Review
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Probiotic Use in Horses – What is the Evidence for Their Clinical Efficacy?

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The gastrointestinal microbiota is extremely important for human and animal health. Investigations into the composition of the microbiota and its therapeutic modification have received increasing interest in human and veterinary medicine. Probiotics are a way of modifying the microbiota and have been tested to prevent and treat diseases. Probiotics are proposed to exert their beneficial effects through various pathways. Production of antimicrobial compounds targeting intestinal pathogens, general immune stimulation, and colonization resistance are among these mechanisms. Despite widespread availability and use, scientific, peer-reviewed evidence behind commercial probiotic formulations in horses is limited. Additionally, quality control of commercial over-the-counter products is not tightly regulated. Although promising *in vitro* results have been achieved, *in vivo* health benefits have been more difficult to prove. Whether the ambiguous results are caused by strain selection, dosage selection or true lack of efficacy remains to be answered. Although these limitations exist, probiotics are increasingly used because of their lack of severe adverse effects, ease of administration, and low cost. This review summarizes the current evidence for probiotic use in equine medicine. It aims to provide veterinarians with evidence-based information on when and why probiotics are indicated for prevention or treatment of gastrointestinal disease in horses. The review also outlines the current state of knowledge on the equine microbiota and the potential of fecal microbial transplantation, as they relate to the topic of probiotics.

Key words: *Bifidobacterium*; Fecal microbial transfaunation; *Lactobacillus*; Microbiota.

The concept of the human microbiota was first introduced to the scientific community by Joshua Lederberg in 2001. He defined the microbiota as 'the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease'.[1] Since then, interest in the intestinal microbiota has culminated in large-scale endeavors such as the Human Microbiota Project,[2] and has also made its way into veterinary medicine.

Abbreviations:

CVM	Center of Veterinary Medicine
EFSA	European Food and Drug Authority
FAO	Food and Agricultural Organization
FDA	Food and Drug Authority
FMT	fecal microbial transplantation
GRAS	generally regarded as safe
IECs	intestinal epithelial cells
LAB	lactic acid-producing bacteria
QPS	qualified presumption of safety
WHO	World Health Organization

Equine Intestinal Microbiota and its Effect on Health

The horse's colon and cecum are large fermentative chambers inhabited by a diverse microbiota consisting of bacteria, protozoa, and fungi.[3,4] The intestinal microbiota has enormous impact on the health and performance of horses.[3,5] Although single pathogens can cause disease, gut microbiota dysbiosis, a shift in the microbiota as a whole, is increasingly being identified as a cause of a wide range of diseases.[6] In humans and other species, various gastrointestinal diseases such as inflammatory bowel disease,[7] diabetes,[8] atherosclerosis,[9] and rheumatoid arthritis[10] have been associated with gut dysbiosis. Alterations in the equine intestinal microbiota are associated with acute colitis, equine grass sickness, and laminitis.[4,11–15]

Over the last decades, most studies have focused on hind gut fermentation processes related to fiber digestion, lactic acidosis and laminitis, and were conducted using culture-dependent or molecular methods.[14,16–22] A thorough understanding of the composition of the equine microbiota could not be reached because of the technical limitations of these methods. Recent advances in culture independent microbial identification and bioinformatics have opened doors towards understanding the composition of the intestinal microbiota.[4,23–26] The information obtained from these studies so far is based on small numbers and has statistical limitations because of different analyses of the data.

The microbiota is unique for each horse, but certain phyla predominate in all healthy animals.[4,23] *Firmicutes* is the predominant phylum in feces accounting for 46–70% of identified sequences. *Bacteroidetes, Proteobacteria*, *Verrucomicrobia, Actinobacteria,* and *Spirochaetes* constitute between 0% and 15% each.[23,27] Substantial shifts in the phylum level occur in horses with gastrointestinal disease. Healthy horses have a greater abundance of *Actinobacteria* and *Spirochetes* whereas diarrheic horses have a greater abundance of

