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Fecal Microbiota Transplantation in Small Animals

Literature review

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Abstract

Fecal Microbiota Transplantation (FMT) is an emerging therapeutic tool for dysbiosis in veterinary medicine. FMT has proven to be effective in human recurrent *Clostridium difficile* infection (rCDI), where its protocols and research have been the foundation of the recent studies on FMT treatment in veterinary medicine. It is important to understand the effects of the gut microbiome on both local and systemic processes, and how we can use the information obtained from the microbiota to treat diseases. In this literature review, the application and effects of FMT in veterinary medicine, and its promising results in GI disorders as well as the current research on non-GI disorders, will be further investigated. Furthermore, donor selection criteria, criteria of the recipient, and preparation of fecal material necessary for successful FMT treatment will be presented.

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1 Abbreviations

FMT – Fecal Microbiota Transplantation

rCDI – Recurrent Clostridium Difficile Infection

DI – Dysbiosis Index

GI – Gastrointestinal

SCFA – Short Chain Fatty Acids

E.g – Example given

C. difficile – Clostridium difficile

C. hiranonis – Clostridium hiranonis

CE – Chronic Enteropathy

IBD – Inflammatory Bowel Disease

NHD – Non-hemorrhagic Diarrhea

AHDS – Acute Hemorrhagic Diarrhea Syndrome

E. coli – Escherichia Coli

CKD – Chronic Kidney Disease

ISAPP – International Scientific Association of Probiotics and Prebiotics

CD – Chronic Diarrhea

CCECAI – Chronic Canine Enteropathy Clinical Activity Index

NRE – Non-Responsive Enteropathy

IRE – Immunosuppressant-responsive enteropathy

DM – Diabetes Mellitus

AD – Atopic Dermatitis

TRD – Tylosine Responsive Diarrhea

CDI – Clostridium Difficile Infection

2 Introduction

Gastrointestinal diseases are commonly seen in animals, and complete resolution of the clinical signs can be challenging, especially in chronic processes. Dogs and cats are frequently presented with diarrhea, vomitus, and acute-and chronic enteropathies, when the imbalance and/or altered composition of the gut microbiome – called dysbiosis – is a common pathological background [1]. The role of the gut microbiome is essential in physiological and pathological processes that occur in the body, and recent studies reveal its importance in immunological activities, metabolism, brain health, and behavioral development [2]. To be able to evaluate the gut microbiota and use the information obtained from it is proven to be an important factor in the management, as well as the diagnosis of disease.

The study of the gut microbiota and microbiome is a complex matter, and the understanding of the microbiological composition and its abundance is crucial. The recent study of bacterial composition and profile with the use of 16S rRNA sequence analysis and Dysbiosis Index (DI) is an important step in the diagnosis of dysbiosis. To this day, a lot of pet animals suffer from acute and chronic intestinal diseases, where the majority of treatment is revolved around antidiarrheal agents, dietary change, pro- and prebiotics and/or antibiotic treatment, often without improvement. To restore the microbiota diversity and the normal flora in animals suffering from dysbiosis, beside the usage of pre-, pro-, and synbiotics, fecal microbiota transplantation (FMT) is a relatively new therapy that has gained interest in veterinary medicine in recent years [3]. Antibiotic resistance causes significant concerns, and limiting the use of antibiotics and finding other alternatives to therapy is an important step in human and veterinary medicine [3]. The use of FMT creates an opportunity to use the beneficial bacteria, and their metabolites in wide range and high amount, to improve gastrointestinal health and to improve immunity in a natural matter, and by that limiting the use of antibiotics [3]. It creates an opportunity to provide an alternative therapeutic intervention, either as standard therapy, supplementary therapy, or if conventional therapy fails. In veterinary medicine, FMT is relatively new and the understanding of the application, mechanism of action, and efficacy are in an early stage, thus it is proven to be a promising research field worthy of further investigation and exploration [3]. In this literature review, the aim is to increase understanding of the importance of the gut microbiome's effect on physiological and pathological processes, and the therapeutic advantages of using FMT in dysbiosis. Furthermore, this review should be a well understanding overview of the

administration of FMT, accessible to a wide range of veterinarians, from specialists to students.

3 Methods

Search engines such as Google Scholar, PubMed, and ScienceDirect have been mostly used to obtain material for this literary review. Most of the studies used have focused on dysbiosis, common gastrointestinal diseases in dogs, and the effect of FMT as therapy. Additionally, the focus has revolved around studies seeking to explain the history, indications, mechanism of action, ways of administration, preparation of fecal material, and donor selection associated with FMT therapy in human and veterinary medicine.

Since FMT is a relatively new therapeutic technique in veterinary medicine, special emphasis has been given to studies published in the most recent years, mainly from 2015 until 2022 (present). However, to provide a thorough background for recent discoveries, older and fundamental studies concerning the gut microbiota and microbiome, bacteriology, and gastrointestinal diseases are also included.

4 The gut microbiome

The gastrointestinal (GI) tract of mammals contains a great number of microorganisms, called the gut microbiota, while the gut microbiome is referred to as the mutual genome of the organism, consisting of the microbiota and its function [4]. The gut microbiota consists of various microbes such as viruses, protozoa, fungi, and bacteria composing a complex ecosystem [5]. Recent studies done by DNA-sequencing technology show that every part of an animal's body contains a certain type of microbiota, such as the skin, GI tract, oral and nasal cavity, respiratory system, urinary tract, and the reproductive tract [1]. These microbes and the host relates in a symbiotic fashion, being beneficial and dependent on each other [5]. A good example of this symbiosis is that the epithelium of the mucous membrane lining the gut wall produces mucous that feeds the bacteria, meanwhile, the bacteria produce short-chain fatty acids (SCFA) that give nourishment to the epithelium itself [5]. A study done by Swanson et al (2011) shows by metagenomic sequencing reads obtained from fecal samples, that > 98% of the intestinal microorganisms consist of bacteria [6]. The most common bacteria found in the canine GI tract arise from the five phyla: *Fusobacteria, Proteobacteria, Bacteroidetes, Actinobacteria,* and *Firmicutes* [5]. The number and type of bacteria vary along the GI tract and depends on the pH levels in the various part of the tract, the nutritional availability, oxygen tension, and the intestinal motility [1]. In the GI microbiota of healthy dogs and cats the *Bacteroidetes, Actinobacteria, Fusobacteria,* and *Firmicutes* are the most abundant bacteria, and a healthy gut is generally characterized by the *Bacteroidetes*- and *Firmicutes* profiles [1]. The phylum *Firmicutes* comprise many significant species, with *Clostridia* being one of the most prevalent [4]. *Bacilli*, *Faecalibacterium, Ruminococcus,* and *Erysiopelotrichi* are other important bacterial classes within the *Firmicutes* phyla [4].

4.1 The function of the microbiome

The gut microbiome affects the physiological functions of the host, contributing to host metabolism, the immune system, brain health, and function, as well as protection against pathogens [5]. The gut microbiota can affect the host locally and systemically, referred to as the gut-organ axis, and the metabolites produced influence important functions of the intestines and other organs, including the heart, brain, and kidneys [4, 7]. The immune system's development is influenced by the colonization of microorganisms that happens in the gut during birth [1]. The development of the immune system and the local protection of

antibodies from the gut microbiota is important in the relationship between the gut microbiome and the immune system [1, 5]. This significantly effects the overall health of the animal, and the ability to recognize the beneficial bacteria, as well as protection against the pathogenic bacteria that can cause infection [1, 5]. In the intestinal lumen, the immune system works by having macrophages that kill the intrusive bacteria, B-cells that present the intrusive bacteria triggering bacterial destruction, or T-helper (Th) cells that aid in protection against inflammation of the gut wall [5]. The balance between Treg (regulatory T-cell) and Th17 cells is important in the homeostasis of the intestinal lumen, and if there is a reduced number of Treg cells, the uncontrolled T cells will trigger intestinal inflammation by responding to bacterial antigens [5]. The gut microbiota has proven to be important in the development of the immune system and the modulation of immune response, as well as it has significant effect e.g in obesity disorders [3]. For example, an increase in *Firmicutes* levels is shown to have an effect on the ability to harvest energy and therefore weight gain, and was proven by transferring fecal material from an obese donor to an underweight recipient [3]. The diversity of the gut microbiota assures the existence of biochemical pathways and enzymes the host otherwise does not possess [2]. The bacteria produce energy and substrates that are important in the proliferation of bacteria as well as to the host itself, along with the fermentation of non-digestible carbohydrates and alcohols [2]. One of the most important metabolic pathways is the production of SCFAs (acetate, propionate, and butyrate) and gasses that happen in the colon and gives energy to the intestinal epithelium as well as other tissues [2]. The transformation of primary- and secondary-bile acids is another important process of the microbiota and is essential in the absorption of lipid-soluble vitamins and dietary fats in the gut [2].

4.1.1 Some important functions of the bacteria in the gut microbiota

Clostridium hiranonis (C. hiranonis) is an important bacterium in converting primary bile acids into secondary bile acids (e.g deoxycholic- and lithocholic-acids) [4]. As explained earlier, this is an important metabolic pathway of the microbiota, and secondary bile acids have several functions in the dog´s colon [4]. Secondary bile acids can inhibit the formation of *Clostridium difficile (C. difficile)* spores, while primary bile acids will promote the formation of *C. difficile* which can lead to dysbiosis [4]. Additionally, some of the functions of the secondary bile acids are to help regulate homeostasis by acting as signaling molecules by binding to G protein-coupled bile acid receptor 1 (GPBAR-1), and through binding to

farnesoid X-receptor they can maintain glucose concentrations [4]. Meanwhile, a decrease in secondary bile acids is seen in dogs suffering from chronic enteropathies (CE) or after antibiotic treatment [4]. A decrease in secondary bile acids is caused by a decrease in *C. hiranonis*, causing an increase in primary bile acids leading to dysbiosis [4].

Faecalibacterium and *Ruminococcus* of the *Firmicutes* phyla are important bacteria in the fermentation of dietary carbohydrates into SCFAs (acetate, propionate, and butyrate) [4]. SCFAs have many functions in the canine GI tract, some of them being an important growth factor and source of energy for the epithelial cells [4]. They also conduct intestinal motility and modify the immune system [4]. Examples of the latter are the ability of butyrate to generate immunoregulatory T-cells, and the ability of acetate to regulate intestinal motility [4].

5 Dysbiosis

5.1 Definition of dysbiosis

When there is an imbalance and/or altered composition of the microbiota, it is referred to as gut dysbiosis, and this is associated with functional changes in the metabolome, microbial transcriptome, and proteome, that is the complete sets of metabolites, transcripts (RNA) and proteins expressed by the microbiota, respectively [5]. A sign of dysbiosis is seen with an increased abundance of mostly the members of *Enterobacteriaceae* family, due to alterations in the bacteria homeostasis that occurs in dysbiosis [5]. *Enterobacteriaceae* family is a facultative anaerobic bacteria, which plays a role in the oxygen composition in the intestinal tract [5]. If there is an increase in free oxygen due to e.g increased permeability of the gut wall, it will lead to an expansion of *Enterobacteriaceae* family due to decreased availability of the anaerobic bacteria, leading to dysbiosis [5].

5.1.1 Dysbiosis in diseases

Local and/or systemic diseases can cause or can be caused by alterations in the gut microbiota, and are therefore associated with dysbiosis [5]. Inflammation, metabolic syndromes, breed, pregnancy, abnormal behavior, age, sex, neurological disorders, diabetes

mellitus (DM), and obesity are all described to be associated with changes in the gut microbiome [1, 2, 4].

