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The equine intestinal microbiome

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Abstract

The equine intestinal tract contains a complex microbial population (microbiota) that plays an important role in health and disease. Despite the undeniable importance of a 'normal' microbiota, understanding of the composition and function of this population is currently limited. As methods to characterize the microbiota and its genetic makeup (the microbiome) have evolved, the composition and complexity of this population are starting to be revealed. As is befitting a hindgut fermenter, members of the Firmicutes phylum appear to predominate, yet there are significant populations of numerous other phyla. The microbiome appears to be profoundly altered in certain disease states, and better understanding of these alterations may offer hope for novel preventive and therapeutic measures. The development and increasing availability of next generation sequencing and bioinformatics methods offer a revolution in microbiome evaluation and it is likely that significant advances will be made in the near future. Yet, proper use of these methods requires further study of basic aspects such as optimal testing protocols, the relationship of the fecal microbiome to more proximal locations where disease occurs, normal intra- and inter-horse variation, seasonal variation, and similar factors.

Keywords: microbiota, microbiome, gastrointestinal

Introduction

Horses are hindgut fermenters with a unique gastrointestinal tract anatomy compared to other domestic animal species. As a non-ruminant herbivore, the intestinal tract relies heavily on the colon and cecum and their activity as fermentation chambers, where fibrolytic bacteria produce short chain fatty acids that account for 65% of the horse's energy production (Al Jassim and Andrews, 2009). Gastrointestinal disease is a leading cause of morbidity and mortality in horses, and a wide range of clinical conditions has been ascribed to alterations in the gastrointestinal microbial population (microbiota), including various types of colic, colitis, and laminitis. However, the complex workings of the gastrointestinal microflora, and its interaction with the local and systemic immune responses and equine host as a whole are poorly understood. It is likely that gastrointestinal microflora alterations can be manifested in a wide range of clinical conditions.

Despite the importance of the intestinal microbiota in horses, it has received limited study, particularly in comparison to ruminants. Much of the available research in horses has focused on hindgut fermentation (Collinder *et al.*, 2000; Varloud *et al.*, 2007; Respondek *et al.*, 2008; Muhonen *et al.*, 2009), yet even with these studies, there is only limited understanding of the microbial population structure and function.

Although understanding of the equine intestinal microbiota has been limited, it is hard to deny that it plays a critical role in health and disease. Defining and studying those specific roles has been a challenge because of the complexity of the population and the inherent limitations of traditional methods for assessment of microbial populations. Recent advances in culture-independent microbial identification methods and bioinformatics are opening the door to a revolution in our understanding of

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Table 1. Estimates of the bacterial population of the equine intestinal tract

Method	Organism(s)	Site	Log_{10} CFU/g	Reference
Culture	Total anaerobes	Colon	7.7	Muhonen *et al.* (2009)
	Lactobacilli		5.9	
	Streptococci		5.8	
	Total anaerobes	Colon	6.8–7.4	Respondek *et al.* (2008)
	Lactobacilli		5.5–6.7	
	Streptococci		5.7–6.4	
	Total anaerobes	Stomach	4.76–8.82	Varloud *et al.* (2007)
	Lactobacilli		<1–7.74	
	Streptococci		<1–7.73	
	Total aerobes	Feces	8.64	Garrett *et al.* (2002)
	Total anaerobes		8.56	
	Total bacteria	Duodenum	6.4	Mackie and Wilkins (1988)
		Jejunum	7.5	
		Ileum	7.6	
	Total bacteria	Stomach	9.1	de Fombelle *et al.* (2003)
		Jejunum	8.7	
		Cecum	7.7	
		Feces	8.5	
	Total bacteria	Feces	11.5	Furet *et al.* (2009)
Quantitative real-time PCR	Total bacteria	Cecum	11	Milinovich *et al.* (2008)

the equine intestinal microbiota; the collection of trillions of micro-organisms, their genomes (the microbiome) and their interaction with their environment. These advances have the potential to lead to unprecedented depth and breadth of information about the nature of the intestinal microbiome, factors that influence the microbiome, the clinical relevance of microbiome changes and ultimately ways to harness the intestinal microbiome for prevention or treatment of disease.

