

## Review

# Equine Fecal Microbiota Transplantation: Possible Mechanisms and Future Perspectives

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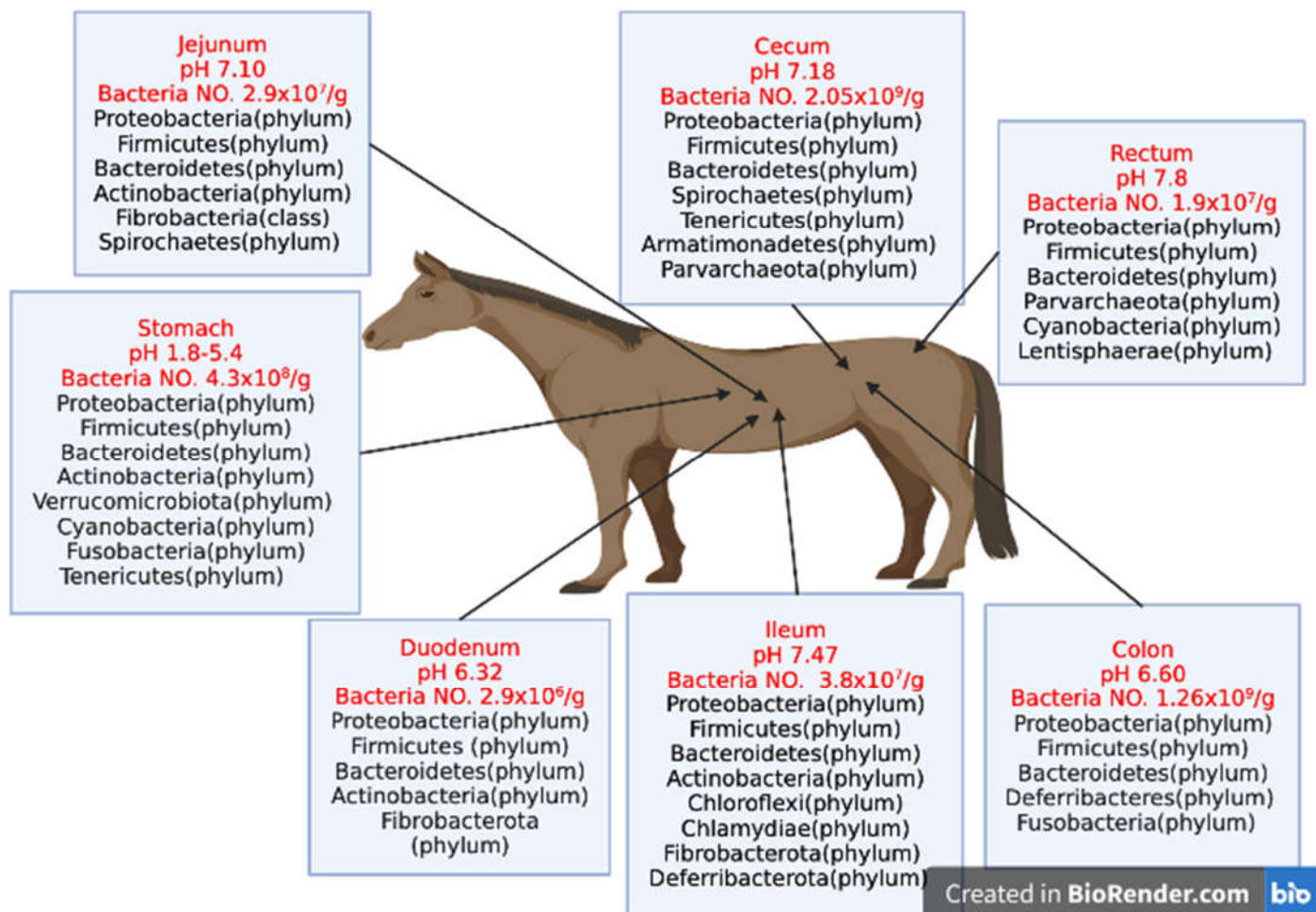
**Simple summary:** Fecal microbiota transplantation (FMT) is a newly adopted therapy in horses that holds promising results in treating various equine gastrointestinal issues, but its wider application is limited by safety concerns and limited understanding of its therapeutic mechanisms. This paper explores the potential mechanisms of equine FMT and discusses its future prospects, including more efficient and safer methods for selecting donor horses, improving FMT efficacy by choosing and pretreating recipient horses, proper stool storage, and its potential uses beyond gastrointestinal disorders, such as behavior modification and obesity treatment.

**Abstract:** Equine fecal microbiota transplantation (FMT) is an emerging therapy for restoring gut microbiome balance in horses. An imbalance in the gut microorganisms, known as dysbiosis, can cause inflammation and metabolic disruptions. FMT, which involves transferring gut bacteria from a healthy donor to a diseased recipient, has shown positive results in treating gastrointestinal diseases in horses, but is still largely limited to research purposes due to safety concerns and lack of understanding of its mechanisms. This paper aims to shed light on the possible mechanisms of FMT in horses and discuss future perspectives for its clinical application. Further research is needed to develop more effective and safer FMT techniques for horses.

