VETERINARIA

Faecal microbiota transplantation in 16 dogs with idiopathic inflammatory bowel disease

Faecal microbiota transplantation (FMT) is an experimental, non-pharmacological medical treatment, which is being used to restore phylogenetic diversity in the microbiota. In human medicine it has been shown to be an effective treatment for recurrent *Clostridium difficile* infections. Therefore, in veterinary medicine FMT has recently been tested as a treatment for multiple gastrointestinal diseases, unresponsive to common medical therapies, such as idiopathic inflammatory bowel disease (IBD), chronic enteropathy, chronic colitis and gastrointestinal infections caused by *Clostridium perfringes*. The aim of our study was to perform FMT in dogs with idiopathic IBD unresponsive to common therapies and evaluate clinical response, using the CCECAI classification index, at follow-ups after 1 month and 3 months. Sixteen dogs with idiopathic IBD were included in this study. Nine animals underwent transplantation with fresh emulsion via an endoscopic procedure and five of these animals were also given the transplant orally. Seven dogs were only administered the oral transplantation using frozen capsules. The data showed clinical improvement in most of the patients after transplantation, whether performed orally or endoscopically. The lack of a complete assessment of the microbiota by pyrosequencing and the absence of a control group represent the two main limitations of this work. Nevertheless, FMT appears to be a concrete and promising possibility for the palliative treatment of idiopathic IBD unresponsive to common therapies, worthy of further study and investigation.

Enrico Bottero[1],
Med Vet

Elena Benvenuti[2]*,
Med Vet

Pietro Ruggiero[2],
Med Vet

INTRODUCTION

The fundamental role that the intestinal microbiota plays in the regulation of metabolism, in the functioning of the immune system and in maintaining intestinal homeostasis has been well-established in human medicine[1,2,3]. Recognition that the gastrointestinal microbiota is intimately involved in the wellbeing of the host has led to the idea of manipulating intestinal microorganisms to improve health. Faecal microbiota transplant (FMT) or faecal bacterial therapy, is a non-pharmacological medical treatment being investigated for this purpose. In this procedure faecal material, containing the microbiota from the distal intestine, is transferred from a healthy donor to an ill patient[1,2,4]. One of the main objectives of a faecal transplant is to restore the phylogenetic diversity of the microbiota, since an alteration in this is a causal or contributory factor for intestinal disease. In human medicine the use of FMT for the treatment of recurrent *Clostridium difficile* infections is consolidated and has a clin-

> FMT is now a consolidated practice in human medicine, used to restore the phylogenetic diversity of the intestinal microbiota.

[1] Poliambulatorio Veterinario Argentina, via Levà 58, Taggia (IM)
[2] Centro Veterinario Specialistico, via Sandro Giovannini 51, Roma
* Corresponding Author (info@endovet.it)

ical efficacy of 90%[2,5,6]. There are also numerous studies on the effectiveness of FMT in the treatment of dysbiotic conditions such as idiopathic inflammatory bowel disease (IBD) and metabolic syndrome[1,7,8,9], diabetes[2,7,9] and autism[2,8,9]. In veterinary medicine FMT has recently been used to treat a range of gastrointestinal disorders not responsive to common medical therapies such as idiopathic IBD, chronic enteropathies, chronic colitis and *Clostridium perfringes* gastrointestinal infections[9,10,11,12,13]. These were mostly case reports, drawn from conference abstracts and not from scientific articles, in which small groups of patients were transplanted using enemas in the descending colon without the help of endoscopy. To date there have been no randomised studies regarding the use of FMT in a large number of patients. The aim of our study was to assess the response and clinical effectiveness of FMT in idiopathic IBD not responsive to common medical treatment in 16 dogs.

MATERIALS AND METHODS

The study population consisted of 16 dogs with idiopathic IBD unresponsive to common therapies. Nine subjects were first transplanted endoscopically and then orally, while the remaining seven dogs received only transplantation *per os*.

> **The candidates for FMT were dogs that were at least 2 years old which had idiopathic IBD unresponsive to usual treatments and which had undergone endoscopic and histological investigations for at least 1 year.**

The selection of recipients

To be eligible for transplantation the recipient dog had to be over 2 years old, have had chronic gastrointestinal symptoms and have been subjected to endoscopic and histological examination of the gastrointestinal tract for at least 1 year. The following inclusion criteria were assessed in each patient that entered the study:

Past medical history: classified according to the Canine Chronic Enteropathy Clinical Activity Index (CCECAI)[15], evaluated at the time of the endoscopic examination.

Laboratory tests: blood count, biochemical profile, urinalysis and specific gastrointestinal tract investigations: trypsin-like immunoreactivity, folate and cobalamin. The analyses had to have been performed within a month prior to the transplant; infectious enteropathies, endocrine disorders and neoplastic diseases were excluded.

Stool analysis: the presence of a parasitic intestinal disorder was excluded by examination of faecal samples taken on 3 successive days or by treating the animal with fenbendazole 50 mg/kg SID for 5 days in the 15 days prior to transplantation (Panacur® tablets 250 mg, MSD Animal Health Srl, Milan, Italy).

