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A Protocol for Fecal Microbiota Transplantation Using Freeze-Dried Capsules: Dosage and Outcomes in 171 Dogs with Chronic Enteropathy

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Abstract: Background: In veterinary medicine, fecal microbiota transplantation (FMT) shows promise for treating chronic enteropathy (CE) in dogs, but standardized protocols for dosage, preparation, and administration are lacking. This study aimed to evaluate the efficacy of freeze-dried FMT capsules (cFMT) and to investigate the existence of a possible optimal dosage for dogs with CE. Methods: A multicenter prospective study was conducted on 171 dogs with CE, treated with freeze-dried FMT capsules (100 mg for dogs $\leq$ 10 kg, 200 mg for dogs > 10 kg). The dosage of freeze-dried FMT material was expressed in different ways, to investigate the effect of putative active principles. Clinical outcomes were assessed by classifying dogs as responders (R) or non-responders (NR) based on veterinary evaluations from a questionnaire, along with changes in the CIBDAI score and variations in 15 clinical signs of chronic enteropathy (CE). Data were collected before and 15 days after treatment. Results: Of the 111 dogs included in the final analysis, 82% showed a positive clinical response, with no significant differences in clinical response between capsule sizes or dosage, irrespective of how it was expressed. Conclusion: Effective dosage range for cFMT administration in dogs affected by CE was defined. The oral administration of 100 mg of freeze-dried cFMT daily for a month was shown to be sufficient to achieve an 80% response rate. Further studies are needed to explore additional factors that may influence the overall effectiveness of cFMT in treating CE.

Keywords: cFMT; dog; freeze-dried capsules; lyophilization; dosage; chronic enteropathy

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1. Introduction

Fecal microbiota transplantation (FMT) is a therapeutic approach in which fecal material from a healthy donor is transplanted into the gastrointestinal tract of a patient to restore microbial balance. While the exact mechanisms of FMT remain incompletely understood, it has been demonstrated to address intestinal dysbiosis by enhancing the abundance and diversity of beneficial bacteria, thereby restoring the functional integrity of the gut microbiota [1].

In human medicine, FMT has been established as an effective treatment for recurrent *Clostridioides difficile* infection (rCDI) [2] and shows potential for a wide range of other

applications in different clinical settings, including infectious disease, gastroenterology, endocrinology, oncology, and neurology [3–8].

While research on FMT in veterinary medicine is still in its early stages, the preliminary results are promising. In companion animals, FMT is primarily used as an adjunctive therapy for chronic enteropathy (CE) [9–11]. Studies have shown that FMT can significantly reduce Canine Inflammatory Bowel Disease (IBD) Activity Index (CIBDAI) and Canine Chronic Enteropathy Clinical Activity Index (CCECAI) in large groups of dogs with enteropathy, suggesting its potential as an effective treatment option [12,13].

A major challenge for FMT in veterinary medicine is the lack of standardized protocols for stool preparation, donor selection, and administration. Ongoing research is focused on determining optimal procedures and dosages. In 2024, the Companion Animal FMT Consortium, an international group of veterinary gastroenterology experts, published the 'Clinical Guidelines for Fecal Microbiota Transplantation in Companion Animals'. These guidelines highlight the necessity for additional research to standardize FMT procedures [14].

In this study, we aimed to address some of the existing knowledge gaps by evaluating the most effective dosage for oral, freeze-dried FMT in capsule form (cFMT) for dogs with chronic enteropathy. Furthermore, we analyzed the possible minimum effective dose of cFMT required to improve CE clinical signs.

Therefore we have defined FMT dosage in several ways by taking into account: (a) the total weight of freeze-dried material administered daily, including bulking agents; (b) the weight of freeze-dried material excluding bulking agents, to investigate the efficacy of components of exclusively fecal origin, considering viable cells, viruses, cell components, and secondary metabolites as a whole; (c) the number of viable bacterial cells in the cFMT preparation, since microorganisms interact with host immunological pathways and are engaged in mechanisms such as direct competition with pathogens, production of antimicrobials, restoration of both secondary bile acids and short-chain fatty acid metabolism, and repair of the gut barrier [1,15,16]; (d) the number of total bacterial cells (viable + dead), since the components of dead cells can also exert an anti-inflammatory response in the gastrointestinal tract [17].

2. Materials and Methods

2.1. Study Design

This is a multicenter prospective cohort study conducted across several first opinion veterinary clinics or referral centers located in Italy, between January 2021 and September 2024. The study was conducted on privately owned dogs within the "Pet FMT Project" (https://www.eubiome.it/en/progetto-pet-fmt, last accessed 31 October 2024). The owners were informed of the purposes of the study and signed an informed consent form. The study received the official approval of the Animal Welfare Committee of the University of Padova (OPBA Prot. n. 369551, on 31 July 2020).

All patients received freeze-dried FMT treatment in oral capsule form. The fecal material used for the transplant was obtained from four healthy donors.