**Keywords:** fecal microbiota transplantation; horse; gut microbiota

## 1. Introduction

The collective term for all microorganisms, including bacteria, fungi, and viruses, is referred to as "microbiota." Advances in culture-independent RNA-sequencing technology, such as 16S rRNA sequencing, and data analysis techniques have revealed that every part of the horse's body is home to a unique microbiota. For example, body sites, such as skin[1], gastrointestinal[2,3], respiratory[4], and reproduction tract[5,6], harbors certain microbiomes. To our knowledge, the gut microbiota of humans and animals has the most diverse microbial community. Bacteria, among them, are the most deeply and widely studied. In the past, bacteria were thought of solely as agents of disease, but recent research has shown they play a significant role in the host's physiology. The bacterial populations in the horse's digestive system vary depending on factors such as pH levels, gut motility, oxygen levels, and nutrient availability along the gastrointestinal tract[2] (Figure1).



**Figure 1.** Anatomy of gut microbiota distribution in the equine gastrointestinal tract, including bacteria type, number, composition, as well as ideal pH level (Figure created using data from Ericsson et al[2]). (Created with BioRender.com).

The equine digestive system is home to approximately  $10^{15}$  bacteria cells[7], consisting of over 108 bacterial genera[8,9] and 7 distinct phyla[3,10]. The most abundant bacteria belong to the *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* phyla, which are the most stable and dominant in the gastrointestinal tract of healthy adult horses[10–12]. The most abundant genera at the genus level are *Succinivibrio* and *Fibrobacter* in healthy horses[13].

The horse gut microbiota is used for food digestion and nutrient absorption[14]. The majority of microbiomes colonize in the cecum [15], where microbial fermentation takes place supplying 60-70% of energy for the horse body[16,17]. The gut microbiota has a strong connection to gastrointestinal illnesses, such as colitis[11], and diarrhea[18]. In addition, new evidence suggested that the gut microbiome can have an impact on health and disease beyond just the intestine by affecting host behavior and impacting other organs, due to its role in their functioning[19,20].

The horse gut microbiome is a highly intricate biological system, composed of archaea, bacteria, yeast, fungi, viruses, parasites, and protists[21–24]. Its precise architecture is hard to characterize completely because the gut microbiota has its own unique differences in individual horses although its uniformity happens among different groups of horses. This phenomenon also results in microbial communication that changes and keeps its relative balance based on individual and group horses. Because of such dynamic feature of equine gut microbiota, its status is usually described in temporal way [10,12]. Having the right composition and proportion of gut bacteria is crucial for its role in defending against pathogens and its involvement in various metabolic processes. The horse gut

microbiome is a dynamic and evolving system that changes throughout the animal's life and can be affected by various factors[25].

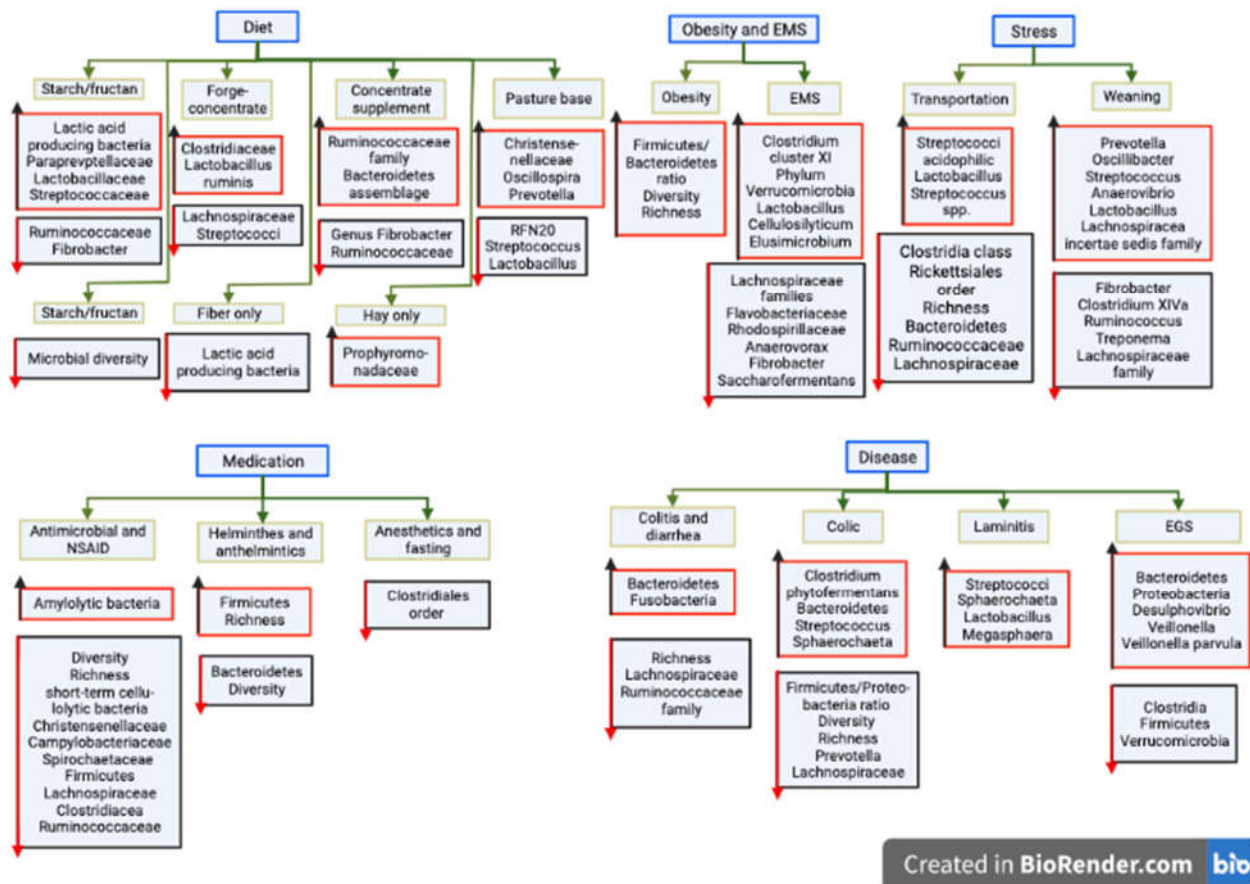
Diversity is an important parameter of healthy gut microbiota, including  $\alpha$ -diversity (diversity of species within a given sample) and  $\beta$ -diversity (measurement of similarity or dissimilarity of two communities). In terms of bacterial species, the equine gut microbiota is distributed throughout the different locations of the gastrointestinal tract, which is specialized to perform explicit function. In the stomach, microbial diversity depends upon the presence and absence of *Lactobacillus spp.*[26]. Compared to the colon bacterial community, the small intestinal microbiota (i.e., duodenum, jejunum, and ileum) is much simpler with medium pH level. The small intestine colonized by low number of commensal microbiota communities is also the main digestion site of protein, soluble carbohydrate, and fat[27]. Cecum microbiota is much more complex, highest number and diversity, than any other sections, and predominated by *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*. Its composition changes with dietary intervention[28]. In horses, it is hard to establish an ideal concept of healthy gut microbiota. However, in general, an ideal state of healthy equine gut microbiota is characterized by *Firmicutes*-dominated microbial profiles, followed by relative abundance of *Bacteroidetes*, *Proteobacteria*, or *Verrucomicrobia* [11,13,29]. It's important to note the interaction between the horse gut microbiome and the immune system, which is crucial in maintaining the animal's overall health. This relationship enables the body to distinguish between beneficial bacteria and coexist with them, while also protecting it from infections caused by opportunistic bacteria[30].