Dietary history: the patient had to have had an adequate dietary trial (a single-protein or hydrolysed home-made or commercial diet) for at least 3 weeks and the clinical response had to be known; patients with diet-responsive enteropathy were excluded.

Drug history: the patient had to have undergone an antibiotic trial with metronidazole at a dose of 10 mg/kg BID (Stomorgyl® tablets, Merial Italia Spa, Padua, Italy) and/or tylosin tartrate at a dose of 15 mg/kg BID (Tylan® soluble, Elanco, Florence, Italy) for at least 3 weeks and the clinical response had to be known. Patients with antibiotic-responsive enteropathy were excluded. For the patients suffering from idiopathic IBD that entered the study, information was required on the medical immunosuppressive therapy administered (active principle, dosage, route of administration and duration of therapy) and the clinical response to the therapy.

The subjects that had followed this clinical process and had not responded to treatment were considered to have unresponsive idiopathic IBD and represented the population studied. The owners were informed and gave consent to the use of the planned palliative therapy.

Recipients dogs were divided into four categories based on weight:

A: dogs weighing < 10 kg
B: dogs weighing between 10 and 20 kg
C: dogs weighing between 20 and 40 kg
D: dogs weighing > 40 kg

The selection of donors

A donor dog had to be at least 6 months old, be in a good general condition, having a normal Body Condition Score and a normal faecal score. The past and recent history of each donor dog was determined. The clinical history was taken to ensure that there had not been more than five episodes a year of various signs, including nausea, loss of appetite, vomiting, diarrhoea, weight loss, abdominal colic and constipation. In the owner's subjective assessment these episodes had to be considered unusual and should not have occurred in the preceding 6 months. Dogs with chronic metabolic, endocrine and oncological diseases (metabolic syndrome, diabetes, Cushing, etc.), autoimmune diseases (atopy, anaemia, lupus, etc.) and/or allergies were ex-

> **Donor dogs were selected after a thorough clinical examination, careful evaluation of the animal's present and past clinical history and detailed haematological and faecal investigations.**

VETERINARIA

cluded. Donors could not have any kin relationship with the recipient and must not have had any kind of medical therapy, antibiotic or dietary supplement in the preceding 3 months. In addition they had to have normal basic laboratory investigations (blood count, biochemical profile, electrophoresis, urinalysis) and gastrointestinal function (trypsin-like immunoreactivity, folate, cobalamin, pancreas-specific lipase). All donor dogs were dewormed with fenbendazole 50 mg/kg SID for 5 days (Panacur® tablets 250 mg, MSD Animal Health Srl, Milan, Italy) at least once in the last 3 months and all had negative results of investigations for parasites in the stool (performed on stool of 3 consecutive days within 15 days of making the transplantation) and of enzyme-linked immunoassay for *Giardia* spp. The donor underwent stool culture tests in the month prior to transplantation and had to result negative for *Salmonella, Campylobacter* spp., *Clostridium difficile* and *Clostridium perfringens*. Donors had to be fed with the same diet for at least 3 months and individuals given raw food in their diet were excluded.

Donor dogs were divided into four categories based on their weight (A: dogs weighing < 10 kg, B: dogs between 10 and 20 kg, C: dogs between 20 and 40 kg, D: dogs weighing > 40 kg). Each dog was used as a donor only for recipient dogs belonging to the same weight category. Other characteristics were identified as being preferred, but not determinant, for choosing donors. Ideally, the donor should come from the same geographic region as the recipient, should be of the same breed as the recipient, should be given the same type of food as that given to the recipient, should have reliable and trustworthy owners and should live in an area convenient for delivery and eventual storage of faecal material by the veterinarian.

Preparation of the material for transplantation
The donor's faeces were collected within 30 minutes of defecation, placed in a hermetic container and refrigerated at 3 °C if the external temperature was greater than 25 °C. The faecal emulsion for endoscopic transplantation had to be prepared within 6 hours of collection of the faeces and transplanted within 12 hours. The following amounts of faeces were used for the different weight categories:
categories A and B: 60 to 80 g of fresh faeces;
categories C and D: 100-150 g of fresh faeces.
For each patient the faeces were mixed with 75-100 ml (categories A and B) or 150-200 ml (categories C and D) of saline (sodium chloride 0.9%®, Eurospital, Trieste, Italy) and 50-75 ml of enrichment solution (low-fat yoghurt)[10]. After blending all the constituents of the mixture at minimum velocity (Innoliving - Innofit, 250W), the emulsion was filtered through a sieve (Fig-

Figure 1 - Preparation of the faecal emulsion. After having blended the emulsion of faecal material, it is filtered through a sieve with small pores.

ure 1) yielding a solution of variable volume from 100 to 250 ml, corresponding to about 10 ml/kg of faecal solution per patient.

Figure 2 - Preparation of the transplant for oral administration. After having blended and filtered the faecal emulsion, the solution is introduced, via syringe, into a plastic bag to form ice-cubes.