In human medicine, studies have shown that causes of dysbiosis have been associated with a shift in the *Firmicutes-Bacteroidetes* ratio, which has been seen in people suffering from obesity, and in people with diabetes type 2 there is an increase in non-butyrate-producing *Clostridia* strains and a decrease in SCFA-producing *Clostridia* strains [4]. If the patient suffers from immunodeficiency, inflammation, infection, or exposure to antibiotics, toxins, or sudden dietary changes, this can contribute to a shift in the relative bacterial abundance leading to dysbiosis [8]. These factors can disturb the normal microbiota, causing a significant reduction of the healthy bacteria which allows the pathogenic bacteria to colonize, grow, and eventually cause dysbiosis leading to pathological reactions [1].

The microbiota of individuals suffering from intestinal dysbiosis, compared to healthy animals, shows changes in the amount, function, and variety of bacterial species [4]. These changes can cause damage to the intestinal barrier of the GI tract, which can lead to the translocation of pathogens and eventually the development of diseases [4]. Dysbiosis may have a negative impact on the host, due to changes in the bacterial metabolites [4]. Types of dysbiosis and its local consequences are presented in *Table 1* [4].

Table 1. *Types of dysbiosis and its local consequences [4]*

Type of Dysbiosis	***Consequences***
• Abnormal substrates stored in the intestinal lumen (medication, undigested nutrients etc) [4].	Osmotic/secretory diarrhea do to an increase in bacterial species [4].
• Improper function of the microbiome due to lack of commensal bacteria (C. hiranonis) [4].	Decreased or lack of conversion of secondary bile acids from primary bile acids caused by bacterial overgrowth (*C. difficile, C. perfringens, E.coli*) [4]
• Increase in total number of bacteria in the small intestine [4].	Activation of inflammatory reactions and osmotic/secretory diarrhea due to increased production of bacterial metabolites [4].
• Increase in the number of mucosa-adherent bacteria [4].	Increased inflammatory reactions due to increased adhesion of the bacteria [4].

Dysbiosis is described to be a contributing factor in the development of enteropathies [9]. Studies done by Suchodolski et al (2012) show that dysbiosis is a common feature of several GI disorders, e.g intestinal bowel disease (IBD), acute non-hemorrhagic diarrhea (NHD),

acute hemorrhagic diarrhea syndrome (AHDS) and food-responsive CE and non-food responsive CE [9] [10]. AHDS and NHD are acute enteropathies that are shown to cause big alterations in the canine microbiota, with an increase of *E.coli and C.perfringens*, and a decrease in SCFA-producing bacteria such as *Actinobacteria* and *Firmicutes* [4]. A long-term dysbiosis can lead to chronic enteropathies; a common GI disease in domestic dogs defined as the persistence of vomiting and diarrhea for three or more weeks [9]. In chronic intestinal diseases, the mechanism and actions of the gut microbiota are greatly impaired, and the local GI ecosystem will be damaged by the colonization of pathogens [9]. The most common chronic enteropathy associated with dysbiosis is inflammatory bowel disease (IBD) [4]. In IBD, studies have shown that there is an increase in *E.coli,* which is described to be a mucosa-adherent bacteria, and a decrease in *Fusobacteria, Clostridiales, Bacteriodaceae,* and *Prevotellaceae* [4].

Dysbiosis has also systemic consequences that are identified in human beings, such as heart diseases, IBD, liver diseases, chronic kidney diseases (CKD), brain disorders, diabetes, respiratory diseases, and cancer, illustrated in *Figure 1* [11].

Few studies are available about the systemic consequences of gut dysbiosis to distant organs in dogs, e.g canine atopic dermatitis, obesity, and diabetes mellitus. Canine atopic dermatitis (AD) is a genetically inherited disease with complex pathogenesis, including immunological modulations, impaired barrier function, and the gut microbiota [12, 13]. How the intestinal microbiota affects extra-intestinal organ functions is not completely understood, but recent studies suggest that there are close links between the intestinal microbiota and the pathogenesis of atopic dermatitis, such as inflammation and immunity [14]. Obesity as well as diabetes mellitus can also develop as a consequence of intestinal dysbiosis in dogs and cats [15]. The high abundance of *Enterobacteriaceae* in addition to fecal alterations of unconjugated bile acids are seen in dogs with diabetes mellitus, and in cats with diabetes mellitus type 2 there is a decrease in butyrate-producing bacteria [15].

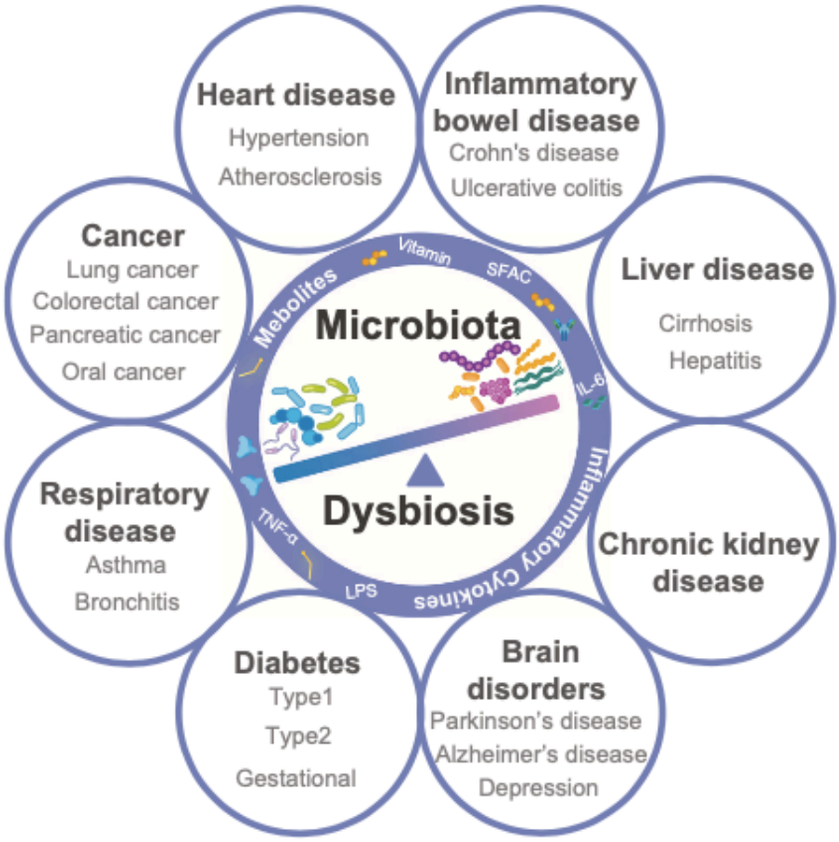

Figure 1. *Systemic consequences of dysbiosis in humans [11]*

5.2 Diagnosis of dysbiosis

The gut microbiome is the largest one of the different body sites that have their own microbiota [2]. The genome of the gut microbiota is 150 times larger than animals' own genome, and it's composed of 10^{13}-10^{14} cells, containing more than 1000 bacteria species [2]. Modern technologies have contributed to understanding the diversity of microbial colonies, such as next-generation sequencing [2]. Analysis of the bacterial 16S rRNA gene by molecular-phylogenic technique creates a detailed index of the bacteria groups present in the GI-tract, showing that the two phyla *Bacteroidetes* and *Firmicutes* compose more than 90% of the bacteria´s present [2].

A Dysbiosis Index (DI) is used to assess the canine or feline fecal microbiome by qPCR reactions, based on the seven most important bacterial groups found in the gastrointestinal tract and summarizes them together with the total bacterial count, into a single number (DI) 5]. The Dysbiosis Index can quantify dysbiosis and is used by veterinarians to observe the patient´s response to treatment, e.g FMT, as well as the progression of the disease [4, 5]. It can also assess the abnormal- or normal conversion of bile acids in the gastrointestinal tract,

based on the abundance of *C. hiranonis*, which is the major contributor to abnormal microbiota [16]. The Dysbiosis Index has been used in several clinical studies, and reference intervals are obtained from healthy dogs and cats from countries all over the world [16]. An article (Canine and Feline Microbiota Dysbiosis Index) published by Texas A&M University;, shows the reference intervals in *Table 2* [16].

Table 2. *Reference intervals of the 7 bacterial groups and the Dysbiosis Index in dogs and cats [16].*

Bacteria	**Function**	**Normal abundance in dogs**	**Normal abundance in cats**	**Change observed in dysbiosis**
Faecalibacterium	Anti-inflammatory, production of SCFA	3.8-8.0	3.8-8.4	Decreased
Turicibacter	Production of SCFA	4.6-8.1	4.4-9.0	Decreased
Streptococcus	Overgrowth associated with dysbiosis	1.9-8.0	1.6-5.2	Decreased
E. coli	Pro-inflammatory	0.9-8.0	1.4-7.0	Increased
Blautia	Production of SCFA	9.5-11.0	Not measured	Decreased
Fusobacterium	Production of SCFA	7.0-10.3	Not measured	Decreased
C. hiranonis	Conversion of primary to secondary bile acids	5.7-7.1	4.5-7.1	Decreased
Dysbiosis index		< 0 normal 0-2 equivocal > 2 dysbiosis		

Data expressed log DNA/gram of feces

The seven bacteria taxa *Faecalibacterium, Turicibacter, Streptococcus, E. coli, Blautia, Fusobacterium,* and *C. hiranonis* are the bacteria that have shown the best specificity and sensitivity, and are therefore always the bacteria that is used to measure the Dysbiosis Index [17]. The Dysbiosis Index is a mathematical algorithm where DI is measured from the mean of each class of the prototype (healthy and diseased) to the closeness of the test sample, into a single value [17]. Interpretation of DI should always be done together with the abundance of the bacteria individually [4]. A normal microbiota is indicated with a DI < 0 [4]. A minor change in the microbiota is indicated as equivocal, meaning the DI is between 0-2, and dysbiosis is shown with DI > 2 [4]. A more mathematical understanding of the DI is defined

by AlShawaqfeh et al. (2017) [17] as *"the difference between the Euclidean distance between the test sample and the healthy class centroid, and the Euclidean distance between the test sample and the diseased class centroid"*, and can be calculated with the formula [17]:

$$\boldsymbol{DI}\,(\boldsymbol{z};\boldsymbol{\mu C_D},\boldsymbol{\mu C_H}) = \|\,\boldsymbol{z} - \boldsymbol{\mu C_H}\,\|_2 - \|\,\boldsymbol{z} - \boldsymbol{\mu C_D}\,\|_2\,,$$

Where $\boldsymbol{\mu}C_D$ means the Centroid of the Diseased sample, and $\boldsymbol{\mu}C_H$ means the Centroid of the Healthy sample [17]. When DI = 0, it means that the center of both classes (diseased and healthy) is in equal line with the test sample [17]. The further distance between the sample and the classes, the higher the DI, meaning a higher deviation from normobiosis [17].

Figure 2 [16] shows that most of the dogs with DI > 2 have a decrease of *C. hiranonis*, and is typically found in dogs suffering from chronic enteropathies, exocrine pancreatic insufficiency (EPI), and dogs treated with tylosine and metronidazole (antibiotics) [4]. Dogs having DI > 2 and a normal abundance of *C. hiranonis*, are typically found in dogs treated with omeprazole (proton-pump inhibitor) and dogs fed with raw food diet, particularly Biologically Appropriate Raw Food (BARF) [4].

