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Fecal microbiota transplantation and its effect on dogs with chronic enteropathy

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LIST OF ABBREVIATION

ARE	Antibiotic Responsive Enteropathy
BARF	Biologically Appropriate Raw Food
BCS	Body Condition Score
CCECAI	Canine Chronic Enteropathy Clinical Activity Index
CE	Chronic Enteropathy
DI	Dysbiosis Index
DTRE	Dysbiosis Treatment-Responsive Enteropathy
EPI	Exocrine Pancreatic Insufficiency
ESBL	Extended-Spectrum Beta-Lactamase
F	Female
FISH	Fluorescent In Situ Hybridization
FMT	Fecal Microbiota Transplantation
FRE	Food responsive enteropathy
GI	Gastrointestinal
IBD	Inflammatory Bowel Disease
IRE	Immunosuppressant-Responsive Enteropathy
M	Male
MCS	Muscle Condition Score
NGS	Next Generation Sequencing
NRE	Non-Responsive Enteropathy
PLE	Protein-Losing Enteropathy
rRNA	Ribosomal Ribonucleic Acid
SCFA	Short-Chain Fatty-Acid
qPCR	quantitative real-time Polymerase Chain Reaction

I. INTRODUCTION

1. The gastrointestinal microbiota and microbiome: definitions and characteristics

The gastrointestinal (GI) microbiota includes the wide variety of microorganisms in the intestinal tract. Microbiota refers to all the living microorganisms in a specific environment, like bacteria, archaea, fungi, protists and algae.

The GI microbiome includes the microbiota, their functions as "theatre of activity", a concept introduced by Berg, and the genetic elements of non-living organisms (Berg et al., 2020). Viruses, phages, and extracellular DNA being non-living, are not considered part of the microbiota, but are included in the microbiome. The “theatre of activity” refers to the molecules produced by those organisms and by the host, including the nucleic acids, proteins, lipids, polysaccharides and metabolites. The microbiome and the microbiota have been frequently analysed for characterizing the wide variety of microorganisms, especially for the intestinal bacteria. The dog's GI microbiota is comprised of 10^{12} to 10^{14} bacterial cells, roughly ten times the number of the dog's own cells (Pilla et al., 2020). Recent advancements in molecular techniques, such as next generation sequencing (NGS), have enhanced our understanding of the canine GI tract, revealing it to have a rich and complex microbial ecosystem comprising over 100 bacterial genera (Minamoto et al., 2019).

The majority of GI bacteria cannot be cultured using standard plating techniques, as they require complex and specific environment for growing (Suchodolski et al., 2016). Molecular methods are nowadays enabling the identification of the bacteria, such as NGS, fluorescent in situ hybridization (FISH) to visualize the translocation of bacteria into the mucosal epithelium or the quantitative PCR (qPCR), a tool frequently used for sequencing the bacterial 16S ribosomal RNA (rRNA) gene. For sequencing all given genomic DNA from a given sample, Shotgun Metagenomic Sequencing can be used (Quince et al., 2017).

The small intestine harbours a mixture of aerobic and facultatively anaerobic bacteria, while in large intestine anaerobes are found (Suchodolski et al., 2016). In a healthy dog, eubiosis, which signifies a balanced and harmonious state of the microbiota, is characterized by the predominance of specific bacterial taxa such as *Clostridium hiranonis*, *Faecalibacterium*, *Streptococcus*, and *Turicibacter*, all of which belong to the *Firmicutes* phylum. Additionally, it includes the presence of *Blautia*, *Proteobacteria* (*Escherichia coli*), and *Fusobacteria* (Suchodolski et al., 2011).

The development of the microbiome starts right at birth, if not during foetal life (Garrigues et al., 2022). It is interesting to note that each animal possesses a unique microbial profile heavily influenced by its diet (Simpson et al., 2002). Moreover, the breed, age, sex, living environment, nutrition, medical treatments, stress, pregnancy and diseases can also influence on the composition. Adequate hydration and a stress-free environment also play significant roles in supporting a healthy microbiome in dogs (Baritugo et al., 2023).

2. The functions of the intestinal microbiome

The network of the intestinal microbial ecosystem forms an intimate relationship with the host. A harmoniously balanced intestinal microbiota forms a protective barrier against opportunistic pathogens like *E. coli* through competition for resources such as oxygen, luminal substrates, and space. Germ-free rodent studies have demonstrated the critical role of bacteria in modulating immunity, particularly when the immune system is compromised and the host becomes susceptible to pathogens like *Listeria monocytogenes* (Khosravi et al., 2015). In recent studies, it is shown that the intestinal microbiota can interact with other organs such as brain, kidney, skin (Celi et al., 2017, García-Belenguer et al., 2021).

In healthy individuals, the bacteria contribute to the synthesis of essential vitamins including riboflavin (vitamin B2), biotin (vitamin B7), folic acid (vitamin B9), cobalamin (vitamin B12), and vitamin K, while also producing bacteriocins that have the capacity to inhibit the growth of pathogenic bacteria (Suchodolski et al., 2016).

Some bacteria strains are also worth mentioning separately. *Clostridium hiranonis (C. hiranonis), also known Peptacetobacter hiranonis* is of particular importance in bile acid metabolism, and its normal function is essential for the intestinal health (Ziese et al., 2021). This bacterium possesses the capability to convert primary bile acids into secondary bile acids. This metabolic process has been shown to have anti-inflammatory effects, inhibit the germination of pathogenic bacterial spores such as *Clostridioides difficile*, and modulate insulin and glucose metabolism through the activation of glucagon-like peptide 1 (Pavlidis et al., 2015).

The phylum *Firmicutes* play a crucial role by generating metabolites, the short-chain fatty acids (SCFA) such as acetate, propionate and butyrate through the fermentation of carbohydrates such as starch, pectin and inulin, which act as a source of energy for the growth of the intestinal mucosa and also for the host (Arpaia et al., 2013).

Another facet of metabolite conversion involves tryptophan, an essential amino acid crucial for serotonin synthesis, as it serves as the sole precursor (Duboc et al., 2013). It supports the development of the central and enteric nervous system, as well as modulates the inflammatory response. The unabsorbed tryptophan from the small intestine serves as a metabolic substrate for the bacteria in colon. *Lactobacillus* can produce serotonin from tryptophan. *Firmicutes* and *Proteobacteria* can also convert tryptophan into indole that can attenuate the inflammation in enterocytes and macrophages, as well as improves intestinal permeability and increases mucin production (Duboc et al., 2013).

3. Definition of dysbiosis

Dysbiosis refers to a disturbance in the overall diversity and abundance of intestinal bacterial groups, which can result from various factors (Pilla et al., 2022). The underlying results of maldigestion and malabsorption stemming from chronic inflammation can be diarrhea, vomiting, abdominal discomfort, indigestion, bloating, hyporexia and weight loss (Sung et al., 2022). This imbalance in bacterial diversity, along with the proliferation of pathogenic bacteria, is linked to gastrointestinal dysfunctions, including chronic

enteropathy (CE), exocrine pancreatic insufficiency (EPI) or parvovirus enteritis in puppies. The excessive proliferation of pathogenic bacteria, such as *E. coli* or *Clostridium perfringens (C. perfringens)*, disrupts intestinal homeostasis, ultimately leading to dysbiosis (Pilla et al., 2022).

Furthermore, there are significant differences of abundance of bacteria, depending on the dog's body condition score (BCS). For instance, in overweight dogs with a BCS of 6/9, there is a correlation of an increased amount of *Fusobacterium perfoetens* (Chun et al., 2020).

Apart from pathological conditions, various treatments can significantly induce significant dysbiosis, especially broad-spectrum antibiotics. The administration of antibiotics such as **tylosin** (Manchester et al., 2019) and **metronidazole** (Pilla et al., 2020), has been observed to induce dysbiosis that can persist for weeks or even months in dogs. These treatments often result in a shift in bacterial diversity. For example, following the administration of tylosin, there is a notable decrease in *Fusobacteria* as early as day seven, which are known for their production of SCFA (Manchester et al., 2009). Similarly, metronidazole reduces *Fusobacteria* levels, and their normal abundance may not fully recover even four weeks after discontinuing metronidazole (Pilla et al., 2020). A reduction in *C. hiranonis* can also accompany, which is closely linked to the improper conversion of bile acids. Moreover, the impact of **omeprazole**, a proton pump inhibitor, a reduction in *Helicobacter spp*. and an increase in *Firmicutes* and *Fusobacteria* are seen, coupled with an upsurge in the overall bacterial count within the duodenum. The dysbiosis usually normalizes within two weeks after discontinuation of omeprazole (Garcia-Mazcorro et al., 2012).

Ultimately, diet exerts a significant influence on the gastrointestinal microbiome, as illustrated by dogs on the BARF (biologically appropriate raw food) diet, who were provided with higher quantities of protein and fat, while receiving significantly lower amounts of nitrogen-free extract and fiber (Schmidt et al., 2018). The qPCR assays showed a significantly increased abundance of pathogenic bacteria *E. coli* and *C. perfringens*. Additionally, there was a higher presence of *Lactobacillales*, *Enterobacteriaceae*, *Fusobacterium*, and *Clostridium* in the BARF. A higher dysbiosis index can be observed but with normal amount of *C. hiranonis*.