Preparation of the faecal emulsion to be frozen for oral transplantation

For recipients in categories A and B, 100 g of faeces were emulsified, within 6 hours of collection, with 200 ml of warm saline, while for those in categories C and D, 100 g of faeces were emulsified with 100 ml of physiological saline; the two components were mixed manually using a wooden spoon. The material was then blended at

> The solution for the transplant is produced from fresh faeces that are emulsified and then filtered; frozen capsules are used for oral transplantation.

low speed using an electric blender (Innoliving -Innofit, 250 W) for 2-3 minutes. The contents of the blender were passed through a narrow-pore strainer and collected in a container. Using a syringe with a wide nozzle, the emulsified material, of semi-liquid consistency, was aspirated from the container and introduced into a plastic bag for making ice cubes (Figure 2). Each portion contained approximately 1.5 g of faecal material for subjects in categories A and B and about 3 g of faeces for dogs in categories C and D.

Transplantation via the endoscopic route

Prior to the transplantation the patient was fasted for 24 hours with regards to food and for 6 hours with regards to fluids.

An endoscopic examination was performed with the patient under inhaled general anaesthesia, in left lateral decubitus; after a macroscopic evaluation of the mucosa, lavage was performed with warm physiological saline, first in the duodenum and then, in suitably prepared patients, also in the ileum and colon. Depending on the

> Transplantation performed by the endoscopic route enables the solution containing the faecal microbiota to be instilled directly into the duodenum and/or ileum and colon of the recipient.

weight of the recipient animal, from 40 to 100 ml of saline were used for the duodenum, from 30 to 60 ml for the ileum and from 60 ml to 120 ml for the colon[10,11]. The lavage was performed, over 10-15 minutes, using an infusion bag under pressure (1 l bag, pressure infusor; Alcyon, Marene, CN, Italy) connected via tubing to the working channel of the endoscope. After the colon had been washed, the transplant was delivered to the duodenum. The faecal emulsion contained in a 60 ml syringe was introduced into the working channel of the endoscope and the material was distributed homogeneously, starting from the most distal part of the duodenum that could be reached (Figure 3). When possible, following the duodenal transplant, material was also transplanted in the ileum and colon (Figure 4).

At the end of the transplantation the patient was maintained under anaesthesia for 15 minutes with gradually decreasing minimum inhalatory anaesthetic concentrations (MAC for isoflurane at 0.8 to 0.6) as stimuli were no longer present at this point.

After recovering consciousness, the patient was kept under medical supervision in hospital for 2-4 hours before being returned to its owner. The owner was asked to assess the general state and behaviour of the patient in the 48 hours following the transplantation. This initial follow-up assessment was categorized into three performance classes:

1: Subject in good condition (normal behaviour and attitude). Defecation similar to that before the transplant or improvement in the consistency of the faeces. No vomiting, loss of appetite, colic or other gastrointestinal symptoms.

Figure 3 - Patient (case n. 2) undergoing a faecal transplant by the endoscopic route. Introduction of the faecal solution through the working channel of the endoscope.

2: Subject not in a good condition. Patient worse than before the transplant (excluding the recovery from anaesthesia): looser consistence of faeces, gastrointestinal or other symptoms not previously present.

3: Subject in a serious condition. Patient dejected, anorexic, with severe diarrhoea and/or dehydration requiring admission to hospital and intensive care.

Between 48 and 72 hours after the transplant, a full blood count and basic blood-biochemistry tests were performed in all recipients.

Seven days after the transplantation (when possible) the stool culture was repeated to exclude the presence of bacterial infections. According to the owners' willingness, after 1 week, five of the nine patients that underwent transplantation endoscopically also received an oral transplant through the administration of frozen faecal material from the same donor (Figure 5). This material was given on alternate days in the first month and then every 72 hours in the second and third months.

In the seven subjects undergoing transplantation solely by the oral route, the material was administered every 48 hours in the first month and then every 72 hours in the second and third months. These subjects also had clinical follow-up and laboratory analyses 48-72 hours after beginning the treatment and, when possible, stool culture 7 days after the first dose to exclude the presence of bacterial infections. Attempts were made not to modify the patients' ongoing therapy or diet but in those subjects for which it was desirable to make changes, these are described individually. All the dogs were followed up, with evaluation of the CCECAI after 1 and 3 months (Tables 1 and 2).

RESULTS

Sixteen dogs were recruited into this study: nine were transplanted endoscopically and seven were trans-
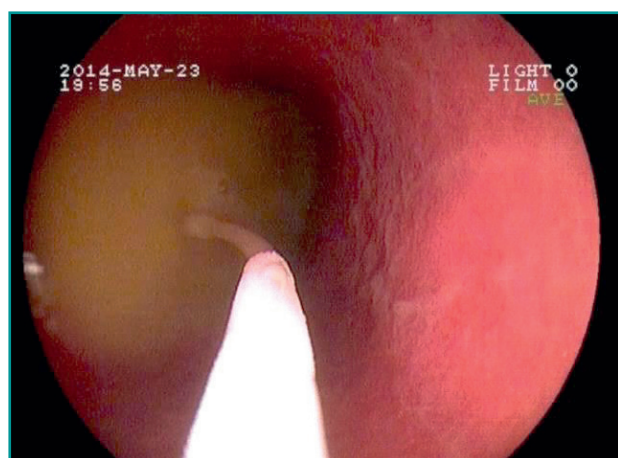

Figure 4 - Endoscopic image of the faecal transplant. After lavage with physiological saline instilled through the working channel of the endoscope, the faecal emulsion is delivered to the ileum.