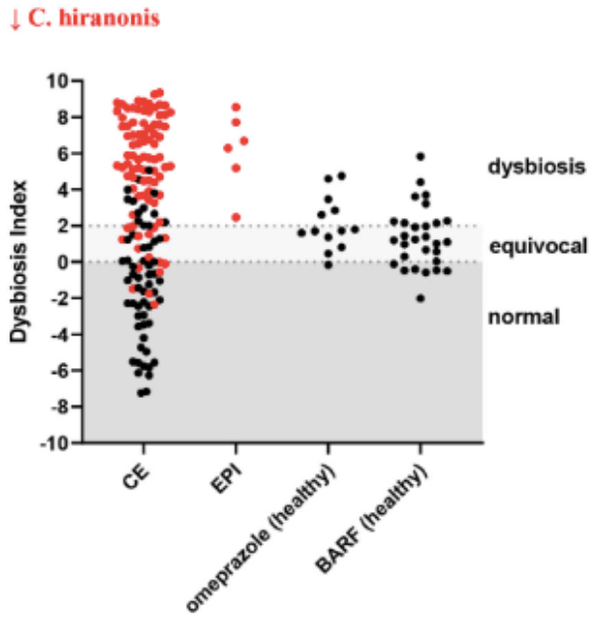

***Figure 2**. Dysbiosis Index in dogs diagnosed with chronic enteropathy and exocrine pancreatic insufficiency, and in healthy dogs treated with omeprazole and BARF diet, in correlation with abundancy of C. hiranonis. Obtained from Texas A&M University (Canine and Feline Micobiota Dysbiosis Index) [16].*

Table 3 shows a fecal sample from a dog sent in for DI interpretation with real-time PCR. This is a patient of Dr. Pápa Kinga (Department of Internal Medicine, University of Veterinary Medicine, Budapest), which presented to the clinic with signs of chronic enteropathy. From the fecal sample sent in, the DI is 1.6, meaning it is equivocal, and it's recommended to do a new sample after a few weeks. The sample shows a decrease in the abundance of *C. hiranonis*, which is consistent with reduced or absent conversion of primary to secondary bile acids in the intestine. The other 6 bacteria were normal.

Table 3. *Dysbiosis Index Interpretation of a dog with dysbiosis by Dr. Pápa Kinga*

Bacteria	**Result**	**Reference value**	**Change observed**
C. hiranonis	0.1	5.1-7.1	Decreased
Faecalibacterium	4.4	3.4-8.0	Normal
Turicibacter	6.8	4.6-8.1	Normal
Streptococcus	4.1	1.9-8.0	Normal
E. coli	4.6	0.9-8.0	Normal
Blautia	10.1	9.5-11.0	Normal
Fusobacterium	10	7.0-10.3	Normal
Dysbiosis Index Interpretation: 1.6			

As mentioned earlier, Dysbiosis Index can be used to observe the patient's response to treatment, e.g FMT [16]. Figure 3 illustrates the changes in the DI of a patient treated with FMT after a long-term antibiotic treatment [16]. The graph shows that after FMT treatment the DI decreases while the abundance of *C. hiranonis* increases.

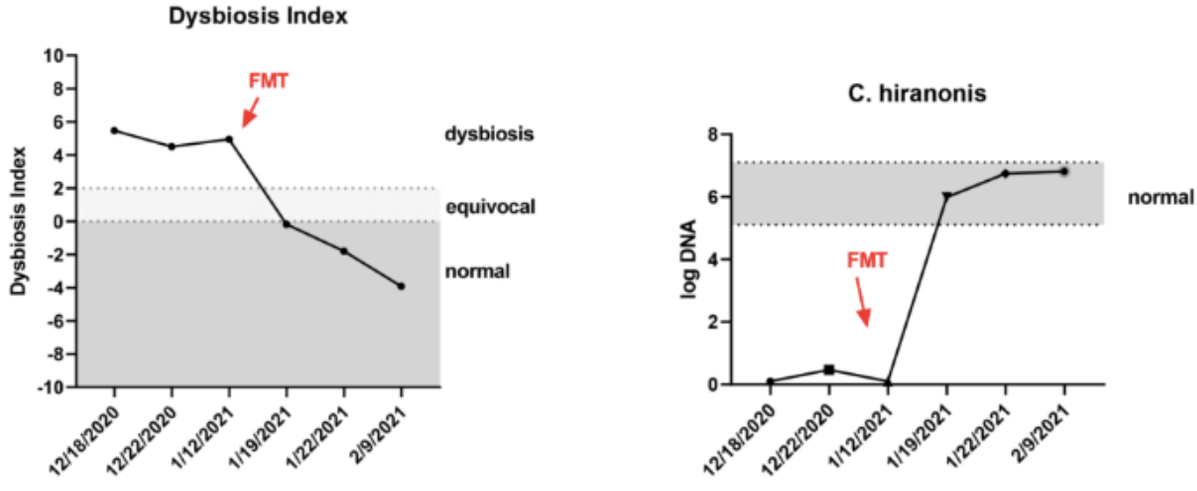

Figure 3. *Dysbiosis Index observed in a canine patient treated with Fecal Microbiota Transplantation due to dysbiosis caused by a long-term antibiotic therapy [16].*

5.3 Treatment options

There are a few therapeutic approaches to change the gut microbiota in case of dysbiosis, including pre-, pro-, syn- and postbiotic supplements, therapeutic diets, and pharmacological treatments like antibiotics and FMT [9]. The therapeutic interventions are not always resolutive and depend highly on the patient's response, which is quite variable [9]. The use of FMT is emerging in the treatment of dysbiosis, and will be further described after the following chapters [9].

5.3.1 Prebiotics, probiotics, synbiotics, and postbiotics

Probiotics are live microorganisms, whilst prebiotic*s* is ingredients that give nourishment to the live microorganisms. They can be given separately or together (synbiotics) to support the microbiota of the gut [18].

Probiotics are defined by World Health Organization (WHO) as *"live microorganisms that when administered in adequate amounts confer a beneficial health effect on the host"* [19]. They comprise indigenous- and exogenous bacteria that interact with several mechanisms within the host and enhance mucosal health [18]. *Enterococcus faecium* (both *E. faecium* NCIMB 10415 E1707 and *E. faecium* NCIMB 10415 E1705)*, Bifidobacterium sp. animalis* and *Lactobacillus acidophilus* are some of the bacterial strains that have proven their efficacy as probiotics [18]. They can produce fatty acids, acetic acid, and lactic acids, induce mucus/mucin production and compete with pathogenic bacteria by interfering with the adherence to the mucosa of the intestinal tract, as well as having an antimicrobial and immunomodulatory effect [4, 18].

Prebiotics are defined by the International Scientific Association of Probiotics and Prebiotics (ISAPP) as *"a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health"* [20]. Important functions of prebiotics are the production of SCFA which enhances the growth of bacteria, and the binding of harmful bacterial metabolites (e.g psyllium's ability to bind to bile acids) [4]. Prebiotics consists mostly of different length of fiber compounds, including disaccharides, oligosaccharides or polysaccharides, and inulin which is a long-chain prebiotic [18]. The most common prebiotics are fructans, consisting of fructo-oligosaccharides (FOS) and inulin, galacto-oligosaccharides (GOS) as well as

resistant starch (RS), and glucose-derived oligosaccharides [20]. Fructans work by fermenting bacteria, and their chain length determines which bacteria that can ferment them [20]. Galacto-oligosaccharides mainly stimulate *Lactobacilli* and *Bifidobacteria* [20]. Resistant starch is a type of starch that is resistant to the digestion of the upper gut, and it produces high levels of butyrate – which has a great health benefit and shows the highest incorporation with *Firmicutes* [20].

Synbiotics is a product that combines probiotics and prebiotics, which benefits the host by improving both the microorganisms and their nourishment [18].

Postbiotics are defined by ISAPP as *"a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host"* [21]. Postbiotics are an emerging treatment method. The name postbiotics refers to the microorganisms being inactivated or dead, and the inanimate substances used may be intact cells or fragments of the microbe e.g the cell wall [21].

The use of pre-and probiotics in veterinary medicine is relatively new but it is broadly spread in human medicine [2]. In several studies, probiotics have been shown to promote benefits in the pet's diet, through the support of the gut microbiota and protection against pathogens and enteric infections [2].

5.3.2 Antibiotics

Tylosin and metronidazole are antibiotics frequently used in the treatment of chronic dysbiosis, where diet change has not been successful [2]. Adult dogs suffering from tylosin-responsive chronic diarrhea (TRD) respond well to tylosin, but the mechanism of action is not totally known [2]. Metronidazole is more commonly used together with diet change and as nutritional therapy, which makes its real effect more difficult to comprehend [2].

The use of antibiotics in the treatment of dysbiosis and other chronic enteropathies has its contraindications [2]. Immoderate use of antibiotics can lead to antibiotic resistance, as well as disturb the balanced ecosystem of the gut microbiota, by decreasing the population of beneficial bacteria and promoting an increase of the pathogenic bacteria and antibiotic-resistant strains [2]. These alterations in the bacteria diversity can lead to the onset of dysbiosis [2].

5.3.3 Therapeutic diets

Dietary change is often, and should always be, the first line of treatment in case of dysbiosis [16]. A veterinary prescription diet contains highly digestible feed that reduces the undigested nutrients in the lumen of the GI tract, and by that reduces the proliferation of bacteria [16]. A novel diet containing only one protein source the patient never has been exposed to before (e.g Rabbit or duck meat), or a diet containing hydrolyzed protein (e.g hypoallergenic from Royal Canine), will reduce the pro-inflammatory responses that occur in the host when the immune system is hypersensitive against a food antigen, as well as facilitate digestion [16, 22]. In dogs suffering from food-responsive-diarrhea (FRD), hypoallergenic diets have been shown to improve the microbiome composition, but do not seem to have any major effect on the microbiome of healthy dogs [22]. Research shows that changes in the microbiome composition are mainly related to big changes in the composition of macronutrients, like high-fiber or high-protein diets [22].

6 Fecal microbiota transplantation

6.1 Definition

Fecal microbiota transplantation (FMT) describes the medical process of transferring fecal matter from a healthy donor, into the recipient's gut most commonly suffering from dysbiosis [23]. FMT (also known as fecal transplantation, fecal bacteriotherapy, or fecal transfaunation) is transferring beneficial bacteria, but also important viruses, food antigens and fungi to the patient suffering from alimentary tract disease [24]. It can be done by ingesting oral capsules, via colonoscopy, enema, or nasogastric tube [23]. This procedure has been used in human medicine for several years, and the first report of FMT in the treatment of pseudomembranous colitis is dated back to 1958 [23, 25], and today FMT is used to treat GI- as well as non-GI disorders [23]. In veterinary medicine, this therapy has gained popularity, but its relatively new with limited scientific study [24].

6.2 History of fecal microbiota transplantation

The first forms of FMT in the treatment of gastrointestinal disorders have been used in Chinese medicine since the fourth century CE [4]. They used different forms of FMT including fermented, dried, infant-derived, and fresh fecal products [4]. In Europe in 1696, a German physician named Franz Paullini published a book describing the use of human and animal feces as medical treatment [4]. In human medicine, FMT is most commonly used in the treatment of recurrent *C. difficile* infection (rCDI), and was first described after a study done by Ben Eiseman in 1958 [4, 23]. Furthermore, FMT has been used in patients suffering from IBD, ulcerative colitis, Colorectal cancer, and Crohn´s disease, but with limited success [4]. Intestinal dysbiosis can affect other organs located outside the GI tract, known as non-GI disorders [23]. Some preliminary studies have shown that treatment with FMT in non-GI diseases such as hepatic encephalopathy, cancer, some neurologic disorders, psoriasis, autism spectrum disorders, and metabolic syndrome had an effect in addition to the conventional treatment, but this requires more research [23].

Even though FMT is a new medical technique in veterinary medicine, the transfer of GI microbiota is not new [23]. Some mammalian species, like lagomorphs, are coprophagic, meaning they are feeding on their own excrement [26]. A lot of important nutrients like amino acids, vitamins, minerals, and trace elements are not utilized in the GI tract and therefore excreted in the feces, which explains the nutritional significance of coprophagy [26]. This behavior is not only seen in rodents and lagomorphs, but also to a lesser degree in dogs, as well as piglets and foals [26].