4. Dysbiosis index

The alteration in the intestinal composition of the microbiota is interpretated with the value of dysbiosis index (DI), performed at the Gastrointestinal Laboratory at Texas A&M University with real-time PCR targeting the 16s RNA of bacteria, based on the fecal abundance of 7 bacteria: *Clostridium hiranonis, Faecalibacterium, Turicibacter, Streptococcus, E.coli, Blautia* and *Fusobacterium* in dogs (AlShawaqfeh et al., 2017) and *C. hiranonis*, *Faecalibacterium*, *Turicibacter*, *Streptococcus*, *E. coli*, *Bacteroides* and *Bifidobacterium* in cats (Sung et al., 2022). The values of DI represent the diversity for each bacterial group and additionally a final number that expresses the extent of intestinal dysbiosis. Although a sequencing approach is valuable for characterizing the microbiota, it primarily offers information about relative microbiome changes between different groups. This expensive and time-consuming tool lacks quantitative data that restricts its practical clinical use for individual cases (Sung et al., 2022)

The DI correlates negatively with species richness: in dogs, a DI surpassing 2, and in cats, a DI exceeding 1, signals a significant and highly specific shift indicative of dysbiosis. Conversely, a DI ranging from 0 to 2 in dogs and from 0 to 1 in cats denotes a moderate alteration in the fecal microbiome (AlShawaqfeh et al., 2017, Sung et al., 2022). Notably, some dogs with chronic enteropathies exhibit a DI less than 0, but with certain bacterial taxa falling outside the reference intervals, suggesting a milder form of dysbiosis (AlShawaqfeh et al., 2017).

As shown in *Table 1* below, dysbiosis in dogs is characterized by an increase in potentially enteropathogenic bacteria such as *Streptococcus* and *E. coli*, alongside a reduction in beneficial SCFA-producing bacteria like *Faecalibacterium*, *Turicibacter*, *Blautia*, and *Fusobacterium*, as well as *C. hiranonis*.

Table 1. The functions and alterations in bacterial populations, as assessed through Dysbiosis Index during dysbiosis in dogs.

	Functions	Change in dysbiosis
Clostridium hiranonis	Conversion of primary to secondary bile acids	↘
Faecalibacterium	Anti-inflammatory & Production of SCFA	↘
Turicibacter	Production of SCFA	↘
Streptococcus	Overgrowth associated with dysbiosis	↗
E. coli	Pro-inflammatory	↗
Blautia	Production of SCFA	↘
Fusobacterium	Production of SCFA	↘

5. Dysbiosis in chronic enteropathy

Dysbiosis is closely associated with GI dysfunctions (Félix et al., 2022) such as chronic enteropathy (CE). In cases of CE in dogs, characteristic clinical symptoms include persistent diarrhoea lasting for more than three weeks and the presence of mucosal inflammation detected through histopathological examination. Dysbiosis is also frequently manifest in cases of CE, a prevalent condition, with its severity often reflected in a high DI.

Significantly distinct bacterial abundances, including those of total bacteria and the seven specific bacterial taxa, can indeed be observed between healthy animals and those suffering from CE.

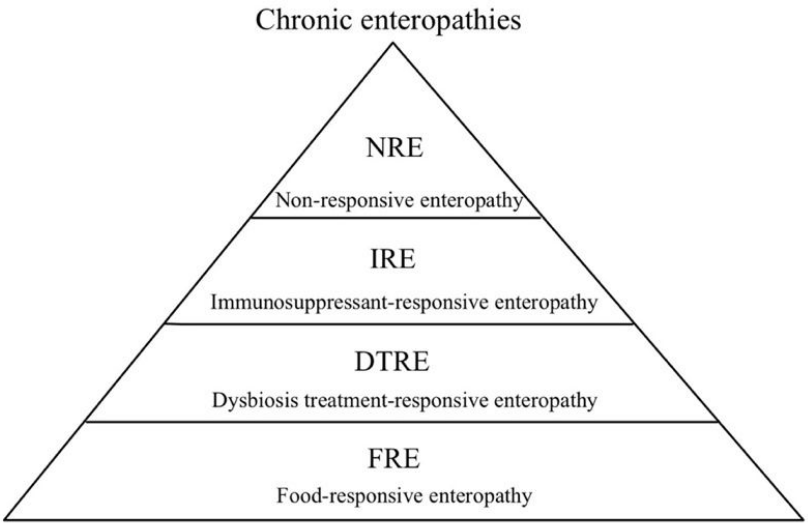

Figure1. Visual representation of the four distinct forms of chronic enteropathies categorized according to their response to treatment (Dandrieux et al., 2016), modified by Dr. Kinga Pápa.

To understand the underlying cause of CE, a systemic approach to treatments is essential. CE is characterized by four distinct categories based on their

response to treatment: food-responsive enteropathy (FRE), dysbiosis treatment-responsive enteropathy (DTRE), immunosuppressant-responsive enteropathy (IRE), and non-responsive enteropathy (NRE) as shown in *Figure 1* (Dandrieux et al., 2016), modified by Dr. Kinga Pápa.

For patients who experience a clinical improvement solely through dietary changes, a diagnosis of FRE is typically established.

In cases where dietary modifications do not yield positive results, microbiome modulation become the second-line treatment. When addressing dysbiosis and aiming to restore a healthy, balanced intestinal microbiome in CE, there exists a spectrum of treatment options to choose from. If a dog responds favourably to dysbiosis treatment such pro-, pre-, post- or synbiotic besides highly digestible, hypoallergenic diet, or in narrow spectrum of cases antibiotics, as tylosin or metronidazole, a diagnosis of dysbiosis treatment-responsive enteropathy (DTRE) is made.

On the other hand, idiopathic inflammatory bowel disease (IBD) may be the underlying cause of CE. Symptoms include recurrent chronic diarrhoea, weight loss, vomiting, and in the most severe forms protein-losing enteropathy (PLE) can develop. In such cases, patients are considered immunosuppressant-responsive, and drugs like prednisolone or cyclosporine are administered.

The final category of CE is NRE, encompassing patients with no response to any of the therapeutic approaches discussed earlier. In NRE, a potential novel additional treatment option is Fecal Microbiota Transplantation (FMT), which will be discussed in greater detail in the following chapter.

Each of these treatment approaches targets specific mechanisms, and often a combination of treatments is employed to achieve the most favourable outcome.

6. Fecal microbiota transplantation

FMT is a therapeutic procedure involving the transfer of a fecal sample from a healthy animal into the gastrointestinal tract of a diseased recipient, which can be achieved through methods such as oral capsules, endoscopy, or rectal enema. The primary objective of FMT is to restore the integrity of the

intestinal barrier and alleviate inflammation by harnessing the metabolic activities of the introduced bacteria.

Despite its established success in treating *Clostridioides difficile* infection in humans (Li et al., 2016, Tariq et al., 2018), FMT remains an emerging therapy in veterinary medicine, and consensus-based guidelines are yet to be established for various aspects, including fecal sample preparation, donor selection criteria, administration methods, dosing, interval between administrations, frequency, and route of administration (Chaitman et al., 2021). This lack of standardized protocols poses a significant limitation to the widespread adoption of FMT in canine medicine.

It's worth noting that, to date, there have been no reports of serious adverse effects associated with FMT, although this may be due to limited data availability (Berlanda et al., 2021). Currently, efforts are underway to develop guidelines by an international group of experts known as the Companion Animal Fecal Bank Consortium (Toresson et al., 2023).

In clinical practice, FMT has shown varying levels of success in dogs with CE. Successful cases have demonstrated an increase in the diversity of beneficial bacteria, such as *C. hiranonis* and *Faecalibacterium*, accompanied by a decrease in *E. coli*. Improvement in fecal scores can often be observed within two to three days following FMT, but it may be followed by relapses, particularly if the underlying pathology persists. Consequently, multiple FMT sessions may be required, supplemented by anti-inflammatory treatment and dietary modifications (Chaitman et al., 2020).

Let’s have a comparative overview of FMT techniques based on findings from ten studies in dogs available in the veterinarian literature till writing of the manuscript. FMT has been applied in various canine conditions, including post-weaning diarrhea in puppies (Burton et al., 2016), IBD in adult dogs (Bottero et al., 2017, Nina et al., 2019), parvovirus infection (Pereia et al., 2018), intermittent large bowel diarrhea (Sugita et al., 2019), acute diarrhea (Chaitman et al., 2020), chronic-recurring pasty large bowel diarrhea (Diniz et al., 2021), acute hemorrhagic diarrhea syndrome (Gal et al., 2021), and chronic enteropathy (Toresson et al., 2023, Carapeto et al., 2023).

The preparation of fecal samples for FMT can involve using fresh samples, samples that are frozen and thawed before application, or lyophilized samples.