2.2. Study Populations

The study population consisted of 171 dogs diagnosed with chronic enteropathy (CE) that did not respond satisfactorily to an elimination diet, probiotics, and/or immunosuppressive treatment. All dogs included in the study were required to be examined by the referring veterinarians both before and 15 days after the FMT procedure. Additionally, each referring veterinarian, in collaboration with the animal owner, had to complete an online questionnaire at the time of the FMT request and again 15 days after the procedure. The online FMT request could not be completed unless the questionnaire was fully filled out.

The questionnaire collected the following information: animal weight and body condition score (BCS) [18,19]; primary clinical signs observed; agitation status; hematology and clinical chemistry results; parasitology, including Giardia positivity and treatment history since puppyhood; Vitamin B12 and folate levels (if available); dietary trials conducted; current diet at the time of the FMT request; use of probiotics and immunosuppressive drugs, including the duration of treatment; and any history of antibiotic use. Inclusion criteria for dogs affected by CE required a minimum of three weeks of intermittent or persistent clinical signs, such as vomiting, diarrhea, anorexia, or weight loss, and a minimum work-up by the referring veterinarian to exclude extra-digestive diseases. Antibiotic and probiotic treatments, if any, were suspended two and five days prior to FMT, respectively. Exclusion criteria included initiating immunosuppressive treatment less than 4 weeks prior to FMT, starting a new immunosuppressive treatment or increasing the dose of maintenance therapy concurrently with FMT, failure to complete the 30-day FMT regimen, changes in diet during the treatment, or incomplete medical records.

2.3. Donors Selection

The donor dogs were deemed healthy based on physical and clinical examinations. They ranged in age from 1 to 6 years, had a body condition score within the acceptable range (4–6/9), and had to satisfy multiple criteria, including no travel history outside the local area; no chronic diseases, allergies, or immune-mediated conditions; and no history of vomiting, diarrhea, or antibiotic exposure within the last 6 months. Their fecal consistency was normal, and they showed no behavioral abnormalities. The Bristol fecal chart was used for fecal scoring. Although it has not been validated in dogs, it has been in humans and appears to perform adequately in dogs [20].

In addition, the donor dogs underwent the following tests: complete blood count, serum biochemical analysis, intestinal function tests (serum cobalamin and folate concentrations), pancreatic enzymatic immunoassays (pancreatic lipase immunoreactivity, trypsin-like immunoreactivity), and an endocrine test (serum cortisol levels). Fecal floatation tests were negative for parasitic eggs, and ELISA fecal tests (Fecal Dx®, IDEXX) showed no evidence of parasites (Giardia, Cryptosporidium, Ancylostoma, Ascarididae, Trichuridae). Real-time PCR analysis (Canine Diarrhea PCR Profile Plus Panel, IDEXX) confirmed the absence of pathogenic infectious agents or toxins (Canine Enteric Coronaviruses, Canine Parvovirus-2, *Canine distemper*, *Canine circovirus*, *Clostridium perfringens netE/netF* genes, *C. difficile toxin A* and *toxin B*, *Campilobacter coli*, *Campilobacter jejuni*). Further testing with cultural methods (Diarrhea Profile C Panel, IDEXX) revealed no pathogenic microbes (*Salmonella* spp., thermophilic *Campylobacters*, hemolytic *Escherichia coli*, coagulase-positive species from genus *Staphylococcus* spp., *Klebsiella* spp., *Proteus* spp., *Yersinia* spp.).

The donors underwent a full rescreening annually. Additionally, tests for parasites, enteropathogens, and toxins were conducted every 6 weeks using the Fecal Dx®, Canine Diarrhea PCR Profile Plus Panel, and Diarrhea Profile C Panel (IDEXX). We collected donor feces from 4 donors: a 4-year-old male Cardigan Welsh corgi dog, a 6-year-old male dachshund dog, a 4-year-old female mixed breed dog, and a 5-year-old female Bernese mountain dog. Fecal samples were collected by the donor's owner into sterile containers and frozen at −20 °C within an hour. The frozen samples were transported weekly to the laboratory to be immediately processed. The delivery box contained 10 kg of dry ice to keep the fecal samples frozen. Upon arrival, the laboratory staff conducted a visual inspection to verify whether the samples were still covered by dry ice and to check for any signs of thawing.

2.4. FMT Preparation

Freeze-dried fecal microbiota were prepared following a protocol previously described, with few modifications [13]. Briefly, fecal material was homogenized with saline solution (1:2, e.g., 50 g of stool in 100 g of elution medium) in a blender bag to remove particles larger than 270 microns. The filtrate was amended with 5% trehalose and frozen within an hour in shelves, with a thickness of ten millimeters. The freeze-drying was performed for 72 h, with a condenser temperature of −50 °C and a pressure of 0.05 mbar. Each batch of capsules was prepared from a single-donor freeze-dried material, collected for 4–6 weeks, using size 1 (200 mg) or size 4 (100 mg) delayed-release capsules (DRcaps, Capsugel). The delayed-release coating helps live microorganisms survive the harsh acidic environment of the stomach and reach the intestines. Capsules were manually filled and stored at −80 °C until needed. Total cell count and viable cell count were performed by flow cytometry for each batch of capsules (BS ISO 19344:2015) [21].