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Fusobacteria.[4] There is also relatively greater abundance of the order *Clostridiales* in healthy horses as compared to diseased horses, suggesting their importance for the health of the equine gastrointestinal tract.[4] Interestingly, there was no difference in the relative abundance of *Lactobacillales* between healthy and diseased horses. The order *Lactobacillales* contains the majority of lactic acid-producing bacteria (LAB) commonly used as probiotics.[4] Despite these data, the gut microbiota remains difficult to interpret because of its complexity. There is difficulty in differentiating cause and effect, poor understanding of the function of different components of the microbiota and problems assessing interaction of the microbiota with the horse. A detailed review of the composition of the equine microbiota in health and disease is beyond the scope of this article and can be found elsewhere.[5]

Microbial composition and function are known to change along the gastrointestinal tract with changes in the most dominant phyla accounting for the major differences.[24,28] In one study, the most dominant phyla of the large intestine were Firmicutes and Bacteriodetes, whereas in the ileum Firmicutes and Proteobacteria dominated.[24] The core microbiota of different regions differed not just in composition, but also in abundance.[27] For clinical cases and in a research setting, fecal samples are mostly obtained. Biologically relevant differences likely exist among compartments of the gastrointestinal tract, complicating research, interpretation, and clinical applications.

Treatment modalities such as prebiotics, probiotics, antimicrobials, and fecal microbial transfaunation (FMT) are being explored to manipulate the microbiota composition. The goal ultimately is to achieve disease reduction, elimination, or prevention. These treatment options hold remarkable promises, but investigations are still in their infancy. Once a better understanding of the equine intestinal microbiota is reached, the approach of modifying the microbiota could become a therapeutic procedure for equine diseases.

Probiotics: Definition and Regulations

Metchnikoff first defined probiotics as 'live microorganisms which exhibit a health promoting effect' in 1908.[29] In 2008, the Food and Agricultural Organization (FAO) and World Health organization (WHO) modified this definition to its current form: '*live microorganisms, that when administered orally at adequate concentrations, provide a beneficial effect beyond that of their nutritional value*'.[30]

In the United States, probiotics, also called 'direct fed microbials', can be classified as a drug, in which case they need to be approved by the Food and Drug Authority (FDA). There currently are no approved probiotics for horses. Alternatively, probiotics can be classified as a dietary or feed supplement 'generally regarded as safe' (GRAS). In the latter case, they do not need to go through the process of drug approval. The producers are responsible for providing an expert opinion on why the product should be considered as GRAS. The Center for Veterinary Medicine (CVM) of the FDA then can approve or reject this status (www.fda.gov.com). Although the FDA has regulatory responsibility, the ultimate responsibility lies with the manufacturing company. The FDA only requires that supplements be labeled in a truthful and not misleading manner. Labels need to be reviewed by the CVM before marketing. The labels need to contain information to identify the feed additive and details on its safe and effective use. Expressed or implied claims that a feed additive can be used to cure, treat, or prevent disease may identify intent to offer the product as an animal drug and are not allowed. However, CVM permits the use of meaningful 'health' information on the label of some animal feed products. For example, 'gastrointestinal health' claims on horse feed fall under this policy (www.fda.gov.com). Consequently, in North America, there are numerous probiotic products for use in horses on the market that can be obtained over the counter, and claim to benefit the horse in various ways. However, peer-reviewed published studies proving the efficacy of these products are limited, or in most cases lacking.

In the European Union, probiotics are considered feed additives, and are classified at a regulatory level as zootechnical additives in the category of gut flora stabilizer for healthy animals. Only probiotics complying with regulation (EC)1831/2003 may be placed on the market. Authorizations are granted for specific animal species, specific conditions for use, and for 10-year periods. Currently 4 commercial products are approved for use in horses in the European Union (www.efsa.europe.eu). Biosprint, Levucell, and YeaSacc, all containing *Saccharomyces cerevisiae*, are registered under the claim of improving fiber digestion. Only ColiCure, containing *Escherichia coli,* is licensed under the claim of improving fecal consistency and odor. Studies outlining the efficacy of these products are not published in peer-reviewed journals. Although the EFSA has judged the evidence to be adequate for licensing, the published evidence and data that can be reviewed are weak.