Fresh samples are typically used within 48 hours to maintain their natural composition, or can be stored at 4 °C for up to one week, while frozen samples can be stored at temperatures ranging from -20 to -80°C for later use, often with the addition of glycerol to protect the bacterial composition. The use of frozen samples helps overcome geographic limitations and facilitates the wider adoption of FMT in veterinary clinical practice (Tuniyazi et al., 2022). To create a homogenous slurry, fecal samples are typically blended and mixed with saline at ratios such as 1:1, 1:2, 1:3, or 1:4 depending on the required consistency, respectively. Some studies also employ a sterile sieve to remove particles and achieve a more uniform structure (Gal et al., 2021).

FMT can be administered through different routes, including oral or via the lower GI tract, using methods like colonoscopy or enema. Oral administration can involve the use of capsules produced through the lyophilization of fecal material, as demonstrated in a study by Carapeto et al. (2023), where one capsule was administered daily for sixty days.

The frequency of FMT administration through the lower GI tract varies among studies, with some employing a single administration (Diniz et al., 2020, Gal et al., 2021), two administrations within 48 hours (Pereira et al., 2018), three administrations with ten to twenty days between each (Chaitman et al., 2020, Toresson et al., 2023), or even nine administrations spaced over six months (Nina et al., 2019).

FMT can be used as a standalone treatment or in combination with other therapies, such as metronidazole (Chaitman et al., 2020), and it may also be accompanied by dietary modifications (Toresson et al., 2023).

While the precise mechanisms of FMT remain uncertain, several hypotheses have been proposed. First, donor strains may outcompete recipient strains for available niches (Kelly et al., 2016). Second, there may be increased competition for nutrients between donor strains and pathogens. Third, the production of antimicrobials, such as bacteriocins, by donor strains can eliminate pathogens (Baktash et al., 2019). Lastly, increased production of secondary bile acids by donor strains may help restore the microbiome (Weingarden et al., 2016).

In human medicine, FMT has shown promise in treating recurrent *C. difficile* infections (Li et al., 2016, Tariq et al., 2018), ulcerative colitis (Costello et al.,

2019) and IBD (Basson et al., 2020). Additionally, it is an emerging therapy for extraintestinal disorders. This includes combating weight gain (Alang et al., 2015), addressing hepatic encephalopathy (Bajaj et al., 2017), promoting responses in immunotherapy-refractory melanoma patients (Baruch et al., 2021), as well as halting the progression of type 1 diabetes (de Groot et al., 2021).

Regarding the preparation of samples for treating recurrent *C. difficile* infections, fresh, frozen, or freeze-dried samples have shown similar success rates (Staley et al., 2017).

For the route of administration in freeze-dried formats, successful outcomes have been achieved both through enema (Lee et al., 2016) and with capsules (Gulati et al., 2020).

7. Selection and criteria of donor dogs

The selection criteria for donor dogs in FMT are a crucial aspect of the process, requiring careful consideration (Toresson et al., 2023).

Donor selection is a critical step in all studies involving FMT in dogs, and it involves a comprehensive evaluation of the donor's health status. This evaluation includes a detailed history and thorough physical examination, along with laboratory screening (Chaitman et al., 2020).

To be considered an eligible donor, the dog must exhibit overall good health, including being up-to-date on vaccinations and free of parasites. Donors should not be fed a raw food diet, as this can introduce potential pathogens into their microbiome and BARF diet may lead to dysbiosis (Schmidt et al., 2017). Laboratory screenings must demonstrate normal results in terms of haematological and biochemical parameters. Fecal samples should test negative for parasites like *Giardia* and roundworms, and the donor should be free of diseases such as parvovirus and distemper. Additionally, screening is done to exclude enteropathogens like *Salmonella spp* or *Campylobacter spp.*

Donor dogs should be adults without any systemic diseases or chronic illnesses, with no history of GI diseases, and they should not have undergone antibiotic treatment for at least 6 months (Toresson et al., 2023).

Assessment of the donor's dysbiosis index is essential to ensure that there is no dysbiosis present. Donors should exhibit high levels of bacteria that SCFA-producing, as a decrease in SCFA-producing bacteria is commonly observed in chronic enteropathies (AlShawaqfeh et al., 2017).

Furthermore, to prevent the transmission of antibiotic-resistant bacteria, such as extended-spectrum beta-lactamase (ESBL)-producing bacteria during the FMT process, donors are screened to confirm the absence of such bacteria in their samples (DeFilipp et al., 2019, Tuniyazi et al., 2022).

8. Extended-spectrum beta-lactamase producing bacteria

E. coli is a commensal bacterium commonly found in the GI tract of healthy animals and humans. While most *E. coli* strains are harmless, some have the capacity to develop mechanisms to inactivate antibiotics, thereby posing challenges for therapies against multidrug-resistant bacteria (Deepti et al., 2010).

The detection of multidrug-resistant bacteria is strongly recommended before applying FMT to avoid any spreading of resistance from the donor dog to the recipient and a critical concern for therapies failure against them (Chong et al., 2011).

The presence of ESBL-producing bacteria in the bowel can cause chronic inflammation, or its multiplication in the urinary tract, the initially uncomplicated cystitis can turn into life-threatening sepsis if not detect (Huber et al., 2012).

As an ESBL-producing bacteria, a group of enzymes produced by Escherichia coli. These enzymes serve as formidable defences against beta-lactam antibiotics such as penicillin, cephalosporins, and monobactams. ESBLs function by hydrolysing the beta-lactam ring, rendering these antibiotics ineffective (Chong et al., 2011).

In addition to resistance to beta-lactam antibiotics, ESBL-producing bacteria may also exhibit resistance to other antibiotic classes, including fluoroquinolones, aminoglycosides, and sulfamethoxazole/trimethoprim (Coque et al., 2008). This multifaceted resistance profile limits the available therapeutic options (Deepti et al., 2010).

The mechanism of resistance is primarily based on plasmid-mediated enzyme production, which can be transmitted through direct contact with an infected animal's bodily fluids, such as blood, urine, wounds, and, in our case, feces. Indirect transmission can also occur through contaminated equipment or surfaces. Horizontal transmission between bacteria is possible, further exacerbating multidrug resistance. Additionally, there is a risk of zoonotic transmission, where multidrug-resistant bacteria can spread between animals and humans (Deepti et al., 2010).

II. AIMS OF THE STUDY

This study had several primary objectives. Firstly, it aimed to investigate the efficacy of FMT in the treatment of dogs suffering from chronic enteropathy. The focus was on assessing the impact of FMT on the clinical signs exhibited by the recipient dogs. The study also involved a comparison of the dysbiosis index before and after FMT application. To achieve therapeutic outcomes, FMT was complemented with dietary modifications and medications, including budesonide, prednisolone, probiotics, and metronidazole.

Secondly, the research addressed the limited availability of veterinary literature on FMT and the absence of evidence-based guidelines or consensus regarding donor criteria and FMT methods. To address this gap, the study adhered to strict criteria for donor selection, with excluding ESBL-producing bacteria.

Lastly, the study aimed to assess the effectiveness of sample preparation, storage techniques, administration frequency, and intervals.

Overall, our goal was to improve the knowledge regarding FMT in dogs and provide useful information for FMT studies.

III. MATERIALS AND METHODS

1. Selection of the donor dogs

To select suitable donors for the FMT in our study, two healthy adult dogs (**Balu**, a 8-year old male Gordon setter and **Saci** a 4 year-old female Giant Schnauzer) were we carefully evaluated. Our donor dogs met specific criteria to ensure their overall health and suitability for the FMT process. They underwent a standard clinical examination to confirm their overall health. Additionally, comprehensive examinations, including blood tests, fecal tests, and the measurement of the dysbiosis index were performed.

1. Deworming and heartworm treatment: both donor dogs were up-to-date with deworming (tablet composed of praziquantel, pyrantel) and monthly heart prevention (tablet containing afoxalaner and milbemycin).
2. Vaccination: yearly vaccination of DHPPi/L4R are checked, ensuring they were free of any infective diseases, including canine distemper, (D) adenovirus (H), parvovirus (P), live attenuated parainfluenza virus (Pi), canine leptospirosis (L4) and inactivated rabies (R) vaccine.
3. Diet: commercially available complete and balanced dry dog food.
4. History of GI diseases: no history of significant gastrointestinal diseases.
5. Antibiotics and immunosuppressants: neither of the donor dogs had been subjected to antibiotic treatments or immunosuppressant drugs in the 12 months leading up to the study.
6. Blood tests in Praxislab laboratorium: the haematology examination was done with Siemens Advia 2120 instrument. The following biochemical parameters were controlled by Beckman Coulter AU 480 biochemical instrument: albumin, globulin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), alkaline phosphatase (ALKP), gamma glutamil transferase (GGT), total bilirubin, alpha amylase, lipase, creatine kinase (CK), lactate dehydrogenase (LDH), triglyceride, cholesterol, glucose, fructosamine, urea and creatinine, iron, iron-binding capacity, c-reactive protein, as well as electrolytes.
7. Fresh fecal samples were sent to the DuoBakt Laboratory and stored in a refrigerator at 4°C both during transport and before analysis, and were

processed within 24 hours: fecal parasitology and bacteriology (for *Salmonella*, *Campylobacter*, *Shigella*, *Yersinia spp*) examination were conducted. Also, *Clostridioides difficile* A+B toxin and antigen were measured as well as *Cryptosporidium* (Ag) ELISA were performed. *Parvo-Corona-Giardia* rapid pet side combo test was also done.