Once removed from the freezer, a 1 g silica gel canister was added to the container and the capsules were delivered to the patient's home at room temperature.

2.5. FMT Administration Protocol

The patient's owner administered one capsule daily to the dog for one month.

For dogs weighing > 10 kg, the dosage was 200 mg daily (monthly amount 6 g, corresponding approximately to 24 g of fresh feces), whereas for dogs weighing ≤ 10 kg, the dosage was 100 mg daily (monthly amount 3 g, corresponding approximately to 12 g of fresh feces).

The quantity of starting fecal material was adopted from the European Consensus Conference on Fecal Microbiota Transplantation in Clinical Practice [22], where a minimum amount of 30 g of feces is required for the preparation of a single FMT treatment in humans, since the yield of freeze-drying process is 25%.

2.6. Variables and Data Sources

To investigate the existence of a possible link between FMT dosage and clinical response, we acquired data regarding (Figure 1):

- The clinical response, as assessed through a global clinical evaluation conducted 15 days post-treatment, which classified subjects as either "responders" (R) or "non-responders" (NR) based on their improvement. Subjects exhibiting positive clinical outcomes following fecal microbiota transplantation (FMT) were labeled as responders (R), while those showing no significant improvement were classified as non-responders (NR). This classification was made by veterinarians based on their professional judgment during a clinical examination. The evaluation process involved completing a questionnaire that included the assessment of animal weight, body condition score (BCS), the CIBDAI score, and 15 clinical signs associated with chronic enteropathy (CE);
- The clinical response, calculated as the variation of the mean value of 15 clinical signs of CE (see Table 1 for the complete list): for each indicator, a score ranging from 1 to 5 indicated the rising severity of the related clinical sign in the active phase of the disease;
- The clinical response, calculated as the variation of the CIBDAI score: a score ranging from 0 to 3 indicated the rising severity of the related clinical sign for each indicator included in the CIBDAI, both before and after FMT, and the total composite CIBDAI score was then obtained as the sum of the single scores;
- The type of capsule (size 4 or size 1, renamed from now on as "S" or "L") received daily by each subject;

- The dose of freeze-dried FMT material administered to each subject daily, expressed as mg/kg body weight (BW); this dose includes fecal material, trehalose, and sodium chloride (see "FMT Preparation and Administration");
- The dose of freeze-dried fecal material administered to each subject daily, expressed as mg/kg BW, i.e., the quantity of freeze-dried FMT material, discarding trehalose and sodium chloride contribution;
- The quantification of alive bacterial cells administered daily with the FMT treatment, quantified as Active Fluorescent Unit (AFU)/kg BW by flow cytometry analysis;
- The quantification of total bacterial cells administered daily with the FMT treatment, quantified as Total Fluorescent Unit (TFU)/kg BW by flow cytometry analysis.

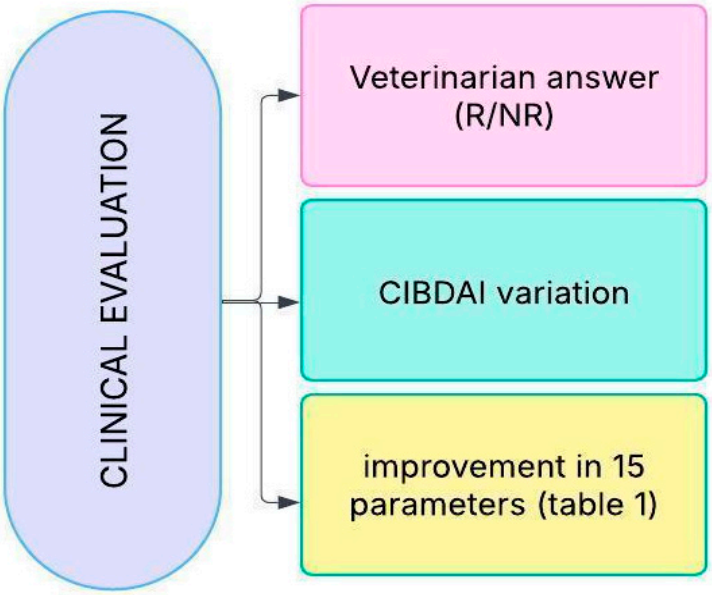

Figure 1. The three methods used to evaluate the clinical response. Arrows reflect different aspects of treatment effect: (1) **Veterinarian answer** (Responder/Non-Responder, R/NR), based on professional judgment during clinical examination; (2) **CIBDAI variation**, calculated from the difference in total CIBDAI scores pre- and post-treatment; and (3) **Improvement in 15 clinical parameters**, calculated as the variation in the mean severity scores of 15 clinical signs associated with chronic enteropathy.