Figure 5 - Oral transplantation. The dog's owner can administer the frozen capsules directly to the patient or give them mixed with food.

planted orally. The patients that underwent endoscopic transplantation were four German shepherd dogs, two medium-sized mongrels, one Cane Corso, one Cocker and one Argentine Mastiff. Six of the dogs were male, of which one was castrated, and three were females, all

> The CCECAI clinical score can be used to characterise the gastrointestinal signs present and monitor the patient's clinical course in the mid-term and long-term.

spayed. Their mean age was 5.4 years (range, 2.5 - 11 years). All patients (100%) had diarrhoea, while 90% had weight loss, 33.3% loss of appetite and 22% had vomiting. The clinical and histological diagnosis was idiopathic IBD; microscopic histological examination revealed that the intestinal inflammatory infiltrate was lymphoplasmocytic in seven dogs, eosinophilic in one and lymphoplasmocytic and neutrophilic in the remaining dog. Sixty-six percent of the dogs had hypoalbuminaemia, which was mild in three cases (2.1 g/dl; normal reference range: 2.3-3.9 g/dl). None of the patients enrolled in the study was suspected of having concomitant diseases such as pancreatitis. At the time of transplantation, 55% of dogs were being fed with a hydrolysed diet, 33% with a home-made diet and 12% with a commercial single-protein diet. As far as concerns the animals' drug history, all patients had been treated with prednisolone (Vet-Solone® tablets, Bayer, Milan, Italy), 55.5% had also been treated with cyclosporine (Atoplus® capsules, Novartis Animal Health, Origgio, VA, Italy) and 12% with budenoside (Rafton® capsules, Falk Pharma GmbH, Germany); metronidazole therapy had been used in 88% of the patients, and all patients had also received tylosin.

At the time of the transplant all subjects were receiving pharmacological treatment with immunosuppressants, antibiotics (metronidazole or tylosin) or both.

The average score of the CCECAI evaluated before transplantation was 11.4, which corresponds to severe disease.

The donors used for the endoscopic faecal transplants were mongrels (2 cases), a Golden retriever (1 case), Sardinian shepherd dogs (4 cases), Cocker (1 case), and an Irish setter (1 case). All recipients were administered the faecal solution by endoscopy into the duodenum; in 44% of patients this was the only site of administration, in another 44% of patients the material was transplanted in the duodenum, ileum and colon and in 12% in the duodenum and colon. The proportion of faecal solution was divided between the various districts as described above.

In the first follow-up after transplantation one patient was found to have diarrhoea.

The analyses carried out after 48-72 hours were unchanged; stool culture, performed for 80% of the dogs, did not show the presence of pathogens.

The average clinical scores (CCECAI) found after transplantation were 7.4 (at 1 month) and 7.8 (at 3 months) (Table 1).

> **The aim of FMT is to introduce a new microbial population which can complete or enrich the native microbiota that is altered or destroyed.**

Seven dogs were given their transplants only by mouth, through the administration of frozen capsules (Figure 5): one German shepherd dog, one Maremma sheepdog, one Pinscher, one Dachshund, one English bulldog, one Belgian shepherd dog and one Bolognese. The average age of patients included was 6 years (range, 3-11 years). There were six males, of which one castrated, and one spayed bitch.

All the patients had diarrhoea, 42% had loss of appetite, 42% had vomiting and 28% had weight loss; the clinical and histological diagnosis was idiopathic IBD. The microscopic inflammatory infiltrate found on histological examination was lymphoplasmocytic in four dogs and

Table 1 (first part) - ENDOSCOPIC TRANSPLANTATION The table presents data on all the patients that received an endoscopic transplant								
Breed	**Sex**	**Age (years)**	**Main GI symptoms**	**Diagnosis**	**Hypoalbuminaemia**	**Treatments given**	**Diet**	**CCECAI**
German shepherd dog	m	3	Unformed faeces, failure to gain weight deficit	Severe eosinophilic IBD	No	Hydrolysed diet, metronidazole, tylosin, prednisolone, azathioprine, cyclosporine	Hydrolysed	12
German shepherd dog	fs	2.5	Loose stools, weight loss	Moderate IBD with lymphangectasia	Yes, mild	Hydrolysed diet, metronidazole, tylosin, prednisolone, azathioprine, cyclosporine	Commercial single protein	13
Cane corso	m	6	Unformed faeces, weight loss, loss of appetite, pruritus	Severe lymphoplasmocytic and neutrophilic IBD	No	Home-made diet, metronidazole, tylosin, prednisolone, azathioprine, cyclosporine	Home-made single protein	10
German shepherd dog	fs	6	Recurrent diarrhoea, weight loss, loss of appetite	IBD	Yes	Metronidazole, tylosin, prednisolone, ranitidine	Hydrolysed	11
German shepherd dog	mc	11	Diarrhoea, weight loss	IBD	Yes	Metronidazole, tylosin, prednisolone, azathioprine, cyclosporine, ranitidine	Home-made single protein	14
Mongrel, medium-sized	m	5	Abdominal colic, vomiting and recurrent diarrhoea	IBD	Yes, mild	Metronidazole, budesonide, tylosin	Hydrolysed	9
Mongrel, medium-sized	fs	11	Diarrhoea, weight loss, nausea	IBD	No	Tylosin, prednisolone, omeprazole, probiotics	Home-made single protein	12
Cocker	m	1,5	Diarrhoea, weight loss, colic	IBD	Yes	Metronidazole, tylosin, prednisolone, cyclosporine, ranitidine	Hydrolysed, highly digestible, low fat	12
Argentine mastiff	m	3	Diarrhoea, failure to gain weight, vomiting	IBD	Yes, mild	Metronidazole, tylosin, prednisolone, ranitidine	Hydrolysed	10