Fecal microbiota transplantation (FMT) aims to reconstruct a healthy gut microbiota after disrupted by medical intervention or harmful bacteria invasion, which has been widely studied since approved its usage for treating human *Clostridium difficile infections* (CDI) by the US Food and Drug Administration in 2013[31]. Our knowledge of FMT is far from complete, particularly in horses, although it is gradually becoming a medical option in equine clinics for treating gastrointestinal disorders such as colitis[32,33]. Studies showed that FMT helps restore microbial balance of the dysbiosis gut in horses via transplanting stool microbiota from a healthy individual into the gastrointestinal tract of a diseased patient, whose illness may be caused by a disorder associated with disruption of the intestinal microbiota[32].

Despite positive results, the main barriers to the widespread clinical use of FMT in horses are safety concerns and the difficulty of explaining the treatment's mechanisms to horse owners. Therefore, in this paper, we aim to discuss possible mechanisms and future perspectives of equine FMT to facilitate its therapeutic usage.

### 1.1. Factors influencing equine gut microbiota

There are many factors that can contribute to disruption of equine gut microbiota[25] (Figure 2). For example, diet (starch/fructose, forge-concentrate, concentrated supplements, and pasture-based management)[28,34–38], obesity and equine metabolic syndrome (EMS)[39–41], stress[9,42], medication (antibiotics, nonsteroidal anti-inflammatory drugs (NSAID) and anesthetics and fasting)[42–44], and diseases (colitis, and diarrhea, colic, laminitis, and equine grass sickness (EGS))[11,13,29,45–49].



**Figure 2.** Factors influencing equine gut microbiota, including diet, obesity/EMS, stress, medication, and disease (Figure created using data from Garber et al[25]). (EGS: Equine grass sickness; EMS: Equine Metabolic Syndrome). (Arrows, upward: increased relative abundance; downward: decreased relative abundance). (Created with BioRender.com).

Equine gut microbial community is also reported to be influenced by other factors including exercise[50], season[12], social interaction[51], breed[52], age[53], sex[54], and pregnancy[55].