In veterinary medicine, the use of FMT in dogs and cats is limited, but the practice of FMT has been used for many years in ruminants and, especially, horses [9]. In ruminants, the use of FMT has been used since the seventeenth century in the treatment of ruminal acidosis in sheep and cattle, and is commonly called ruminal transfaunation [4]. FMT has also been used in the treatment of chronic diarrhea in horses, and in newborn chicks to increase their resistance against enteric pathogens [4]. A study performed by Teng et al (2020) revealed that early FMT in newborn Yorkshire piglets improved their intestinal mucosal health, the gut microbiota, the mucosal immunity of the intestinal tract, and the functions of intestinal development [27]. The piglets received fecal microbial transplantation daily for 10 days, and after 21 days there was a significant increase in IgM and IgG of the jejunal mucosa, as well as IgG of the ileal mucosa, and a decrease in the relative abundance of *Proteobacteria* [27].

FMT treatment in calves suffering from chronic diarrhea (CD) was also proven to be successful in a study performed by Islam et al (2022), where 70% of the treatments were successful after 7 days following rectal FMT [28]. The use of FMT in the reduction of porcine circovirus in nursery pigs has proven to be successful, as well as the treatment of parvovirus in dogs and colitis in horses [3].

6.3 Mechanism of action

The principle of using FMT, alongside probiotics, prebiotics, synbiotics or postbiotics, is the intention of modifying the intestinal microbiota and by that enhancing the health of animals [29]. In veterinary medicine, the mechanism of how FMT improves the health of the patient is not fully understood, but research shows that it relates to the rebuilding and re-establishment of the intestinal microbiota, and the ability of the FMT recipient to maintain and adopt the donor's microbiota [29]. Taking ruminal transfaunation as an example, studies have shown that the benefits arrive from the recolonization of the beneficial anaerobic bacteria found in the rumen, which will lead to the restoration of the normal function of the fermenting process [3]. It is also found that the digestive capacity of the host is affected by its ability to metabolize complex carbohydrates and that this is improved by increased diversity of the host's microbiome [3]. In human medicine, antibiotic-refractory CDI has been the main indication for FMT treatment over the past few years, where the mechanism and effect of FMT have shown to be clear [30]. This is why the use of FMT in the treatment of antibiotic-refractory CDI has been the center of studies of FMT in other GI or non-GI disorders, in both human and veterinary medicine [30]. In addition to how *C. difficile* infection develops due to antibiotic therapy, Tuniyazi M et al (2022) [1] proposed a few hypotheses that are used to explain the mechanism of FMT, including 1) increased production of secondary bile acids, 2) competitive niche exclusion, 3) increase competition for nutrition, and 4) production of antimicrobials.

6.3.1 Description of the mechanism of FMT through the example of *C. difficile* colitis

To maintain optimal gut barrier and mucosal immune system of the GI tract, the gut microbiota is continuously sending signals [30]. The specialized mucus layer, immunoglobulins, antimicrobial peptides, and mucosal lymphocytes all comprise the mucosa defense system within the intestinal lumen [30]. The mucosal immune response is

decreased after antibiotic therapy, causing a collapse of the mucous layer that separates the microbiota from the epithelial cells, which reduces the production of antimicrobial peptides leading to a rise in the germination of *C. difficile* [30]. When *C. difficile* is in contact with the epithelial cells, the toxins TcdA and TcdB are produced, which inactivates important cellular signaling pathways, leading to the colonization of *C. difficile* [30]. The local mucosal immune response is important in the defense mechanism against systemic infections [30]. If the local immune response fails to work, systemic infection happens due to the translocation of the bacteria across the gut wall [30]. The use of FMT will reestablish the gut barrier by the production of mucin, and antimicrobial peptides and provide signals for the regeneration of the epithelial cells [30]. Furthermore, beneficial bacteria from FMT that produce butyric acid increase interleukin-10 production as well as generate regulatory T-cells, which will lead to increased mucosal immune response and decreased inflammation [23].

6.3.2 Increased production of secondary bile acids

In the life cycle of *C. difficile*, bile acids have an important role, and the host environment and nutrient conditions is important in the germination of its spores [30]. Bile acids arrives from the cholic acid class and the chenodeoxycholic acid class, where cholic acid class stimulates germination of spores, whiles chenodeoxycholic acid class inhibit the germination of the spores [30]. It has been reported in *C. difficile infection* (CDI) patients that went through FMT that they rebuilt *Firmicutes*-levels and secondary bile acid metabolism after treatment [1]. The microbial changes associated with fecal transfer create an unfavorable environment for the growth of *C. difficile* by the establishment of secondary bile acids over primary bile acids [23]. The feces of dogs with chronic enteropathy consist of a low amount of *C. hiranonis*, which is a bacteria that converts primary bile acids to secondary bile acids [23]. This explains the decreased amount of secondary bile acids in chronic enteropathies and its role in dysbiosis [23]. The mechanism of production of secondary bile acids is illustrated in *Figure 4* [1].

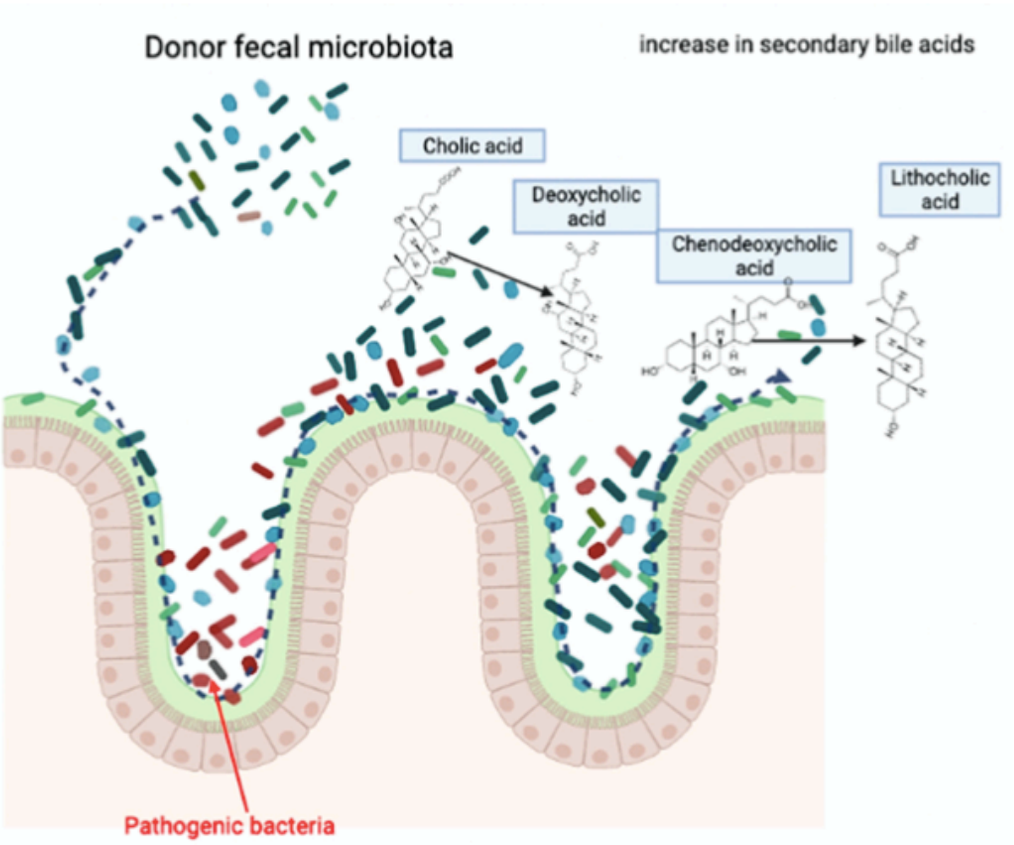

Figure 4. *Mechanism of production of secondary bile acids* [1]

6.3.3 Competitive niche exclusion

Competitive niche exclusion means that the bacterial strains of the fecal donor compete with the recipient's pathogenic bacteria strains for the same niches in the intestinal tract [1]. The strains of the fecal donor will more successfully occupy these niches (locations) by the colonization of resistance barrier and thereby maintain the healthy gut microbiota [1]. This method has been shown to be fortunate in FMT treatment of human CDI patients, by introducing non-toxigenic strains of *C. difficile* that will compete with the toxigenic strains (TcdA and TcdB) of *C.difficile* [1]. This mechanism is illustrated in *Figure 5* [1].

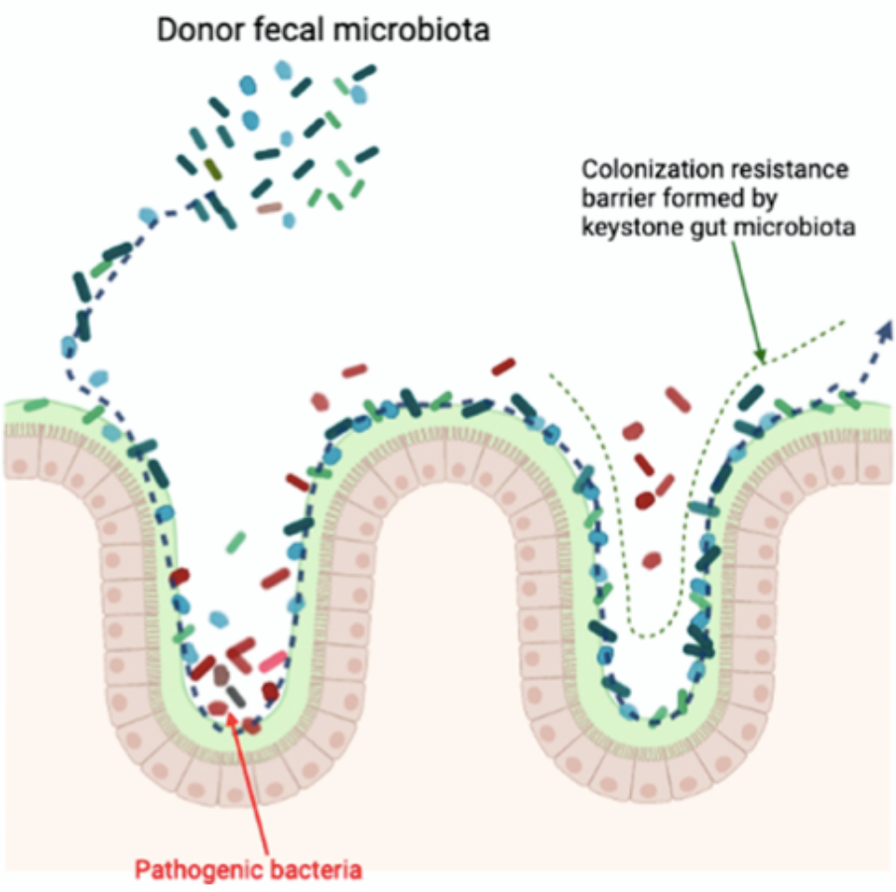

Figure 5. *Mechanism of competitive niche exclusion* [1].

6.3.4 Increased competition for nutrition

Increased competition for nutrition is a form of indirect interaction, in many ways similar to niche exclusion, where both mechanisms compete with pathogenic strains [1]. In this mechanism, the donor fecal strains will compete with the recipient pathogenic strains for nutrition, and in this way reduce the survival opportunities of the pathogenic bacteria [1]. This mechanism is illustrated in *Figure 6 [1]*.