8. The dysbiosis index, were conducted at the Gastrointestinal Laboratory at Texas A&M University with qPCR: min. 4 grams of fecal sample is required to be frozen during the storage and the transport from the Praxislab laboratorium.
9. The detection of ESBL producing bacteria was done at the Microbiological Department of the University of Veterinary Science. The samples were stored at -80°C until the process. This involved inoculating the fecal samples of the donor dogs onto MacConkey agar, a specialized medium designed for the isolation of enteric bacilli based on lactose fermentation. The key components of this agar, including bile salts and crystal violets, function to inhibit the growth of Gram-positive bacteria, allowing only Gram-negative bacteria to thrive.

   Moreover, this agar provides a means to differentiate between bacteria capable of fermenting lactose to produce lactic acid and those that cannot. Lactose-positive bacteria result in colonies with a pink coloration due to the lowered pH caused by lactic acid production, while lactose-negative bacteria yield yellow colonies. This is an essential characteristic observed during the examination process, with colonies of *E. coli*, *Klebsiella*, and *Enterococcus* displaying the distinctive pink color. To specifically select for the growth of multidrug-resistant colonies, 2µg/mL of the antibiotic cefotaxime was added into the medium.

2. Preparation of the fecal samples

To prepare for FMT, fecal samples were collected naturally during defecation from two carefully chosen donor dogs under strict contamination-free conditions, with precautions taken to minimize grass and ground contamination. These samples were then rapidly transported within 6 hours in sealed containers under cooled conditions, and their weight was recorded.

During the preparation and also the administration of FMT we used necessary equipment such as masks and protective gloves in an isolated room to minimize contamination risks.

Figure 2. Preparation of a frozen sample mixed with saline and glycerol.

Three types of samples were prepared depending on the time of administration.

1. If used directly, there was no need for storage. Fresh samples were mixed with saline in a 1:1-2 ratio to create a slurry.

2. If intended for later use within 6 months, the samples were frozen at -80°C, thawed in the fridge at 4°C overnight before the day of administration, and then mixed with saline in a 1:1-2 ratio.

3. Frozen samples also stored at -80°C but mixed with saline and glycerol in a 1:1 ratio to protect bacterial cells from the low temperature before the

storage, as illustrated on *Figure 2*. For every 100 mL of mixed stool and saline, 12 mL of 86 % glycerol was used. Same thawing technique as 2.
The samples were divided into small portions before freezing.

3. Selection of the recipient dogs

The selection of our 5 recipient dogs was based on their presentation of chronic diarrhea and confirmed diagnoses of chronic enteropathies (CE), such as IBD and PLE.

Kira, a 7-year-old Havanese Bichon, was diagnosed with CE, FRE, and bile acid dysmetabolism. Luna, a 10-month-old Weimaraner Vizsla, was diagnosed with CE. Merlin, a 2.5-year-old Golden Retriever, has CE and IBD. Aston, a 7-year-old Boston Terrier, is dealing with CE and IBD. Picúr, a 13-year-old Havanese Bichon, has severe PLE and IBD.

Prior to considering FMT, we conducted a comprehensive diagnostic workup to establish a definitive diagnosis and to assess the health of the recipients:

1. Thorough history, physical examination was done and data of the body weight (BW), the fecal score, the body condition score (BCS) and the muscle condition score (MCS) based on WSAVA Nutritional Assessment Guidelines were recorded, as shown on *Table 2*. The canine chronic enteropathy activity index (CCECAI) according to Allenspach's table (2007) was also evaluated.

Table 2. BW, CCEAI, BCS and MCS of the 5 dogs
CCECAI: 0-3 clinically insignificant, 4-5 mild, 6-8 moderate, 9-11 severe, >12 very severe
BCS: 1-3 too thin, 4-5: ideal, 6-9 too heavy Fecal score: 1: very hard and dry, 7: watery
MCS: 0: severe muscle loss, 1: moderate muscle loss, 2: mild muscle loss, 3: ideal

Name	BW	CCECAI (0-27)	BCS (1-9)	MCS (0-3)	Fecal score (1-7)
Kira (F)	8kg	1	5	3	1
Luna (F)	16kg	6	3	3	3
Merlin (M)	29kg	7	3	2	4
Aston (M)	4.4kg	9	2	1	6
Picúr (M)	7.3kg	18	2	1	4

2. FRE, DTRE including conventional treatment of dysbiosis were ruled out and patients were unresponsive to therapies, such as dietary changes, probiotics, fenbendazole, antibiotics, and steroids.

3. Blood tests to assess overall health were conducted on 5/5 animals: the haematology examination, biochemical parameters: albumin, globulin, total protein, ALT, AST, GLDH, ALKP, GGT, total bilirubin, alpha amylase, lipase, CK, LDH, triglyceride, cholesterol, glucose, fructosamine, urea and creatinine, as well as electrolytes.
4. Fecal score to evaluate fecal consistency based on Nestlé Purina fecal score chart and parasitological examination to rule out parasitic infections.
5. Ultrasonography performed on all dogs.
6. Endoscopy and histopathology to exclude gastrointestinal tumors and establish the diagnosis, performed on Merlin and Aston, but not on Luna and Picúr. Endoscopy and histopathology were performed on Kira two years ago.
7. Measurement of the dysbiosis index through samples sent to the Gastrointestinal Laboratory at Texas A&M University.

It is important to note that FMT was not administered as a unique treatment but was combined with dietary modifications, anti-inflammatory drugs, and immunosuppressant drugs as part of a comprehensive therapeutic approach, as seen on *Table 3*.

Table 3. Diagnosis, ongoing treatments and the amount of FMT provided to the 5 dogs

Name	Diagnosis	Other treatments	FMT
Kira (F)	CE, FRE Bile acid dysmetabolism	Home-made diet, Prednisolone, Probiotic	2x
Luna (F)	CE	Hydrolized protein diet, Budesonide, Probiotic	2x
Merlin (M)	CE, IBD	Hydrolized protein diet, Budesonide, Probiotic , Vit B12	2x
Aston (M)	CE, IBD	Hydrolized protein diet, Budesonide, Probiotic ,Vit B12	3x
Picúr (M)	Severe PLE, IBD	ULF diet, Prednisolone, Probiotic, Vit B12, Metronidazole, Enrofloxacin, Samylin, Spironolactone, Clopidogrel, Calcium & Magnesium salts	4x

4. FMT procedure

For every five dog undergoing FMT, we utilized the following equipment: a sterile 60 mL syringe, a sterile 120cm, 14-inch French, Levin type nasogastric duodenal PCV-tube with orifices at the extremity, some paraffin and a

container with warm water to warm up the fecal sample before the administration to match the recipient's body temperature.

The preparators wore gloves and masks for hygienic purposes and to minimize contamination risks.

The type and the quantity of fecal material in the form of a slurry, was administered with 5-6 mL/kg of the recipient, as illustrated in *Table 4*.

Table 4. The recipient dogs with the type and quantity of fecal sample according to their body weight. The sample with glycerol added prior the freezing is marked with a "+ G".

	FMT 1	FMT 2	FMT 3	FMT 4
Luna	Frozen + **G** 80mL – 16kg	Frozen 80mL – 17,5kg	-	-
Kira	Fresh 45mL – 8kg	Frozen + **G** 40mL – 8,3kg	-	-
Merlin	Frozen + **G** 150mL – 29kg	Frozen + **G** 150mL – 30kg	-	-
Aston	Frozen + **G** 20mL – 4.4kg	Frozen 22mL – 4.4kg	Frozen 25mL – 4.7kg	-
Picúr	Frozen 35mL – 7,3kg	Frozen 35mL – 7kg	Frozen 30mL – 6.7kg	Frozen 30mL – 6.5kg

All samples were administered rectally as shown on *Figure 3*. To achieve this, we placed the fecal material in a syringe connected to the sterile tube, which was lubricated with paraffin to facilitate rectal introduction. The length of the tube inserted corresponded to the distance from the anus to the last rib. The material was administered slowly, over 10 minutes. FMT was performed 2 to 4 times, with intervals of 2 to 4 weeks between administrations.