Composition of the equine intestinal microbiota

Culture-dependent assessment

Initial studies assessing the equine intestinal microbiota relied on bacterial culture, despite an assumption that a significant percentage of organisms was unculturable using standard techniques. Bacterial culture has been used to provide an overall assessment of the intestinal microbiota and for targeted assessment of specific populations such as coliforms, lactobacilli or streptococci (Muhonen *et al.*, 2009). Some of the earliest studies of the equine intestinal microbiota used culture to evaluate the impact of exogenous factors such as carbohydrate overload, where an increase in lactic acid bacteria (LAB) and decrease in Gram-negative bacteria was associated with the onset of laminitis (Garner *et al.*, 1978).

Assessment of the reported total microbial counts in culture-based studies (Table 1), which are typically many logs less than the true estimated microbial population, demonstrates a major weakness in reliance on culture data, since culture can only detect a small fraction of the microbiota. Providing detailed assessment of diverse bacterial populations is also challenging with culture, making it difficult to know whether the inability to detect changes or differences in the microbiota truly reflects a lack of differences between samples or populations or limitations in test sensitivity. For example, trimethoprim-sulfadiazine administration was reported to have 'very little' influence on fecal streptococci, *Bacteroides* and *Veillonella* counts, with a concurrent 10-fold decrease in coliform counts (Gustafsson *et al.*, 1999). Another study reported no impact of trimethoprim-sulfadiazine, but large increases in coliforms, *Bacteroides, Clostridium perfringens,* and *Streptococcus* spp. after administration of oxytetracycline (White and Prior, 1982). Given the role of antimicrobials in colitis in the horse and the known impacts of antimicrobials on the microbiota in other species, culture-based studies reporting no impact of an antimicrobial must be interpreted with caution.

The impact of dietary supplementation has been similarly assessed, with significant changes in total anaerobes, lactobacilli, and streptococci identified in response to supplementation with short chain fructooligosaccharides (Respondek *et al.*, 2008).

Some insight can be obtained from culture-based studies, and changes detected by culture certainly indicate significant changes in the microbiota. Yet, limitations need to be considered and there are few (if any) situations where culture provides optimal data for microbiota assessment. Culture can at best provide a superficial overview of selected components of the microbial population and at worst, be misleading. Culture is perhaps best reserved for targeted detection of specific, known, culturable organisms, particularly those that are present at low abundance and where enrichment culture may be superior to newer broad range molecular methods.

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Toward a new era: use of non-culture-dependent methods

Given the problems cultivating and identifying many gastrointestinal micro-organisms, as well as limitations in throughput for conventional culture methods, evolution of testing methods led to the application of a variety of non-culture-dependent approaches. There is currently no Gold Standard for microbiota assessment and available tests all have inherent advantages and limitations, as have been discussed elsewhere (O'Sullivan, 2000; Sekirov *et al.*, 2010). Despite some limitations, the evolution of culture-independent testing methodology has greatly expanded our understanding of the microbiota.

Some studies have used a hybrid approach, with preliminary culture followed by identification of organisms using Sanger sequencing. For example, selective LAB culture followed by 16S rRNA gene cloning and sequencing was used to assess the diversity of LAB in equine feces (Willing *et al.*, 2009), identifying *Streptococcus bovis, Streptococcus equinus*, *Lactobacillus salivarius,* and *Lactobacillus mucosae* as the most common species. This approach provided more information about LAB diversity, yet only investigated a specific culturable group of organisms and is therefore inadequate for broader assessment of the microbiome.

