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Clinical Guidelines for Fecal Microbiota Transplantation in Companion Animals

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KEYWORDS
• Fecal microbiota transplant • Fecal transplant • Dysbiosis • Microbial-directed therapeutics
• Companion animals • Microbiome • Microbiota • Clinical guidelines

KEY POINTS
- Fecal microbiota transplantation (FMT) is the transfer of feces from a healthy donor to a diseased recipient aimed at directly modifying the recipient's gut microbial ecosystem to confer a health benefit to the recipient.
- FMT is a safe, well-tolerated, minimally invasive procedure that can be performed in any veterinary practice type.
- Establishing a fecal donor program is feasible for veterinarians, and donor screening guidelines, which can be modified on a case-by-case basis, are included herein.
- Fresh feces should be utilized for FMT whenever possible. Specific recommendations for FMT product processing and preparation provided here can be tailored to meet the availability of personnel and equipment resources for each practice.
- Currently in veterinary medicine, the Companion Animal FMT Consortium recommends FMT as an adjunctive microbial-directed therapeutic for canine parvovirus enteritis, canine acute diarrhea, and chronic enteropathy in both dogs and cats.

Video content accompanies this article at https://www.advancesinsmallanimalcare.com/.

INTRODUCTION

Evidence is rapidly mounting that modifying the gut microbiota is important for treating diseases and maintaining health. Fecal microbiota transplantation (FMT) utilizes donor feces to modify the recipient's gut microbial ecosystem to confer a health benefit in patients suffering from gut dysbiosis. Investigation into the utility of FMT is growing in diverse fields such as infectious disease [1], gastroenterology [2], endocrinology [3], neurology [4], and oncology [5]. FMT for recurrent *Clostridioides difficile* infection represents the first successful human clinical application of directly modifying the gut microbial ecosystem to restore health [6].

Currently in veterinary medicine, microbiome research surrounding FMT is in its infancy [7,8]. Mechanistic studies related to longitudinal changes in gut microbial communities, microbial engraftment, host–microbe interactions, FMT dosing, and administration routes are limited in companion animals. The paucity of information in companion animals provides evidence that further investigation into the role gut microbes and their metabolism play in disease states and how microbial-directed therapeutics, like FMT, can be used clinically are required.

The Companion Animal FMT Consortium, a unique international group of veterinary experts, was established to develop the first clinical FMT guidelines for companion animals. The Companion Animal FMT Consortium aims to increase accessibility of FMT as a microbial-directed therapeutic for dogs and cats by simplifying and demystifying the process of performing FMT in clinical practice. The information presented in later discussion is intended to serve as a summary of the technical aspects of FMT, current small animal therapeutic indications of FMT, and important considerations for establishing a fecal donor program. These FMT clinical guidelines are intended for veterinarians in a variety of clinical practice types and can be modified and adapted as needed to align with financial and technical resources available to individual practitioners.

CLINICAL GUIDELINES DEVELOPMENT PROCESS

The Companion Animal FMT Consortium aimed to develop the first veterinary clinical guidelines for FMT in dogs and cats to provide veterinarians with evidence-based recommendations for performing FMT in a variety of clinical practice settings. Aligned with methodology utilized by human FMT consensus statements for clinical practice [9], the following steps were completed: selection of experts to serve on the Companion Animal FMT Consortium; identification of main topics for clinical guidelines and formation of working groups; development of statements germane to topics with critical evaluation of evidence available; development of consensus on clinical statements through an electronic

modified Delphi process [10], and virtual meetings to develop the final version of clinical statements.

Members of the Companion Animal FMT Consortium were selected based on their expertise in FMT in dogs and cats and represent diverse backgrounds from academia and private practice. The FMT Consortium is composed of 21 global experts from 19 institutions, with each member having an active role in the development of the clinical FMT guidelines presented herein. Each member was assigned based on their expertise to one of the following working groups: (1) donor selection and screening; (2) FMT preparation; and (3) FMT clinical applications and dosing. Members of each working group assigned a lead to coordinate their group and provided the FMT Consortium chair (J. Winston) finalized clinical statements for their assigned topic. The quality of evidence for each statement was adapted from the Grading of Recommendations Assessment, Development and Evaluation system [11,12]. The definitions utilized for quality of published evidence are provided in Box 1.

Statements provided by each working group were uploaded to an online voting system (Qualtrics, Provo, Utah, USA) by the FMT Consortium chair. A modified Delphi method was used to achieve consensus on all statements [10]. Each set of topic statements underwent multiple rounds of revisions based on the group's anonymous feedback during online voting. Each time statements were uploaded and sent out for the Companion Animal FMT Consortium members to evaluate. After multiple rounds, the modified Delphi method resulted in consensus on all statements provided herein.

For each statement, experts rated their level of agreement as follows: (1) agree strongly, (2) agree with reservations, (3) undecided, (4) disagree, or (5) disagree strongly. If experts selected anything other than "agree strongly," they were asked to add comments to explain their reservation and/or disagreement with the statement and specifically explain how to improve the statement. For each statement, a predetermined threshold of 80% or greater of experts "agreeing strongly" was required for the statement to be included in the clinical FMT guidelines. All statements not reaching 80% agreement were revised and rated again in additional rounds of online voting. Once the statements were finalized for each topic (achieving 80% or greater agreement), the working group members provided commentaries for each statement based on the available evidence. Working group leads then provided the final sections to the Companion Animal FMT Consortium chair and clinical guidelines were complied.

BOX 1
Definition of the Quality of Evidence

QoE	Definition
High	At least one properly designed RCT or equivalent
Moderate	At least one well-designed clinical trial, without randomization; evidence from cohort or case series; or equivalent
Low	Opinions of respected experts, based on clinical experience, descriptive single case reports, or reports of expert committees

Abbreviations: QoE, quality of evidence; RCT, randomized clinical trial.

CLINICAL GUIDELINES FOR FECAL MICROBIOTA TRANSPLANTATION IN COMPANION ANIMALS

The clinical guidelines for FMT in companion animals are divided into 3 sections: (1) donor selection and screening; (2) FMT preparation; and (3) FMT clinical applications and dosing. Each section consists of a series of statements to provide veterinarians with general recommendations relevant to the topic. Evidence germane to the topic is also provided as commentary below each statement when available, including the quality of evidence (definitions in Box 1).

Part 1: Donor Selection and Screening

In this section, general recommendations for FMT donor selection and screening are provided. It is important to note that additional considerations depending on geographic location (ie, infectious disease screening), financial resources (ie, additional bloodwork to screen donor), and other factors should be considered on a case-by-case basis. Box 2 provides a summary of the fecal donor screening recommendations.

Health status

Statement. Donors should be clinically healthy (no abnormalities identified on comprehensive physical examination and history).

Quality of evidence: Low.

Comment. While there are no data regarding the impact of general health of the FMT donor on FMT success or safety, general clinical status is a readily identifiable indicator of overall health. It is plausible that an animal that does not appear clinically normal could pose an elevated risk for pathogen shedding or have an altered gastrointestinal (GI) microbiota, known as

BOX 2
Fecal Donor Selection and Screening

Health status

- Clinically healthy (no abnormalities on comprehensive physical examination and history)
- Acceptable BCS (4–6/9)
- No history of current (within last 4 months) or chronic GI signs
- If acute GI (<2 weeks) develop, wait 3 months and then rescreen
- Permanently exclude any donor with a history of chronic GI signs (>3 weeks)

Age, signalment, and environment

- Minimum 12 months old and younger than <75% of expected lifespan
- No exclusion of breeds
- No exclusion for farm/wildlife exposure, dog parks, boarding, and so forth; unless GI signs present
- Exclude donor if hospitalized or boarded for longer than 8 to 10 hours in previous 4 weeks

Feline fecal donors

- Indoor cats from single-cat-household preferred
- Ideally 6 weeks in household prior to screening

History of drugs that induce dysbiosis

Minimum duration to exclude donor since treatment with medications that potentially induce dysbiosis:

- Antimicrobials (oral/parenteral): At least 6 months and rescreen donor
- Acid suppressants (anything changing gastric pH): At least 2 weeks
- Exclude donors that have received raw food diets/treats within last 30 days

Microbiome screening

- DI should be performed where available to exclude animals with abnormal microbiomes
- Next-generation sequencing based technologies for the assessment of the microbiome are not recommended, as they are not validated, and there is no standard interpretation of results for individual animals
- Routine bacterial culture is not an effective tool for assessing the diversity of the entire microbiome

Infectious disease screening

It is *recommended* to test for the following infectious diseases in donors:

- *Salmonella* (fecal culture or PCR)
- *C. jejuni* (PCR or culture by experience laboratory with identification on a species level)
- *Giardia* (IFA, SNAP test, coproantigen, or centrifugal flotation with zinc sulfate, PCR, or a combination of these)
- *Cryptosporidium* (IFA or antigen testing)
- Other intestinal parasites (centrifugal fecal flotation, coproantigen tests, Baermann, and PCR)

Additional testing for feline fecal donors:

- *Tritrichomonas foetus/blagburni* (PCR on fresh fecal sample)
- Enteric coronavirus (PCR; performed once for cats if an indoor cat from a single-cat-household)
- FIV and FeLV (SNAP Triple Test or FeLV antigen ELISA, and FIV antibody testing; Regarding FeLV testing cat should be indoor and not have contact with known infected cats for at least 6 weeks prior to testing)

It is *optional* to test for the following infectious diseases in fecal donors:

- *C. perfringens* netF-toxin gene (PCR; optional as occurrence is rare in clinically healthy dogs)
  - *Note*: It is not recommended to screen for *C. perfringens* enterotoxin and alpha toxin genes as the clinical significance is unknown in dogs and cats
- *C. difficile* (PCR; optional as evidence of pathogenicity is weak in dogs and cats and the zoonotic potential is unclear; depending on clinician's preference)

Frequency of fecal donor screening

- Screened every 6 months, potentially more frequently based on risk/environment (endemic area)

Abbreviations: GI, gastrointestinal; PCR, polymerase chain reaction.

dysbiosis. There are few practical limitations to excluding clinically abnormal animals, given the large pool of clinically normal animals, so the precautionary principle supports a requirement that donors be clinically normal. There may be some situations where clinical abnormalities could be deemed irrelevant (eg, mild orthopedic abnormalities or dental disease) and the animal may be selected as a donor. However, the default should be for animals to be clinically normal and not receive any medications.