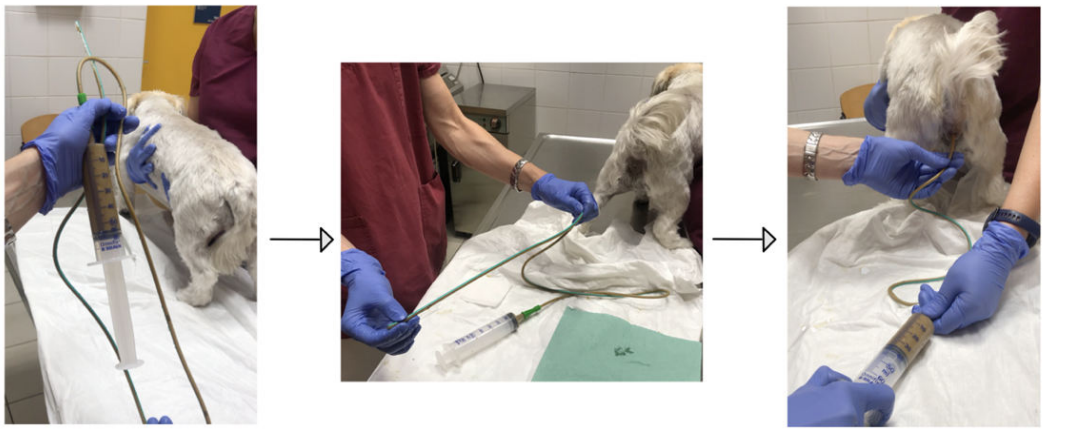

Figure 3. Rectal FMT procedure from fresh sample on the recipient Kira weighing 8kg

To prepare the recipient dogs for FMT:

1. They were encouraged to walk before the FMT administration to empty the GI tract.
2. A 12-hour fasting period was required prior to FMT to clear the GI tract.
3. FMT was conducted in a calm environment without sedation. Dogs were given 30 minutes to 1 hour to retain the material to enhance colonization. Immediate walking after FMT was then avoided.
4. Dogs were closely monitored for any GI side effects during and right after FMT, including vomiting, abdominal pain, or diarrhea.
5. After the FMT, the owners were requested to provide feedback regarding fecal consistency and the time of the first defecation.

IV. RESULTS

1. Donor selection

a. Blood and fecal analysis

The parameters from the blood examinations of both donors were in the normal range. According to the fecal tests, both donor dogs were healthy. The *Parvovirus* and *Corona*virus tests and the *Salmonella*, *Shigella*, *Yersinia enterocolitica* were negative. They had a normal intestinal flora, with aerobic culture, a negative *Cryptosporidium* (Ag) ELISA test and *Clostridioides difficile* was not cultured. *Clostridioides difficile* A+B toxin and antigen test was negative. Moreover, they were free of worm eggs and protozoon and the *Giardia* test were also negative.

b. Dysbiosis index

The DI reference is lower than 0, and the lower the DI is, the more diverse the microbiota is.

Both dogs have an optimal DI and a normal range in the values of the seven taxa groups as shown on *Table 5*, meaning that there is no shift in the overall diversity of intestinal microbiota. Plus, their abundance of *C. hiranonis* is normal.

Table 5. DI results of donor dogs from Gastrointestinal Laboratory, Texas A&M University. The unit for the abundance of the 7 taxa groups is log DNA/g.

	Results of Balu	Results of Saci	Reference value
Dysbiosis index	-1	-4.9	<0
C. hiranonis	6.3	6.7	5.1-7.1
Faecalibacterium	6.8	6.2	3.4-8.0
Turicibacter	6.8	7.5	4.6-8.1
Streptococcus	5.6	4	1.9-8.0
E. coli	7	3.8	0.9-8.0
Blautia	10.1	10	9.5-11.0
Fusobacterium	7.8	7.3	7.0-10.3

c. ESBL-producing bacteria detection

This plate in *Figure 4* is an example of how the inoculation of the fecal sample looks like in a MacConkey agar with 2µg/ml of cefotaxin. Reference strains of antibiotic resistant bacteria and antibiotic sensitive bacteria are inoculated on the 48168 and ATCC 225922 parts of the plate, respectively. Cefotaxin resistant bacteria can grow, whereas cefotaxin sensitive bacteria cannot. On the 310614, a fecal sample from a positive control shows growth of ESBL-producing bacteria. Their colonies grow and colored in pink due to the production of lactic acid. Finally, fecal samples of the donor candidates, Saci and Balu were inoculated onto the last part 310626 and 310626 on the other plate, respectively, where no ESBL-producing bacteria were found, as no colonies could grow. With this method of detection, the two donor dogs Saci and Balu used for the experience were both free of ESBL-producing bacteria.

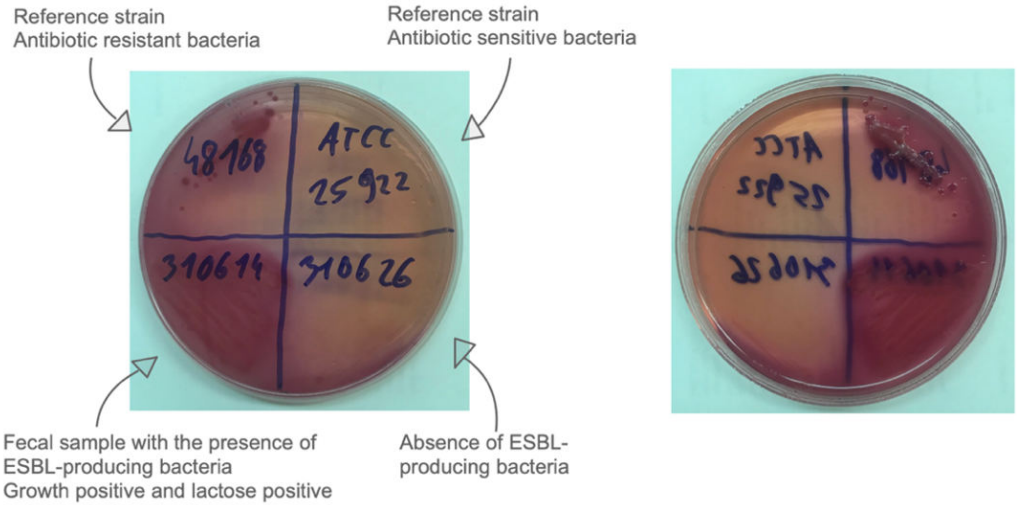

Figure 4. Antibiotic resistance test to detect the presence of ESBL-producing bacteria.

2. Fecal preparation and the FMTs procedure

Regardless the type of the sample (fresh, frozen at -80°C, frozen -80°C with saline and glycerol), the consistency of the slurry before the administration did not show any difference in homogenecity. 5-6mL/kg was administered for each patient. We did not encounter any technical issues with the chosen equipment (50 ml-syringe, 14-inch French Levin type nasogastric duodenal PCV-tube, paraffin). We skipped the sieving step, as our blending process was efficient. There was only once where the tube became partially obstructed at its tip, but we quickly resolved this by gently removing the particle with a needle. It took ten minutes on an average for one FMT.

3. Recipient dogs

a. Tolerance of FMT

The owners of the 5 dogs provided us with information about their dogs' reactions within 24 hours after the sedation-free FMT procedure. The first defecation occurred after 8 to 12 hours later. They tolerated well except for **Aston**, **Luna** and **Picúr**. **Aston** and **Luna** experienced soft feces and diarrhea after the administration of the frozen sample with glycerol. However, they had a normal fecal consistency when the frozen sample without glycerol was used. **Picúr** had multiple problems during and after the FMTs, even though his attitude improved for a few days after his third and fourth FMTs.

b. Clinical signs

Clinically all the patients improved permanently after FMT administered simultaneously with conventional treatment. Although **Picúr** with severe PLE improved after each FMT only for one week, his clinical signs did not improve and unfortunately, he is in a very bad clinical condition at the time of writing the thesis.

c. DI, BW, CCECAI, BCS, MCS, fecal score before after FMT

For the five recipient dogs, DI measurements were taken before FMT (*Figure 5*). However, we did not monitor the post-FMT dysbiosis index for **Merlin** and **Aston** as their indexes were normal before FMT. In addition, their body

weight (*Figure 6*), the CCECAI (*Figure 7*), the BCS (*Figure 8*), the MCS (*Figure 9*) and the fecal score (*Figure 10*) were taken before and after FMT.

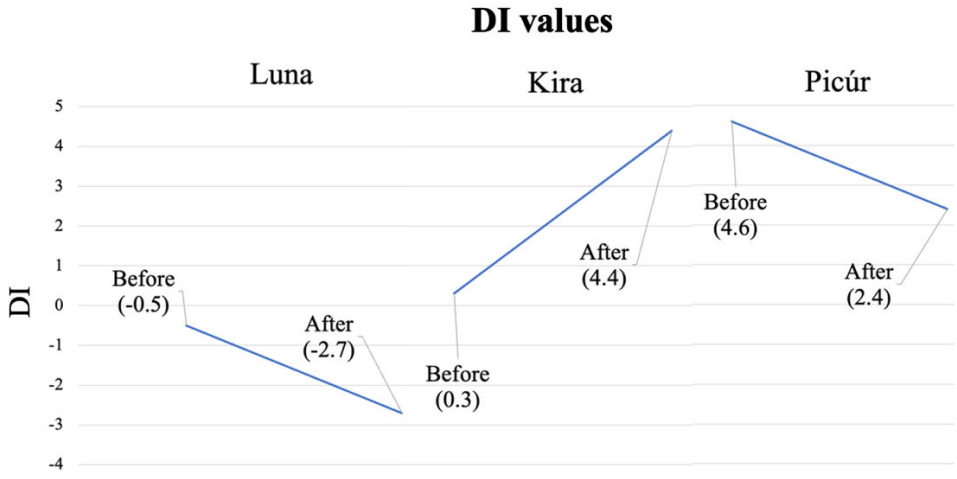

Figure 5. Dysbiosis index (DI) before/after FMTs of Luna Kira and Picúr.