lymphoplasmocytic and eosinophilic in the other three patients; no patients had hypoalbuminaemia. None of the patients enrolled in the study appeared to have any concomitant diseases, such as pancreatitis. At the time of transplantation, 42% of the dogs were on a hydrolysed diet, 42% on a single-protein diet and 16% of cases were being given a highly digestible diet. All patients had been treated with metronidazole in association with prednisolone. Before transplantation, the average CCE-CAI score was 8.57. The donors used for the oral transplants were Golden retrievers (3 cases), Sardinian shepherd dogs (2 cases) and Cockers (2 cases).

At the first follow-up, 48 hours after the transplant, none of the patients had had side effects or changes in blood-biochemistry. One month after transplantation pharmacological therapy was suspended in four patients, whereas antibiotic treatment was continued in two cases. One patient (case number 3, Table 2, first part) was given steroids after 2 weeks and the faecal transplant by mouth was suspended. This patient was excluded from the clinical evaluation of the CCECAI at 1 and 3 months.

DISCUSSION

Faecal microbiota transplantation, also known as faecal bacteriotherapy, or faecal infusion, is an experimental, non-pharmacological medical procedure, used as compassionate care for patients with chronic enteropathy unresponsive to common drug therapies. This procedure was used for the first time in human medicine for the treatment of pseudomembranous colitis in 1958 in four patients[16], while in 1983 it was used for the treatment of *Clostridium difficile*[11]. Interest in this procedure has increased: in human medicine it is now an established practice for the treatment of *Clostridium difficile* and in veterinary medicine its efficacy is being investigated in gastrointestinal disorders refractory to common medical therapies[13]. Unlike probiotics, which are given mainly to help to restore the metabolic or immunological activity of the subject's native microbiota, the goal of a faecal transplant is to introduce a complete, new community of microorganisms that balance or even replace the altered or destroyed native microbiota[18]. The mechanism underlying this change and the accompanying resolution of clinical signs is not, however, entirely clear.

The primary aim of our study was a clinical evaluation of faecal transplants in idiopathic IBD unresponsive to common therapies in dogs. We studied 16 patients with IBD refractory to medical therapy; seven cases were given only oral transplantation, while nine were transplanted endoscopically.

Table 1 (second part) - ENDOSCOPIC TRANSPLANTATION							
Donor	Site of transplant	First post-transplant follow-up 48-72 hours	Oral transplant	Post-transplant treatment	CCECAI at 1 month	CCECAI at 3 months	Therapeutic changes at 1 month post-transplant
Mongrel, m, 4 years, 20 kg	Duodenum-ileum-colon	1	No	Hydrolysed diet, tylosin	7	12	No
Mongrel, m, 4 years, 20 kg	Duodenum-ileum-colon	1	No	Home-made diet, tylosin	5	8	No
Golden retriever, fs, 3 years	Duodenum-ileum-colon	1	No	Home-made diet, tylosin	4	8	Changed to hydrolysed diet
Sardinian shepherd dog, m, 6 years	Duodenum-colon	1	Yes	Home-made diet, tylosin, prednisolone	7	6	Steroids at minimum effective dose and tylosin on alternate days
Sardinian shepherd dog, m, 6 years	Duodenum	1	Yes	Prednisolone, cyclosporine, tylosin	8	6	Gradual decrease of steroids and cyclosporine
Irish setter	Duodenum	1	Yes	Tylosin, prednisolone, probiotics	6	5	Prednisone on alternate days
Sardinian shepherd dog, m, 6 years	Duodenum	1	No	Tylosin, metronidazole prednisolone	12	10	Addition of azathioprine and probiotics
Cocker, f, 6 months	Duodenum	2	Yes	Tylosin, omeprazole prednisolone	10	10	Cyclosporine
Sardinian shepherd dog, m, 6 years	Duodenum-ileum-colon	1	Yes	Tylosin and tapered prednisolone	8	6	No

The diagnosis in patients included in this study was made by endoscopy and histopathology over at least 1 year; the choice of a clinical history of such a long period, although excluding patients that did not respond to common therapies in less time, ensured that the individuals included had undergone an appropriate therapeutic process. In addition, the unsatisfactory clinical course of these patients facilitated and stimulated the owners' acceptance of the transplant procedure. Unfortunately it was not possible to enrol patients that had undergone the same treatment protocol, given the significant variability in the use of medications and supplements during the IBD; this is one of the limitations of our study. Although the CCECAI is a clinical score for all chronic enteropathies, the authors preferred its use to that of the Canine Inflammatory Bowel Disease Activity Index (CIBDAI)[14] created by Jergens in 2003 to classify IBD. In the authors' opinion, the CCECAI is more complete and suitable for characterising clinical signs and for both mid-term and long-term follow-up.