Bacterial Strains Used as Probiotics

There are many important factors for choosing a microorganism for the development of probiotic. The most recent FAO/WHO guidelines state that potential probiotic strains should be able to survive the gastric environment, have antimicrobial properties, adhere to mucus and epithelial cells and have properties to be able to withstand the rigors of production.[30] Not all LAB have probiotic properties, and even different strains of the same species can have different properties making it necessary to evaluate probiotics on a strain basis.[31]

Both bacteria and yeast are used as microbial feed additives (Table 1). The bacteria that comprise commercial probiotics constitute <1% of all intestinal microorganisms in total. Depending on the species and

the segment of the gastrointestinal tract evaluated, their relative abundance can be much higher.[4,27] Many probiotics for horses are designed to target the large colon of the horse where many diseases occur. The most commonly used genera for probiotics, *Lactobacillus*, *Bifidobacterium,* and *Enterococci*, are not the most abundant species in the large colon of the horse.[4,24,27] This observation suggests that these species have less influence on the gastrointestinal health of horses. The approach of using lactobacilli or other LAB as probiotics, mainly used in humans and small animals, might be futile in horses. The focus of probiotic studies should be placed on other more abundant species. Studies investigating the effect of abundant bacterial species, such as members of the *Clostridia* class, are lacking to date. These classes could theoretically have a better effect than current probiotic strains.

There is debate about whether or not strains isolated from the target host have better survival and colonization capabilities because of host specificity.[32] *Lactobacillus* species from healthy equine feces or gastric epithelium were highly adherent to cells of the equine digestive tract.[33] On the other hand, enterococci with probiotic properties isolated from healthy horse feces were shown to adhere best to human and canine mucus.[34,35] The same phenomenon was seen in LAB isolated from humans and dogs.[36] Thus, it appears that the ability of probiotics to colonize the intestinal tract is not always host-specific, indicating that strains should be chosen based on their probiotic properties, not their origin. Technical instability of a bacterial strain is important for commercial production. This is often a limiting factor, but by evaluating strains already in commercial production this limitation can be overcome.[37]

Antibiotic resistance is an important selection criterion for bacterial strains intended for use as probiotics. Transferrable resistance genes present in a probiotic bacteria's chromosome or on plasmids may be transferred horizontally to the indigenous flora and to opportunistic pathogenic bacteria. Thus, ideally, probiotics should not harbor potentially transferable resistance genes, especially those conferring resistance traits of high clinical relevance. Because most probiotic strains are members of the human or animal indigenous flora, the presence of antibiotic resistance determinants must be systemically evaluated.[38] In Europe, all probiotics on the market undergo an evaluation of their resistance gene content before they are considered for the QPS standard followed by potential marketing of a product (www.efsa.europe.eu). In North America, such a regulation is not currently in force.

The possibility of resistance transfer is related to the genetic basis of the resistance mechanism. Horizontally transferred antibiotic resistance genes, particularly those carried within mobile genetic elements are most likely to be transmitted. Differentiation of the antibiotic resistance mechanisms involved is therefore of extreme importance.[39]

Enterococci often carry mobile genetic elements containing multiple resistance genes coding for resistance against clinically relevant antibiotics.[40,41] For example, 66% of enterococci isolated from aquatic cultures displayed acquired antibiotic resistance other than penicillin. Nineteen percent of isolates were resistant to vancomycin and 32% of the *E. faecium* strains were multi-drug resistant (>2 antibiotics).[42] This creates potential concerns because of the common use of enterococci in probiotics and limited data regarding susceptibility of enterococci in most probiotic products.

Data on antibiotic resistance determinants are mostly available for lactobacilli and scarce for other bacterial species. The most common resistance genes code for tetracycline resistance but chloramphenicol, macrolide, aminoglycoside, and beta-lactam resistance genes also have been identified.[38] Transfer of genes has been shown to occur *in vitro* and in animals models among *Lactobacillus* strains and from lactobacilli to different gram-positive bacteria, including staphylococci.[43,44] Lactobacilli also can acquire resistance genes from other gram-positive bacteria.[45] Taken together, these results support the hypothesis that probiotics can act as antibiotic resistance traffickers in an *in vivo* situation.