To avoid problems with loss of fastidious organisms or those that are unculturable using conventional approaches, molecular methods completely independent of culture have been increasingly used. Sequencing of 16S rRNA gene PCR products without prior enrichment culture can provide a broader assessment of the microbiome by detecting organisms that do not grow in selected enrichment broths, yet equine studies have been limited. One study evaluated 272 sequences from four intestinal compartments of five grass-fed horses (Daly *et al.*, 2001). Ninety-two percent of bacterial species belonged to two major phyla, namely the low G-C% Gram-positive bacteria (Firmicutes) (72%) and the *Cytophaga/Flexibacter/Bacteroides* (CFB) group (20%). As part of a terminal restriction fragment length polymorphism (TRFLP) study, small clonal libraries were created from PCR products, but only 67 sequences from a combination of six horses were evaluated (Willing *et al.*, 2009). Few conclusions can be obtained from such a small dataset, but there were similarities with the earlier study, notably a predominance of the Firmicutes phylum, which accounted for 73% of sequences from horses on one diet and 46% on another. The Bacteroidetes phylum was less common but accounted for up to 49% of sequences. While studies involving cloning and sequencing of 16S rRNA gene PCR products started to provide important, novel information, limitations in throughput with cloning-based methods hamper detailed assessment of the microbiome. In addition, lack of clear information about quality control screening of sequences (e.g. chimera checking) and limited phylogenetic analysis impact on the depth of information these initial studies provided; however, they started to provide novel depths of research to help understand the complex equine intestinal microbiome.

A different approach to evaluating PCR products is denaturing gradient gel electrophoresis (DGGE). This method provides information to compare samples or populations, but is limited in the scope of information it can provide and is rather subjective. DGGE has also been used to assess LAB diversity (Endo *et al.*, 2007, 2009), with one study reporting significant differences in LAB between horses, with relative stability of the LAB population within horses over time (Endo *et al.*, 2007). DGGE assessment of 16S rRNA gene PCR products was used to assess the effects of penicillin or anesthesia on the fecal microflora, reporting inter-horse variation with little intra-horse variation, even following antimicrobial therapy or treatment (Grønvold *et al.*, 2010). The failure to identify an effect of penicillin was surprising and raises questions about whether there are truly few discernable effects of penicillin and anesthesia, or whether the DGGE method that was used was of limited ability to detect differences. An additional limitation of DGGE is that while it may be able to detect differences between samples, characterizing those differences and determining what accounts for them may be difficult or impossible. Thus, while DGGE is able to determine whether there are differences between samples, it provides limited information about what those differences actually represent.

While more limited in scope, fluorescent *in situ* hybridization (FISH) can provide more specific information in some circumstances. The ability to specifically target organisms can allow for increased sensitivity of evaluation, particularly with low-abundance bacteria. This was shown in a study of the equine stomach that reported significant differences in *Streptococcus* spp. distribution in different stomach regions, while high throughput sequencing did not identify any differences between regions (Perkins *et al.*, 2012). FISH has also been used to assess changes in the cecal microbiota in oligofructose-induced laminitis, where a proliferation of *Streptococcus lutetiensis* was identified prior to the onset of laminitis with shifts in lactobacilli and *Escherichia coli* occurring after the onset of disease (Milinovich *et al.*, 2008). This suggested a causative role of *S. lutetiensis,* with incidental or secondary changes in those other populations. Somewhat similar data were obtained in another study of oligofructose-induced laminitis, with proliferation of *S. bovis/equinus* complex in cecal fluid and feces before the onset of laminitis (Milinovich *et al.*, 2007). Thus, FISH can be particularly useful when a specific group is being targeted, but is of less use for broader characterization of the microbiome.