Table 1. The 15 clinical signs of CE and the evaluation score range.

Clinical Signs	Not at All (1)	Little (2)	Moderate (3)	High (4)	Very High (5)	I Don't Know
Diarrhea						
Mucus in stool						
Blood in stool						
Vomiting						
Reflux						
Nausea						
Pica						
Wight loss						
Loss of vitality						
Lack of appetite						
Restlessness						
Itching						
Abdominal pain						
Flatulence						
Borborigmi						

All the data about the listed variables were collected by means of a digital survey filled by the veterinarian before the start of the FMT and 15 days after the end of the treatment.

2.7. Statistical Methods

The possible association between dose and response was assessed using all the three different characterizations of the response, i.e., R/NR labels, variation of CE clinical signs and CIBDAI variation, and the four definitions of dose (see Section 2.6). As a preliminary analysis, an investigation was conducted on the consistency of the R/NR labeling with clinical signs, specifically examining the changes in the 15 CE clinical signs and the variation in CIBDAI before and after FMT. To achieve this, a t-test was employed to assess the presence of statistically significant differences between subjects labeled as NR and those labeled as R, with regard to the post–pre FMT variation of the two indicators, particularly verifying that the NR class corresponded to a small improvement that was statistically inferior to that of the subjects labeled as R.

For R/NR response, a Chi-squared test and a Fisher's test were used to check for differences in response between subjects having received the "S" or "L" capsules. For the remaining three dose descriptions, a Kruskal–Wallis test between values in the two groups was performed.

When considering the variation of the mean of CE clinical signs, the correlation between the dose and the difference of the mean of scores after and before FMT was computed, and a regression model was built for each dose definition. The same analyses were performed for the association between dose and CIBDAI variation.

3. Results

3.1. Animals Included in the Study

A total of 171 dogs were initially selected for inclusion in this study. However, following data review, 111 dogs were ultimately included in the final analysis (Figure 2). The exclusion of 60 dogs was due to incomplete or missing data: 55 cases had missing or incomplete post-treatment veterinary assessment data, and 5 cases lacked pre-treatment weight data. Only dogs with complete and valid data for both pre- and post-treatment assessments were included in the final analysis (Figure 2). The median age was 6.5 years. The most common breed was mixed breed dogs (17%) followed by German shepherd (14.4%), chihuahua (5.4%), French bulldog (4.5%), and golden retriever (3.6%). In total, 57 dogs were male and 43 were female. The most common clinical sign was diarrhea (92.5%), followed by various signs of abdominal discomfort (borborygmi, flatulence), weight loss, and nausea. The CIBDAI median prior to treatment was 5 (range 0–16), which decreased to 2 (range 0–15) after FMT. The detailed characterization of the selected population is included in Table 2.

Table 2. Characterization of the selected population at baseline.

Age (years)	Mean	7.41
	Median	6.59
	SD	4.02
	Min	1.49
	Max	17.07

Table 2. *Cont.*

Duration of disease (weeks)	Mean	110.5
	Median	77.64
	SD	95.76
	Min	5.29
	Max	541.57
CIBDAI	Mean	5.41
	Median	5
	SD	3.33
	Min	0
	Max	16
Sex (%)	F	43
	M	57
Top 5 breeds (%)	Mixed breed dogs	17.12
	German Shepherd	14.41
	Chihuahua	5.41
	French Bulldog	4.5
	Golden Retriever	3.6

