropathy
Vecchiato et al. 2025	Chronic enteropathy, no improvement with diet,	20 dogs	Enema 2-5 g/kg b.w.	Clinical improvement in 17 dogs within 90 days and clinical improvement in 10 dogs within one year after FMT	Significant improvement was observed in CIBDAI, dysbiosis index (DI), and primary (PBAs) and secondary (SBAs) bile acids in feces
Pérez-Accino et al. 2025	Diagnosed chronic enteropathy	7 dogs	Enema, single dose in 6 dogs, three doses in one dog	Improvement of the clinical index up to 10 weeks, varied changes in the composition of the microbiome after FMT	Clinical indicators CCECAI and microbiota sequencing (16S rRNA) were assessed

methods, transplantation of the intestinal microbiome has both advantages and disadvantages. Significant advantages of food content transplantation include a reduction in the number of pathogenic strains as well as an increase in the diversity of bacterial flora and metabolites. The disadvantages of food content transplantation include low impact on adherent bacteria, recurrence of dysbiosis in the event of mucosal inflammation, possible transfer of pathogens and genes, and neoplastic activation. Transmission of obesity or diabe-

Table 2. Adverse events in patients who underwent fecal microbiota transplantation.

Short-term adverse events	Long-term adverse events
bloating/spasm	obesity
gaseousness	immune-mediated disorders
diarrhea	immune thrombocytopenia
irregular bowel habit	rheumatoid arthritis
irritable bowel syndrome	inflammatory bowel disease
constipation	irritable bowel syndrome
abdominal pain, tenderness	
fever	
nausea	
aggravation of inflammatory	
bowel disease	
gram negative bacteremia	
bowel perforation	
belching	
death	
hematochezia	

tes genes to donors has been observed in both humans and animals (Alang and Kelly 2015, Dailey et al. 2019, Wei et al. 2024). In turn, there are numerous reports on the positive effects of the intestinal microbiome in the treatment of many metabolic and neurological diseases (Abenavoli et al. 2019, Amabebe et al. 2020, Mosquera et al. 2024). Even though FMT has shown promising results, with minor adverse events, in the treatment of various gastrointestinal disorders in dogs, safety concerns and a lack of complete understanding of the therapeutic mechanisms are the main reasons for limiting its wider, widespread use (Wang et al. 2016, Park and Seo 2021, Marrs and Walter 2021). Due to the lack of a satisfactory explanation of the mechanisms occurring during the transplantation of intestinal microbiome, human transplantation, e.g. in the USA and Poland, has routinely been only performed, until now, in individuals with recurrent or refractory gastrointestinal infection by *Clostridioides difficile* bacteria (Merrick et al. 2020).

Currently, clinicians' opinions on the prospects for fecal transplants are divided. There are many studies in the available literature that confirm the efficacy of this form of therapy in patients with chronic enteropathy (Bottero et al. 2017, Pereira et al. 2018, Alves 2023, Winston et al. 2024). However, the wide variety of FMT protocols used makes it difficult to develop a clear opinion on the usefulness of FMT in the treatment of enteropathies. The evaluation of the effectiveness of transplant administration requires future long-term studies assessing the impact of this form of enteropathy therapy on the immune system and the occurrence of long-term adverse events (Salavati 2022, Zikou et al 2024). However, observing the advances in research into probiotics and symbiotics, it is probable that FMT, in its present form, may represent a transitional form of treatment, the ultimate goal of which will be the administration of well-characterised, clinically studied and therapy-specific probiotics (Limdi et al. 2006, Jergens and Heilmann 2022, Xia et al. 2024).

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