Another 16S rRNA gene method that has been used is TRFLP, although results have been variable. Willing *et al.* (2009) used TRFLP to evaluate the impact of high forage or high carbohydrate diets on the fecal microbiome

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Table 2. Phylum distribution in studies of the equine fecal microbiome as assessed by next generation sequencing of 16S rRNA gene PCR products, excluding unclassified sequences

Reference	Shepherd *et al.* (2012)	Costa *et al.* (2011)
Method	V4 region	V3–V5 region
Firmicutes	70%	64%
Bacteroidetes	5.9%	14%
Proteobacteria	6.0%	12%
Actinobacteria	3.4%	4.0%
Spirochaetes	NR	3.4%
Verrucomicrobia	6.6%	NR
TM7	2.9%	NR

NR, not reported.

(Willing *et al.*, 2009). Greater microbial stability with reductions in LAB and *S. bovis/equinus* complex was identified in horses on a forage-only diet. This study also showed significant variation between horses, similar to the DGGE-based study of the impact of penicillin and anesthesia (Grønvold *et al.*, 2010).

Quantitative oligonucleotide hybridization can also be used to assess selected bacterial communities. While unable to characterize the microbiome in depth, this method can provide relative quantitative data regarding specific targeted populations, such as the predominance of Spirochaetaceae (15.6%), *Cytophaga–Flexibacter–Bacteroides* assemblage (14.5%) and *Eubacterium rectale–Clostridium coccoides* group (14%) that was reported in healthy horses (Daly and Shirazi-Beechey, 2003). This method has also been used to assess the influence of diet on the relative abundance of certain microbial populations. Significant differences were identified between horses on different diets, with increased abundance of Clostridiaceae cluster XIVa (Lachnospiraceae) and *Bacteroidetes* assemblage (*Cytophaga–Flexibacter–Bacteroides*), and decreased abundance of *Fibrobacter* spp. and Ruminococcaceae in concentrate-fed horses versus grass-fed horses (Daly *et al.*, 2011). The increased abundance can be explained by saccharolytic properties of Lachnospiraceae, which would logically lead to their increased abundance in horses fed higher carbohydrate diets. Similarly, as *Fibrobacter* spp. are obligate fibrolytic organisms, decreased abundance in horses fed a higher concentrate diet is understandable.

Quantitative real-time PCR can be used to quantify the entire microbial population (without differentiation) or selected bacterial populations. This method was used in a study of the effect of penicillin on the fecal microbiome, with no significant differences identified, perhaps in part because of the small sample size (Grønvold *et al.*, 2010). However, various changes in the proportions of *Bacteroides*-like, *C. perfringens*-like, and *Enterococcus* spp. groups were identified. Furet *et al.* (2009) used this method to compare the fecal microbiota of different animal species, focusing on selected *Clostridium* clusters, LAB, coliforms, and enterococci. The main advantage of this method is probably the ability to provide an assessment of overall bacterial counts and to target specific low-abundance organisms that might be overlooked in broad range 16S rRNA gene sequence-based studies.

The next generation: high throughput sequencing

Availability of next generation sequencing and refinement of bioinformatics platforms has revolutionized the study of gastrointestinal microbiology. Studies have been performed in a wide range of animal species, with unprecedented depth of study and the first true ability to understand the composition of the complex and critical microbial populations. While such studies are now being performed and reported in horses, the scope of published research is still rather narrow and some published data have limitations. Regardless, these preliminary studies unsurprisingly demonstrate the complexity of this microbial environment.

The first published equine study evaluated the fecal microbiome of grass-fed horse that were presumably clinically normal, although no clinical data or information about other factors that might affect the microbiome (e.g. recent antimicrobial exposure) were provided (Shepherd *et al.*, 2012). Further, only two horses were studied and these were pooled, providing no information about inter-horse variation and limiting extrapolation to the broader horse population. The results are also somewhat questionable since no chimera checking or other post-sequencing filtering was reported, something that raises concerns since the authors reported 38% of sequences unclassified at the Phylum level. Rather than truly novel phyla, these likely represent chimeras or sequence artifacts. Regardless, some general information can be obtained by disregarding the unclassified sequences and focusing on high taxonomic (i.e., phylum) level data. The most notable finding was the predominance of the Firmicutes phylum (70% of sequences after unclassified organisms were removed, Table 2), which was consistent with earlier non-culture dependent studies (Daly *et al.*, 2001; Willing *et al.*, 2009). No other phylum accounted for more than 7% of sequences.