Table 1. Bacterial genera and yeasts typically used as probiotics.

Saccharomyces (*yeast*)
Lactobacillus
Bacteroides
Escherichia coli
Enterococcus
Bacillus
Nitrobacter
Nitrosomonas
Streptococcus
Rhodobacter
Fusobacterium
Butyrivibrio
Rhodobacter
Clostridium
Eubacterium

Genera are bacteria unless stated otherwise.

Fields outlined in gray indicate genera that have been evaluated as probiotics in horses.

Probiotic Survival in the Equine Gastrointestinal Tract

Colonization is superior to mere survival in the gastrointestinal tract, because probiotics could act beyond the period of administration. Generally, host-specific strains are believed to be able to colonize the gastrointestinal tract of the indigenous host for longer periods of time. Indeed, colonization of the adult equine gastrointestinal tract with *L. rhamnosus* LGG of human origin was shown to be poor.[46] After a 5-day course of probiotic administration at 3 different dosages (1×10^9, 1×10^{10}, 5×10^{10}), fecal recovery in 21 adults was shown to be 71%, 29%, and 86% after

24 h for each dose, respectively. After 48 h, the probiotic was recovered from the feces of 14%, 14%, and 56%, whereas 3 days after administration only 1 horse in each of the lower 2 dosage groups remained positive. Fecal recovery was longer in foals where the probiotic could be recovered up to day 9 after administration in some foals.[46] This suggests that the immature gastrointestinal flora of foals could facilitate probiotic survival. Additionally, foals and adults showed a lack of dose response, making it difficult to determine an ideal dose to use.[46]

Similar results were obtained when administering *Saccharomyces boulardii*.[47] After administration of 10×10^9 cfu/g to 3 horses and 20×10^9 cfu/g to 2 horses for 10 days, fecal samples were negative for *S. boulardii* on day 20. On day 5, all horses had viable *S. boulardii* in their feces.[47] Similarly, *S. cerevisiae* has been shown to survive but not colonize the ceca and colons of horses.[17,48,49] This indicates that any beneficial effect of probiotics might not continue beyond the period of administration, making long-term or repeated treatment a necessity.

In the above studies, probiotic survival was only assessed by analyses of fecal samples. By this approach, colonization can only be detected if the animal is shedding the bacterium at the time of sampling. Whether certain bacteria are shed persistently or intermittently in horses, and how many samples are required to detect intermittent shedding historically has been a topic of great debate. The number of fecal samples necessary to establish *Salmonella* shedding, for example, has not been conclusively determined so far and likely depends on prevalence of the bacterium.[50] For probiotic bacteria, there currently is no information on intermittent or persistent shedding available in the literature.

Mechanisms of Action of Probiotics

There are 4 main mechanisms of action by which probiotics prevent colonization of the digestive tract by pathogenic strains or prevent disease: (1) modulation of the host innate and acquired immune system, (2) antimicrobial production, (3) competitive exclusion, and (4) inhibition or inactivation of bacterial toxins (Fig 1 A and B).

Many reported mechanisms of action of probiotics are based on *in vitro* studies only, and extrapolation of these results to *in vivo* conditions is controversial. Some evidence also has been generated by *in vivo* studies done in laboratory animals or humans.

Immune Modulation

Probiotics can influence the host's immune system as live or dead bacteria through their metabolites, cell wall components, or DNA.[51] Probiotics and their metabolic products are recognized by conserved recognition receptors by IECs and gut-associated immune cells. The effects include fortification of the intestinal barrier by maintaining tight junctions, supporting survival of intestinal epithelial cells (IECs) and their growth, and induction of IgA and β-defensin production, resulting in suppression of growth of pathogens as well as systemic and local anti-inflammatory effects.[51]