Statement. Donors should have an acceptable body condition score (BCS: 4–6/9).

Quality of evidence: Low.

Comment. The current scientific literature does not provide information on the impact of a fecal transplant from a donor with an abnormal BCS (Canine ideal BCS: 4–5; Feline ideal BCS: 5) on the gut microbial ecosystem of the recipient. Similarly, there are no data available documenting BCS increases in canine FMT recipients from overweight or obese donors. People with a significantly increased body mass index, obesity, or type 2 diabetes are not considered acceptable fecal donors due to their altered gut microbiota compared to individuals with normal body condition [13]. In a clinical trial involving dogs, slight gut microbiota variations were observed between obese and lean individuals [14].

Statement. Donors should not have a history of current (within the last 4 months) or chronic GI signs (eg, vomiting, diarrhea, weight loss, dysorexia, melena, or hematochezia).

Quality of evidence: High.

Comment. Studies indicate that GI disorders in dogs and cats frequently lead to shifts in their gut microbiota, and some causes of GI disease can presumably be transmitted through FMT [15]. Any pathogen that can be transmitted through the fecal–oral route should be assumed to be transmissible via FMT, and some causes of acute GI disease in dogs and cats have fecal–oral transmission potential. In chronic enteropathies, gut microbiota changes can be quite significant and persistent, while in acute gastroenteritis and canine acute hemorrhagic diarrhea syndrome (AHDS), the alterations may be minor and only transient [16–20].

Statement. If acute GI signs (<2 weeks) develop, wait 3 months and then rescreen donor.

Quality of evidence: Low.

Comment. Data obtained from dogs indicate that the gut microbiota undergoes only minor and transient changes during episodes of acute GI disease [21]. After the resolution of clinical signs, the gut microbiota of these dogs characteristically returns to normal within a few weeks [21]. This has been demonstrated in dogs with acute diarrhea and AHDS [15,17,19]. However, there is a lack of comprehensive large-scale studies assessing the duration of infectious agent presence in the GI tract of dogs and cats after an episode with acute GI disease, and it is known that some infectious agents can be shed for weeks. Therefore, the precautionary approach would dictate a restriction period before use as a donor.

Statement. Permanently exclude animals as donors with history of chronic GI signs that have lasted more than 3 weeks, which may suggest possible underlying chronic enteropathy.

Quality of evidence: Low.

Comment. Numerous clinical investigations have demonstrated that the gut microbial ecosystem is altered in a substantial proportion of animals with chronic enteropathies when compared to healthy individuals [16,22]. Given that the objective of FMT is to restore a healthy gut microbial ecosystem in the recipient, it is imperative to exclude dysbiotic animals as donors.

Age, signalment, and environment

Statement. Donors should be a minimum of 12 months and younger than less than 75% of their expected lifespan (eg, <12 years for a breed expected to live 16–17 years).

Quality of evidence: Low.

Comment. The physiologic gut microbiota of dogs younger than 1 year is different compared to that of adult dogs and cats [23,24]. In human medicine, there is evidence that the gut microbial ecosystem changes negatively in older subjects due to various inflammatory processes in the body [25]. In dogs, one study has shown minor alterations in intestinal functional markers in older dogs [26]. The age at which a dog or cat can confidently be assumed to have a stable adult gut microbiota is unknown, but based on expert opinion, 1 year as a cutoff was chosen.

Statement. No exclusion of breeds.

Quality of evidence: Low.

Comment. Studies demonstrating breed-specific changes in the gut microbiota are lacking for both cats and dogs. There is no evidence that specific breeds have an altered gut microbiota compared to other breeds.

Statement. *For donors with farm/wildlife exposure, dog parks, boarding, and so forth:* There is no reason to exclude the donor as long as the animal is healthy with no GI signs.

Quality of evidence: Low.

Comment. The risk of acquiring infectious agents, transmission of enteropathogenic and multidrug-resistant bacteria from other dogs/cats is probably increased in animals that are in close contact with many other individuals (as it is the case in the named circumstances) [27]. However, there are no large-scale studies documenting the degree of risk increase, which is why dogs/cats kept in these circumstances should not be systematically excluded as donors. Testing for certain infectious agents (*see later section*) is, therefore, important.

Statement. Exclude animals that have been hospitalized or boarded for longer than 8 to 10 hours in the previous 4 weeks as this could have increased the risk of acquiring infectious agents.

Quality of evidence: Low.

Comment. To minimize the risk of acquiring infectious agents due to close contact with potentially sick animals, based on expert opinion, it is recommended to temporarily exclude such animals as potential donors.

Statement. Indoor cats from single-cat-households are preferred to minimize risk of infections.

Quality of evidence: Low.

Comment. Cats are prone to harbor parasitic and infectious diseases, regardless of whether they live indoors and/or outdoors. However, hunting of wild animals or scavenging significantly increases their risk of acquiring certain parasites [28]. A comprehensive meta-analysis of parasitic infection risks in indoor and outdoor cats found that limiting outdoor access could help reduce parasitic infections [29]. In geographic regions where the prevalence of free-roaming cats is very high and it is difficult to identify strictly indoor cats, free-roaming cats could be screened as potential donors as described in later discussion.

Statement. Feline fecal donors should ideally be at least 6 weeks in the household prior to undergoing screening.

Quality of evidence: Low.

Comment. As the feline leukemia virus (FeLV) p27 antigen point-of-care test can take up to 6 weeks after infection to become positive [30], a corresponding waiting period should be applied before screening donors. Important to note, no new cats should be introduced within the household during these 6 weeks. If the population of strictly indoor cats is low, the inclusion of free-roaming cats might be a necessity; however, ideally cats would be kept inside for a period of 6 weeks prior to donation.

History of antimicrobials, acid suppressants, and other drugs that induce dysbiosis

The following are the minimum required waiting times (since end of administration) prior to considering fecal donation for each potential dysbiosis-inducing drug.

Statement. *Antimicrobials (oral/parenteral):* At least 6 months and rescreen donor for intestinal dysbiosis.

Quality of evidence: Moderate.

Comment. The aim is to have a fecal donor with a healthy gut microbial ecosystem. Research has demonstrated that antimicrobials have a detrimental impact on the gut microbiota of dogs and cats, resulting in dysbiosis [31]. This is especially true for antimicrobials with anaerobic spectrum, such as metronidazole and tylosin, which can cause severe intestinal dysbiosis [32,33]. Studies indicate that the duration required to recover a normal gut microbiota following cessation of antimicrobial therapy can vary greatly among individuals and may sometimes take several months [24,32,33].

Statement. *Acid suppressants (anything changing gastric pH):* At least 2 weeks.

Quality of evidence: Moderate.

Comment. The reason for acid suppressant administration should be determined to evaluate additional risks. Research suggests that omeprazole and esomeprazole may result in temporary dysbiosis, yet it has been observed that the abundance of the bile acid converting bacterium *Clostridium hiranonis* (newly named *Peptacetobacter hiranonis*) remains within the reference range [34,35]. The gut microbiota typically returns to normal

within 1 to 2 weeks after the cessation of treatment [34–36]. These findings have been established in canines and also apply to cats [37]. Studies on the effects of H_2-receptor antagonists on the gut microbiota are lacking.

Diet and other supplements

Statement. Exclude animals that have received raw food diets or raw treats within the last 30 days.

Quality of evidence: Moderate.

Comment. Animals consuming raw food or raw treats are at an increased risk of harboring pathogens and extended spectrum beta-lactamase (ESBL)-producing microorganisms in their feces. Studies suggest that the feces of dogs that were fed a raw diet often contain enteropathogenic bacteria, such as *Campylobacter jejuni*, *Salmonella*, and *Listeria monocytogenes*, at a higher frequency than the feces of dogs that were fed a commercial diet [38,39]. Additionally, the number of these bacteria was higher in dogs that were fed raw diets containing chicken [39]. More than 50% of dogs that were fed a raw diet harbor ESBL-bacteria in their feces [40–42]. Moreover, evidence showed that dogs on a raw diet have an altered gut microbiota [42]. Therefore, it is advised to avoid transferring fecal material containing enteropathogens or multidrug-resistant bacteria, and animals on a raw diet should be excluded as fecal donors. Animals can be switched to processed/cooked diets and then screened after 1 month on the new diet.