Broader assessment of the fecal microbiome of healthy horses and horses with idiopathic colitis has more recently been reported, as part of a study evaluating 6 normal and 10 diarrheic horses, and a total of greater than 195,000 sequences (Costa *et al.*, 2011). As with the above-reported study, the Firmicutes phylum predominated (Table 2); however, other major phyla (particularly Bacteroidetes) were represented in greater abundance than reported above. There were profound differences in the fecal microbiome between groups, with normal horses having significantly greater relative abundance of Actinobacteria and Spirochetes and diarrheic horses having significantly more Fusobacteria. An interesting

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finding was greater abundance of the Clostridiales order in healthy horses, perhaps indicating the important and often overlooked role of commensal clostridia in gastrointestinal health. In contrast, there was no difference in the abundance of the order Lactobacillales, which contains LAB. While only one study, these data indicate that colitis is associated with profound alterations in the fecal microbiome, and that broad assumptions about groups such as Clostridia and LAB may need to be reassessed.

While most of the focus is on the lower gastrointestinal tract, study of the equine stomach has been reported. Perkins *et al*. (2012) reported evaluation of biopsies from the squamous, glandular and antral regions of the stomach of six horses, along with biopsies of ulcerated sites. A diverse microflora was evident with an average of 78–128 genus level (5% divergence) operational taxonomic units (OTUs) per horse. The Firmicutes, Proteobacteria, and Bacteroidetes phyla predominated. Various species were identified, with *Lactobacillus* spp., *Moraxella* sp., *Streptococcus* sp., *Sarcina* sp., *Eubacterium* sp., *Actinobacillus* sp., *Acinetobacter* sp., and *Veilonella* sp. being most abundant in different samples. The small sample size limits some of the comparisons that can be made, but differences were apparent between endoscopic samples from fasted horses and post-mortem samples from non-fasted horses. The latter group was dominated by Firmicutes while endoscopic samples from fasted horses had a much more diverse phylum-level distribution, with Firmicutes representing a minority of sequences and larger populations of Proteobacteria and Bacteroidetes. Whether this reflects profound changes from short-term fasting, differences in sampling technique or unidentified patient factors is unclear. Samples clustered by horse, not gastric region, suggesting more inter-horse variation than intra-horse variation, a seemingly common theme in studies of the equine intestinal microbiome using different methods and different sample sites.

Limitations in microbiome assessment

Many limitations in assessment of microbiomes have been overcome through the use of culture-independent methods. Advances in bioinformatics now facilitate interpretation of large and complex datasets, allowing for vast depth of study. Yet, some limitations remain. One major concern is the size and variability of the equine intestinal tract. Studies assessing the intestinal microbiome typically involve feces because of the ease of sampling. However, the structure and function of the intestinal tract change dramatically from stomach to rectum, and there is no reason to suspect that the microbial population does not similarly change. Thus, evaluation of feces may only partially reflect the composition of more proximal components, something that is of clear relevance since disease does not occur in the rectum. With the exception of rare experimental studies evaluating horses with cannulae placed into different intestinal locations (something that may have its own limitations because of the potential for an altered local environment) or terminal investigations, studies will focus on feces. While understandable and fully necessary from a practical standpoint, there is inadequate information about how well feces reflect the composition of proximal intestinal locations. Research in humans (Zoetendal *et al*., 2008) and dogs (Suchodolski *et al*., 2005) has shown that the composition of the gastrointestinal flora is site specific, and varies significantly along the gastrointestinal tract. Limited equine study is available and current data are somewhat contradictory. A FISH-based study comparing bacterial species in feces and the cecum concluded feces was not an adequate model for proximal segments (Milinovich *et al*., 2007). In contrast, a study using real-time PCR to assess the abundance of cellulolytic (*Ruminococcus flavefaciens* and *Fibrobacter succinogenes*) and non-cellulolytic (*S. bovis*) bacterial species in cecum, colon, and rectum of horses determined that feces was a suitable model for studying the composition of these particular bacteria in the colon and cecum (Hastie *et al*., 2008). A study of various intestinal tract compartments of euthanized horses using 16S rRNA gene PCR and TRFLP analysis reported a large degree of variation (Schoster *et al*., unpublished data). Thus, further information about the ability of feces to adequately reflect the microbiome of proximal locations is required.