Anti-inflammatory effects can be achieved by the modification of cytokine production by IECs and effects on cells of the innate immune system, such as macrophages and dendritic cells.[52] Probiotics and their products influence T_{reg} cells, the subset of T-cells that plays an important role in the down-regulation of misdirected immune responses. Some probiotics are able to induce production of T_{reg} cells, thereby exerting anti-inflammatory properties.[53] Although probiotics often exert an anti-inflammatory action, certain probiotic bacteria also can up-regulate inflammatory mediator production[54] and increase adhesion of pathogens.[55]

Within the intestinal wall, B-cells differentiate into plasma cells and secrete IgA antibodies. These then are transported into the lumen and are important for mucosal-associated immunity. Certain probiotic strains are able to influence IgA production.[56–58] Systemically, probiotics can influence immunoglobulin production by altering systemic isotype profiles.[54]

Probiotics have diverse effects on the immune system. Such effects can be stimulatory or inhibitory depending on the biological features of individual probiotic strains. Most of the above studies, assessing the effects of probiotics on the host, have been conducted using human or laboratory animal cell lines, but equivalent studies are lacking for horses. Given the conservation of the immune system across all species, it is likely that similar effects would occur in horses.

Antimicrobial Production

Probiotic strains produce various substances that are effective against microbes. Fatty acids, lactic acid, and acetic acid are produced in large quantities. Additional antimicrobial substances that are produced in much smaller amounts by LAB include formic acids, free fatty acids, ammonia, hydrogen peroxide, diacetyl, bacteriolytic enzymes, bacteriocins, antibiotics, and several undefined substances.[59]

Competitive Exclusion

Probiotic strains adhere to epithelial cells in the host and interfere with pathogen adherence by blocking receptors or by increasing mucin production. *L. rhamnosus* GG decreased the adherence of pathogenic bacteria such as *Salmonella, E. coli,* and clostridia to pig intestinal mucus.[60] This mechanism, however, appears to be specific to certain probiotic strains, and some strains increased the adherence of pathogenic bacteria to human intestinal mucus.[61] *In vitro*, probiotics can prevent pathogenic bacteria from invading the epithelial cell. In contrast to the former described effect, this feature seems to be a widespread trait among probiotic bacteria.[51]