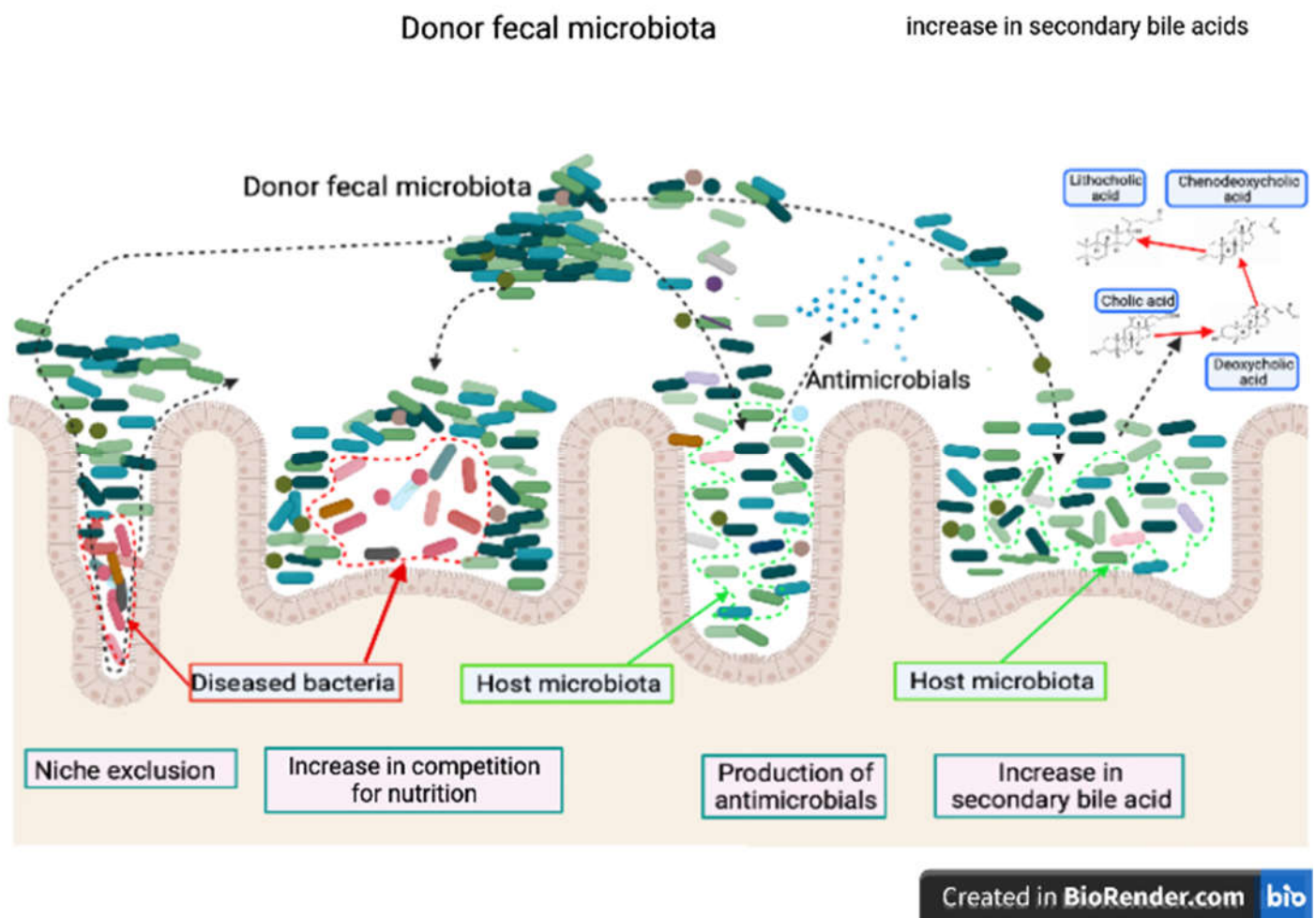
When the horse gut microbiome is disturbed by these factors, the diversity and abundance of normal gut bacteria decrease, particularly keystone bacteria that play a role in providing resistance against colonization. Impaired gastrointestinal wall would easily cause the colonization of diseases-leading bacteria and various disease reactions.

### 1.2. Mechanism of fecal microbiota transplantation in horses

The purpose of FMT is to reconstruct the disrupted gut microbiota and restore its composition and function. Gut microbiota in healthy horses is commonly composed of certain phylum and genus with a high-level of taxonomic and functional diversity. In healthy horses, most of these bacteria are beneficial and interact with each other and with other organs to support and maintain a healthy immune protection in the host.

Although the exact mechanism of FMT is still imprecise, four relevant hypotheses have been proposed (Figure 3): (1) niche exclusion, (2) increased competition for nutrition, (3) production of antimicrobials, and (4) increase in secondary bile acid.





**Figure 3.** Possible mechanisms of equine fecal microbiota transplantation. (Created with BioRender.com).

Competitive niche exclusion is a possibility for FMT's mechanism. The intestinal tract has limited space in the abdomen, causing the intestinal folds to maximize surface area and create niches. However, these niches provide a haven for pathogens and can render treatments ineffective. Healthy donor stool microbes can replace both healthy and diseased microbes in the recipient's gut, thereby occupying these niches.