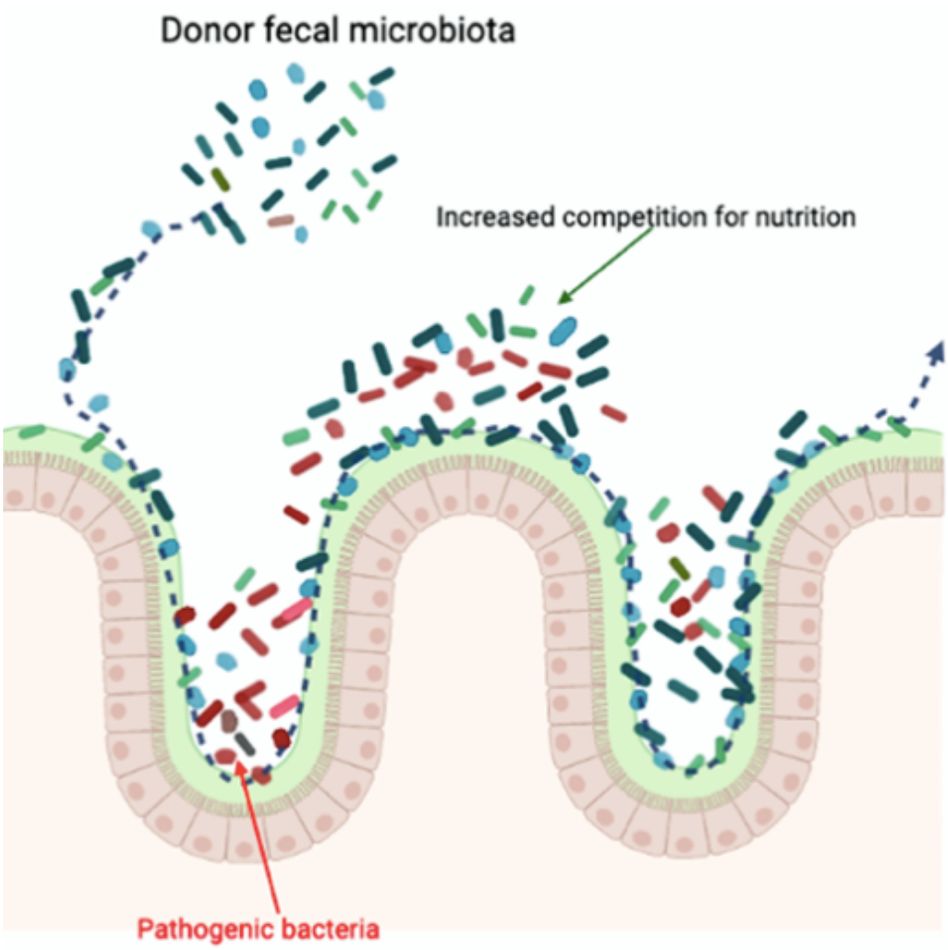

***Figure 6**. Mechanism of increased competition for nutrition* [1]

6.3.5 Production of antimicrobials

Production of antimicrobials is another form of competitive interaction, where the origin of bacteriocin production is the interaction between the donor's bacterial strains and the recipient's pathological strains [1]. *Figure 7* illustrates this mechanism [1].

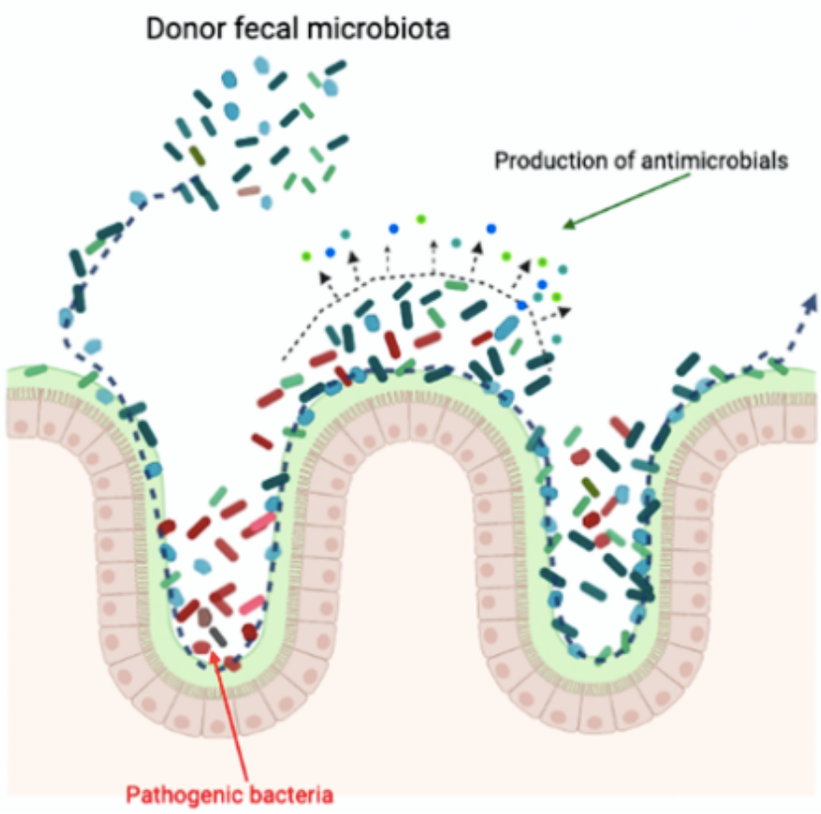

***Figure** 7. Mechanism of production of antimicrobials* [1].

6.4 Indications

In veterinary medicine, we can divide the use of FMT into three groups, including prophylactic use, therapeutic use, and stimulation of pathogen-specific immunity [3]. Prophylactic use of FMT can be used prior to expected exposure to pathogens and/or disease by providing a beneficial microbiome to the animals [3]. Therapeutic use of FMT is when the goal is to treat clinical signs or cure ongoing diseases, and may be used in diseased pets associated with dysbiosis, such as IBD, idiopathic diarrhea, and acute and chronic enteropathy, and is the most commonly used method among the three groups [3, 29]. The use of FMT in the stimulation of pathogen-specific immunity is an immunostimulatory tool and can be compared to the mechanisms used in vaccinations, and aids in increasing the transfer of immunoglobulins [3]. This application is new and more research is needed, as the use of live pathogens increases the risk of adverse effects [3]. In practice, FMT is relatively new with few scientific studies, and it is more commonly used as a last resort when there are no other options in the therapy of GI diseases in a patient [29].

6.5 The use of FMT in different disorders of small animals and in experimental mice models

In human medicine, FMT has proven to be successful in the treatment of CDI, and research suggests that it can be useful in many other GI diseases, such as IBD and irritable bowel syndrome (IBS) [30]. The use of FMT in the treatment of autoimmune diseases, nervous system development, and metabolic problems have also been suggested, but more research is needed [30]. The use of FMT in human medicine in the future is believed to be associated with complications with antibiotic treatment and multidrug-resistant pathogens (such as rCDI), e.g extended-spectrum beta-lactamase (ESBL) producing bacteria, vancomycin-resistant enterococci (VRE), carbapenem-resistant Enterobacteriaceae, and others [30]. Under antibiotic pressure, these pathogens colonize the GI tract during intense medical care [30]. One example of this is in patients receiving allogeneic hematopoietic stem cell transplantation, where they receive intensive antibiotic therapy leading to a rise in potentially pathogenic bacteria and loss of diversity of the gut microbiota, which can potentially lead to blood-born infections [30]. The use of FMT in these disorders will have the ability to restore gut microbiota, cure infectious diseases and eliminate the factor of causing antibiotic resistance [30].

6.5.1 The use of FMT in GI disorders

Over the years there has been an increase in interest in the use of FMT in veterinary medicine, but the practice is limited due to few studies reporting the effectiveness and application of FMT in the treatment of dysbiosis [9]. As mentioned, FMT may be used in animals suffering from acute or chronic enteropathies [30]. The most common GI diseases treated with FMT in veterinary medicine will be further described in the next chapters.

6.5.1.1 Inflammatory bowel disease

A common cause of chronic and recurrent gastrointestinal disease in dogs is idiopathic inflammatory bowel disease (IBD) [31]. For it to be a common GI disease in dogs, there are limited reports on the treatment with FMT and its efficacy [31]. One case report by Niina A et al (2019) reported the changes in microbial diversity after repeated, long-term treatment with FMT in one dog suffering from IBD [31]. A 10-year-old neutered male Toy poodle

with diagnosed IBD from histopathology was submitted to the case study due to his history of prolonged vomitus and diarrhea, treated with antidiarrheal agents and antibacterial medicine without improvement [31]. The dog received periodic long-term rectal enema with fresh feces from a healthy donor, and 16S rRNA sequence analysis was performed before and after the FMT [31]. The study showed improved clinical symptoms and microbiome diversity of the recipient, and there were not observed any side effects during the FMT treatment [31]. In another case series, 16 adult dogs diagnosed with IBD were treated with FMT by duodenoscopy and/or oral transplant in the form of frozen capsules [32]. The Canine Enteropathy Clinical Activity Index (CCECAI) was used to measure the clinical response before and after FMT administration, and improvement of dysbiosis was seen in most of the dogs [32].

6.5.1.2 Non-responsive enteropathy and immunosuppressant-responsive enteropathy

Non-responsive enteropathy (NRE) is a form of chronic enteropathy where dogs do not respond to immunosuppressant treatment, as supposed to immunosuppressant-responsive enteropathy (IRE) [33]. NRE can be difficult to treat, and usually antibiotics, immunosuppressants, therapeutic diet, and antidiarrheal agents have been administered without improvement [34]. Shiba is a dog breed that is predisposed to chronic enteropathies and research shows that it might be associated with small cell intestinal lymphoma, and that they also have a poorer prognosis than other dog breeds [34]. One study was done on a Shiba dog diagnosed with NRE where immunosuppressant drugs had failed to work [34]. The dog was treated with one single FMT in addition to chlorambucil treatment [34]. The FMT was administered into the cecum and colon, and it lead to a fast recovery of clinical signs and the abnormalities found on histopathology, and it corrected the gut dysbiosis [34]. This study suggests that FMT treatment, possibly in addition to chlorambucil, is a good option for NRE, especially in Shiba dogs, due to them being predisposed to CE and having poorer prognosis compared to other breeds [34]. Another case report about a dog suffering from relapsing chronic diarrhea improved after oral FMT treatment [35]. This dog received continuous low-dose prednisolone as the only therapy that had an effect, and in this study, the authors wanted to evaluate clinical improvement and the possibility to reduce or completely stop prednisolone treatment by FMT [35]. The dog received frozen oral capsules with strict monitoring for 21 days, and continuous follow-up for 18 months [35]. After 21 days the canine inflammatory bowel disease activity index (CIBDAI) score improved to clinically

insignificant, bloating rapidly improved and the dyschezia was completely resolved [35]. During the 18-month follow-up, there were no adverse effects observed and only mild relapses, and there was no need for an increase in the prednisolone dose [35]. The study concluded that they could not reduce or completely stop prednisolone treatment, but after FMT there were only mild relapses where they never needed to increase the prednisolone dose, as they had to do before FMT treatment [35].

6.5.1.3 Parvovirus infection

A study published regarding treatment with FMT in puppies with parvovirus infection showed promising results [36]. 66 puppies with diagnosed parvovirus infection were evaluated and treated at two different veterinary hospitals [36]. The dogs were assigned randomly into two groups, one group was treated with standard treatment (STD) consisting of IV fluids and antimicrobials, and the other group was treated with STD and FMT applied with enema [36]. The study showed that dogs treated with FMT + STD had a shorter hospitalization time, faster recovery from diarrhea, and lower mortality rate compared to the group only receiving STD [36]. The procedure was proven to be safe, and there were no adverse effects observed [36].