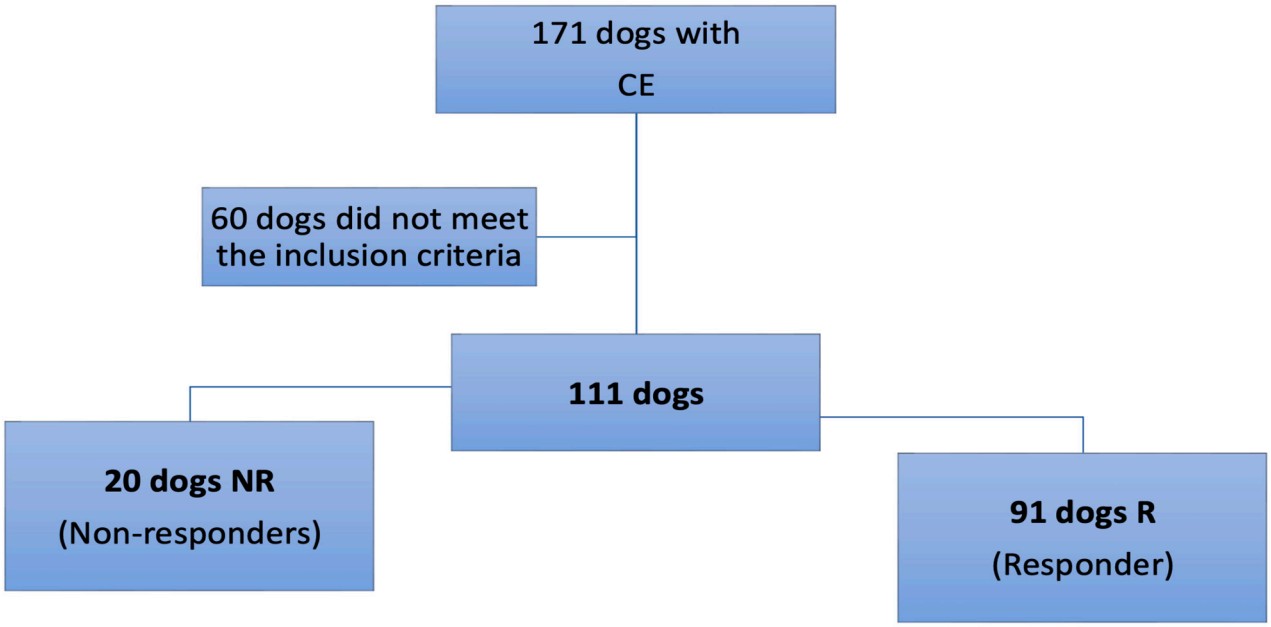

Figure 2. Animals included in the study.

As shown in Table 3, the prevalence of each of the 15 clinical signs decreased after FMT (for the full data about pre- and post-FMT CIBDAI values and clinical signs scores for each included dog see Supplementary Table S1).

Table 3. Prevalence of each clinical sign in the population of included dogs, pre- and post-FMT.

Clinical Sign	Prevalence PRE	Prevalence POST
Diarrhea	92.5%	58.8%
Borborygmi	75.0%	47.0%
Flatulence	68.0%	48.0%
Mucus in stool	67.0%	41.2%
Weight loss	64.8%	19.6%
Nausea	60.6%	33.3%
Abdominal pain	58.8%	25.7%
Vomiting	58.5%	21.6%
Loss of vitality	57.5%	20.4%
Restlessness	56.2%	23.3%
Loss of appetite	51.9%	26.2%
Reflux	42.9%	22.2%
Blood in stool	41.9%	11.8%
Itching	40.6%	30.4%
Pica	40.4%	24.5%

3.2. Coherence of R/NR Labeling with Clinical Signs

To avoid data collection bias prior to the subsequent analyses, an investigation was conducted to assess the consistency of the R/NR (response/no response) labeling provided by the veterinarian in relation to the change in the 15 CE clinical signs and the variation in the CIBDAI before and after FMT. This evaluation aimed to determine whether the categorization of response accurately reflected the clinical outcomes observed in the patients. The analysis demonstrated a consistent relationship between the labeling and the two quantitative indicators. This is illustrated by the fact that both the mean of CE clinical signs and the CIBDAI showed a statistically lower improvement (with medians close to 0) in the NR group compared to the R group (Figure 3). Specifically, the t-test results yielded a *p*-value of 0.039 for the 15 clinical signs and a *p*-value of 0.0018 for the CIBDAI.

3.3. Administered Dose Quantification

The dosage administered to the included subjects was quantified for each dose type we evaluated. For freeze-dried FMT material, a range of 3.774–64.516 mg/kg BW (mean: 14.398) was quantified, while for freeze-dried fecal material, i.e., the FMT material, discarding trehalose and sodium chloride contribution, a range of 1.314–32.615 mg/kg BW (mean: 7.423) was obtained. For this last quantification, three additional subjects had to be excluded due to missing data.

The quantification of alive bacterial cells administered daily with the FMT treatment led to a range of 2.600×10^8–7.778×10^9 AFU/kg BW (mean: 1.902×10^9), while for the count of total bacterial cells, we obtained a range of 6.067×10^8–1.944×10^{10} TFU/kg BW (mean: 4.067×10^9).