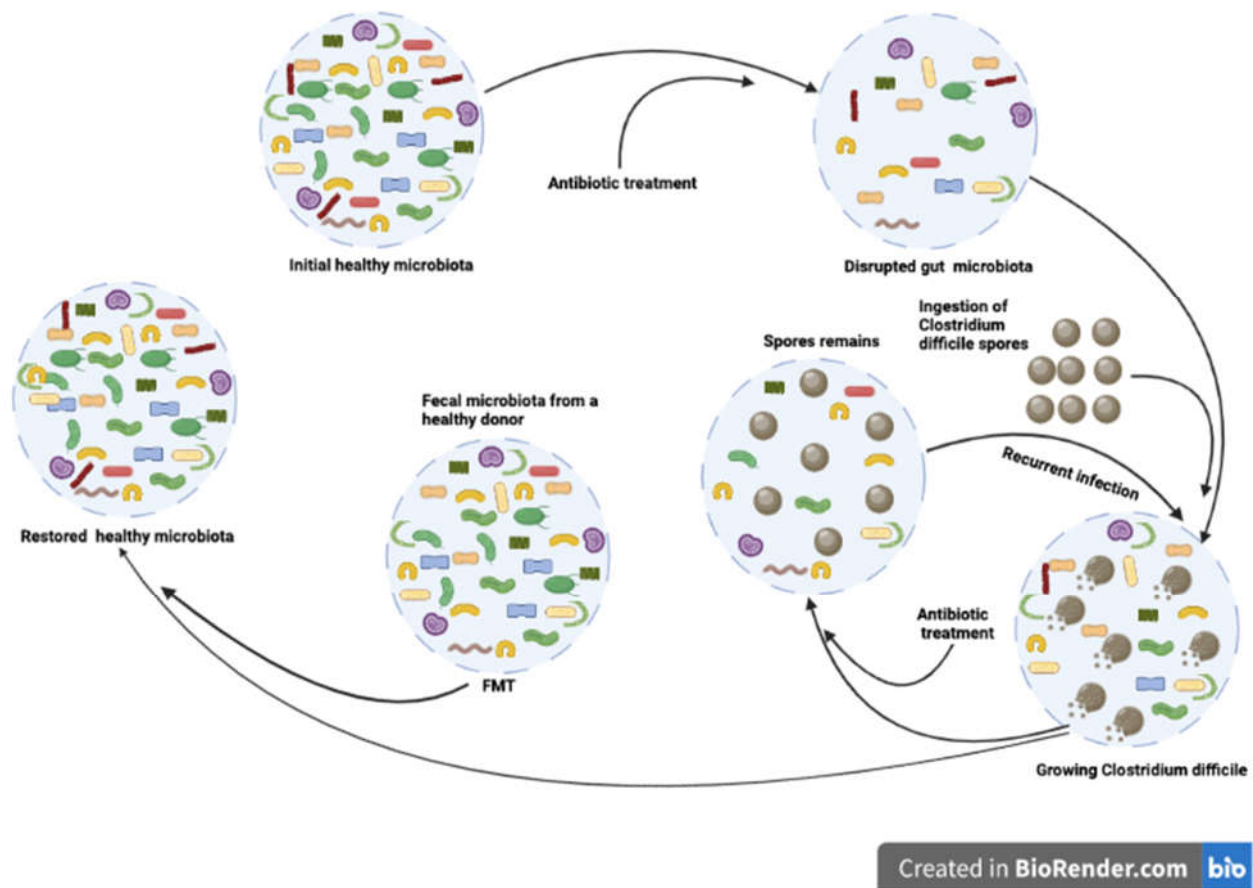
In human clinical settings, FMT is frequently used for treating CDI. During treatment, the donor's fecal matter not only interacts directly with the recipient's native gut microbiome, but also indirectly affects it by competing for nutrients with pathogens. For instance, the introduction of non-toxic *C. difficile* strains can lower the likelihood of CDI recurrence in patients[56]. Sometimes, the fecal microbiota strains from a healthy donor may outcompete the recipient's pathogenic bacteria for available nutrients. This mechanism is similar to competitive niche exclusion. Both of them are aimed to reduce surviving opportunities for pathogenic microbiomes.

Another potential mechanism of FMT treatment is increased production of antimicrobials [57]. This mechanism is also competition-based, and the interaction between the recipient and donor gut microbiota is the source of bacteriocin production [58]. Under the normal condition, the volume of bacteriocins produced by gut microbiota is sufficient for eliminating or deactivating pathogenic and opportunistic microorganisms. However, when the gut flora is disbalanced, the volume of bacteriocin production is diminished and unable to stop harmful agents from colonization and proliferation. Transferring healthy donor microbiota could increase the relative abundance of healthy gut microbiota as well

as bacteriocin production, and restore the dynamic elimination of pathogenic and opportunistic microorganisms.

Bile acids are crucial for gut metabolism, signaling, and the composition of the microbiome. The liver produces primary bile acids[59], but gut microbes modify them into various forms, including secondary bile acids, which have been associated with various diseases such as cirrhosis, inflammatory bowel disease, and cancer[60]. Increased secondary bile acid production is the last potential mechanism of FMT [61]. Transferring healthy donor microbiota can change the bile acid metabolism in the recipient related to the altered composition of gut microbiota [62]. One example of the impact of FMT on bile acids is the observed decrease in primary bile acids and increase in secondary bile acid production after FMT treatment for CDI. Studies have also shown that FMT can restore the *Firmicutes* phylum and secondary bile acid metabolism in CDI patients[63]. Both in vivo and vitro studies have demonstrated that increased production of secondary bile acid can prevent the germination and growth of *C. difficile* spores[64,65].

CDI is a well-known cause of acute diarrhea in both adult horses and foals[66–68]. While FMT has not been studied as a treatment for CDI in horses, its mechanism of treating CDI in human patients has been well researched. It serves as an illustration of the combination of exclusion of pathogens and increased competition for nutrients (Figure 4). Normally, a healthy intestinal internal environment is mostly populated with highly diverse and benign microbiomes. They can prevent pathogenic and opportunistic bacteria such as *C. difficile* from overgrowing. However, when the host is treated with antibiotics for various reasons, the gut microbiota become less diverse and result in a state of dysbiosis, which allows colonization of *C. difficile*. *C. difficile* is a spore-forming bacterium, when ingested via contaminated water or food, it arrives in the intestinal tract, proliferates and produces toxins which leads to CDI. However, common CDI treatment generally involves prescription of antibiotics such as metronidazole or vancomycin[69]. Such medicines could eliminate *C. difficile*, but its spores could remain in the intestinal tract, and result in rendering recurrent CDI (rCDI). In CDI and rCDI patients, transferring the stool material from a healthy donor to the recipient's intestinal tract can restore the normal gut flora both in composition and function.



**Figure 4.** An example of possible mechanisms of fecal microbiota transplantation for CDI treatment. (Created with BioRender.com).

### 1.3. Fecal microbiota transplantation in horses

FMT has gained popularity in veterinary clinical practices in recent years. However, studies about the application of equine FMT are only a few. So far, it has been used as investigational treatments for gastrointestinal diseases such as colitis and diarrhea.