All the parameters evaluated showed an overall improvement in the clinical status of the patients transplanted, whether endoscopically or orally.

In the group of transplanted endoscopically, at the 3-month follow-up, only one patient (case 1, Table 1, first part) had an unsatisfactory condition with a CCECAI score comparable to the initial one, while in another case (case 7, Table 1, first part), a clinical improvement was seen only 40 days after the transplant. This fact could

be explained as a random finding, a physiological fluctuating trend of chronic inflammatory diseases, or as indicating only a partial response to the transplant given endoscopically. In support of this hypothesis it should be noted that neither subject was given oral transplantation after the endoscopically administered transplant. Another hypothesis is that more severe cases of idiopathic IBD benefit less from FMT. Indeed, in human medicine, in some studies[19,20], the response to transplantation in patients with idiopathic IBD was better in those with moderate disease than in those with severe disease. In one series of five cases[19], only one patient, whose initial condition was less severe than that of the others, showed a good response to the transplant for at least 3 months. It is interesting to note that, also in the group of dogs given only oral transplants, two subjects (case 1 and case 4, Table 2, first part) that started with higher CCECAI scores had different clinical responses at 3 months: excellent in the first case, moderate in the second; This further demonstrates the complexity of both the therapeutic management of idiopathic IBD and that of understanding FMT.

One variable that needs to be investigated in more depth is the site of delivery of the transplant. In our series the transplant was performed only in the duodenum in some dogs while in others material was also transplanted in the colon and ileum, in all cases using the same quantity of transplanted faeces; this choice was primarily influenced by operational difficulties in

Table 2 (first part) - ORAL TRANSPLANTATION								
The table presents data on the patients that underwent exclusively oral transplantation								
Breed	**Sex**	**Age (years)**	**Main GI symptoms**	**Diagnosis**	**Hypoalbuminaemia**	**Treatments given**	**Diet**	**CCECAI**
Maremma sheepdog	fs	7	Unformed faeces, weight loss, loss of appetite, vomiting	Severe lypmphoplasmocytic and eosinophilic IBD	No	Single-protein diet, metronidazole, tylosin, prednisolone, methylprednisolone, azathioprine, cyclosporine	Commercial single-protein	11
Bolognese	m	4	Loss of appetite, vomiting, unformed faeces, pruritus	Moderate/ severe IBD	No	Hydrolysed and single-protein diet, metronidazole, tylosin, prednisolone, azathioprine	Commercial single-protein	8
German shepherd dog	m	7	Loss of appetite, unformed faeces, weight loss	IBD	No	Highly digestible diet, metronidazole, tylosin, amoxicillin + clav. acid, prednisolone	Commercial highly digestible	8
Pinscher	mc	6	Abdominal colics, diarrhoea	IBD	No	Prednisolone, metronidazole, cyclosporine	Hydrolysed, home-made	12
Dachshund	m	3	diarrhoea	IBD	No	Tylosin, metronidazole, prednisolone	Commercial single-protein	8
English bulldog	m	4	Diarrhoea and borborygmi	IBD	No	Tylosin, metronidazole, prednisolone	Hydrolysed	8
Belgian shepherd dog	m	11	Diarrhoea, vomiting	Eosinophilic IBD	No	Tylosin, metronidazole, buscopan	Hydrolysed	5

reaching the ileum or inadequate preparation of the colon. In the opinion of the authors the ideal procedure would be to perform the transplant in all accessible districts, but because of the limited number of cases of this study this view cannot be supported by statistically significant data.

Some studies in human medicine[20] have highlighted the importance of the quality and composition of the donor's microbiota and how variable the composition of the microbiota is between subjects after the transplant. The work cited earlier showed a dynamic variation of the flora with the presence of peaks of some families, suggesting that the colonisation by the donor's microbiota is a gradual process. These considerations confirm the complexity of understanding the different variables in the course of FMT and raise the hypothesis that repeated transplants may be more effective than a single transplant performed once.

At present there is no work in veterinary medicine that has investigated these variables systematically and especially faecal transplantation from several donors to the same recipient. In our study, no patient was subjected to transplantation with different donors, since all the dogs underwent only one endoscopic transplant and, when they received subsequent continued oral treatment, the stool always came from the same donor. The choice of not changing donor was made to reduce the number of variables to be included in the evaluation of results; however, the possibility of changing donor should certainly be investigated further in subjects that show an inadequate or incomplete clinical response. In addition, we chose to use donors with a body weight

similar to that of the recipients, although there are no descriptions in the literature of an association between body weight and composition of the microbiota, because we considered that this would have reduced any still unidentified variables.

> **Endoscopic transplantation seems to have a higher probability of success if associated with oral transplantation.**

An interesting point is the different clinical response between subjects that, after endoscopic transplantation, also received oral transplants and those in which these integrations were not given.