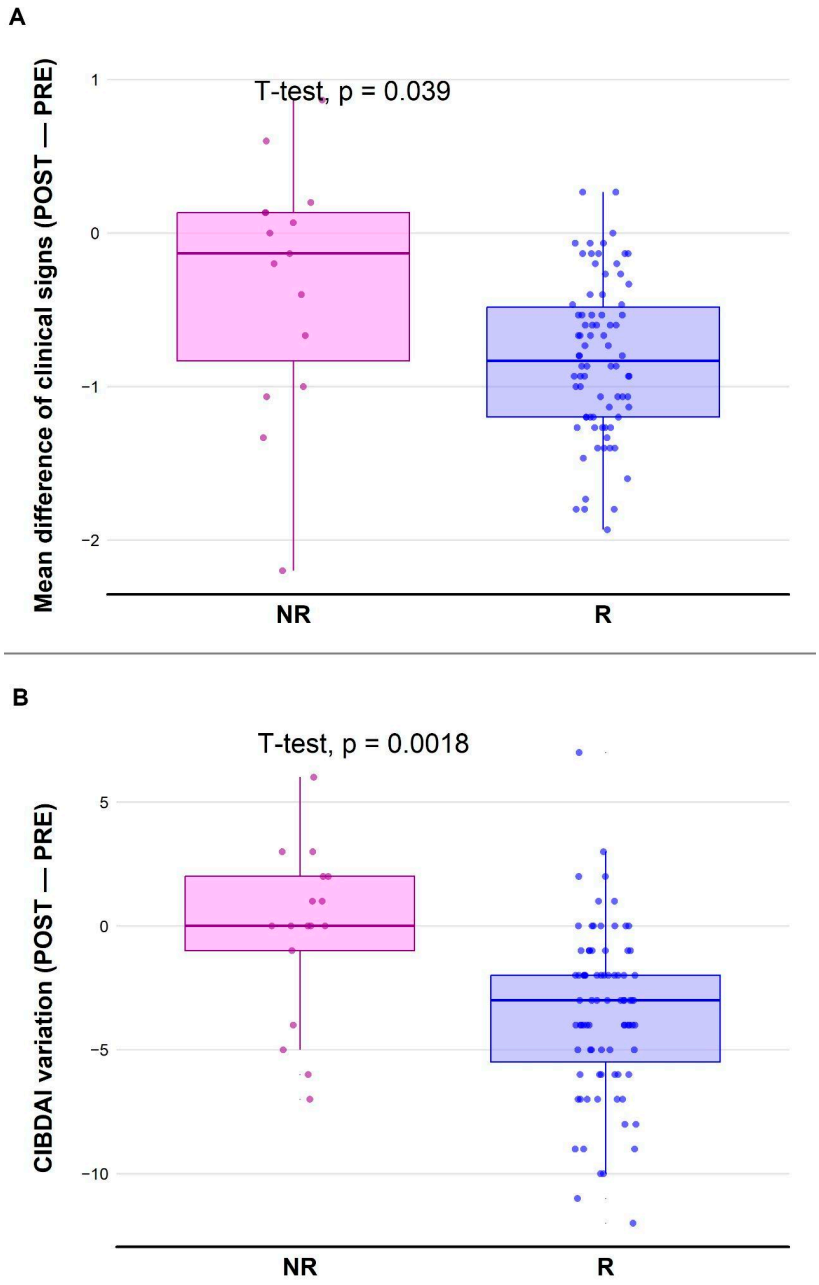

Figure 3. Boxplot showing, for NR and R subjects, (**A**) the variation of the mean value of 15 clinical signs of CE and (**B**) the variation in the CIBDAI, both calculated as the difference between the value post-FMT and before FMT.

3.4. Clinical Efficacy of Capsule FMT (cFMT)

3.4.1. Relationship Between Capsule Size and Response

The distribution of subjects having received the "S" or "L" capsules according to the response (NR/R) to the FMT (Table 4) clearly showed that the efficacy of the treatment was comparable for both the doses and, considering data all together, the total positive response rate was 82%. Although some differences were observed in the efficacy rate of the two doses, the Chi-squared test (p = 0.4901) and Fisher's test (p = 0.4213) showed no significant difference between them.

Table 4. Contingency table showing the distribution of subjects having received the "S" or "L" capsules according to the response (R/NR) to the FMT.

Capsule Size	NR	R	%R
S	4	28	87.50%
L	16	63	79.75%

Analogously, no significant difference was found between the doses received by subjects responding to FMT and those who showed no improvement after treatment, as indicated by the Kruskal–Wallis test results for the four dose types (Figure 4): total freeze-dried FMT material (p = 0.81), freeze-dried fecal material (p = 0.56), alive bacterial cells (p = 0.25), and total bacterial cells (p = 0.32). These findings indicate that the dosage, evaluated through both capsule size and the four measures of FMT dose, does not significantly influence treatment response.