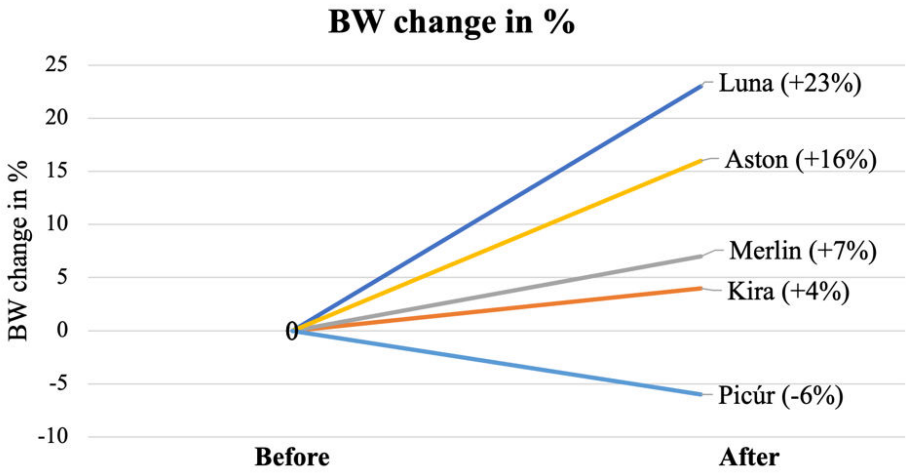

Figure 6. Body weight (BW) change in % before/after FMTs of the 5 dogs.

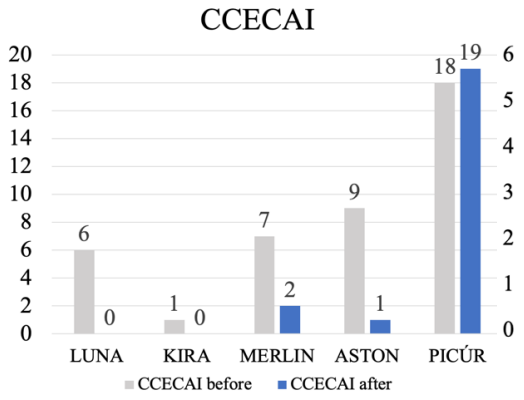

Figure 7. Canine chronic enteropathy activity index (CCECAI) before/after FMTs.
0-3 clinically insignificant, 4-5 mild,
6-8 moderate, 9-11 severe, >12 very severe

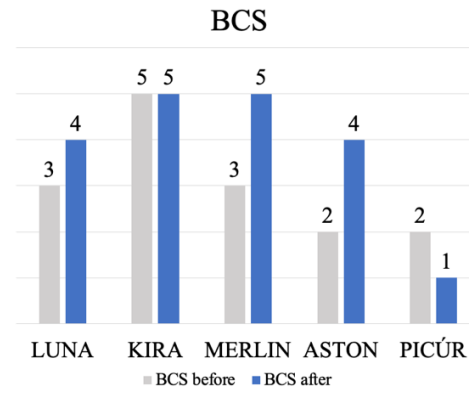

Figure 8. Body condition score (BCS) Before/after FMTs
1-3 too thin, 4-5 : ideal, 6-9 too heavy

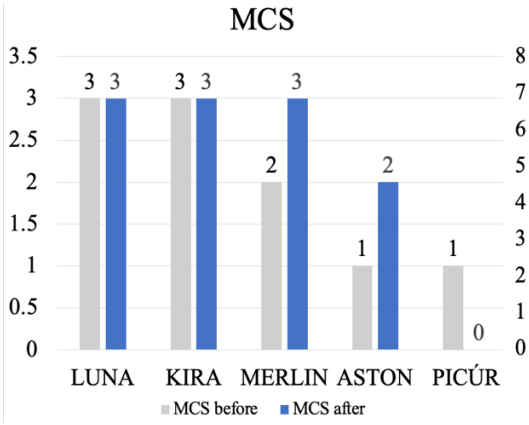

Figure 9. Muscle condition score (MCS) before/afterFMT
0: severe muscle loss, 1: moderate loss, 2: mild loss, 3: normal

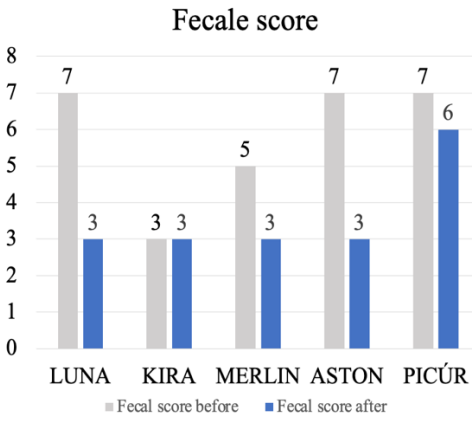

Figure 10. Fecale score before/after FMTs
1: very hard and dry, 7: watery

In 4/5 dogs, an increase in body weight is seen (23% for **Luna**, 4% for **Kira**, 7% for **Merlin**, 16% for **Aston**), whereas **Picúr** lost 6% of the initial weight. For the same 4/5 dogs, a decrease in CCECAI, an increase in BCS, and either no change or an increase in MCS are observed.

The ideal fecal score is 3-4 (normal consistency), 1 with severely constipated and 7 with severely diarrhea. Fecal score is improved in 4/5 dogs, and in one dog (**Kira**) it was normal (3) before FMT and did not change after FMT. **Luna** and **Picúr**'s DIs improved, along with positive changes in clinical signs for **Luna**, but not for **Picúr**, even though the quantities of the 7 taxa increased. **Kira**'s DI did not improve, but her clinical signs showed improvement, along with an increase in *C. hiranonis*. **Merlin** and **Aston's** DI were not recorded after their FMTs, but both exhibited improvements in their clinical signs.

d. Seven taxa groups before and after FMT

1. Luna's case

DI before and after: -0.5 and -2.7			
Unit Log DNA/g	**Before**	**After**	**Reference value**
C. hiranonis	5	5.5	5.1-7.1
Faecalibacterium	6.1	6.5	3.4-8.0
Turicibacter	5.5	7.5	4.6-8.1
Streptococcus	6.6	4.2	1.9-8.0
E. coli	3.7	6.7	0.9-8.0
Blautia	10.1	9.9	9.5-11.0
Fusobacterium	8.6	8.9	7.0-10.3

Table 6. DI and amount of the seven taxa of Luna measured at the Gastrointestinal Laboratory of Texas A&M University.

Prior to FMT, the amount of *C. hiranonis* was lower than the normal ranges as shown on *Table 6*. After the first FMT, Luna had already a normal consistency of stool. She seemed to play more and move more.

2. Kira's case

DI before and after: 0.3 and 4.4			
Unit Log DNA/g	**Before**	**After**	**Reference value**
C. hiranonis	0.2	0.1	5.1-7.1
Faecalibacterium	4.4	3.9	3.4-8.0
Turicibacter	5.2	5.5	4.6-8.1
Streptococcus	1.2	3.2	1.9-8.0
E. coli	6.3	7.8	0.9-8.0
Blautia	9.3	8	9.5-11.0
Fusobacterium	6.9	7.9	7.0-10.3

Table 7. DI and amount of the seven taxa of Kira measured at the Gastrointestinal Laboratory of Texas A&M University.

The amounts of *C. hiranonis* and *Blautia* mildly decreased after the FMT, while *Fusobacterium* increased within normal ranges as shown on *Table 7*. After the first FMT, Kira tolerated it well, so no diarrhea or vomiting, but after the second FMT, Kira vomited later at home.

3. Merlin's case

DI before and after: -3.6 and NA			
Unit Log DNA/g	**Before**	**After**	**Reference value**
C. hiranonis	5.8	-	5.1-7.1
Faecalibacterium	7.3	-	3.4-8.0
Turicibacter	5.6	-	4.6-8.1
Streptococcus	4.4	-	1.9-8.0
E. coli	4.5	-	0.9-8.0
Blautia	9.8	-	9.5-11.0
Fusobacterium	9.4	-	7.0-10.3

Table 8. DI and amount of the seven taxa of Merlin measured at the Gastrointestinal Laboratory of Texas A&M University.

Merlin's intestinal microbiota are in normal values even before FMT as shown on *Table 8*, although he has clinical signs of chronic enteropathy, such as diarrhea.

After the FMTs, Merlin has shown significant improvement in clinical signs and is currently in remission.

4. Aston's case

DI before and after: -3.2 and NA			
Unit Log DNA/g	**Before**	**After**	**Reference value**
C. hiranonis	6.7	-	5.1-7.1
Faecalibacterium	5.1	-	3.4-8.0
Turicibacter	4.6	-	4.6-8.1
Streptococcus	2.5	-	1.9-8.0
E. coli	7.8	-	0.9-8.0
Blautia	9.3	-	9.5-11.0
Fusobacterium	10	-	7.0-10.3

Table 9. Amount of the seven taxa of Aston measured at the Gastrointestinal Laboratory of Texas A&M University.

The decreased amount of *Blautia* is associated with intestinal dysbiosis, as shown on *Table 9*. Aston used to have diarrhea after every meal and presented severe emaciation before the FMTs. He put on weight and the frequency of diarrhea decreased after the FMTs, along with other treatments such as hypoallergenic diet, prednisolone and synbiotics. He tolerated the FMTs well.