No clear recommendation can be given for dietary supplements. Although probiotics can shift the gut microbiota, clinically significant changes are not observed [43]. Currently, there is not enough evidence to provide a statement regarding the administration of probiotics to fecal donors.

Microbiome screening

Statement. Dysbiosis index (DI) should be performed where available to exclude animals with abnormal microbiomes.

Quality of evidence: Moderate.

Comment. The canine and feline fecal DI are validated and standardized tests designed for assessing microbiome shifts [16,22]. A recent meta-analysis of 27 studies revealed that the canine DI, which is calculated based on core bacteria such as *C. hiranonis* (newly named *P. hiranonis)*, is a potential useful biomarker of intestinal functionality [44]. Additionally, robust correlation between untargeted metagenomic sequencing and the DI has been demonstrated [45]. The DI is a reliable indicator of shifts in the fecal microbiota in dogs and cats, providing better comparisons across individuals, as well as within the same individual over time, due to its superior reproducibility and analytical sensitivity in comparison to sequencing techniques [45]. The DI for donors should be below 0, and the abundance of all included bacterial taxa should be within their reference ranges. See "Disclosure" section for conflict of interest statement.

Statement. Next-generation sequencing-based technologies for the assessment of the microbiome are not recommended, as they are not validated and there is no standard guidance for interpretation of results for individual animals.

Quality of evidence: Low.

Comment. While recent technologies permit detailed investigation of the microbiota, there is a poor understanding of "normal" at the individual level and its variations [46,47]. There is an inadequate understanding of the gut microbiota to define what constitutes normal/healthy (or acceptable) and abnormal (or unacceptable) results for FMT donors.

Statement. Routine bacterial culture is not an effective tool for assessing the diversity of the entire microbiome, but culture can still be used to test for specific enteropathogens.

Quality of evidence: Moderate.

Comment. Fecal culture is a diagnostic technique used to identify specific or opportunistic enteropathogenic bacteria and fungi in dogs and cats. Many commercial veterinary diagnostic laboratories offer this service to assess the microbial composition of the feces and provide their own interpretation of the balance between normal and abnormal microbial populations. However, this approach has limitations, as aerobic culture-based methods do not adequately represent the mostly anaerobic intestinal microbiota [48]. A study comparing results from 3 commercial laboratories on bacterial culture with the canine DI showed that fecal cultures are not useful for identifying dysbiosis in dogs [49]. In fact, the interpretation of culture results can be misleading.

Infectious disease screening (recommended)

Statement. It is recommended to test for the following infectious diseases in fecal donors:

- *Salmonella* (recommend fecal culture or polymerase chain reaction [PCR])

- *C. jejuni* (recommend PCR or culture by experienced laboratory with the identification on a species level)
- *Giardia* (recommend immunofluorescence assay [IFA], SNAP test, coproantigen, or centrifugal flotation with zinc sulfate, PCR, or a combination of these)
- *Cryptosporidium* (recommend IFA or antigen testing)
- Other intestinal parasites (recommend centrifugal fecal flotation, coproantigen tests, Baermann, and PCR)

Additional testing in cats includes the following:

- *Tritrichomonas foetus/blagburni* (recommend PCR on fresh fecal sample)
- Enteric coronavirus (recommend PCR; performed once for cats if an indoor cat from a single-cat-household)
- Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) (recommend SNAP Triple Test or FeLV antigen enzyme-linked immunosorbent assay [ELISA], FIV antibody testing; Regarding FeLV testing, cat should be indoor and not have contact with known infected cats for at least 6 weeks prior to testing)

Quality of evidence: Moderate.

Comment. The listed organisms have been shown to potentially induce GI disease as well as diseases in other organ systems. Therefore, animals harboring these organisms should be excluded [50].

Optional infectious disease screening

Statement. It is optional to test for the following infectious diseases in fecal donors:

- *Clostridium perfringens* netF-toxin gene (PCR; optional as occurrence is rare in clinically healthy dogs)
  - *Note*: It is not recommended to screen for *C. perfringens* enterotoxin and alpha toxin genes as the clinical significance is unknown in dogs and cats.
- *C. difficile* (PCR; optional as evidence of pathogenicity is weak in dogs and cats and the zoonotic potential is unclear; depending on clinician's preference)

Quality of evidence: Moderate.

Comment. Current evidence suggests that netF, a beta-pore-forming toxin, is likely the major virulence factor in *C. perfringens* strains responsible for canine AHDS [51,52]. Another toxin, *C. perfringens* enterotoxin (CPE) has been found more often in the feces of dogs with AHDS than in the feces of dogs in a control group [53]. Although AHDS is not a transmissible disease, the role of specific toxins in its development remains unclear, and netF-encoding *C. perfringens* strains are rarely detected in healthy dogs; therefore, it seems prudent to avoid factors/procedures that could increase the risk of exposure to these particular *C. perfringens* strains [51,53,54].

C. difficile is a common pathogen in humans, often leading to pseudomembranous colitis associated with antibiotic use [55]. Companion animals have been shown to carry the same or similar *C. difficile* ribotypes as those found in people, suggesting interspecies transmission [56,57]. However, a recent study demonstrated that interspecies transmission of *C. difficile* occurs infrequently in households with people infected with *C. difficile* [57]. The prevalence of *C. difficile* in dogs is estimated to be as high as 19%, but it rarely produces clinical signs [55]. There appears to be an association between *C. difficile* carriage and intestinal dysbiosis in dogs [58,59].

Frequency of fecal donor screening

Statement. Fecal donors should be screened every 6 months, potentially more frequently based on risk/environment (endemic area).

Quality of evidence: Low.

Comment. There are no clinical trials showing the resilience of the gut microbiota over 6 months or longer, but expert opinion suggests that the gut microbiota remains relatively stable in dogs and cats over the course of several months, provided there are no significant environmental changes or illnesses. Testing should be more frequent in endemic areas at the discretion of the veterinarian.

Part 2: Fecal Microbiota Transplantation Preparation

In this section, general recommendations for fecal collection and FMT preparation are provided. It is important that these recommendations need to be adjusted and optimized according to individual resources and facilities. Considerations include available personnel and equipment (ie, availability of −80°C freezer, lyophilizer, and so forth). Fig. 1 provides an overview of FMT product preparation and processing.

Fecal collection

Statement. Personal protective clothing, gloves, face mask, and eye protection should be worn.

Quality of evidence: Low.

Comment. Fecal samples should be handled in accordance with your local health and safety rules. In

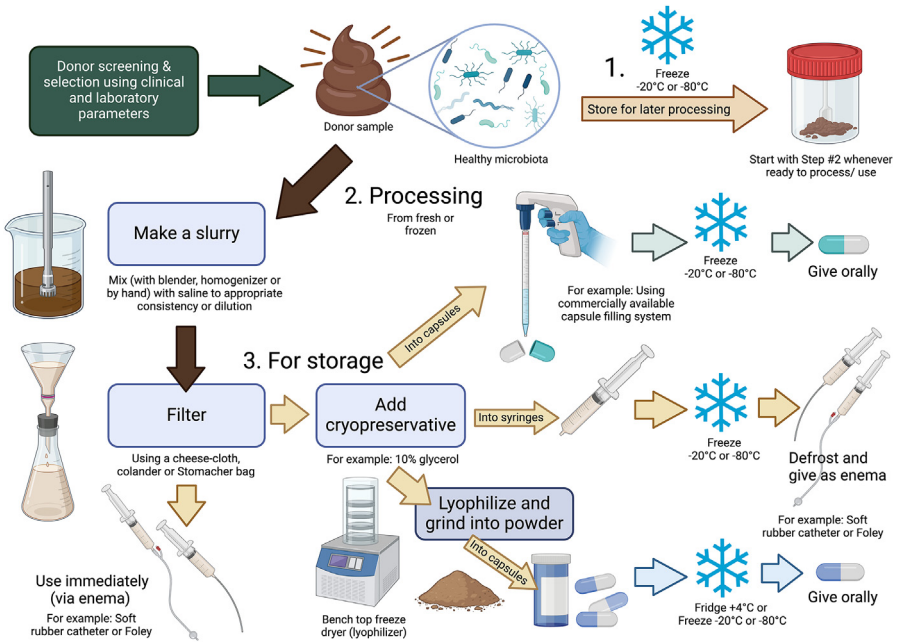

FIG. 1 Overview of FMT product preparation and processing. After a fecal donor screening and selection is complete, naturally voided feces can be used for FMT preparation and processing. Freshly void feces is ideal for FMT administration. If use of fresh feces is not feasible, then feces can be stored until processing and/or use (Step 1). Processing steps for fresh feces and/or frozen feces including making a fecal slurry and filtering the FMT product (Step 2). Once the FMT product is prepared, it can be used immediately or can be stored (Step 3). If the FMT product is to be stored, a cryopreservative can be added. FMT capsules can be administered orally and processed fecal slurries can be administered via rectal enema. (Created with BioRender.com.)

addition, unintentional contamination of individuals handling fecal material may occur, depending on the processing method. Despite the absence of documented cases of people getting diseases following contamination during FMT preparation, feces from small animals can potentially harbor infectious zoonotic pathogens. For this reason, the Companion Animal FMT Consortium advises as part of good laboratory practice to minimize the risk of infection by protecting oneself from contact with fecal material.