Disruption of normal gut microbial communities has been associated with CDI and may be a main leading factor for the development of acute, undifferentiated and antibiotic-induced colitis in horses[70]. Although *C. difficile* is native to the large intestine in healthy horses[71], it becomes toxin-producing and disease-causing bacterium when the GI tract loses microbial balance. Diarrhea is a common symptom caused by CDI, as *C. difficile* can produce toxins to demolish the intestinal epithelium [72]. Standard treatment for CDI is antibiotic administration. However, it increases the risk factors for CDI and further leads to the development of rCDI[73,74]. FMT has been used for treating CDI in humans with promising results[75–78]. However, in equine medicine, relevant researches that evaluate the therapeutic efficacy of FMT on CDI recovery are few. According to a previous paper, fresh fecal transplantation was successful in a horse for treating severe CDI, and the stool consistency was returned to normal status after 12 h of post-FMT[79]. However, this was a review paper, not a controlled study. Therefore, further studies with control groups are needed for better evaluating the efficacy of FMT in treating equine CDI.

Colitis in horses is a leading cause of critical illness with an estimated fatality of 25.4% to 35%[80,81]. Without early medical intervention, colitis usually leads to severe complications such as laminitis, coagulopathy, and cardiovascular dysfunction[82]. Studies demonstrated that healthy equine gut microbiota has a significantly greater  $\alpha$ -diversity but lower  $\beta$ -diversity compared to the ones with colitis[83–85]. Although in most cases the exact cause of colitis remains unclear, gastrointestinal dysbiosis suggests that FMT

could be a manageable choice[86]. A recent study of five geriatric horses (> 20 years old) with colitis revealed that 3-consecutive-day FMT induced an increased relative abundance of *Kiritimatiella* (formerly classified as *Verrucomicrobia*)[87]. Moreover, at the end of the study, the fecal microbiota of treatment responders had a higher  $\alpha$ -diversity than prior to treatment and became phylogenetically more similar to that of their donor. Therefore, FMT could help restore the gut microbiota of horses with colitis and resolute diarrhea. However, the biggest limitations of this work were the small sample size and only 3 of the 5 horses responded to the treatment. In addition, this was not a controlled study. Thus, it may need larger case-controlled studies to ensure reliability of these results. In terms of a larger sample size, a study was conducted to better evaluate the FMT efficacy on equine colitis treatments[88]. This work enrolled a total of 22 horses with moderate to severe diarrhea, consistent with a diagnosis of colitis. FMT was performed on 12 horses in 3 consecutive days, while standard care (oxytetracycline, combination of penicillin and gentamicin) without FMT was managed on the rest. The results showed that in all colitis horses improved manure consistency was associated with a greater  $\alpha$ -diversity in fecal microbiota. In addition, compared with standard cared horses, FMT-treated patients demonstrated lower UniFrac distance (a distance metric used for comparing biological communities) which suggested greater normalization of the gut microbiota occurred in these horses. However, in this study the control group was collected from a different hospital, introducing potential bias to their results.

Similarly, several other studies also showed that FMT can improve symptoms of acute and chronic diarrhea in horses[89,90]. In conclusion, FMT may serve as a therapeutic option to reduce diarrhea severity in horses with colitis by improving diversity of gut microbiota.

However, it should be made clear to the horse owners that fecal transplant therapy may not work in some situations even though the symptoms are caused by intestinal dysbiosis. For example, Costa et al.[91], indicated that 7 days of FMT treatment in 6 horses with acute and chronic diarrhea was not sufficient for restoring the disrupted microbiota, where only 4 horses showed improved symptoms while the other 2 horses did not survive. In addition, a recent case-controlled study suggested that FMT failed to prevent Metronidazole induced dysbiosis in horses[92]. The biggest drawback in this study was the authors conducted FMT with metronidazole treatment, which could kill beneficial bacteria in the fecal solution. Therefore, based on clinical practices, we suggest conducting FMT treatment before or after 4-8 h of antimicrobial administration which could maximize the efficacy of fecal therapy.

#### 1.4. Risks and limits of equine fecal microbiota transplantation

Based on limited FMT studies in horses, coupled with promising results from human clinics, we might suggest that FMT is a safe choice for treating gastrointestinal disorders with little adverse effects. However, in addition to risk factors mentioned above[93], the medicinal safety concern in equine is the principal limitation of fecal transplant therapy as a lack of research, practical, duplicatable guidelines and appropriate guidelines. Another major concern in FMT therapy is possible to transmit opportunistic bacteria existing in the donor's intestine without provoking clinical symptoms[94], such as *E. coli*, *Salmonella*, and *C. difficile*.

Presently, the donor selection process is focused on safety by excluding as many risky elements as possible to obtain relatively 'healthy' fecal materials. The concept for healthy gut microbiota has never been defined, maybe never will. Now our main objective is to improve the treatment efficiency of FMT, however, as we rely too much on the donor, it is not always easy to control and anticipate the outcomes.

In addition, the equine gut microbiota also includes other microorganisms such as fungi and viruses, which may have an impact on FMT efficacy. For example, in humans, a previous study showed that fungi might have potential influence on FMT efficacy in



rCDI treatment[95]. However, the impact of fungi and viruses on efficiency of FMT treatment is an undetermined research area in veterinary science, and more studies are needed.