5. Picúr's case

DI before and after: 4.6 and 2.4			
Unit Log DNA/g	**Before**	**After**	**Reference value**
C. hiranonis	0.3	5.5	5.1-7.1
Faecalibacterium	5.6	5	3.4-8.0
Turicibacter	4.4	5.2	4.6-8.1
Streptococcus	5.1	7.9	1.9-8.0
E. coli	7.2	5.7	0.9-8.0
Blautia	9.8	10.2	9.5-11.0
Fusobacterium	7.6	8.1	7.0-10.3

Table 10. DI, Amount of the seven taxa of Picúr measured at the Gastrointestinal Laboratory of Texas A&M University.

The amount of *C. hiranonis* increased eighteen times following four FMTs as shown on *Table 10*. However, Picúr's condition deteriorated. He continues to have severe diarrhea, vomiting and presents ascites due to a severe protein-losing enteropathy. He has pancreatitis and Cushing disease and diabetes mellitus as well.

Picúr experienced diarrhea after the first FMT and vomited during the procedure. Additionally, he had diarrhea again 15 minutes after the second FMT. To address these issues, a loperamide tablet was administered for the

third and fourth FMTs. After the third FMT, he managed to retain the sample for 11 hours without discomfort. However, during the fourth FMT, he displayed some discomfort and expelled a slurry. Subsequently, Buscopan Compositum A.U.V. (metamizole, butyl-scopolamine) was given, and the fourth FMT was performed 20 minutes later. This time, he tolerated it without discomfort and retained the sample for 8 hours.

V. DISCUSSION

1. Donor selection

Currently, there is no consensus on donor screening protocols for FMT in dogs (Toresson et al., 2023). Following the guidelines from Chaitman's study, it is recommended that donors are healthy, physically fit with normal BCS and MCS, with a CCECAI close to 0 (Chaitman et al., 2018). Additionally, a high amount of certain bacterial groups, such as *C. hiranonis*, important for the bile conversion and SCFA production, should be ensured (AlShawaqfeh MK et al., 2017). A history of raw food diet or recent antibiotic treatments in the last 6 months should be excluded, as well as those testing positive for enteropathogens, as *Giardia* (Toresson et al., 2023). Both of our donor dogs met the desired criteria.

It is important to note that in previous studies, the exclusion of ESBL-producing bacteria was not a primary concern for donor selection (Furmanski et al., 2017, Gal et al., 2020, Chaitman et al., 2020). However, in our study, excluding ESBL-producing bacteria was a critical precautionary measure to minimize this risk. Due to the global concern about the spread of antibiotic resistance, they pose significant risks in both human and veterinary medicine (Chong et al., 2011, DeFilipp et al., 2019, Tuniyazi et al., 2022). The transmission of ESBL-producing bacteria through FMT has been reported in cases involving human intestinal colorectal cancer (Fong et al., 2021) and *C. difficile* infection (Schwarz et al., 2013).

2. FMT procedure

The delivery method for FMT can vary between upper and lower GI tract administration. Lyophilized fecal samples in capsules can be taken orally (Carapeto et al., 2023), while lower GI delivery can be accomplished through colonoscopy or enema (Toresson et al., 2023). In our study, we chose to use enema as it was the available preparation method for us.

In human medicine, there is a belief that the effectiveness of frozen or fresh fecal samples is similar (Tang G et al., 2017). Therefore, we used fresh samples when feasible for same-day procedures and frozen samples for storage and later use.

The equipment we used are a sterile syringe and a sterile catheter with a diameter of 14 FG. The length of the catheter introduced corresponded to the distance from the last rib to the anus, as suggested in Toresson's study (2023).

FMT dosage and protocol can vary among small animal clinicians, ranging from 5 mL to 50 mL per kilogram of the recipient's body weight (Salavati et al., 2022). We chose to administer 5 mL per kilogram of body weight.

We skipped the sieving process that is suggested in Gal's study (2021), as we thoroughly blended the fecal samples. During our 13 FMT procedures, we only encountered one issue of stuck catheter, which was easily resolved.

Regarding the preparation of the recipient dogs, we followed the protocol outlined in Toresson's study (2023), including fasting for minimum 6 hours prior to the procedure and a 30-minute walk before FMT. After the procedure, we advised against eating and walking for several hours to ensure prolonged contact between the transplant and the recipient's intestinal mucosa. In our study, they retained for 8 to 11 hours.

In contrast, L. Toresson's study utilized acepromazine to induce relaxation in the animals, we did not need to sedate the animals, and they remained calm throughout the 10-minute procedure, except for Picúr, who required a bowel relaxation treatment, as he did not tolerate the FMT procedure.

We applied 2-4 FMTs with 10 to 20 days between each procedure.

In conclusion, our selection criteria for donors, choice of equipment, type of fecal sample, and procedural approach were effective, resulting in improvements in the overall health of 4/5 dogs with CE.

3. FMT failure

In our study, the long-lasting effect of FMT on 1/5 dog Picúr was unsuccessful. The treatment with FMT is considered as a failure when persistent GI signs are seen and no improvements are observed in the CCECAI, BCS, MCS and fecal score. Several factors may contribute to the failure of an FMT treatment, as listed below.

Donor screening and infections: while a donor is initially considered as suitable, a donor could become infected with a viral or bacterial disease by the time their stool is collected. Such infections could significantly influence the composition of the intestinal microbiota. Additionally, a donor might have an undiagnosed or latent disease that was not detected during the screening and stool testing process. These undetected issues could potentially be transmitted to the recipient (Fischer et al., 2018). In human medicine, similar situations have led to chronic diseases such as obesity, autoimmune disorders, and cardiovascular diseases (Fong W et al., 2020).

To provide greater precision and help ensure that the donors remain suitable throughout the study, it would have been beneficial to conduct periodic blood tests, fecal tests, and measurements of the DI and the abundance of the seven bacterial taxa groups in the donors' samples before each FMT. However, frequent DI measurements can be expensive. We also encountered an issue with one of our donor dogs, Balu, who tested positive for *Giardia* after spending time in a kennel. This underscores the importance of having multiple screened donor dogs available to ensure a continuous supply of suitable donors.

Storage conditions: a study indicates that lyophilized or frozen samples without glycerol have a lower quantity of *C. hiranonis* compared to fresh samples and frozen samples with glycerol. The highest bacterial abundance was observed in the samples with glycerol (Lopes et. al., 2023). In our study, the samples with or without glycerol did not make a difference in the clinical outcome and were effective regardless of the viability of the bacteria, but 2/5 dogs experienced diarrhea after FMTs containing glycerol, whereas this was not the case with FMTs without glycerol.

Recipient factors: in some cases, certain microorganisms present in the recipient may interfere with the effectiveness of FMT. For example, in

humans, *Candida albicans* has been associated with decreased FMT efficacy in treating *C. difficile* infection (Zuo et al., 2018). Additionally, severely infected human patients with *C. difficile* may be less likely to respond favorably to FMT (Fischer et al., 2018). In Picúr's case, his severe pre-existing PLE may have been a significant factor in the failure of his four FMTs.

4. Recipient dogs

a. Physical parameters and clinical signs

In previous studies on FMT in dogs, positive outcomes could be observed with dogs infected with parvovirus (Pereira et al., 2018), on obesity and metabolic syndrome (Zhang Z et al., 2019), in IBD (Niina et al., 2020), in acute diarrhea (Chaitman et al., 2017), chronic diarrhea (Chaitman et al., 2018, AlShawaqfeh et al., 2018) and in chronic enteropathy (Toresson et al., 2023). Although it is difficult to perform a statistical analysis due to the small number of cases with 2 donors and 5 recipient dogs, we could still see the positive effect of FMTs in all of the parameters on the majority of our dogs with CE, as expected. According to Toresson's study (2023), dogs with a higher DI, indicating a more severe shift in the microbiome, may be less likely to respond favourably to FMT. This was the case for Picúr with a DI of 4.6, who had severe PLE, his weight, BCS and MCS did not improve.

In addition to these physical parameters, we observed positive outcomes in the clinical signs, as measured by the CCECAI, as expected with dogs with CE (Innocente et al., 2022). An ideal value is close to 0. The CCECAI improved significantly in 4/5 dogs, meaning that they reached normality in various aspects, including attitude, appetite, stool consistency and frequency, absence of vomiting and weight loss, normal albumin levels, no ascites, no peripheral edema, and no pruritus, as per the clinical scoring index (Allenspach et al., 2007). Unfortunately, Picúr still has a high CCECAI, that midly increased after FMT from 18 to 19.

b. Dysbiosis index and the seven taxa groups

According to Chaitman's study (2018), FMT was shown to decrease the DI in dogs. However, our study yielded slightly different results. 3/5 DI were

measured pre- and post-FMT, as the other 2/5 dogs had a normal pre-FMT DI. 2/3 showed an improvement in DI, while 1/3 did not. Notably, a significant decrease in CCECAI was observed in 4/5 dogs after the FMTs, which aligns with the findings of Jergens et al. (2003), where various parameters were recorded including attitude, appetite, vomiting, stool consistency, frequency, and body weight.