It is also unknown whether the microbiome is relatively stable in the same individual over time, in the absence of obvious factors that would result in microbiome perturbation (e.g. antimicrobial exposure). Longitudinal studies are lacking, with the exception of small studies focused on specific pathogens such as *Clostridium difficile* (Schoster *et al*., 2011). It is likely that the core microbiome remains relatively stable within individuals over time, with greater change in the other micro-organisms, yet this remains to be studied objectively. Yet another aspect to consider is the relationship between luminal micro-organisms and organisms that colonize the intestinal mucosal surface, although it has been reported that bacterial composition does not differ significantly between luminal contents and the mucosal surface in horses (Daly *et al*., 2001).

While feces will presumably remain the main material for equine intestinal microbiome assessment, more study is needed to determine the relationship between intestinal bacterial populations in different intestinal locations in both health and disease.

Factors affecting the equine intestinal microbiome

The small number of studies, the small size of many studies and limitation in methods that have been used

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hamper clear assessment of the composition of the gastrointestinal microbiome in healthy horses and how it is modified in disease. The equine intestinal microbiome certainly can change in response to certain situations, and these changes are sometimes associated with development of disease. This is best demonstrated in carbohydrate overload and oligofructose-induced laminitis, where significant proliferation of LAB species can be identified prior to the onset of disease (Garner *et al*., 1978; Milinovich *et al*., 2006; Crawford *et al*., 2007; Milinovich *et al*., 2007, 2008). The timing of microflora modification, the known impact of overgrowth of LAB on cecal pH and the various potential effects in response to this change (e.g. lactic acidosis, mucosal damage with absorption of hindgut derived endotoxin or vasoactive amines) support the clinical relevance of these microbiological changes (Milinovich *et al*., 2007). Less dramatic dietary manipulation can also have an impact on the microbiota. Willing *et al*. (2009) compared the fecal bacteria from horses submitted to two different diets and observed that horses receiving supplementation with concentrate had 10 times more LAB than horses receiving a forage-only diet. An increase in colonic LAB was also identified in horses fed silage, with a decrease in streptococci (Muhonen *et al*., 2009). Increased abundance of Clostridiaceae cluster XIVa and *Bacteroidetes* assemblage, and decreased abundance of Ruminococcaceae with high concentrate versus hay-based diet has also been reported (Daly *et al*., 2011).

Clearly, infectious colitis is associated with potentially profound microbiome alterations (Costa *et al*., 2011), but differentiating cause versus effect can be a challenge. It also remains to be determined whether the nature and degree of microbiome alteration is similar with colitis of different etiologies.

The impact of factors such as antimicrobial therapy is not well understood. Antimicrobial exposure can undoubtedly lead to colitis, and most, if not all, antimicrobials that are used in horses have been definitively or anecdotally associated with induction of colitis. Yet, the limited equine microbiota studies to date have not provided clear and consistent information. Marked microbiota disruption has been shown with oxytetracycline administration (White and Prior, 1982), but the lack of an apparent impact of other antimicrobials (White and Prior, 1982; Grønvold *et al*., 2010) must be interpreted carefully considering the methods that were used. At this point, it is certainly impossible to say that some antimicrobials have no impact on the microbiota. Study of the effect of antimicrobials and the relative impacts of different antimicrobials is needed, considering the importance of antimicrobial-associated diarrhea in this species.