Statement. Use naturally voided feces, immediately after defecation.

Quality of evidence: Moderate.

Comment. Although this is usually achievable with dogs, it can be more challenging with cats. One study reported that feline feces did not show significant changes in microbiota composition and diversity over a period of 4 days at room temperature [60]. An additional study obtained the same results for a period of 12 hours at room temperature [61]. Although neither study investigated bacterial viability, which might affect the clinical efficacy of the final FMT product, these findings suggest that feline feces naturally voided overnight can be processed the following day. As both studies used healthy cats and similar methodology (DNA sequencing), the recommendation is to process cat feces ideally within 24 hours of defecation with the current state of knowledge.

Statement. Collect feces in clean plastic bags, fecal collecting tubes, or glass containers.

Quality of evidence: Low.

Comment. In a clinical setting, it is most practical to use the aforementioned collecting equipment. Adhering to good laboratory practices ensures the preservation of fecal sample quality by reducing the risk of contamination with foreign material. Veterinary studies have demonstrated the clinical efficacy utilizing samples collected in bags and/or leak-proof containers before processing [18,20].

Fecal handling before processing

Statement. Once fresh feces are naturally voided, it should be processed and either administered to the patient or stored as fast as possible and feasible (preferably within 2–6 hours of defecation).

Quality of evidence: Moderate.

Comment. It should be noticed that one study using feces from dogs [62], and other unpublished studies in dogs and cats from the Companion Animal FMT Consortium members have found a significant decrease in the number of viable bacteria after freezing compared to fresh feces.

Statement. If immediate processing is not feasible, freshly voided feces should be stored refrigerated at 4°C and processed as soon as possible.

Quality of evidence: Low.

Comment. Although there is only anecdotal information in dogs, exposure to oxygen might decrease bacterial viability. Therefore, it would be advisable to keep feces in a zip locked bag or sealed container to reduce exposure to oxygen prior to processing.

Statement. *For feline feces if covered in litter:* Manually remove as much litter from the surface of the feces as possible. This can be done using a wooden tongue depressor, spoon, rubber spatula, and/or gloved hand.

Quality of evidence: Low.

Comment. Litter or foreign material adhered to feces may lead to obstruction of the rectal catheter used for enema administration of FMT. If any such material is left after manual removal, it will be eliminated during the filtering of the fecal slurry.

Fecal processing

Statement. Feces can be processed under aerobic conditions and at room temperature.

Quality of evidence: Moderate.

Comment. Studies in human medicine suggest that anaerobic conditions help to preserve bacterial viability of obligate anaerobes while species richness does not seem to be significantly altered under aerobic conditions [63,64]. However, aerobic processing is notably more practical and easier to conduct. Due to the proven clinical efficacy in both human and veterinary medicine in different diseases, the Companion Animal FMT Consortium supports aerobic processing at room temperature [18,20,65–67]. In veterinary medicine, the potential for the enhancement of FMT efficacy through anaerobic preparation remains uncertain.

Statement. Sterile 0.9% saline (NaCl without additives) or phosphate buffered saline (PBS) can be added to feces to obtain a fecal slurry.

Quality of evidence: Low.

Comment. Although tap water has been described to prepare FMT products, the Companion Animal FMT Consortium prefers using sterile 0.9% saline (routinely available in clinical practice) or PBS to reduce the risk of contamination [68]. In addition, although other dilution media have been reported, such as skimmed milk, most studies have used NaCl or PBS.

Statement. Dilutions of 1:1 to 1:5 (fecal material:solution) have been used to achieve a desired consistency based on FMT method of administration.

For example, if FMT administered via enema, fecal slurry consistency would be based on catheter size utilized for enema (most commonly 1:1–1:5 dilution). If FMT is administered via endoscopy, a smoothie consistency would be required to easily pass through the endoscopic channel (likely 1:5 dilution used).

Quality of evidence: Low.

Comment. Several FMT dilutions are reported both in human and veterinary medicine. No data are available on the viability and efficacy of the individual mixtures. However, when administered as a rectal enema, the consistency should be as thick as possible to keep the volume low and prevent leakage.

Statement. Various methods can be used to homogenize fecal mixture such as:

- Blending using a dedicated kitchen-style blender/mixer or an immersion blender
- Manual kneading in a clean plastic bag or Stomacher® strainer bag (eg, Stomacher® from Seward Ltd., Bohemia NY, USA)
- Mixing with a spoon in a small container (ideal for small amounts of feces)

Quality of evidence: Low.

Comment. Even though evidence from direct comparisons in FMT preparations is lacking, the possible disadvantage of blending (not kneading/other types of mixing) is that undigested material and foreign substances will also be blenderized and might end up in the filtered FMT slurry.

Statement. In order to remove large particles (such as grass and hair) from the fecal slurry, the fecal slurry can be filtered using the following methods:

- A fine kitchen sieve. A wooden tongue depressor, spoon, or rubber spatula can be used to press thicker fecal slurries through the sieve.
- A cheesecloth (highly absorbent, low-lint cotton fabric). Before use, rinse the cheesecloth to remove lint. Then layer it over a container and fill with fecal slurry. Gather corners and twist the cheesecloth to wring out liquid. With gloved hands, squeeze the middle section of the cheesecloth tightly so the fecal slurry drains out. You can also press down on the strained fecal contents with a wooden tongue depressor, spoon, or rubber spatula to press liquid out.
- An alternative is to use a Stomacher® strainer bag (Stomacher® from Seward Ltd.).

Quality of evidence: Low.

Comment. The use of a Stomacher® bag has been reported for the preparation of human FMT [69].

Fecal slurry preparation for storage

Statement. If fecal slurry is not intended for immediate use, it should be immediately frozen and stored for future use.

Quality of evidence: Low.

Comment. Immediate freezing of the fecal slurry is recommended for optimal survival of fecal bacteria. See "Fecal product storage after processing" section for additional details.

Statement. The addition of glycerol as a cryoprotectant is recommended in order to improve bacterial viability upon thawing of fecal slurry.

- Recommended: Add glycerol to a final concentration of 10% (1 mL of glycerol to 9 mL of fecal slurry).

Quality of evidence: Moderate.

Comment. The addition of 10% glycerol improves viability in some bacterial species upon thawing [62]. However, studies have shown clinical improvement without the addition of glycerol [20]. Currently, there is also a lack of clinical studies comparing the FMT effectiveness of adding glycerol or other cryoprotectants.

Statement. Lyophilized FMT products are available commercially. They are prepared by freezing fecal slurries or feces at −80°C and then freeze-dried through sublimation to a powder form. This process uses a commercial lyophilizer and thus is currently only performed in research and commercial facilities.

Quality of evidence: Low.

Comment. In human medicine, lyophilized FMT capsules administered orally are safe and efficacious for recurrent *C. difficile* infection [70]. It appears that spore-forming bacteria are the most important engrafting microbes in lyophilized FMT capsules given orally to people [70]. To date, limited data are available for using lyophilized FMT capsules given orally to dogs and cats [71,72]. Importantly, the shelf-life, based on viability studies, of the commercialized lyophilized FMT products is unknown. Several *in vitro* and *in vivo* engraftment studies are underway in veterinary medicine to investigate the bacterial viability, shelf-life, and clinical efficacy of lyophilized FMT capsules.

Fecal product storage after processing

Statement. After processing, fecal slurries can be stored at −20°C or −80°C for up to 6 months.

- Sealed syringes or conical tubes can be used to store the fecal slurries in aliquots of 50 to 100 mL.
- Fecal slurries can be filled into capsules and then frozen for later administration.

Quality of evidence: Low.

Comment. Although there is some evidence that storage at −80°C can better preserve bacterial diversity compared to −20°C, there is no evidence that this can impact bacterial viability [62]. This recommendation is based on the guidelines used for FMT in humans [9].

Preparation of fecal microbiota transplantation products for administration

Statement. Frozen FMT products should be defrosted in a warm water bath or warming cabinet (up to 37°C/98.6°F) for immediate use, or overnight at fridge temperature before administration to the patient.

Quality of evidence: Low.

Comment. Although defrosting with high temperatures (>40°C) might harm the bacteria, it is unclear if "fast thawing" in a warm water bath or "slow thawing" overnight might influence the viability or efficacy of FMT products. There is clinical evidence for successful FMT after "slow thawing" overnight at fridge temperature in dogs [20].

Statement. Once fecal slurry is thawed, it cannot be refrozen.

Quality of evidence: Moderate.

Comment. Freeze–thaw cycles have been reported to decrease survival of some bacteria [73]. Anecdotal evidence from preliminary results from members of the Companion Animal FMT Consortium supports bacterial degradation caused by thaw and freezing.