It is important to note that we observed varying DI values with different outcomes. **Luna's case** is the most straightforward; her DI improved (-0.5 to -2.7), and her BW and BCS increased. CCECAI after the FMTs from moderate to clinically insignificant CCECAI-score (6 and 0, respectively). In her case, the FMTs had a positive effect across multiple parameters, indicating a successful outcome. **For Kira's case**, positive outcomes were observed in terms of BW and CCECAI, with an unchanged BCS and MCS. However, the DI surprisingly increased from grey zone (0,3) to abnormal (4,6) category. However, 3/7 bacterial taxa groups were not in the normal range before FMT, 1 of those 3 taxa groups reached normal values after the FMTs, showing some improvements but no change in *C. hiranonis* (0,2 to 0.1). **In the cases of Merlin and Aston**, BW, BCS, and MCS increased, and improvements in their CCECAI (moderate (7) for Merlin, severe for Aston (9) to both clinically insignificant (0)) were observed. **In Picúr's case**, he did not reach constant improvement, but he reached better clinical signs only for 5 days after the third and fourth FMT. He displayed intolerance, vomiting, and immediate defecation after the second and fourth FMT, and in his case, bowel-relaxing medication was necessary. His body weight, BCS, and MCS decreased, while CCECAI stayed as very severe, and his overall health deteriorated. Despite these unfavorable parameters, the final DI improved, and there was a significant increase in *C. hiranonis*.

In conclusion, our study suggests that the final DI values do not necessarily correlate with the physical parameters and clinical signs in the recipient dogs. When examining the 7 taxa, we observed improvements after the FMTs, although this was not consistently the case. The taxa that initially fell below the normal ranges tended to reach reference values after the FMTs. Specifically, a low *C. hiranonis* was noted in 3/5 dogs before FMTs, and this decrease has been linked to dysbiosis (AlShawaqfeh et al., 2017). After FMTs,

an increase was seen in Luna's case with 10% increase and a remarkable 18-fold increase in Picúr and a decrease in Kira.

Interestingly, we observed that the amount of *C. hiranonis* alone did not necessarily correlate with positive clinical outcomes, as evidenced by Picúr's case. Conversely, the decreased amount of this bacterium in Kira did not appear to influence the positive outcome of her clinical signs.

In contrast, *Faecalibacterium*, *Turicibacter*, *Blautia*, and *Fusobacterium* were decreased or in the normal range before FMTs, and they increased after FMTs in cases where DI measurements were available. These bacteria are crucial for producing SCFA.

In conclusion, our findings suggest that FMT can influence the composition, abundance, and diversity of the intestinal microbiota, highlighting its potential as a therapeutic intervention for dogs with chronic enteropathy.

c. Limitations and further researches

FMT is generally considered safe (Tuniyazi et al., 2022), but it is not without risks. In our study, we observed mild side effects in 2/5 dogs after the use of FMT containing glycerol, and 1/5 dogs with complications during the procedure. Another potential concern is the transfer of **ESBL-producing bacteria**, which we focused on their absence in our donor screening process. However, our knowledge is limited about the all of the possible pathogens in the sample.

One significant limitation of our clinical study is the use of conventional treatments along with FMT, as our responsibility as veterinarians is to cure the animals. Thus, it becomes challenging to attribute the observed outcomes solely to FMT, as these concurrent treatments may have interacted and influenced the results. It is essential to recognize FMT as a complementary therapy alongside conventional treatments, as it may not exhibit lasting effectiveness without addressing the underlying causes.

In summary, our work provides compelling evidence of the positive effects of FMT in dogs with CE. Given the relatively small sample size and the need for standardized protocols, further research is warranted to refine our understanding of FMT's optimal application in veterinary medicine. Encouragingly, a consortium of international experts known as the

Companion Animal Fecal Bank Consortium is actively working on developing guidelines, which are expected to provide valuable insights into the use of FMT in companion animals and enhance its clinical utility.

VI. ABSTRACT in English and in Hungarian

Dysbiosis refers to an imbalance in the intestinal microbiota composition that can be linked to gastrointestinal dysfunctions. Therapeutic approaches of dysbiosis aim to modulate and support the intestinal microbiome. One of the most promising therapeutic interventions is FMT. FMT involves transferring fecal sample from donor dog into the gastrointestinal tract of a diseased recipient using methods such as oral capsules, endoscopy or rectal enema, with the goal of restoring intestinal barrier integrity.

As a new method, FMT currently lacks standardization in veterinary sciences, and requires further research. The aim of our study was to provide better knowledge on FMT-procedure, as well as introduce FMT to the small animal practice and establish a fecal donor program in Hungary. We intended to further develop the protocol for donor selection through screening to confirm their absence of ESBL (extended-spectrum beta-lactamase) producing bacteria, which is routinely not included in the donor selection procedures. Our study also focused on assessing the effects of FMT in five dogs with CE. During FMT-procedure fresh or frozen fecal samples were prepared and rectally administered to five dogs with chronic enteropathies at intervals of two to four weeks, in total two or three occasions. Two donor dogs were selected for good health based on negative blood tests, fecal tests, favourable fecal microbiota composition analysed with DI, and absence of ESBL-producing bacteria in their feces. The recipient dogs were specifically chosen due to their prior unresponsiveness to conventional therapies. Many of them had unfavourable DIs as well.

The results demonstrated that our fecal preparation techniques provided samples with good condition. FMT was well-tolerated and led to improved fecal quality and the overall condition of patients with chronic enteropathies. Our donor selection procedure keeping strict criteria was appropriate to

prevent transmission of enteropathogens via fecal enema. Selection protocol should focus on exclusion of ESBL-producing E. coli strains that are regularly carrying resistance to the most common antibiotics.
In conclusion, our FMT-study including the investigation of the technique and also its effect has shown promising outcomes. The favourable response of our patients with CE highlights the potential of this technique as a significant part of the complex treatment for this condition. However, the study's limitation lies in its small recipient pool, emphasizing the need for further research involving a larger number of patients diagnosed with chronic enteropathies to thoroughly evaluate FMT's effectiveness.

A dysbiosis a bél mikrobiotikus összetételének kedvezőtlen változása, ami összefüggésbe hozható gyomor-bélrendszeri diszfunkciókkal. A dysbiosis kezelésének a célja a bél mikrobiom helyreállítása és működésének támogatása. Az egyik legígéretesebb gyógykezelési eljárás a FMT. Az FMT során donor kutyából származó bélsármintát juttatunk be a beteg recipiens bélcsatornájába. Ez történhet orális kapszulák beadásával, továbbá endoszkópia vagy végbélen keresztüli beöntés során, azzal a céllal, hogy visszaállítsuk a bélbarrier integritását.
Az FMT az állatorvostudományban egy új eljárás, ami jelenleg még nincs standardizálva, ezért a kisállatokban való alkalmazása további kutatást igényel. Tanulmányunk célja az FMT-vel kapcsolatos ismeretek bővítése, valamint a bélsárdonor program kidolgozása és bevezetése Magyarországon. Célul tűztük ki a donorprogram továbbfejlesztését ESBL (kiterjesztett spektrumú béta-laktamáz) termelő *E.coli* törzsek kizárására irányuló vizsgálattal, ami jelenleg általában nem része a donorok bélsárvizsgálatának. Tanulmányoztuk továbbá az FMT hatásait öt krónikus enteropátiás kutyán.
Az FMT során frissen ürített vagy fagyasztott bélsármintát dolgoztunk fel és adtunk be rektálisan öt, krónikus enteropátiás kutyának két-három alkalommal, két-három hetes időközönként. Két egészséges donor kutyánk volt. A kiválasztás kritériumai voltak a negatív vér- és bélsárvizsgálatok, a bélsár kedvező mikrobióta összetétele – amit dysbiosis indexszel (DI) vizsgáltunk –, valamint ESBL-termelő E. coli mentessége. A recipiens kutyák

közé olyan betegek tartoztak, akik a konvencionális terápiákra nem reagáltak megfelelően. Többük kedvezőtlen dysbiosis indexszel is rendelkezett.

Az eredmények azt mutatják, hogy bélsár előkészítési módszereinkkel jó minőségű mintákat tudtunk előállítani. Az FMT jól tolerálható volt, és javította a krónikus enteropátiás betegek bélsárminőségét és általános állapotát. A donor-jelöltek szigorú feltételeket követő szűrése alkalmas volt arra, hogy megelőzzük az enteropatogének bélsárral történő átadását a FMT során. A vizsgálat fontos része a leggyakrabban használt antibiotikumokra többnyire rezisztenciát mutató ESBL-termelő baktériumok szűrése is.

Összefoglalva, az FMT-módszert kidolgozó és hatásait vizsgáló kutatásunknak ígéretesek az eredményei. A krónikus bélbetegeink kedvezően reagáltak az FMT-re, ezért elmondhatjuk, hogy az FMT lényeges eleme lehet a betegség komplex kezelésének a jövőben. Fontos azonban megjegyezni, hogy a tanulmány lényegi limitációja a kis elemszám. További kutatásokra van tehát szükség nagyobb számú krónikus enteropátiás beteg bevonásával, annak érdekében, hogy pontosabb képet kapjunk az FMT hatékonyságáról.

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