6.5.1.4 Acute Hemorrhagic Diarrhea Syndrome

A study done by Gal et al (2020) performed FMT on 8 dogs diagnosed with AHDS, where 1 dog originated from New Zealand and 7 from South Africa [37]. Patients were included in the study if they had acute onset (less than 3 days duration) of watery bloody diarrhea in large volume [37]. Additionally, the patients included showed hemoconcentration (at or above the reference interval) and total protein concentration (TP) normal or lower than the reference interval [37]. Small animal veterinary internal medicine diplomates were responsible for the enrollment of patients included in the study, which was based on the patient's clinical history (eg. watery bloody diarrhea for less than 3 days), physical examination, CBC (hemoconcentration) and serum biochemistry (serum total protein), abdominal x-rays and urine analysis within normal limits. The fecal samples were tested with 16S-rRNA sequencing at admission, discharge, and 30 days after discharge in both FMT and sham-treated dogs. The FMT treatment was performed with one single colonoscopy. The study concluded that there were no significant changes in the AHDS

clinical score between the sham-treated (control) and FMT-treated from admission to 30 days post-admission and that FMT treatment did not result in clinical improvement [37].

6.5.1.5 Ulcerative colitis

Studies available regarding FMT treatment in cats are very limited. There is one simple case report available where FMT was successful in one 10-year-old cat diagnosed with ulcerative colitis [38]. FMT was administered by enema, and there was observed a fast resolution of clinical signs, with improvements in the fecal color, odor, and consistency. 5 weeks after the first FMT treatment, there was a relapse of diarrhea, and a second FMT was performed where normal stool consistency was gradually observed during a 3-month period [38]. The last follow-up was performed after 11 months, where the cat still had complete resolution of diarrhea and the fecal consistency was normal [38].

6.5.2 The use of FMT in non-GI disorders

6.5.2.1 Atopic dermatitis

As the gut microbiota is involved in the pathogenesis of atopic dermatitis, one study explored the ability to restore gut microbiota with FMT to alleviate AD in mice [13]. 16S rRNA sequencing was used before and after the FMT, and the SCFA content was also measured to determine the metabolite levels of the gut [13]. Blood parameters were also evaluated to assess allergic responses induced by atopic dermatitis (white blood cell count and IgE levels) as well as the amount of mast cells in skin tissue and ileum, cytokine levels (Th1 and Th2), and evaluation of dermatitis score [13]. After treatment with FMT, there was a restoration of the gut microbiota to the level of the state of the donor, and increased levels of SCFAs [13]. There was also a reduction in the IgE levels, mast cells, basophils and eosinophils, and the cytokine balance of Th1 and Th2 was restored [13]. Based on this the study concluded that FMT restored the immunological balance and gut microbiota, as well as suppressed the allergic responses induced by atopic dermatitis, and would therefore be a potential new therapy for canine atopic dermatitis [13].

6.5.2.2 Chronic kidney disease

Chronic kidney disease (CKD) is a disease where there is an accumulation of uremic toxins, and the kidney's ability to reduce its concentration is limited [39]. Many studies have supported the role of the gut microbiota in the production of uremic toxins in CKD complications [39]. A study by Barba et al (2020) explored this theory by treating mice with diagnosed CKD with FMT treatment [39]. The study revealed improvement of the gut microbiota, improved glucose tolerance, and decreased p-cresyl sulfate 3 weeks following FMT, but no improvement in the function of the kidneys [39]. They concluded that FMT limited the buildup of uremic toxins from the cresyl-pathway in the gut microbiota, but that further studies are needed [39].

6.6 Different techniques of FMT transplantation

As mentioned earlier, there are several ways of transferring fecal material, and the most commonly used methods are enema, oral capsules, colonoscopy, and nasogastric tube [4]. In veterinary medicine, there are not enough evidence-based data to recommend one method of administration over the other [29]. *Table 4* summarizes the pros and cons of each delivery method [40]. In human medicine, a recent meta-analysis involving 305 patients collected from 14 different studies concluded that FMT received through the lower GI tract to be more effective than through the upper GI tract in the treatment of rCDI [29]. Another study revealed that FMT administered by oral capsules and colonoscopy to be more superior than via enema and nasogastric tube in the treatment of CDI [23]. In veterinary medicine the way of administration depends on the location of the problem, the size of the patient, the dedication of the owner, and if the procedure requires sedation or not [29]. There are two main groups of FMT, autologous- and allogenic transplantation [4]. Autologous FMT is when the patient's own feces is used for transplantation [4]. This method is used in patients that have undergone e.g allogenic hematopoietic stem cell transplantation, where the use of antibiotics has caused dysbiosis [4]. The patient's feces is collected prior to the antibiotic treatment, to restore the microbial composition after surgery [4]. Allogenic FMT is where the fecal sample is collected from a healthy donor, which has proven to be successful in rCDI patients [4]. Administration of FMT through the lower GI tract is most frequently performed by enema and colonoscopy in human medicine, but also sigmoidoscopy to a lesser degree [40].

6.6.1 Enema

Enema is a procedure where the fecal material is liquified and delivered through the rectum and into the lower parts of the GI-tract [41]. Enema is preferred in veterinary medicine as it is easy to administer and does not require general anesthesia or any special equipment [23]. In human medicine, the recipient needs to lay on their back for 30min after the FMT, and because of this, some veterinarians prefer to sedate the recipient for 30 minutes post-FMT, to limit their urge to defecate and to keep them still during this period of time [23].

6.6.2 Colonoscopy

Colonoscopy is when you use an endoscope through the rectum and into the large intestine, consisting of a tube with a camera placed on it [42]. The camera aids in the inspection of the mucosal wall, and to look for any signs of inflammation [42]. The colonoscopy is usually performed to the terminal part of the ileum, but the degree of inflammation of the mucosal wall will determine if a full colonoscopy is safe or not [42]. Before inspection of the mucosal wall, colon preparation should be performed, where the intestinal content is removed with lavage followed by suction of any residual fecal material [42]. Fecal preparation is important before colonoscopy, but in e.g severe ileus enema can replace the colon preparation protocol [40]. After this, the liquid fecal material is infused into the ileum, cecum, and the four quadrants of the colon (*colon ascendens, colon transversus, colon descendens,* and *colon sigmoideum)* [42]. During colonoscopy, the patient has to be under general anesthesia [37]. Gal et al (2021) made a colon preparation protocol for dogs diagnosed with AHDS that were going to receive FMT by colonoscopy, which was performed under general anesthesia with the use of a nasoesophageal tube [37]. In this protocol, the recipient was given 4ml/kgBW lukewarm water every hour for 8 hours in total. 0,20mg Bosacodyl was dissolved in the lukewarm water at 0hr, which is a laxative that aids in cleaning out the intestine and helps against constipation. In the 1st and 5th hour, one pack of polyethylene glycol was dissolved in lukewarm water, which is an osmotic laxative that increases bowel movements and softens the stool [37]. According to the most recent published studies, colonoscopy has an 84%-93% efficacy, and if it's delivered on the right side of the colon it has an efficacy of 93% by one single infusion [40]. The limitations of colonoscopy are the invasiveness and the required skills of the veterinarian and the equipment needed [40]. Adverse effects reported with colonoscopy are mostly related to the risk of anesthesia, such as reaction to sedative drugs

and cardiovascular events, and using colonoscopy also increases the risk of tissue perforation, bleeding and infection [40].

If the patient suffers from severe colitis, ileus, or other diseases that would make delivery through the lower GI tract difficult, FMT can be delivered through the upper GI tract, with methods such as oral capsules, nasogastric tubes, or esophagogastroduodenoscopy [40]. Delivery through the upper GI tract increases the risk of vomiting and aspiration, and the efficacy is said to be between 81%-86% [40].

6.6.3 Oral capsules

Oral capsules are easy to deliver, cheap and noninvasive, and they were introduced due to limitations of the other delivery methods [4]. They are easy to store and administer, and oral capsules also eliminate risks during procedures that can happen in other FMT treatment methods, such as tissue perforation [4]. Oral capsules are also convenient if there are geographical limitations in case of accessibility to clinics that perform colonoscopies, or if the patient has contraindications towards colonoscopy [40]. The gut microbiota can be prepared into fresh or freeze-dried capsules, or there are “do it yourself” kits available for filling oral capsules with the fecal material of the veterinarian's own in-house donor [43]. Studies have shown that regarding the limitations of oral capsules, it can take up to several hours for the capsules to reach the large intestine, as well as its ability of the organisms to survive through the stomach and small intestines, which makes its efficacy questionable [43].

Table 4. *Different routes of FMT administration [40]*

Route of administration	**Pros**	**Cons**
Enema	Well tolerated	Can be difficult to retain
	Low cost	Inability to reach the right-sided colon
	No need for sedation, can be easily repeated	Modality with the lowest efficacy
Colonoscopy	Useful for differential diagnosis	Risks of the procedure and the anesthesia
	High efficacy rate in rCDI patients	Needs skilled practitioner and special equipment
		Additional cost
Capsule	Noninvasive	Risk of aspiration and vomiting
	Cost and time saving, easy to administer	
Nasogastric tube	Low cost	Discomfort during administration
		Needs x-ray to confirm
		Risk of vomiting and aspiration

6.7 Technical issues associated with FMT

6.7.1 Donor selection

There are very strict procedures in the screening of donor selection in human medicine to prevent the spread of infectious diseases [23]. The donor fecal samples can be collected from two different sources: universal donors and patient-directed donors [4]. Universal donors are donors that have gone through physical examination, they are preferably young and healthy humans with low body mass index (BMI), which voluntarily provide a fecal sample for donor screening to universal pool banks [4]. Patient-directed donors are usually family members or friends of the recipient [4]. The criteria for being a donor revolve mainly about reducing the probability to transmit infectious pathogens, and this is mainly done with a thorough historical and physical examination [4]. They should preferably be less than 50 years old, healthy, and free of diseases (e.g gastrointestinal diseases, diabetes mellitus, allergies, autoimmune diseases, obesity, psychiatric diseases, or cancer), and any recent medical treatment must be reported (e.g antibiotics, corticosteroids, chemotherapy etc.) [4]. They should undergo blood and fecal examination to rule out any underlying disease and

infectious diseases that can be transmitted [4]. A complete blood count (CBC) and serum biochemistry should be analyzed, as well as a fecal sample test to rule out fecal parasites, *C. difficile* and *Helicobacter pylori (H. pylori)* [4].

In veterinary medicine, these screening protocols are mainly based on human donor screening, but the protocols differ between studies [23]. Chaitman J et al. (2021) published a general screening criteria protocol for donor dogs, shown in *Table 5*, to prevent the spread of infectious diseases and to ensure optimal quality of the transplanted feces' metabolome and microbiome [23]. The American company AnimalBiome, also screens the donor's fecal material for *C. difficile* toxin B, *Cryptosporidium spp.*, and *Canine Parvovirus 2* in addition to what's mentioned in *Table 5 [23]*. Contrary to human medicine, the responsible veterinarian can choose the depth of the screening, and if desired can include intestinal function tests like folate and serum cobalamin concentrations, and pancreatic enzyme immunoassays like Canine or Feline pancreatic lipase immunoreactivity (cPLI/fPLI) test and/or trypsin-like immunoreactivity (TLI) test [23]. It is also recommended to feed the donor a hydrolyzed diet, e.g Royal Canine Hypoallergenic or Hills z/d, or a diet with limited ingredients for up to 6 weeks before, and during, the collection of the canine feces [23].