Patient preparation based on fecal microbiota transplantation product

Statement. *FMT capsules to be administered orally:* No special patient preparation is recommended. There is no evidence that fasting is required.

Quality of evidence: Low.

Comment. When using commercially available FMT capsules, adhere to manufacturer's instructions. The Companion Animal FMT Consortium declines to make any recommendations regarding patient preparation, as only few studies report on pre-FMT protocols in people [74] and none in veterinary medicine when oral capsules are used. Pretreatment reported include initiation, continuation, or discontinuation of either proton pump inhibitors or antibiotics before oral FMT. The Companion Animal FMT Consortium does not recommend pretreatment with either of those drug categories before any type of FMT.

Statement. *FMT slurries to be administered via enema:*

- Patients should be motivated to defecate before FMT enema administration.
- Cleansing warm water enema is optional.
- Sedation is usually not necessary but depends on patient temperament.
- There is no evidence that fasting is required.

Quality of evidence: Low.

Comment. In patients without diarrhea, the procedure can be scheduled according to the patients' routine of passing feces. It appears logical that engraftment of the FMT might be better if the colon is empty. In a meta-analysis from human medicine, poor bowel preparation was one of the factors associated with failure of FMT [75]; however, there are no data available for dogs and cats to date, and bowel cleansing was not performed in most available studies. Sedation is only recommended for very excited or anxious animals that are unable to tolerate an enema and will not remain quiet for a short period after FMT administration. Cats might require pregabalin or gabapentin for FMT administration; but if deeper sedation is required, general anesthesia should be preferred to enable protection of the cat's airways. Pretreatment with antimicrobials or proton-pump inhibitors is not recommended.

Statement. *FMT slurries to be administered via endoscopy:*

- No special patient preparation aside from normal endoscopic procedures.

Quality of evidence: Low.

Comment. In people, FMT are frequently delivered via duodenoscopy or colonoscopy [76], whereas this has been rarely reported in dogs [77]. In contrast, most of the veterinary studies have been using rectal enemas [18,20,67]. The Companion Animal FMT Consortium advises that there is limited evidence to make a recommendation on the use of endoscopy to deliver FMT in dogs or cats. However, if endoscopy is clinically indicated, FMT can be administered at the end of the procedure.

Statement. *FMT slurries to be administered via feeding tubes (nasogastric, esophagostomy, percutaneous endoscopic gastrostomy, and gastrostomy tubes):*

- No special patient preparation is recommended. There is no evidence that fasting is required.

Quality of evidence: Low.

Comment. The efficacy of FMT has been reported to be higher with intrarectal (enema) administration compared to other routes for recurrent *C. difficile* infection (rCDI) in people [78]; however, the FMT slurries might be administered orally or intragastrically. Noteworthy, FMT slurries have been given orally with the use of syringes in one canine study [79], but this practice could be associated with a risk of aspiration pneumonia. At this time, the Companion Animal FMT Consortium does not recommend administration of FMT products via feeding tubes.

Part 3: Fecal Microbiota Transplantation Clinical Applications and Dosing

In this section, general recommendations for FMT clinical applications and dosing are provided. Table 1 summarizes the current evidence available for clinical applications and dosing of FMT in companion animals.

Fecal microbiota transplantation indications

Statement. There is a high level of evidence for the use of FMT in acute parvovirus infection and other causes of acute diarrhea in dogs.

Quality of evidence: Moderate.

Comment. Hospitalized puppies with parvoviral diarrhea improved faster and had a shorter hospitalization duration when treated with FMT and standard treatment (eg, intravenous fluids and antimicrobials) as opposed to only standard treatment [67]. The parvovirus-infected puppies received 10 g of feces diluted in a 10 mL saline rectal enema within 6 to 12 hours of being admitted to the hospital. In a study comparing dogs with acute diarrhea treated either with FMT or metronidazole, dogs treated with FMT had a better improvement in fecal scores at day 28 than the metronidazole group [18]. In addition, dogs treated with FMT had an improvement in their DI, whereas dogs treated with metronidazole did not [18].

Statement. There is some evidence for the use of FMT in chronic enteropathy in dogs. The duration of the effect is variable.

Quality of evidence: Moderate.

Comment. In dogs with chronic enteropathies, FMT may be useful as an adjunctive therapy. It has been shown to decrease the Canine IBD Activity Index and Canine Chronic Enteropathy Clinical Activity Index [20,80]. While any dog with chronic enteropathy may respond to FMT, in one study those with a mildly elevated DI were more likely to respond [20]. This finding needs to be further evaluated in a prospective study; therefore, at this time, the Companion Animal FMT Consortium would recommend FMT for any dog with chronic enteropathy regardless of DI result.

Statement. There are anecdotal reports for the use of FMT in cats with acute or chronic enteropathy.

Quality of evidence: Low.

Comment. A cat with ulcerative colitis who failed treatment with conventional therapy responded to 2 rectal enema FMTs within 5 weeks [81]. There was gradual improvement in the stool quality over a 3 month period. The cat had normal feces at an 11 month follow-up.

Fecal microbiota transplantation preparations and technique

Statement. There is a high level of evidence that, despite impact on total and selected bacterial viability, aerobic processing, freezing, lyophilization, and encapsulation of fecal material does not negatively affect safety and clinical efficacy of FMT in people. These findings are likely to be translated onto small animal medicine.

Quality of evidence: Low.

Comment. The impact of aerobic stool processing on clinical efficacy of FMTs in people and small animals is unknown as almost all cohort and randomized clinical trials (RCTs) available in the literature are based on an aerobic homogenization technique. Recent meta-analyses in people with rCDI and Crohn's disease have failed to find any difference in clinical outcomes between FMTs using fresh versus frozen feces [82–84]. Similarly, recent open label single-group or controlled studies demonstrated noninferior safety and short-term clinical efficacy of fresh-frozen, cryopreserved, encapsulated frozen, or lyophilized feces compared to traditional nonoral delivery methods in people with rCDI [70,85]. These findings likely translate onto small animal medicine [86].

Fecal microbiota transplantation route of administration

Statement. There is a high level of evidence in people that the route of administration is not significantly associated with the outcome of FMT for the treatment of GI diseases including CDI, ulcerative colitis, and Crohn's disease. In companion animals, FMT has been administered orally and via rectal enemas, but no study has compared the efficacy of the different routes. Administration via rectal enemas is by far the most common route of administration in humans and companion animals.

Quality of evidence: Low.

Comment. In companion animals, FMT has been administered orally and via rectal enemas, but no study has compared the efficacy of the different routes. The most common route of administration in published studies to date is via rectal enemas in companion animals (see Table 1).

Patient preparation

Statement. There are no studies evaluating the effects of preconditioning the GI tract on patient outcomes including engraftment or improvement of clinical signs of intestinal disease in companion animals. In the absence of such evidence and to maintain good antimicrobial stewardship, the Companion Animal FMT Consortium discourages the use of antimicrobials prior to FMT administration if not otherwise clinically indicated.

Quality of evidence: Low.

TABLE 1
Summary of Studies and Anecdotal Reports Describing Techniques of Fecal Microbiota Transplantation in Dogs and Cats

Author	Species	Study Title	Study Design	Indication	Number of Animals, Frequency of FMT	Route	Technique
Burton et al, [106] 2016	Canine	Evaluation of Fecal Microbiota Transfer as Treatment for Postweaning Diarrhea in Research-Colony Puppies	RCT	Puppies at weaning age, postweaning diarrhea	11 puppies received FMT daily for 5 d, 12 received sham treatment	Oral	10 mL fecal suspension (100 g pooled dam feces mixed with 200 mL 2% fat cow's milk after filtration)
Bottero et al, [80] 2017	Canine	Faecal Microbiota Transplantation in 16 Dogs with Idiopathic Inflammatory Bowel Disease	Case series	IBD refractory to conventional treatment	16 adult dogs with severe, refractory IBD of >1 y duration. Oral treatment group received FMT q48–72h	Endoscopic/ oral	Donor feces were mixed with saline at a 1:1 ratio, filtered and mixed with low-fat yoghurt as enrichment solution. 60–80 g feces for dogs <20 kg BW, 100–150 g for dogs >20 kg BW
Pereira et al, [67] 2018	Canine	Fecal Microbiota Transplantation in Puppies with Canine Parvovirus Infection	Non-RCT	Parvovirus infection	33 received standard treatment, 33 received FMT in addition. FMT administered within 6–12 h of admission and q48 h thereafter	Rectal	Donor feces were mixed with saline at a 1:1 ratio. 10 g feces were administered per puppy
Niina et al, [86] 2019	Canine	Fecal Microbiota Transplantation as a New Treatment for Canine Inflammatory Bowel Disease	Case report	IBD refractory to antibiotic and immunosuppressive treatment over time	One 10 y old toy poodle	Rectal	Donor feces were mixed with lactated Ringer at a 1:1 ratio. The dog received approximately 3 g feces/kg BW. Nine treatments within 6 mo
Sugita et al, [107] 2019	Canine	Successful Outcome after a Single Endoscopic Fecal Microbiota	Case report	Intermittent large bowel diarrhea, 4 mo duration, feces positive for *C. difficile*	One 8 mo old French bulldog	Oral	30 mL fecal suspension (60 g feces diluted in 50 mL tap water after filtration) given orally.