Similarly, the role of the intestinal microbiota in colic is unclear. Various colic risk factors such as diet change and diet composition (Morris *et al*., 1989; Cohen and Peloso, 1996; Hudson *et al*., 2001) can plausibly be related to microbiota alterations, but these have not been adequately investigated.

Therapeutic modification of the microbiome

As more information becomes available about the role of the microbiome in disease, efforts to harness microbiome alteration for therapeutic purposes are inevitable (and indeed desirable). While basic in concept, therapeutic manipulation of the microbiome may be difficult, in part because of the sheer size of the equine intestinal tract and its associated microbiota.

Probiotics are widely used in horses for the treatment or prevention of gastrointestinal disease, but clinical trials have yielded disappointing results (Parraga *et al*., 1997; Kim *et al*., 2001). Whether this is the result of inadequate choice of organisms, inadequate dosing, poor-quality control of commercial products (Weese, 2002; Weese and Martin, 2011) or an inherent inability of orally administered micro-organisms to modify the equine intestinal microbiota is unclear. Producing changes in the microbiota is likely more feasible in young foals since they have a less well-established commensal bacterial population. Microbiota alteration was presumably achieved in a clinical trial using *Lactobacillus pentosus* in neonatal foals; however, probiotic administration was associated with increased risk of diarrhea, perhaps from excessive or undesirable modification (e.g. excessive lactic acid production) (Weese and Rousseau, 2005).

While antimicrobials can have detrimental effects on the intestinal microbiome, selected drugs are also used therapeutically. Metronidazole is widely used for the treatment of idiopathic and clostridial colitis, and efficacy against idiopathic colitis has been demonstrated (McGorum *et al*., 1998). Zinc bacitracin has been shown to have positive effects in the prevention and treatment of experimentally induced colitis (Staempfli *et al*., 1992), but it is rarely used clinically. The positive therapeutic impacts of antimicrobials provide evidence of the clinical value in modifying the intestinal microbiota, yet antimicrobials must be considered a rather broad and crude approach. The impact of these antimicrobials on the microbiota is not adequately understood and while clinical benefits can be derived, it is possible (if not likely) that beneficial components of the microbiota are similarly affected. While a clinical necessity at this point in time, further study of the impact of antimicrobials (both clinically and microbiologically) is needed to refine approaches to the treatment of colitis and potentially develop more specifically targeted therapies that might spare the core, beneficial components of the microbiota.

In humans, fecal microbiota transplantation (also known as fecal transplantation, stool transplantation or fecal bacteriotherapy) has received attention over the past few years, largely because of the successful use of fecal enema administration for treatment of recurrent and

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refractory *C. difficile* infection (Gough *et al*., 2011). Similar efforts have not been reported in horses, and the feasibility of fecal enema therapy in horses is perhaps questionable because of the long distance from rectum to typical sites of dysbiosis and disease. However, there are anecdotal accounts of fecal administration by nasogastric tube in equine medicine for decades. No data are available regarding the efficacy of this approach, but the concept of administering a large and diverse bacterial population to modify the intestinal microbiota is appealing as a potentially effective, safe, cost-effective and non-antimicrobial approach to the treatment of a range of disorders.

Conclusions

While a wide variety of techniques have been used to assess the equine intestinal microbiota and interesting insights have been obtained, it is clear that only a superficial understanding of this complex microbial population exists. Recent advances in next generation sequencing and application of these methods for investigation of the intestinal microbiome in both healthy and diseased horses will likely revolutionize our understanding of the role of the microbiome in health and disease. Yet, the complexity of this population, the apparent variation between horses, differences between different intestinal compartments, heterogenous management of horses (with corresponding variable influences on the microbiome) and a diverse range of diseases that might be attributable to the intestinal microbiome indicate that much is to be learned.

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