Also, there is no guarantee that FMT can treat all gastrointestinal disorders. A recent study documented a temporary effect worked when horses with Fecal Water Syndrome were treated with FMT [96]. However, it should be noticed that the main purpose of FMT is to restore a disrupted gut microbiota. It might not be a logical choice to use FMT for treating horse gastrointestinal diseases without dysbiosis. Therefore, the veterinary professionals should offer a detailed explanation of risks and limitations that may involve FMT treatment to the owners before the procedure.

## 2. Future Prospective

In veterinary medicine, the future of FMT in handling gastrointestinal disorders will be bright, particularly in equine clinical treatments. Horses are extremely subtle to altered gastrointestinal microbial environment and usually present symptoms such as diarrhea, colic or laminitis. Therefore, FMT has enormous potentiality due to its role in restoring the disrupted gut microbiota community to ameliorate such conditions with little adverse events.

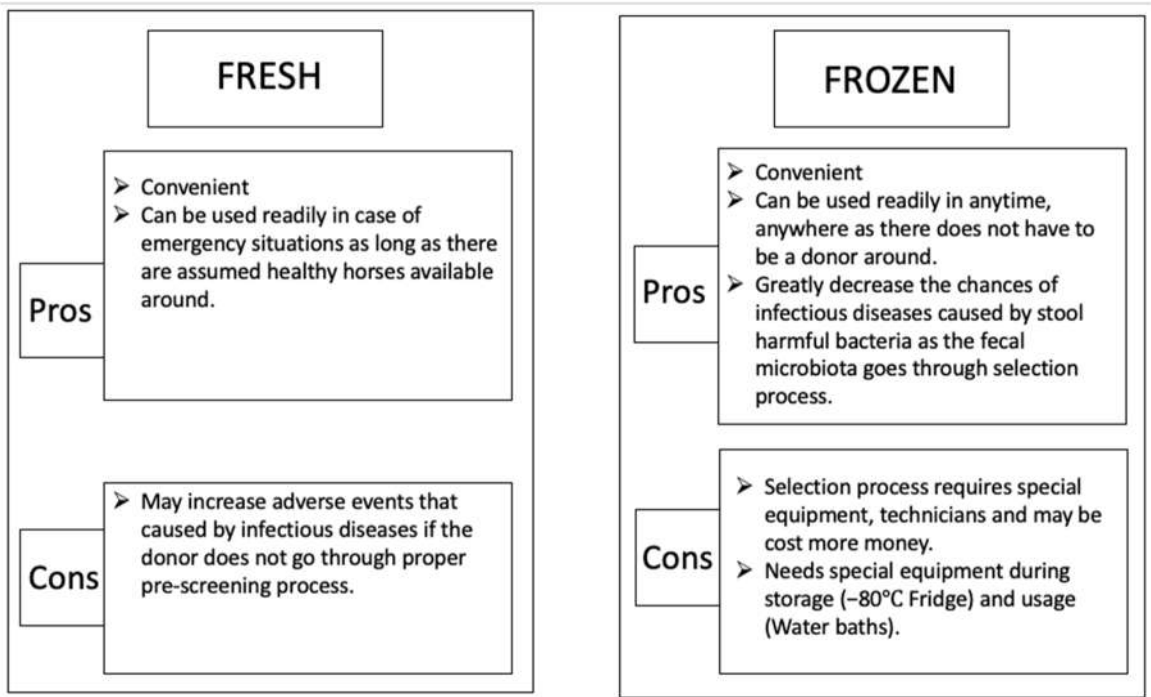
Some veterinary specialist proposed possible steps for conducting equine FMT in recent years based on studies and experience. We are now able to select specific donor's gut microbiota aimed at the recipient horse by 16S rRNA sequencing technique. For instance, a study suggested that FMT responders of colitis horses showed an increased relative abundance of *Kiritimatiella* after treatment[97]. This result indicated that transplanted stool with the highest relative abundance of *Kiritimatiella* might be more efficient for treating horses with colitis. In the future, studies could investigate such associations, a specific disorder and alternations in the intestinal flora before and after conducting FMT, which might significantly improve the efficiency of equine fecal transplant therapy.

Moreover, evidence indicated that gut dysbiosis can induce psychological status by influencing the gut-brain axis. Abnormal behaviors including stereotype actions, abnormal oral and locomotion, and aggressiveness were the most frequent in mentally stressed horses which could impacted by altered gut microbial composition[98]. Indeed, transplanting gut microbiota could transfer the psychological status of the fecal donor. For example, Kelly et al. indicated that experimental rats with depleted microbiomes in the intestine presented anxiety-like behavior by transferring fecal microbiota from depression patients [99], indicating that gut microbiota can transmit mental stress. It has also been reported that certain bacteria such as *Lactobacillus* and *Bacteroides* can alleviate stress and anxiety-like behaviors in mice[100], possibly by restoring specific bacterial metabolites.[101]. In addition, a recent review article in human studies indicated that a decreased depression and anxiety-like behaviors were observed after transplanting healthy fecal microbiota[102]. Although the authors did not discuss how long such therapeutic effects could last, FMT was effective in alleviating symptoms of psychiatric disorders. Equine studies have shown that the intestinal microbiota change dramatically after transportation and sports events[103,104]. Thus, the gut microbiome may serve as a therapeutic target for managing and preventing abnormal behaviors in horses. For example, creating a stool bank using feces from horses with normal physical and behavioral health, and then administering it to stressed horses, especially those involved in frequent sports events and transportations, could be a new approach to improve the wellbeing of horses.