		Transplantation in a Shiba Dog with Non-responsive Enteropathy during the Treatment with Chlorambucil		(PCR and toxins A and B)			Equivalent to approx. 2.5–3 g feces/kg BW
Chaitman et al, [18] 2020	Canine	Fecal Microbial and Metabolic Profiles in Dogs With Acute Diarrhea Receiving Either Fecal Microbiota Transplantation or Oral Metronidazole	Non-RCT	Uncomplicated acute diarrhea of <14 d duration	18 dogs; 11 dogs received a single FMT, 7 dogs received metronidazole 15 mg/kg q12 h for 7 d, 14 healthy control dogs	Rectal	2.5–5 g fresh feces per kg BW recipient, blended with 60 mL 0.9% NaCl until homogenous. For very large dogs a larger volume of saline may be needed to obtain sufficiently liquefied fecal solution
Gal et al, [77] 2021	Canine	One Dog's Waste is Another Dog's Wealth: A Pilot Study of Fecal Microbiota Transplantation in Dogs with Acute Hemorrhagic Diarrhea Syndrome	Case series/ uncontrolled clinical trial	Canine AHDS	8 dogs; 4 received a single enteral FMT, 4 received placebo	Rectal via colonoscopy	Donor feces were blended with sterile saline at a ratio of 1:4 and filtered through a sieve. 10–15 mL/kg fecal slurry was administered into the ascending colon during colonoscopy
Sugita et al, [108] 2021	Canine	Successful Outcome after a Single Endoscopic Fecal microbiota Transplantation in a Shiba Dog with Non-responsive Enteropathy during the Treatment with Chlorambucil	Case report	Refractory chronic enteropathy	8 y old male neutered Shiba Dog	Rectal via colonoscopy	100 g donor feces were dissolved in 100 mL saline. The solution was filtered through a gauze pad. 50 mL were administered during colonoscopy into the cecum and colon
Niina et al, [103] 2021	Canine	Fecal Microbiota Transplantation as a New Treatment for	Uncontrolled clinical trial	IBD (FMT as add on treatment)	9 dogs received a single rectal FMT	Rectal	3 g/kg donor feces were dissolved in Ringer's solution and filtered through a gauze pad.

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TABLE 1
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Author	Species	Study Title	Study Design	Indication	Number of Animals, Frequency of FMT	Route	Technique
		Canine Inflammatory Bowel Disease					10 mL/kg fecal slurry were administered rectally
Salavati Schmitz, [100], 2022	Canine	Observational Study of Small Animal Practitioners' Awareness, Clinical Practice and Experience With Fecal Microbiota Transplantation in Dogs	NA	Mixed	Summary of FMT practices performed by study participants (155 small animal practitioners)	Variable	Summary of practices: Volume of FMT: 5–50 mL/kg Total volume of FMT: 20–300 mL Weight of FMT: 2-5 g/kg Total weight of FMT in grams (often diluted in water or saline): 1–50 g
Cerquetella et al, [109] 2022	Canine	Case Report: Oral Fecal Microbiota Transplantation in a Dog Suffering From Relapsing Chronic Diarrhea—Clinical Outcome and Follow-Up	Case report	Relapsing chronic diarrhea (FMT as add on treatment)	6 y old male Labrador retriever, 5 capsules/10 kg body weight for 5 consecutive days	Oral	Frozen capsules (size #00) containing 650 μL fecal slurry
Marclay et al, [110] 2022	Canine	Recovery of Fecal Microbiome and Bile Acids in Healthy Dogs after Tylosin Administration with and without Fecal Microbiota Transplantation	RCT	Tylosin-induced intestinal dysbiosis	22 dogs, 10 control dogs (placebo treatment), 6 dogs received a single rectal FMT, 6 dogs received 2 FMT capsules PO q 24 h for 14 consecutive days	Rectal/oral	Rectal FMT: Donor feces were mixed with sterile saline at a ratio of 1:4, filtered, cryopreserved with glycerol at a final concentration of 10% and stored at −80°C for a maximum of 2 mo. Aliquots were thawed at 37°C water bath and 10 mL/kg were administered rectally Oral capsules: Frozen capsules (size #00) containing fecal sediment

Collier et al, [111] 2022	Canine	Investigating Fecal Microbial Transplant as a Novel Therapy in Dogs with Inflammatory Bowel Disease: A Preliminary Study	RCT	IBD (FMT as add on treatment)	13 dogs, 7 dogs received a single rectal FMT, 6 dogs received placebo	Rectal	Fecal samples from 5 donor dogs were pooled at a total of 50 g. Feces were blended with sterile saline at a ratio of 1:5, filtered using a sieve, stored in 60 mL catheter tip syringes at −20°C for a maximum of 3 mo. Recipients received 10 mL/kg using a rubber catheter
Alves et al, [112] 2023	Canine	Faecal Microbiome Transplantation Improves Clinical Signs of Chronic Idiopathic Large Bowel Diarrhoea in Working Dogs	RCT	Large bowel diarrhea (suspect stress-induced colitis)	30 large breed working dogs, 15 dogs received psyllium husk orally for 30 consecutive days, 15 dogs received a single rectal FMT	Rectal	50–60 g of fresh donor feces was blended with 250 mL of saline and filtered using a gauze. 60 mL of this slurry was rectally administered using a 60 mL catheter tip syringe and a 12 French red rubber catheter
Toresson et al, [20] 2023	Canine	Clinical Effects of Faecal Microbiota Transplantation as Adjunctive Therapy in Dogs with Chronic Enteropathies-A Retrospective Case Series of 41 Dogs	Case series/ uncontrolled clinical trial	Dogs with chronic enteropathy that failed prior conventional medical treatment	41 dogs received between 1 and ≥5 FMTs	Rectal	5–7 g/kg of recipient's body weight of fresh frozen feces. Feces was thawed 4–24 h in a fridge. 2–120 mL of sterile saline was added and the mixture was blended. Saline was added until a desirable consistency was reached (a consistency that could be passed through the syringe and rectal catheter with mild-to-moderate pressure)

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TABLE 1
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Author	Species	Study Title	Study Design	Indication	Number of Animals, Frequency of FMT	Route	Technique
Sugita et al, [79] 2023	Canine	Pilot Evaluation of a Single Oral Fecal Microbiota Transplantation for Canine Atopic Dermatitis	Clinical trial	Atopic dermatitis	12 dogs with atopic dermatitis, receiving a single oral FMT 20 healthy control dogs	Oral	60 g of feces were dissolved in 50 mL of tap water. Solution was filtered through medical gauze pad. 15–50 mL of this solution were administered orally using a syringe (equivalent to 2–12 g/kg of donor feces administered)
Lin et al, [113] 2024	Canine	Effects of Fecal Microbial Transplantation on Police Performance and Transportation Stress in Kunming Police Dogs	RCT	Effects of FMT on performance and transportation stress in Kunming police dogs	20 male Wolf Cyan Kunming puppies (45–55 d old) received oral FMTs daily for 14 consecutive days	Oral	Resuspended precipitates of FMTs were used at different dilutions
Rojas et al, [71] 2024	Canine	Microbiome Responses to Oral Fecal Microbiota Transplantation in a Cohort of Domestic Dogs	Uncontrolled clinical trial	Dogs with chronic diarrhea, vomiting, or constipation	54 dogs with chronic diarrhea, vomiting, or constipation	Oral	2 capsules containing lyophilized donor feces for 25 d given with food
Lee et al, [105] 2024	Canine	Safety Profile and Effects on the Peripheral Immune Response of Fecal Microbiota Transplantation in Clinically Healthy Dogs	Case series/ uncontrolled clinical trial	Healthy dogs	10 healthy dogs were treated with a single rectal FMT. AEs and effects on peripheral immune responses were observed	Rectal	Donor feces were mixed in a ziplock bag by kneading with 2.5 mL of nonbacteriostatic sterile saline solution per gram of feces and 30% glycerol to a final glycerol concentration of 10%. The fecal slurry was filtered through mesh sieves, and

							stored in 60 mL catheter tip syringes at −80°C for a maximum of 6 mo. FMTs were thawed in a warm water bath at 37°C and administered rectally using a red rubber catheter. Dogs received between 2.5 and 5 g/kg of donor feces (weight before processing/dilution) body weight
Vecchiato et al, [114] 2023	Canine	Fecal Microbial Transplantation Effect on Clinical Outcome and Fecal Microbiota and Metabolome in Dogs with Chronic Enteropathy Refractory to Diet	Uncontrolled clinical trial	Dogs with food refractory chronic enteropathy	20 dogs with chronic recurrent GI signs that failed a 2 wk dietary trial with hydrolyzed diet or homemade single protein diet—received 1–2 FMTs 2–4 wk apart	Rectal	2.5–5 g/kg donor feces (fresh: processed within 4 h from collection) mixed by hand in zip bag with 1:1 ratio of nonbacteriostatic sterile saline solution and filtered through fine kitchen sieve
Winston, [99] 2023, Unpublished data	Canine	Scientific and Clinical Assessment of Fecal Microbiota Transplantation to Enhance Weight Loss in Obese Dogs (SLIM Pilot Study)	Randomized, blinded clinical trial	Obese but otherwise clinically healthy dogs	19 obese dogs received a single induction dose (20 capsules; 5 capsules from each fecal donor), followed by once weekly maintenance dose (12 capsules; 3 capsules per fecal donor) for a total of 12 wk	Oral	Feces from 4 lean donors was processed by diluting feces with 1:4 nonbacteriostatic saline. The fecal slurry is filtered in a stomacher bag and double centrifuged. Glycerol is added to a 10% final concentration. Final fecal slurry was pipetted into size 0 delayed released capsules and double