Of the five dogs that underwent oral transplantation after endoscopic transplantation, four (80% of patients) showed satisfactory results and only one had an unsatisfactory outcome. These data suggest that oral administration plays a positive and decisive role in maintaining a good clinical outcome. Also in human medicine[1,12,19] it has been hypothesised that, in cases of more severe IBD, continuous transplantation is more effective than a single transplant. These considerations arise both from clinical assessment and from evaluation of the microbiota in transplanted patients by various techniques, primarily pyrosequencing. The main limitation of our study was the inability to evaluate changes in the microbiota by specific, advanced analytical methods. The only control parameter in our study was of a clinical nature and stool examinations, performed before and after transplantation, were not aimed at iden-

Donor	First post-transplant follow-up 48-72 hours	Post-transplant treatment	Stool culture after 7 days	CCECAI at 1 month	CCECAI at 3 months	Therapeutic variations at 1 month post-transplant
			Table 2 (second part) - ORAL TRANSPLANTATION			
Golden retriever, fs, 3 years	1	Single-protein diet, tylosin, metronidazole	0	2	3	Metronidazole suspended, diet and tylosin continued
Golden retriever, fs, 3 years	1	Single-protein diet, tylosin		4	6	No
Golden retriever, fs, 3 years	1	Highly digestible diet, prednisolone	0	Not evaluated	Not evaluated	Steroid treatment started after 2 weeks and oral FMT suspended
Cocker, f, 6 months	1	Tapered budesonide	0	11	8	Various dietary changes
Cocker, f, 6 months	1	Tylosin, metronidazole		6	5	No
Sardinian shepherd dog m, 6 years	1	Tylosin, prednisolone	0	7	5	Steroids suspended
Sardinian shepherd dog, m, 6 years	1	All withdrawn	0	7	6	All withdrawn

tifying the microbiota but rather at the need to exclude the presence and onset of bacterial infections that could be dangerous for the patient and/or potentially for humans. In humans there are specific analytical parameters that are related to clinical response; for example, it has been demonstrated that the relative abundance of Enterobacteriaceae and Lachnospiraceae in the faeces of patients with ulcerative colitis is associated with a worsening of clinical signs and hence reduced transplant efficacy[19].

Again in humans, with recurrent *Clostridium difficile* infection treated with faecal transplantation, the recipient microbiota, evaluated by pyrosequencing, was very similar to that of the donor 45 days after transplantation[20]; the mechanism underlying this change, which also accompanied the resolution of clinical signs, remains unclear.

> **Molecular tests, such as pyrosequencing, should be performed in order to gain a more precise estimate of the number of micro-organisms transplanted and their taxonomic composition.**

It is not known what really happens in patients in whom an improvement does not occur or lasts only a short time. The available data show that the microbiota in subjects who do not improve is different from that of the donor. It is probable that patients with more severe disease have greater difficulty in maintaining a stable microbiota and this is also presumably the case in our series. In humans this difficulty is manifested mainly in patients with ulcerative colitis or Crohn's disease, whereas the clinical improvement following treatment of *Clostridium difficile* persists for a long and sometimes forever[20,21]. Monitoring patients by faecal pyrosequencing, performed at regular intervals (2-4 weeks), would help to understand the effectiveness of transplantation and to prevent recurrence of the disease, but was not done in our study because of the high costs involved[10,11]. Furthermore, the nonrandomised subdivision of patients into two therapeutic groups (endoscopic transplantation vs oral transplantation) may have biased the comparative study between the two methods.

In our study, subjects with clinically less severe disease were included in the group of patients that underwent only oral transplantation. The choice of including patients with less severe symptoms in this group was aimed at understanding whether transplantation could be an option even in patients with an acceptable response to therapy, with the goal of reducing or even suspending pharmacological treatment. In six patients steroid therapy was discontinued after 1 month, while dietary therapy and/or antibiotic treatment was continued. Only

in one case was it necessary, due to worsening clinical signs, to suspend the transplant and administer steroid therapy after 2 weeks. Given that pyrosequencing was not performed, it was not possible to determine whether this clinical deterioration was related to the transplant or the physiological variability in the course of idiopathic IBD.

In four patients, all treatment was suspended after 1 month, except continued oral transplants administered on alternate days. In the authors' opinion, oral transplantation may be a good therapeutic option to limit the use of antimicrobial therapies. In addition, psychological rejection of oral intake of faecal material is less in the dog than in human beings and hence this procedure could be continued for long periods.

There are some methodological considerations to be made regarding oral transplantation. First, it is not yet known whether and, if so, how the microbiota changes outside the donor's body after being exposed to oxygen[24]. Although there are no standardised collection and storage procedures, the technique should be designed to minimise manipulation of the material and the transplantation should be carried out as soon as possible after collecting the material.