Currently, the process of selecting a donor for FMT mainly focuses on excluding as many pathogens as possible for increased safety, but there is no standard agreement on how to choose a donor horse. Future studies or clinical applications may involve evaluating microbial diversity and a desirable ratio of *Bacteroidetes* to *Firmicutes*, which are signs of a healthy gut microbiota. In addition, behavioral evaluations are recommended in the donor selection process, as behavioral abnormalities tend to affect the composition of the fecal microbiota[105]. The criteria for donor screening may be expanded in the future if

new pathogens are found to disrupt the composition of healthy gut flora or can be transmitted through fecal transplantation.

The storage of fecal material is a critical aspect in FMT. In many cases of equine FMT, fresh feces are used directly for the procedure without testing for pathogens or drug-resistant bacteria, which could pose a risk to the recipient horse. Research in human studies has shown that frozen feces can be just as effective as fresh ones[106]. This has greater implications in veterinary practices, as the use of pre-screened, readily available frozen feces is both cost- and time-effective, and safer than using fresh feces. The use of frozen feces in horse FMT can overcome geographical limitations, making it more widely available in equine clinical practices (Figure 5). However, it's important to note that the feces must be stored in appropriate conditions. Regular freezing condition (e.g., -20°C) could impair the viability of the microbial population[107].



**Figure 5.** Pros and cons of using fresh or frozen stools for fecal microbiota transplantation.

When creating a stool bank for equine FMT, several important factors should be taken into consideration. Strict screening and selection of donor horses should be performed, including stool microbial culture analysis, antibiotic resistance testing, and necessary hematological exams to minimize risk. Stool banks not only save time and make FMT easier to perform in equine clinics and ranches, but also reduce costs for donor selection and stool handling. Additionally, detailed information about the donor horses should be recorded[108], allowing for easy tracking during and after the FMT procedure and ensuring the safety of the stool bank samples. Therefore, establishing an equine stool bank may be a preliminary groundwork for FMT application in the future.

Unlike other animals, horses are adored for their sports abilities such as jumping and running. New evidence has indicated that gut microbiota plays an important role in human performance ability[109,110]. Results showed that higher relative abundance of lactic-utilizing bacteria in the gut is related to better sport capacity. Although there is a lack of such studies, an in vitro study identified that lactate-utilizing bacteria are present in the equine gut microbiota community[111]. While gut microbiota is reported to be not an indicator predictor of horses in endurance races[112], it is possible that lactic-utilizing bacteria colonized in the intestine can enhance equine performing ability. Therefore, establishing a stool bank using samples from high performance athlete horses rich in these bacteria may be used as a natural stimulator in sports events. Obesity is a growing health

issue in horses, as it is linked to metabolic disorders like insulin imbalances, high lipid levels, and laminitis[113–115]. Studies have shown that gut microbiota can change in overweight horses after weight loss[116], leading to a significant increase in the alpha-diversity of their fecal microbiota. Given these findings and the impact gut microbiomes have on fitness, using lean horse feces, selected based on Body Condition Score (BCS), as a treatment option for weight loss in overweight horses may be a safe and cost-effective approach.

In equine clinics, the recipients are usually not subjected to any pretreatment during FMT process. However, results from human and mice studies showed that antibiotic pretreatment may enhance FMT efficacy[117–119]. This is due to commensal bacteria in the gastrointestinal tract acting as a protector that stops other microbiomes from residing[120]. Antibiotic treatment prior to FMT is aimed to alter the gut microbiota in recipients to increase colonization efficacy by disrupting the colonization resistant barriers that are provided by the receiver's indigenous gut bacteria. However, using antibiotics in horses is very dangerous and leads to severe conditions such as colitis[121–124], diarrhea[125,126], colic[127], laminitis[128], etc. Hence, researchers found a potential alternative for eradicating the recipient gut microbiome in horses: polyethylene glycol (PEG 4000). One study demonstrated that administering 40 ml/kg of PEG was effective in cleaning the bowel in human subjects[129]. Another recent study revealed that giving 425 g/l of PEG through oral-gastric gavage at 20-minute intervals could empty the intestine and decrease the microbiome by 90% after four consecutive bowel cleanings in mice[130]. Although FMT has already been successful in treating horses without pre-treating the recipient's gut microbiome, it may still be worth exploring the efficacy of PEG in equine FMT as it can increase its effectiveness by reducing the need for repeated treatments, which would greatly improve equine welfare.

### 3. Conclusion

FMT is a promising treatment option for treating gastrointestinal microbiota related disorders in equine clinics and has been used for digestive tract disorders including colitis and diarrhea. Successful FMT is reliant on selecting the most proper donor candidate and the best content of stool materials that plays a crucial role as a regulator otherwise disrupted gut microbiota in the patients. In horses, FMT is becoming a potential choice of treatment, understanding how FMT works is an urgent demand for both veterinary specialists and horse owners. For this regard, we described possible mechanisms of equine FMT to deepen our understanding and to facilitate its therapeutic usage as well as future perspectives that may help direct equine FMT related studies.

The future of FMT in equine will be bright with an increasing number of medical workers and owners preferring to use it as a primary therapeutic option as microbiome modulation and manipulation are the minimum aggressive treatment compared to other methods such as antibiotic intervention. An evidence-based systematic and practical procedure, and a specific disorder of the recipient (a disease-based approach) that defines 'healthy' donor gut microbiota in different diseases may also increase FMT efficacy and reduce potential adverse events.

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