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TABLE 1
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Author	Species	Study Title	Study Design	Indication	Number of Animals, Frequency of FMT	Route	Technique
							encapsulated in a size 00) gelatin capsule. Fecal capsules stored at −80°C
Furmanski et al, [81] 2017	Feline	First Case Report of Fecal Microbiota Transplantation in a Cat in Israel	Case report	Ulcerative colitis	10 y old female spayed Abyssinian cat, 2 rectal FMT enemas	Rectal	5 g of donor feces were blended with nonbacteriostatic sterile saline solution at a 1:6 ratio. The suspension as filtered through a strainer yielding a large particle-free slurry. 30 mL of fecal slurry were administered using a 60 mL catheter-tip sterile syringe with an 8 FR 2 way standard sterile balloon silicone-coated latex Foley catheter
Rojas et al, [72] 2023	Feline	Microbiome Responses to Fecal Microbiota Transplantation in Cats with Chronic Digestive Issues	NA	Chronic vomiting, diarrhea and/or constipation (FMT as add on treatment to standard care)	46 cats received daily oral FMTs	Oral	1–2 capsules containing lyophilized feces q24 h for until a minimum of 50 capsules were administered
Procoli, unpublished data, 2024	Feline	Cats with Food and Steroid Refractory Chronic GI Signs and Dysbiosis (Based on Fecal DI)	NA	Diarrhea, weight loss	5 cats	Rectal	2.5 g/kg fresh frozen donor feces mixed by hand in zip bag with sterile nonbacteriostatic saline solution at 1:2 ratio then filtered on a

							fine kitchen sieve and frozen with 10% glycerol at −20°C for max 3 mo. Max volume enema 25 mL. Administered after thawing at 37°C on day of procedure
Marsilio, unpublished data, 2024	Feline	NA	NA	Therapy-resistant diarrhea in kittens	5 kittens	Rectal	Donor feces are processed by removing litter, mixing feces with 2.5 mL/g of nonbacteriostatic saline and 30% glycerol to a final glycerol concentration of 10%. The fecal slurry is filtered through mesh sieves and stored in 60 mL catheter tip syringes at −80°C for a maximum of 6 mo

Abbreviations: BW, body weight; DI, dysbiosis index; FMT, fecal microbiota transplantation; IBD, inflammatory bowel disease; NA, not applicable; RCT, randomized clinical trial.

Comment. Preconditioning of the bowel refers to procedures or treatments to prepare the bowel for the administration of an FMT with the goal to improve engraftment and, by extension, the outcome of a patient. Preconditioning can entail fasting, bowel lavage, and treatment with antimicrobials. The European Consensus Conference on FMT in humans recommends preconditioning with oral antimicrobials before FMT administration for patients with rCDI only and with the goal to reduce the abundance of *C. difficile* [9]. However, preconditioning with antimicrobials has shown to negatively affect engraftment in patients with irritable bowel syndrome [87]. The most common indication for FMT in companion animals is currently chronic enteropathy, which is a distinctively different disorder than rCDI. Therefore, the Companion Animal FMT Consortium advises against preconditioning of the GI tract with antimicrobials, and their use should be strictly limited to situations where clinically indicated for other reasons. The effect of bowel lavages and cleansing enemas on FMT engraftment and patient outcome is unknown; therefore, the Companion Animal FMT Consortium does not recommend such procedures unless required for patient procedure preparation. To reduce residual fecal matter in the recipient prior to FMT and possibly prolong FMT retention, the Companion Animal FMT Consortium recommends fasting patients prior to FMT administration.

Fecal microbiota transplantation dosing

Statement. No evidence-based dosing regimen for administration of FMTs in any form (fresh, frozen, or lyophilized) or through any route (oral or rectal) can be provided at this point.

Quality of evidence: Low.

Comment. The techniques for FMT administration in the literature and among members of the Companion Animal FMT Consortium vary widely. For FMTs administered via rectal enemas, some FMT Consortium members prefer smaller volumes of concentrated fecal slurries to increase the retention time, while others prefer larger volumes to increase mucosal surface contact. There are currently no studies supporting either technique. To provide guidance, the Companion Animal FMT Consortium summarized doses and techniques that have previously been published and/or used by members of the FMT Consortium (see Table 1). It is important to note that the administration of rectal enemas may cause vomiting and subsequently aspiration, especially in cats. The effect is mostly volume dependent. Companion Animal FMT Consortium members routinely administer the following fecal slurry volumes via rectal enema to dogs and cats:

- Medium-to-large-sized dogs: 10 to 20 mL/kg body weight of the recipient
- Small dogs and cats: 5 to 10 mL/kg body weight of the recipient

Statement. While some preliminary data exist that FMT may be of value for some extra-GI diseases (eg, diabetes mellitus and obesity), the level of evidence or even anecdotal reports are scarce. The routine use of FMT for the treatment of extra-GI diseases cannot be recommended at this point.

Quality of evidence: Low.

Comment. It has recently been suggested that in complex extra-GI diseases, precise manipulation of microbes and microbial metabolism may be more tractable than modulating host physiology, in part, due to the plasticity of the microbial ecosystems [88]. The gut microbiota influences the pathogenesis of metabolic diseases like diabetes mellitus, metabolic syndrome, and obesity through mechanisms such as the production of bacterial metabolites (eg, short-chain fatty acids, secondary bile acids, and indole metabolites) that can compromise intestinal barrier integrity, promote chronic inflammation, and affect glucose homeostasis; however, these sequelae may be potentially reversible by FMT [89,90].

FMT studies in humans with type 2 diabetes mellitus have shown minimal clinical effects but significant shifts in gut microbial communities, indicating a complex relationship between the microbiota and metabolic health [91]. In type 1 diabetes mellitus (T1DM), FMT has been observed to prolong beta cell function, with microbial composition and certain biomarkers predicting the preservation of this function, highlighting the potential of microbiome modulation in the management of T1DM [92]. A meta-analysis and various clinical trials on metabolic syndrome and obesity have shown mixed short-term benefits of FMT, including improved hemoglobin A1c (HbA1C) levels and lipid profiles in some cases, but no consistent effects on obesity, illustrating the nuanced impact of FMT on metabolic parameters [93]. A small pilot, prospective, randomized, double-blinded, controlled veterinary clinical trial on diabetic dogs showed that FMT decreased water consumption and had a modest effect on host metabolism but did not change key diabetic indicators, expanding the interest in microbiome intervention in dogs [94].

For obesity, there are multiple human placebo controlled RCTs that highlight the important role that the gut microbiota play in obesity and metabolic disease and demonstrate that engraftment of lean microbes into an obesogenic gut ecosystem is possible [95,96]. Dysbiosis is noted in obese companion animals [97,98], indicating that microbial targeted intervention, such as FMT, may be beneficial. Currently in veterinary medicine, there are 2 ongoing clinical trials (SLIM studies) evaluating the scientific and clinical utility of FMT to enhance weight loss in obese dogs and cats. The SLIM studies are the first to evaluate the efficacy of FMT as an adjunctive therapy for canine and feline obesity management and will shed light on the role(s) that the gut ecosystem plays during treatment and recovery from an obesogenic disease state [99].

Fecal microbiota transplantation frequency

Statement. Repeated FMT treatments can be beneficial in dogs with chronic enteropathy, but the specific number of FMTs and the administration frequency are dependent upon individual patient factors.

Quality of evidence: Low.

Comment. In a survey for veterinarians assessing FMT practices in small animal patients, approximately two-thirds of participants reported that FMTs were routinely administered to patients more than once, yet the frequency of repeated administrations ranged from daily to every 2 weeks [100]. Repeated FMTs may improve engraftment and clinical response after the initial treatment in some dogs, particularly in patients with more severe dysbiosis. In a retrospective study examining the clinical effects of adjunctive FMT therapy in 41 dogs with chronic enteropathy, a median of 3 FMTs was administered to each dog via rectal enema, with most dogs receiving treatments at 10 to 20 day intervals [20]. Additionally in 74% of FMT responders, further clinical improvement was observed after receiving a second FMT, as compared to the first [20]. Factors such as clinical response to FMT, adverse effects, patient tolerance of the procedure, and client factors should be considered when determining the number and administration intervals of FMTs in individual patients.

Statement. Fewer total FMTs may be required for acute diarrhea in dogs, with one study in dogs with parvovirus infection requiring an average of 1.8 (range 1–3) transplants until improvement of diarrhea.

Quality of evidence: Moderate.