In human medicine, Ianiro G. (2014) described that maintaining anaerobiosis would be indicated in order to allow transmission of anaerobic strains of bacteria and that the method of inoculating the microbiota (capsules, endoscopy, enema) may also affect the survival of the bacteria. In a study by Marks S. (2014), faecal matter was collected and frozen within 8 hours, subsequently thawed and endoscopically administered to patients with recurrent *Clostridium difficile* infections with success rates similar to those in patients treated with fresh faecal material. In this regard, in our study the fresh faecal material, for both oral and endoscopic transplantation, was prepared within 6 hours of collection. In a study of humans with clostridium infections[22], the authors used human microbiota that was filtered and mixed with a cryoprotective substance and then frozen and stored at -80 °C until use. This process eliminates the odour of faeces and reduces the volume. This material was as effective as fresh faecal material in the treatment of clostridium infections. The authors also performed sequencing studies, demonstrating the stability of the implanted microbiota, with the composition of the recipient's microbiota altering in the direction of that of the donor's. Transplantation of fresh faecal material is not easy to perform because it requires close collaboration and coordination between the animal's owner and the veterinarian. The possibility of using frozen faecal material also for the endoscopic transplantation should be further investigated in veterinary medicine, too.

There are various ways of performing FMT in humans: by duodenoscopy, ileo-colonoscopy, enema or orally[1,23,25]. Although methods in veterinary medicine have not yet been standardised, in the authors' opinion, a combination of different transplant modes could be the right choice. In fact, in our study, the patients that first underwent endoscopic and then oral transplantation were those with the best clinical responses. To date there is no evidence in veterinary literature that one technique is more effective than another. Even in human medicine, there is no statistical difference in the therapeutic results of treatment administered endoscopically in the duodenum or delivered by enema to the descending colon.

According to some studies[1,24] it is more important to have an accurate estimate of the number of micro-organisms transplanted in each endoscopic procedure rather than the exact taxonomic composition of the bacterial phyla. This consideration should also be applied to the quantity contained in each oral capsule to determine whether there is a minimum effective or "therapeutic" quantity. Unlike the situation in recurrent *Clostridium difficile* infections, in which the native microbiota is severely damaged by multiple antibiotic treatments and the proliferation of enterotoxigenic clostridium is facilitated by the destruction of the commensal microbial flora, in IBD this destruction does not occur. However, patients with chronic inflammatory disease are frequently subjected to antibiotic therapy and dysbiosis is a major cause and/or consequence of the ongoing pathological process[1,2,10]. This consideration is the rationale for using faecal transplantation in IBD, particularly in patients receiving antibiotic therapy. In some human studies, patients were given antibiotic treatment prior to transplantation[17]. We did not use this strategy systematically, but at the time of transplantation about 90% of our patients were receiving antibiotic therapy with one or more of the following active substances: metronidazole/spiramycin and/or tylosin tartrate.

This potential aspect of the procedure also needs further investigation and standardisation. In addition, most of the studies[2,5,17,19] on faecal transplantation in human medicine recommend stopping antibiotic treatment after transplantation. In our study antibiotic therapy was only suspended in four out of 16 patients, all of which belonged to the group undergoing oral transplantation and therefore all patients considered not to have severe disease. Whether or not to discontinue antibiotic treatment after faecal transplantation deserves further consideration and not having applied a uniform strategy in this regard is a limitation of our study. In fact, since we did not perform faecal pyrosequencing, it was not possible to determine whether and how the transplanted microbiota was modified by the antibiotic therapy, and it cannot be excluded that the clinical improvement was related to the antibiotic therapy rather than the transplantation. However, this latter hypothesis is unlikely as all patients had previously been subjected to the same antibiotic therapy without response.

While in recurrent *Clostridium difficile* infections it is possible to measure clostridial toxins as markers of therapeutic success, in our study, clinical evaluation was the only follow-up parameter. This and the absence of a placebo-controlled control group are the main limitations of our study. Unfortunately, we were unable to identify an adequate number of owners who were prepared to subject their animals to potentially placebo endoscopic transplantation. To remediate, in part, this defect, we chose to include only subjects with a preceding history of disease for at least 1 year, in order to have exhaustive details on response to treatment prior to transplantation for comparison with the course after transplantation (for up to 3 months). This work is, in fact, a preliminary phase of a broader study: the long-term post-transplant follow-up evaluation is ongoing of subjects in which oral transplants were continued and in those in which the transplants were suspended.

In conclusion, in veterinary medicine, faecal transplantation in idiopathic IBD not responsive to common therapies is a palliative procedure requiring further investigation and greater standardisation. Based on the preliminary results obtained, FMT appears promising and worthy of other studies aimed at identifying the categories of patients that could benefit most from this therapeutic strategy and at improving the procedures for performing it.

KEY POINTS

- Faecal microbiota transplantation (FMT) or faecal bacteriotherapy is an experimental, non-pharmacological medical procedure aimed at restoring the phylogenetic diversity of the microbiota.

- In human medicine, transplantation of the faecal microbiota is an established practice for the treatment of *Clostridium difficile* and its efficacy in gastrointestinal diseases refractory to common medical therapies is being investigated.

- It has been demonstrated that alterations in the number and composition of the microbiota (dysbiosis) occur during the course of chronic intestinal disease; this condition is often worsened by antimicrobial and/or immunomodulatory therapies.

- The qualitative and quantitative characterisation of the faecal microbiota of the recipients and donors can be performed through molecular techniques, such as pyrosequencing.

- At present, there are only anecdotal reports on the use of faecal microbiota transplantation in veterinary medicine for the treatment of chronic enteropathy unresponsive to common medical therapies.

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