Table 5. *Recommended criteria for Canine fecal donors by Chaitman et al (2021) [23].*

History and physical examination	Preferably between 1-10 years old
	No travel history outside the local area
	No health issues in the last 6-12 months
	No history of chronic GI diseases, allergies, or immune-mediated diseases
	Has not received antibiotics in the last 12 months
	Regularly vaccinated according to guidelines
	Fed a balanced diet
	Not overweight or underweight (BCS 4-6)
	Normal fecal consistency
	Deemed healthy on physical examination
Laboratory examination	Normal CBS and serum biochemistry
	Consider evaluation of basal cortisol, thyroxine
	Negative for parasite ovas on fecal flotation, consider empirical deworming with a broad-spectrum drug
	Negative for Giardia oocysts on fecal flotation and ELISA fecal test
	Consider testing for fecal pathogens such as Salmonella spp., Capylobacter spp., etc
Fecal microbiome evaluation	Fecal dysbiosis index less than 0

6.7.2 Criteria of the recipient

A proper historical and physical examination to identify the cause of dysbiosis is very important before starting treatment with FMT, as recurrent diarrhea is likely to happen if the underlying cause is not addressed [23]. The patient should be screened for diseases such as food-responsive enteropathy (FRE), antibiotic-associated diarrhea (AAD), parasitic infections, atypical hypoadrenocorticism etc., and properly treated before treatment with FMT [23]. Patient's receiving antibiotics are not recommended to start treatment with FMT, due to the antibiotics ability to affect the microbiome negatively, and also decrease the effect of FMT treatment [23].

Gal A, et al. (2021) pilot study about FMT in dogs with acute hemorrhagic diarrhea syndrome (AHDS) showed the same donor screening criteria as Chaitman et al. (2021), and they also included criteria of the patient receiving FMT. Patients excluded from the study were patients that were currently on treatment, or treated within the last week prior to presentation, with glucocorticoids, non-steroid anti-inflammatory drugs (NSAIDs), and/or antibiotics [37]. Additionally, patients were excluded if they tested positive for Canine Parvovirus with ELISA test, or showed parasites in the feces after fecal flotation [37].

6.7.3 Preparation of the fecal material

The protocols of preparation of canine fecal samples derive from the protocol used in human medicine [23]. For each FMT procedure about 20g-100g of the donor feces is typically used [23]. After defecation, the fresh feces should be used within 6 hours and during this time it can be safely stored at room temperature [23]. The storage and preparation time should be as short as possible to protect the anaerobic bacteria from exposure to oxygen [23]. After collecting the fecal material, it should be homogenized with saline solution and then sieved thoroughly in order to avoid clogging of tubes and syringes used for infusion [23]. In the homogenized fecal samples, it has been shown that the bacteria can be maintained for up to 1 week at 4°C in a refrigerator, but for a longer storage time, it should be frozen to maintain the viability of the bacteria [23]. Study shows that storage for more than 3 months can only maintain the bacterial viability by adding glycerol (10% of total volume) stored at -20°C in the freezer, and storage longer than 6 months should be stored at -80°C with 10% glycerol [23]. The glycerol added is used as a cryoprotectant, so that during the freezing we ensure the viability of the microorganisms [29]. The frozen fecal samples should be thawed in a

water bath maintaining 37,5 °C (body temperature) and used within 6 hours [23]. Research done by Chaitman et al. (2021) presented a protocol that can be used in the preparation of canine fecal transplants, including the co-writer Gaschen et al. (2018) unpublished studies obtained from the same research study, shown in *Table 6 [23].*

Table 6. *Protocol for preparation of canine fecal material and administration of fecal microbiota transplantation [23].*

Author	***Fecal amount***	***Preparation***	***Administration***	***Sedation of Recipient***	***Post-FMT care***
Chaitman (from Chaitman et al, 2020)	2.5-5g feces per kg BW recipient	Mix fresh feces with 60mL 0,9% NaCl in blender. Blend on high until the stool is liquified. For very large dogs a larger volume of saline may be needed to obtain sufficient liquification.	Rectally via 12-14 French red rubber catheter pushed all the way into the colon	Not necessary in most dogs	Do not feed, restrict activity for 4-6hr post-FMT to decrease risk of defecation
Gaschen (adapted from Kao et al (2018) unpublished observations)	1-2g feces/kgBW recipient	Use feces within 6-12hr of defecation. Mix 1 volume feces with 4 volumes of 0,9% NaCl (20% solution) and filter solid material using guaze or other method. If freesing, add glycerol (10mL per 100mL final solution) and store at -80*C	Rectally via large bore red rubber catheter (5-10mL solution/kgBW recipient)	Mild sedation is beneficial to keep the recipient calm during and 30min after the procedure	Same as above

Collection and storage of donor fecal material is also an important factor in the success of FMT therapy [1]. In human medicine, the use of frozen fecal samples has proven to be just as effective as a fresh stool, but in veterinary medicine fresh stool is more commonly used [1]. The use of frozen stool in veterinary medicine in the future would be more time-and cost-effective, as well as easier to transport over geographical boarders [1]. This would make the pool of donor selection bigger and more available for every veterinarian, eliminate the step in finding their own donor, and easier to choose the correct donor based on screening profiles available [1]. Having a canine stool bank makes it even more important to have strict screening and selection programs for the canine donor to eliminate the risks of pathogens, antibiotic-resistant bacteria, parasites, and other underlying diseases [1]. *Table 7* and *Table 8 illustrate* the positive and negative aspects of using fresh and frozen stool samples [1].

Table 7. *Pros and Cons of Fresh fecal samples [1]*

	Fresh fecal sample
Pros	Convinient
	Easily accessed in case of emergency
	Do not require special clinical skills, can be administered orally by the owner
Cons	Increased risk of infectious diseases and adverse effects if there are noe proper screening of the donor pre-FMT

Table 8. *Pros and Cons of Frozen fecal samples [1]*

	Frozen fecal sample
Pros	Convinient
	Can be used anytime, do not have to be a donor around
	Decrease chances of infectious diseases as it have been through a selection and screening prosess
Cons	Requires special equipment and skills for selection processes, may cost more money
	Needs special equipment for storage (-80*C in fridge) and use of water bath (37*C)

6.8 Possible side effects associated with FMT

Even in high-risk patients, FMT is considered a safe therapy [4]. But, some mild complications have been reported after FMT treatment in human medicine, such as cramps, bloating, diarrhea, abdominal discomfort, constipation, low-grade fever, vomiting, and nausea [4]. Diarrhea and bloating during the first 24 hours post-FMT are the most common side effects, and the patient's stool consistency is usually normal by 1-2 weeks [40]. The biggest controversy in the recent studies of FMT, is the potential risk of transmitting multidrug-resistant bacteria [4]. There have been reported serious complications, such as high-grade fever and multidrug-resistant (MDR) bacteremia with 2 deaths, but only on rare occasions [23]. Due to the lack of data available, there have been no registered moderate or severe side effects after FMT treatment in dogs, only mild cases of diarrhea, constipation, and vomitus [9]. In addition to the risk of transmitting multidrug-resistant bacteria, is the possibility of transferring disease-causing genes, where there are unknown genes transferred from the donor's fecal sample and into the recipient, and consequently causing chronic disease in a later stage [4]. There is always a greater risk if the patient is very sick and immunocompromised, and this also shows the importance of proper recipient-and donor-screening in both human and veterinary medicine [23].

7 Results and Discussion

As previously mentioned, research and published studies regarding FMT in small animals are limited. There are even less published studies about FMT treatment in cats than in dogs, and more research on cats is needed. Most of the study in veterinary medicine regarding indication and methods is extrapolated from human studies, and there are few case reports about the efficacy of FMT available in dogs and cats. More research is needed to make a relevant conclusion. Data obtained in this text arrives from different published articles, 4 case reports, 3 case series, and 2 experimental studies with a focus on the most common canine GI disorders and the effect after FMT therapy. In the study of FMT and its efficacy, live animal experiments have been used. The methods have been mainly humane treatment of sick animals to evaluate their response to treatment, with the owner's consent. The animal chosen for the particular study have undergone thorough physical examination, and the diseases have been diagnosed with histopathology. Some studies included in this review have used laboratory experiments on mice to evaluate the psychological effects of the gut microbiome. These types of live animal experiments are essential in medical research, and the great advantages of the researchers being able to obtain data without the interpretations of others. The disadvantage of live animal experiments is associated with it being time-consuming and it requires great dedication from the owners. Furthermore, online questionnaires have been evaluated through an observational study by Salvati Schmith (2022), for diseases, indications, administration, and effect of FMT from veterinary clinics all over the world. The advantage of this is the ability to widen the subject pool and more easily obtain data, but the risk of submission of inaccurate data is a limiting factor.

Case series regarding chronic enteropathy in dogs have been published, but the interpretation of the clinical responses after FMT treatment have been difficult to prove [23]. This is due to the inhomogeneous population: different clinical presentations, their history and symptoms, as well as the additional treatment these dogs have received before, during, and after FMT treatment [23]. One example is a case series published by Bottero et al (2017) where 16 adult dogs diagnosed with IBD were included in the study [32]. 9 dogs received FMT by duodenoscopy with fresh feces, and 5 of these also received oral transplant in addition to duodenoscopy, and 7 dogs only received frozen oral capsules [32]. CCECAI was used to calculate the clinical response before FMT and after 1 month and 3 months post-

FMT [32]. Clinical improvements were seen in most of the dogs, and there were no differences in regard to the different administration methods used. Nevertheless, the two main limitations of this study were the lack of a control group, as well as the complete determination of the gut microbiota using pyrosequencing [32]. However, in one case report written by Niina et al (2019) one dog received a long-term rectal enema treatment against IBD, and the clinical symptoms improved and the gut microbiome resembled the microbiome of the healthy donor after FMT therapy [31]. There was a high abundance of *Proteobacteria* (52.2%) and *Fusobacteria* was undetectable before FMT treatment. After FMT, the *Proteobacteria* resembled the donor's composition with 3.2% and 24.5% abundance of *Fusobacteria* [31]. Both studies conclude with improvement of clinical signs after treatment with FMT, and this suggests that FMT can be an effective treatment for canine IBD in the future. Nevertheless, more research is needed regarding clinical presentation, best administration method, and duration of treatment.

Two different case reports regarding canine NRE and IRE also concluded with clinical improvement after FMT treatment. Sugita et al (2021) performed one single endoscopic FMT into to the colon and cecum of one Shiba dog diagnosed with NRE in addition to oral chlorambucil treatment, which resulted in remarkable improvement of the clinical symptoms and of the dysbiosis [34]. On day 176 post-FMT chlorambucil treatment was terminated, and there were no reoccurrence of anemia, leukocytosis, hypoalbuminemia or GI-signs after ended treatment and there were no need for further medications [34]. Last control was performed after 288 days and the stool consistency and frequency remained normal, and there were not observed any adverse effects [34]. Cerquetella et al (2022) performed oral FMT therapy on one dog suffering from IRE in addition to prednisolone treatment [35]. 21 days after FMT therapy, there was a significant improvement in bloating and complete resolution of dyschezia, and the CIBDAI score had improved to a score of three (was scored as four before FMT) [35]. There was not observed weight gain or weight loss, the body weight remained within normal limits of 29.8-30.5 kg, and the owner reported that the general condition had improved and the dog seemed healthier [35]. 1.5 year after FMT there was an attempt to stop prednisolone treatment, but it resulted in a clinical relapse, and it was concluded that the dog needed to continue using low-dose prednisolone [35]. In both these case reports FMT was used as an additional treatment to the conventional therapy, and in both cases it lead to improvement of the clinical signs. This suggests that the use of FMT as supporting therapy can be beneficial in chronic enteropathies.

In a case series of 66 puppies with parvovirus infection, 50% received STD treatment and 50% received STD + FMT treatment [36]. FMT+ STD treatment resulted in shorter hospitalization stay with a median of 3 days, compared to a median of 6 days in the puppies only receiving STD treatment [36]. Additionally, they had 61% faster resolution of diarrhea versus 5% in STD treatment, and their mortality rate was 21.2% (7/33) as to 36.4% (12/33) in puppies only treated with STD [36]. There was no difference in the statistics between the two groups, and PCR was positive for parvovirus in all the puppies [36]. There was not observed any discomfort with the treatment, as the FMT was administered by enema, there was no need for sedation, restraint or analgesia [36]. Enema is a cost-effective procedure, and it doesn't require any special equipment or skills, which makes the therapy easy to perform and could be a good tool in the prevention of the high mortality rates usually associated with canine parvovirus [36]. In another case series performed by Chaitman et al. (2020), 18 dogs with acute diarrhea (AD) were submitted to the study where they performed FMT and oral metronidazole therapy to evaluate differences in fecal consistency and resolution of dysbiosis [44]. 11 dogs received FMT by enema and 7 dogs received oral metronidazole treatment for 7 days, and fecal samples were obtained on day 0, 7 and 28 [44]. The fecal consistency improved for both groups after 7 days, but by day 28 the fecal consistency of the dogs that had received FMT was firmer than the ones who were treated with metronidazole [44]. The use of FMT in acute enteropathy has proven to be successful in the published case reports, and should be considered to try as conventional therapy in acute enteropathies before the administration of antibiotics.