Comment. As in dogs with chronic enteropathy, the specific number of FMTs for dogs with acute diarrhea is dependent on the individual patient and is typically based on clinical response, adverse effects, patient tolerance of the procedure, and client-related factors. In a study of dogs with canine parvovirus infection, a mean of 1.8 FMTs were administered [67], as compared to a median of 3 FMTs reported in dogs with chronic enteropathy [20]. A single FMT dose has also produced positive clinical outcomes in dogs with acute uncomplicated diarrhea [18]. Thus, fewer total FMTs might be sufficient in dogs with acute diarrhea as compared to dogs with chronic enteropathy.

Statement. There are currently no data or reports available on the frequency of administration of FMTs for cats with acute or chronic enteropathy.

Quality of evidence: Low.

Comment. Data regarding the frequency of administration of FMTs in cats are not available. As in dogs, the number of FMTs and the frequency of administration should be determined on a case-by-case basis and based on the clinical response to initial FMT, adverse effects, tolerance of the procedure, and client-related factors.

Fecal microbiota transplantation retention times

Statement. No studies on the effect of retention time on the outcome of patients have been conducted in humans or companion animals. Members of the Companion Animal FMT Consortium are generally aiming for a minimum retention time of 30 to 45 minutes.

Quality of evidence: Low.

Comment. Improved engraftment of donated microbes is a theoretic benefit of prolonged retention of transplanted material, though a consensus recommendation for ideal retention time has not been established. For Companion Animal FMT Consortium members, a retention time of at least 30 to 45 minutes is targeted in dogs and cats receiving FMT via rectal enema, but defecation of the transplanted material prior to that time should not be considered a treatment failure. In one study of 41 dogs receiving an FMT via rectal enema, only one dog defecated within 30 minutes of the procedure [20]. The remaining dogs had an owner-reported minimum retention time ranging from 1 to 15 hours [20].

Patient sedation

Statement. Sedation should be considered on a case-by-case basis, with particular consideration given to patients that are anxious, aggressive, intolerant of the procedure, or unable to retain the transplant.

Quality of evidence: Low.

Comment. The decision to use sedation is made on a case-by-case basis and determined by patient temperament. No prospective, controlled studies have neither evaluated whether sedation prolongs retention time in dogs and cats nor evaluated how the use of sedation impacts overall efficacy of the procedure. The majority of the Companion Animal FMT Consortium members (67%) report never or rarely using sedation in dogs receiving FMT, whereas 40% report never or rarely using sedation in cats. For patients with severe colitis-associated rectal pain, local analgesia (eg, rectal suppositories containing a local anesthetic) could be considered if available and applicable to the patient.

Endpoints for fecal microbiota transplantation

Statement. Different endpoints for measuring the success of an FMT need to be considered including quality of life, clinical signs, and/or reduction or discontinuation of concurrent medication such as immunosuppressants or antimicrobials. Further studies are needed to assess biomarker-based endpoints such as the DI or other measures of the intestinal microbiota as tools to assess treatment success.

Quality of evidence: Low.

Comment. While the canine and feline DI might be helpful as biomarker to guide FMT-based therapy in individual cases, the DI does not normalize in every patient and/or long term despite improvement or resolution of clinical signs.

Statement. While FMT is generally considered a safe treatment and often helpful in a variety of primary GI diseases, it should be considered as part of a multimodal treatment approach rather than a sole treatment option.

Quality of evidence: Low.

Comment. While anecdotal reports of FMT as the sole successful treatment of acute or chronic GI disorders exist, most trials in humans and small animals have used FMT as an adjunct treatment in conjunction with other treatment modalities.

Adverse events for fecal microbiota transplantation

Statement. While there is a scarcity of data on adverse events (AEs) in dogs and cats, data in human medicine and reports in veterinary medicine show that FMTs are generally considered safe with few serious side effects reported even in immunocompromised patients (eg, dogs with parvovirus infection) and patients on immunotherapy (eg, corticosteroids).

Quality of evidence: Moderate.

Comment. In a 2021 metanalysis evaluating 9 high-quality studies from which data were collected for 756 FMTs performed in 388 patients for the treatment of *C. difficile* infection, the total pooled rate of AE was 39.3% with most AEs being mild (eg, self-limiting signs such as flatulence, abdominal pain, vomiting, bloating, nausea, constipation, headaches, dizziness, or fever) [101]. In a 2018 Cochrane review evaluating 4 studies with a total of 277 participants with ulcerative colitis, the authors noted that it was challenging to differentiate serious AEs (eg, aspiration pneumonia, bowel perforation, sepsis, or death) related to the FMT itself, the procedure involved with the delivery, or the underlying disease [102]. A total of 7% (10 out of 140) of FMT participants had serious AEs compared to 5% (7 out of 137) of control participants (RR 1.40, 95% CI 0.55–3.58; 4 studies; IO = 0%; low certainty evidence). A total of 78% (50 out of 64) FMT participants had mild AEs compared to 75% (49 out of 65) in the control group (RR 1.03, 95% CI 0.81–1.31; IO = 31%; moderate certainty evidence). As with the predisposition of chronic enteropathy, several factors likely play a role in the development of FMT-related AEs including the method of FMT administration, presence of comorbidities and immunocompetence, concurrent medications, the integrity of the gut mucosal barrier of the recipient, and the rigor of the fecal donor screening process.

Statement. The most commonly reported adverse effects (AEs) associated with FMT in both humans and companion animals include worsening of diarrhea, bloating, flatulence, abdominal pain, nausea, vomiting, and dysorexia. Rarely fever and dehydration have been reported in companion animals.

Quality of evidence: Moderate.

Comment. As described in the statement earlier, the majority of AEs associated with FMT administration

to humans is mild. In studies where FMT has been performed in dogs and cats [18,20,72,86,103], very few have reported AEs although it is unclear if a monitoring protocol was in place to detect AEs in all studies. In an unpublished, uncontrolled study, mild AEs (eg, fever, diarrhea, vomiting, inappetence, and abdominal pain) were described in a group of colony cats and cats with chronic enteropathy [104]. These signs were not observed in a study of client owned, healthy dogs where a monitoring system was in place to detect AEs [105]. Systematic controlled studies are needed to determine the prevalence of AEs in dogs and cats receiving FMT. Additionally, a unified standard for screening of donors for FMT administration to dogs and cats as well as a central repository for reporting FMT-related AEs are strongly recommended to help minimize FMT-related AEs and to better describe their occurrence in veterinary medicine. Until then, clients should be made aware of the possibility, albeit low, of FMT-related AEs.

SUMMARY

The gut microbiota is an intricate and complex ecosystem that has substantial impacts on the host during health and disease [115]. As demonstrated herein, dysbiosis has been noted in a variety of disease states in veterinary medicine and FMT should be considered as microbial-directed therapeutic. To increase accessibility of FMT to dogs and cats, establishment of a fecal donor program should be considered in any practice setting where FMT would be utilized on a regular basis. The Companion Animal FMT Consortium developed these clinical guidelines specifically to provide veterinarians with guidance for fecal donor selection and screening, standardized FMT preparations, and current recommended FMT clinical applications. These clinical guidelines are the first available to provide veterinarians with evidence-based statements to increase the accessibility of FMT as a microbial-directed therapeutic in veterinary medicine.

As we continue to acquire knowledge about the therapeutic potential of FMT in companion animals, rational decisions about how to manipulate gut microbial ecosystems given a specific dysbiotic state will become available. Aligned with the Companion Animal FMT Consortium's mission, to promote accessibility of FMT to veterinarians in diverse practice settings, the FMT Consortium plans to provide updated clinical FMT guidelines for dogs and cats every 5 years as new evidence for FMT emerges in small animal medicine.

CLINICS CARE POINTS

- A fecal donor program can readily be established in any practice type; thus, increasing the accessibility of FMT to dogs and cats.
- FMT processing and preparation can be modified based on the availability of equipment and resources. Ideally, fresh feces should be utilized for FMT whenever possible.
- Substantial evidence for use of FMT in patients suffering from canine parvovirus enteritis, canine acute diarrhea, and chronic enteropathy is currently available. FMT should be considered as an adjunctive therapeutic in these diseases. Active research into other clinical applications for FMT in veterinary medicine are actively underway.
- Although no specific FMT dosing can be recommended at this time, Table 1 provides an overview of FMT formulation, dosing, and frequency of administration based on the available veterinary evidence.

CONTRIBUTORS

The Companion Animal FMT Consortium chair (J.A Winston) planned all the virtual meetings and organized members of the consortium. All consortium members established the main topics. Working groups leads (J.S. Suchodolski, F. Gaschen, K. Busch, and S. Marsilio) orchestrated the development of topic statements. Once statements were finalized, each working group provided supporting evidence and drafted the text of commentary relevant to their statements. J.A. Winston wrote the initial draft of the article. All consortium members read and revised the article for important intellectual content and approved the final article.

DISCLOSURE

J.S. Suchodolski and M. Katherine Tolbert are employees of the Gastrointestinal Laboratory at Texas A&M University, which offers microbiome testing including the DI on a fee-for-service basis. Both authors refrained from contributing to the microbiome screening section of these clinical guidelines.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yasa.2024.06.006.

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