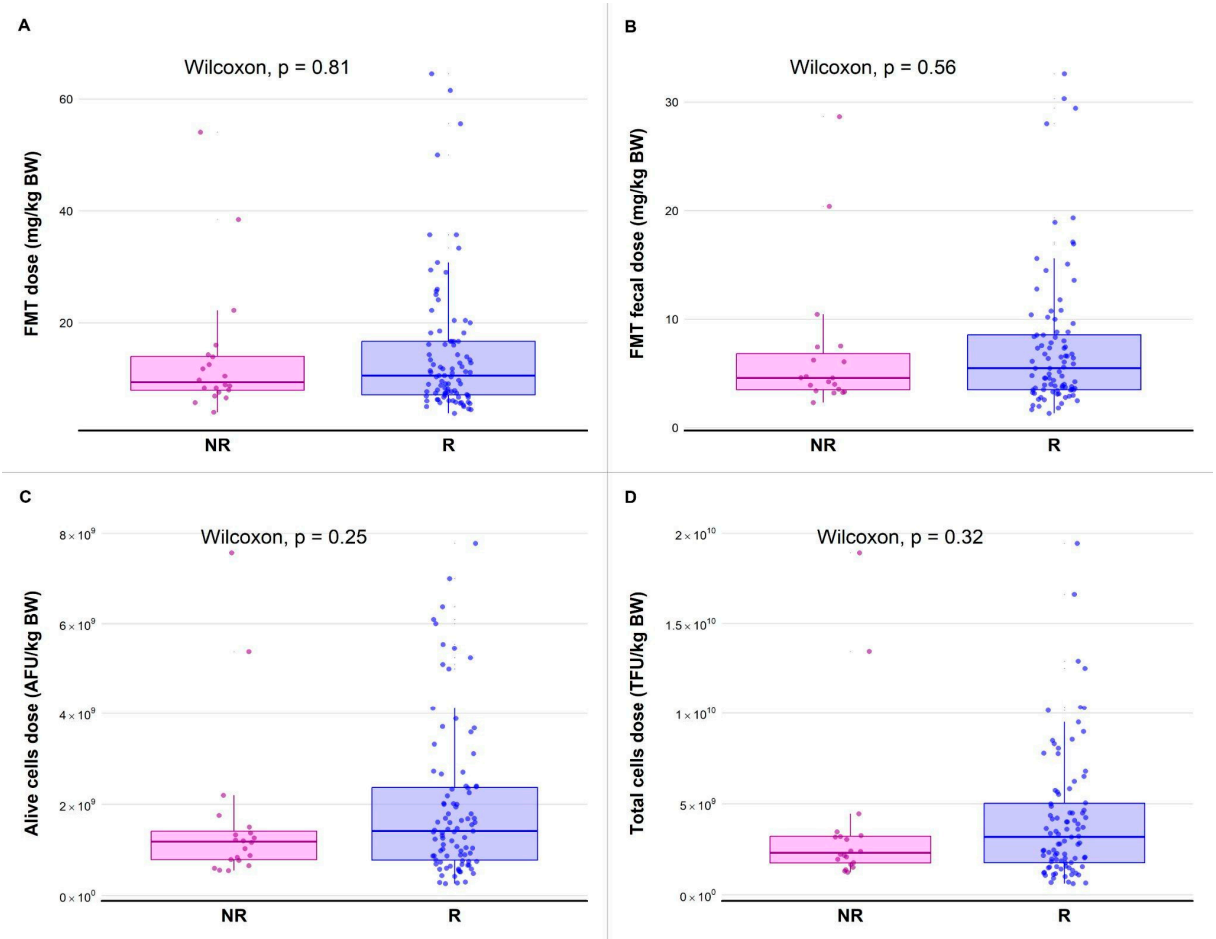

Figure 4. Boxplot showing, for NR and R subjects, the daily dose received in terms of (**A**) total freeze-dried FMT material (including excipients), (**B**) freeze-dried fecal material (excluding excipients), (**C**) alive bacterial cells, and (**D**) total bacterial cells.

3.4.2. Relationship Between Dose and Variation of the Mean of CE Clinical Signs

The analysis assessed the possible influence of the four types of dose—total freeze-dried FMT material, freeze-dried fecal material, alive bacterial cells (AFU), and total bacterial cells (TFU)—on the variation in the mean of 15 CE clinical signs. The total freeze-dried FMT dose exhibited an extremely weak correlation of −0.0996 and a non-significant coefficient (p = 0.345), explaining little variability (R-squared = 0.00993). The correlation

for the fecal material dose was −0.15, with a regression *p*-value of 0.158 and R-squared of 0.02251, indicating minimal variability. The AFU dose showed a correlation of −0.18368, a marginally significant coefficient ($p = 0.0797$), and R-squared of 0.03374. The TFU dose had a correlation of −0.1777 and a marginally significant coefficient ($p = 0.0901$), with R-squared at 0.03158.

Overall, these findings suggest that the assessed doses do not significantly affect the variation in clinical signs, indicating that other factors may be influencing the outcomes.

3.4.3. Relationship Between Dose and Variation of CIBDAI

In evaluating each FMT dose type as an independent predictor, weak negative correlations were observed with changes in the CIBDAI. For the total dose of freeze-dried FMT material, the correlation was −0.0825, with no significant effect on Diff_CIBDAI ($p = 0.417$) and R-squared of 0.0068. The correlation for the fecal material dose was −0.1208, indicating a weak negative relationship, with a simple regression showing no significant effect ($p = 0.241$) and an R-squared of 0.0146. The AFU dose had a correlation of −0.0782, with a non-significant coefficient ($p = 0.442$) and R-squared of 0.0061, suggesting minimal explanatory power. Lastly, for the TFU dose, the correlation was −0.1145, and the simple regression did not reveal a significant effect ($p = 0.259$), with R-squared at 0.0131.

Collectively, these findings demonstrate that the assessed doses are not associated with variations in the CIBDAI scores.

4. Discussion and Conclusions

This study demonstrates that our production procedure and administration protocol of encapsulated microbiota (cFMT) is an effective therapeutic approach for dogs with chronic enteropathy (CE), regardless of the patient's weight. A positive clinical response was observed in 80% of cases. These results are promising and suggest a potential benefit of cFMT as a treatment option for dogs with chronic enteropathy, in line with evidence from several human studies [23–25]. Further research is needed to determine whether the observed response is sustained over the long term.