Contrary to this, the study of Gal et al. (2020) concluded that there were no significant changes in the AHDS clinical score between the sham-treated (control) and FMT-treated dogs from admission to 30 days post-admission, and that FMT treatment did not result in clinical improvement [37]. This indicates that further research is required to evaluate the efficacy of FMT in these disorders, as the small number of cases as well as the few clinical studies done are not enough to make well-established conclusions.

Furthermore, a study was done by Salavati (2022) based on an online survey about FMT used by practitioners. This study significantly contributes to/improves the knowledge of veterinarians about the indications, techniques, and outcomes of FMT in dogs [43]. The online questionnaire was sent out to small animal practices and referring hospitals, to a total of 1881 possible respondents, where the aim of this survey was to collect the results of canine

FMT therapy into one comprehensive article, due to the limited studies found regarding FMT in dogs [43]. They received 115 responses, where the majority arrived from the United Kingdom (52%) with 60 respondents, followed by 17% (n=20) from USA, 9.6% (n = 11) from Italy and 5% (n = 6) from France [43]. With additionally, 2 responses (1.7%) each from Greece, Germany and Sweden, and several other countries (Netherlands, Russia, Ireland, Portugal, Denmark, and Switzerland) provided 1 response each [43]. The primary indications for use of FMT as a therapy collected from all 115 responders is presented in *Table 9* [43]. 5 out of 115 responders used FMT for one single primary indication, while the rest reported the use of additionally several secondary indications, listed in *Table 10* [43].

Table 9. *Primary indications for use of Fecal Microbiota Transplantation collected from 115 responses [43]*

Primary indications for use of FMT	**Number of responses (n)**
Chronic enteropathy	21
Parvovirus	7
Small Intstinal Bacterial Overgrowth (SIBO) and/or bacterial infection	2
Haemorrhagic gastroentereitis and/or AHDS	2
Idiopathic diarrhea in puppies	1

Table 10. *Secondary indications for the use of Fecal Microbiota Transplantation in addition to the primary indications [43]*

Secondary indications for the use of FMT	**Number of responses (n)**
SIBO or other bacterial infections	14
Chronic enteropathy/IBD	11
Protein-loosing enteropathy	9
Acute idiopathic diarrea and/or vomiting in adult dogs	7
Hemorrhagic gastroenteritis/AHDS	7
Idiopathic diarrhea in puppies	6
Viral infections	4
Adjunctive treatment for weight loss	1

The route of administration varied between the upper- and lower- GI tract, and the reported ways of administration are presented in *Table 11,* with enema being the most used method

and oral capsules being the least used [43]. 79% of the patients that received enema used fresh stool for FMT administration, while 46% of the ones who received duodenoscopy, colonoscopy, iloescopy, nasoesophageal/nasogastric tube, and oral capsules used frozen stool preparations [43]. Donor screening and preparation of fecal material were performed by the same protocols demonstrated by Chaitman et al (2021) in *Table 5* and *Table 6* [23]. 67% of the recipients received FMT more than once, and the routine for repeated administrations varied between every 24-48 hours or until improved clinical signs, some performed it every 3rd or 4th day within 14 days, and some repeated it every week for 4 weeks or every 2nd week for 6 weeks [43].

***Table 11**. Route of administration of Fecal Microbiota Transplantation [39].*

Route of administration	**Number of responses (n)**
Enema	26
Duodenoscopy	9
Colonoscopy	6
Ileoscopy	3
Nasoesophageal or nasogastric tube	3
Oral capsules	2

The effect of FMT varied from outstanding to minor response, and 4 respondents (12%) experienced adverse effects [43]. 9% reported an "outstanding response" (with more than 90% success), 33% reported a "good" clinical response (less than 90% response, but more than 50%), and 6% reported a "minor" response (less than 50%). Adverse effects observed in the 4 respondents included the onset of hemorrhagic diarrhea in 75%, flatulence in 25%, and worsening of diarrhea in all 4 respondents. The study concluded that most of the responses showed improvement after FMT, but also that much more study are needed regarding FMT in dogs, and especially in cats [43]. This conclusion is in agreement with our findings gained from our literature review.

7.1 Limitations

In human medicine, the concerns mainly revolve around the long-term risks, as there is limited study about this, and since the therapy is relatively new the long-term risks remain unknown [40]. The importance of donor screening is to eliminate the risk of transmitting infectious pathogens, and a thorough physical and historical examination, and any donor that has diseases (such as cancer, neurological disorders, obesity, diabetes, etc.) is excluded, but diseases can develop at a later time, which remains a concern [40].

Different studies support the approach of FMT in the treatment of canine gastrointestinal diseases and dysbiosis, but the limitations revolve mainly around the lack of guidelines and proper regulations involving donor selection and preparation of the recipient [1]. One of the main concerns is the possibility of transmitting pathological bacteria, such as MRSA and/or drug-resistant *E.*coli, that live sub-clinically in the donor [1]. In addition to the transmission of pathogens, is also other adverse effects such as colic, diarrhea, and constipation [1]. To choose the best route of FMT administration was said to be an important factor in successful treatment, but based on recent studies it may not be that important after all [1]. The most important key elements in regard to a successful treatment are said to depend mostly on the skills of the veterinarian, the equipment, and the dedication of the owner [1].

To this day, FMT in dogs is generally performed with enema or endoscopy, which might be the reason why the use of FMT in dogs is limited, due to its invasiveness and the need for skilled veterinarians [9]. In the future, FMT would be more available and utilized if it existed further standardized method for oral administration, but this requires more research [9].

The GI microbiota consists of other microorganisms such as viruses and fungi, that have proven to be an important factor in the treatment of CDI in human medicine [1]. It was concluded that the abundance of fungi might influence the efficacy of FMT, but this requires more study and remains unknown in the involvement of FMT in veterinary medicine [1].

7.2 Further required studies

The future of FMT in the treatment of canine and feline GI diseases looks very promising, as GI diseases in dogs and cats are quite common [1]. They are very sensitive to dysbiosis, and we commonly see dogs and cats with diarrhea, constipation, colic and chronic gastrointestinal diseases [1]. The limited studies available regarding feline FMT makes

evaluation of its efficacy very difficult in this species, and research of feline FMT in the future should be further explored. Until now, veterinary medicine in regards to FMT treatment is mostly based on research and experience, but there is much room for improvement and exploration in the future [1]. The diagnostic tool of using 16S rRNA sequencing is a promising, relatively new method of choosing the correct donors for each individual recipient, and this method is under rapid development and is becoming more available and less costly to use [1].

For example, a study showed with the use of 16S rRNA sequencing that in the treatment of dogs with IBD they found that the recipient of the FMT showed an increased relative abundance of *Fusobacteria* [1]. In this patient's therapy, it would be beneficial to use a donor with a relatively high abundance of *Fusobacteria* [1]. The relationship between specific diseases and dysbiosis should be further explored and studied before and after FMT treatment to enhance the outcome and efficacy of FMT [1].

Dysbiosis and imbalance in the gut microbiota can affect brain health and cause psychological disorders [1]. Dogs suffering from behavior abnormalities such as anxiety, stress, and aggressiveness can indicate an imbalance in gut microbiota [1].

In an FMT study done with rats, they transferred fecal material from a donor suffering from depression to the intestinal tract of a rat with dysbiosis, where the rat showed anxiety-like behavior post-FMT [1]. In a study done with stressed mice, they reported that *Lactobacillus* spp. ameliorated their social behavior and communication, and *Bacteroides spp.* improved anxiety [1]. Both these studies suggest that behavior abnormalities such as depression and mental stress can be transmitted through gut microbiota and that in the treatment and prevention of abnormal behavior in dogs restoration of gut microbiota could be a treatment option [1]. This is also an element that should be focused on during the donor screening, and mentally sound dogs should be used to prevent the transmission of behavioral abnormalities and used as a treatment for mentally unsound dogs [1]. The *Bacteroidetes* vs. *Firmicutes* ratio should be further inspected in the future, as they are good indicators of healthy gut microbiota [1]. Nowadays the focus lays more on excluding pathogens in the selection of donors to ensure the safety of FMT [1].

8 Conclusion

Due to the limited study available, and FMT treatment being a relatively new method/indication, further study is required to make a definite conclusion about its efficacy. Although, the research available is very promising and the treatment with FMT in puppies with parvovirus infection and dogs with IBD, NRE, and IRE seems promising. To receive FMT through the upper gastrointestinal tract via capsules would be the least invasive and most available method, but more research is required regarding its efficacy. Enema is an easy process most practitioners can perform, but the limitations lay in the need for a donor. Published studies regarding treatment with FMT in cats are very limited, and the few studies available are not scientifically good enough to include in this literature review. On this note, further research regarding FMT in cats are highly needed.

Another important consideration is that – based on the results of studies -, FMT may replace the antibiotic treatment in significant number of cases with GI-issues. In most of the studies mentioned, the recipients had received broad-spectrum antibiotics at some point before the treatment with FMT was tested. Some of them were given antibiotics without improvement, some improved but relapsed at the end of treatment, and some also received additional antibiotic treatments, and different types of antibiotics when one treatment didn't work. Most of the studies showed improvement after FMT treatment and based on this it would be advisable to try treatment with FMT before antibiotic therapy. Treatment with antibiotics can cause life-long problems such as resistance, and it can even be a cause of dysbiosis. The adverse effects reported after FMT treatment are much less severe than the ones following antibiotics, and therefore it could be advisable to try FMT treatment first and evaluate its effect before starting treatment with antibiotics. This does not apply to patients showing signs of sepsis or other life-threatening complications of dysbiosis where antibiotics are an absolute necessity.

As the overuse of antibiotics is a big issue in both human and veterinary medicine, alternative therapies to antibiotic treatment are much needed in the control of diseases and reduction of antibiotic resistance [3]. FMT serves as a great alternative method for antibiotics [4]. It can be used as standard therapy alone, a supplement to conventional treatment, or in case of failure of conventional treatment [4].

The use of FMT in veterinary medicine is relatively new, and there is still uncertainty regarding the mechanism of action, and application methods of modulating the gut

microbiota [4]. Further research and investigation of FMT in veterinary medicine should be explored, and it could be an evolution in the treatment of GI and non-GI diseases in the future.

9 Summary

Dysbiosis in dogs and cats is commonly seen in veterinary practice. The use of pre-, pro-, syn-, and postbiotics, dietary change, and antibiotics have been, and still are, the most common treatments in GI diseases. The use of FMT has proven to be successful in the treatment of rCDI in human medicine, and the use of FMT in veterinary medicine is emerging, where most of the protocols used arrive from human medicine. In this literature review, FMT has been shown to have a good effect in the treatment of parvovirus infection in puppies, IBD, NRE, and IRE in dogs. But, due to the limited studies available, more research is needed in regard to the administration of FMT and to prove its effectiveness, especially in feline GI disorders. To find additional therapy to antimicrobial treatment is a very important subject in both human and veterinary medicine. The use of FMT in GI disorders could limit the use of antibiotics, and therefore also reduce the risk of resistance.

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