Although response to treatment has been defined in different ways, the outcome was comparable. This is a demonstration of the robustness of the method, considering that the veterinarians who joined the project were not part of a single research center but worked independently. We used the veterinarian's classification as the primary outcome measure, as we consider a comprehensive clinical evaluation to be more reliable. Indeed, changes in CIBDAI scores or in the scores of the 15 individual clinical signs may not necessarily correspond to the animal's true clinical status, either at baseline or after treatment. Although the clinical signs included in the CIBDAI are scored on the same numerical scale, they do not all represent the same degree of clinical severity. Moreover, the reliability of the results is reinforced by the large sample size. In fact, the efficacy of FMT was evaluated in over 100 dogs.

This is the first study, to the best of our knowledge, that correlates cFMT dosage to clinical response. Since the biological mechanism underlying the effectiveness of FMT remains unknown, various methods have been proposed to determine the appropriate dosage. These approaches are based on hypothetical mechanisms of action and include considerations such as the presence of secondary metabolites, the total number of microbial cells, and the number of viable cells. Interestingly, the study does not demonstrate a clear dose trend for efficacy, despite the wide dose range, which varied from 17.09 to 32.04 times, according to the dosing method (the minimum variation was found when dosage was expressed as mg/kg BW, the maximum variation was found when dosage was expressed as TFU/kg BW). A similar result was obtained in a human study, where high dose cFMT

(1.25–2.5 $\times$ 10^{12} AFU) and low dose cFMT (2.1–2.5 $\times$ 10^{11} AFU) performed equally well in terms of clinical outcomes and microbiota engraftment [23].

This suggests that factors other than the quantity of fecal material, viable bacteria, or total bacteria likely influence the therapeutic response. Furthermore, the concept of dose–response relationship applies for most of the drugs, but may not be appropriate to describe a complex biological system, which includes replicating microorganisms, such as fecal preparation.

Further research is needed to identify factors that promote microbiota engraftment and the expansion of key microbial taxa essential for maintaining gut health and immune homeostasis. In addition to bacterial content, abiotic factors such as metabolite concentrations (e.g., short-chain fatty acids like butyrate or bile acids) may influence both the success and dynamics of microbiota engraftment [26]. These metabolites play a crucial role in nourishing beneficial microbes, modulating the immune system, and maintaining gut barrier integrity—key processes for reducing inflammation and restoring normal gastrointestinal function in dogs with CE [23]. Their concentrations may be associated with clinical improvement.

Additionally, recipient-related factors—such as age, disease duration, severity, and underlying gut physiology—could have an equally important impact on treatment outcomes as the composition of the fecal transplant itself. A recent study has shown that, at baseline, the severity of dysbiosis assessed through the dysbiosis index was significantly lower for good responders versus poor responders [12].

While this study provides compelling evidence for the clinical benefits of cFMT, several limitations must be acknowledged. The lack of a control group limits our ability to definitively attribute the observed improvements solely to FMT. Furthermore, the short follow-up period (15 days) may not capture the long-term effects of FMT, particularly in chronic conditions like CE, where clinical signs of improvement may require more time. A longer follow-up would have helped to clarify the sustainability of the clinical response and assess the potential for relapse.

For the purpose of this study, we did not analyze the composition of the microbiome, as our primary focus was to assess the clinical response. Previous research has shown that clinical outcomes in similar cohorts are not directly correlated with the microbiome composition. Therefore, microbial profile analysis was outside the scope of this investigation.

In conclusion, our findings support a new protocol for the administration of FMT. We demonstrated that oral administration of 100 mg of freeze-dried cFMT daily for a month (equivalent to a minimum dosage of 3.774 mg/kg BW of freeze-dried cFMT, 1.314 mg/kg BW of freeze-dried fecal material, 2.600 $\times$ 10^{8} AFU/kg BW of live bacterial cells, and 6.067 $\times$ 10^{8} TFU/kg BW of total bacterial cells) significantly improves the clinical signs of CE, with an encouraging 80% clinical response rate observed during and up to fifteen days after cFMT administration. Given the chronic nature of the condition, longer follow-up studies will be needed to better understand the long-term impact of cFMT. However, these results are promising. Notably, the success rate remained consistent for all dogs treated with doses above 100 mg of freeze-dried FMT, regardless of the fecal material weight, bacterial viability, or total bacterial count. This suggests that microbiota composition, engraftment efficiency, and non-bacterial components of the transplant may play more significant roles in treatment outcomes than previously assumed. Further research into the mechanisms of microbiota engraftment, the role of metabolites, and the optimization of FMT protocols will be essential to improving the effectiveness and long-term sustainability of microbiota-based therapies for gastrointestinal diseases in veterinary medicine.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pets2020016/s1, Table S1: Pre- and post-FMT CIBDAI values and clinical signs scores for included dogs.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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