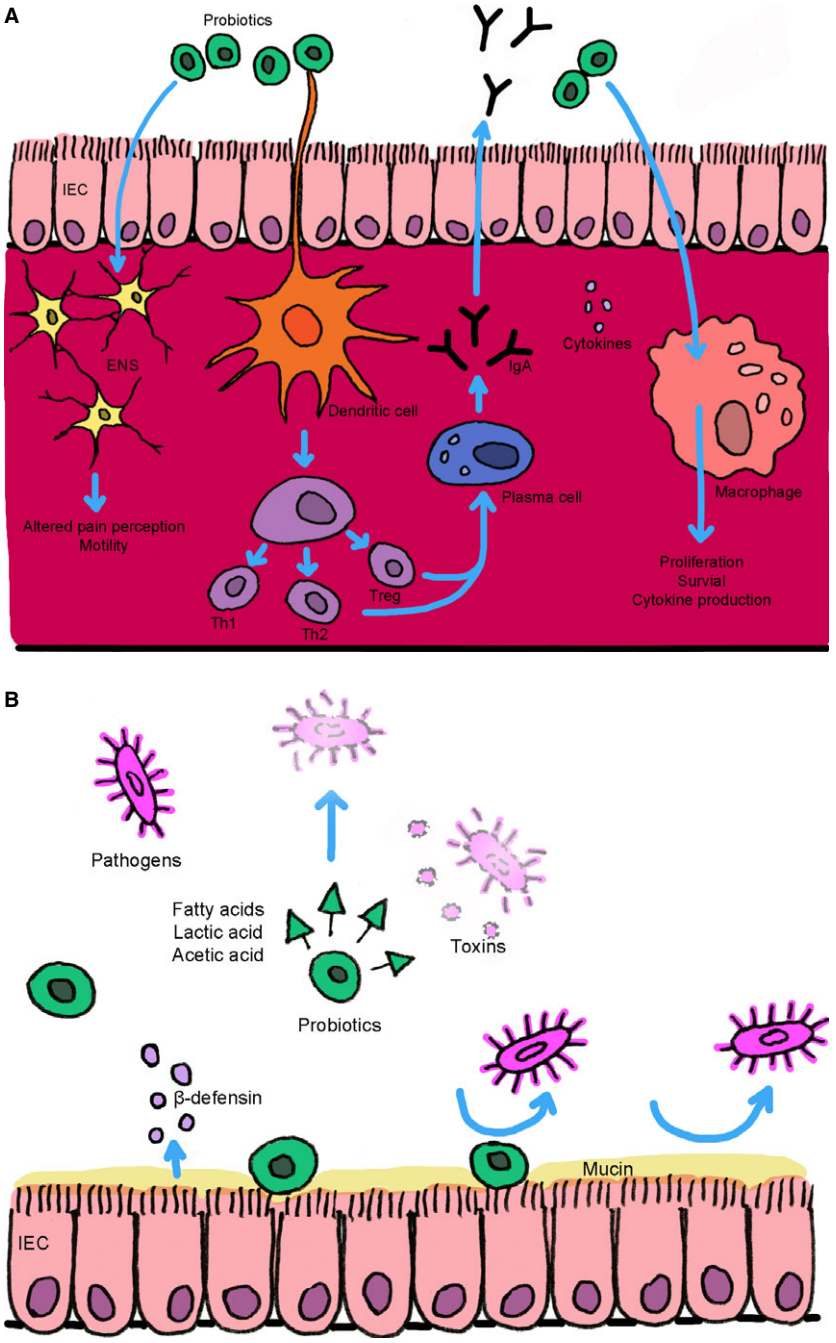

Fig. 1. Mechanism of action of probiotics. Modified from: Thomas and Versalovic 2010.[54] Probiotics can alter pain perception and gastrointestinal motility by interaction with the enteric nervous system. The interaction of probiotics with dendritic cells in the wall of the intestine modulates the T-cell response, which in turn influences differentiation of B-cells and immunoglobulin production. Probiotics also modulate cytokine production as well as proliferation and survival of macrophages (**A**). Probiotics increase β-defensin production by intestinal epithelial cells, enhance mucin production, and contribute to colonization resistance. Probiotics and the substances they produce that have a direct effect on pathogens and their toxins (**B**).

Inhibition or Inactivation of Bacterial Toxins

Toxins are important virulence factors for some enteropathogenic bacteria. Some probiotics block the effects of enteropathogenic bacteria. For example, *S. boulardii* is protective in the murine ileal loop model as well as in cell cytotoxicity assays when challenged with toxigenic *C. difficile* by this mechanism.[62] Lactobacilli can decrease toxin gene expression and toxin production by bacteria including *Salmonella*, *E. coli,* and *C. perfringens*.[63–65] The anti-toxin effect of some probiotics may be beneficial in managing infectious diarrhea.

Probiotics also are able to inactivate toxins by metabolic mechanisms. *Saccharomyces boulardii* releases a protease that can digest *C. difficile toxins* A and potentially can prevent *C. difficile* infection.[66] Inactivation also can occur by physicochemical interaction. Some probiotics can bind to toxins and decrease their bioavailability to the host.[67,68]

Quality Control of Commercial Products

As described earlier, manufacturers of over-the-counter products in North America have no obligation to perform quality control of their products. In Europe, the few licensed products periodically are tested by the EFSA (www.efsa.com). The effect of probiotics might therefore not be predictable because of inadequate content of commercial probiotic formulations.

Many commercial veterinary and human probiotic preparations are not being accurately represented by label claims (Fig 2). Only 9/21 (43%) of human products and 2/23 (8%) of veterinary products from Canada were adequately labeled.[69] Three of the human and 7 of the veterinary products contained inadequate descriptions of the bacterial content, including missing names, unspecified strains, nonexisting names and outdated names.[69] Only 16/21 (76%) human and 5/23 (22%) of veterinary products from Canada provided information on bacterial concentrations.[70]

Quality control of contents also is poor. Only 2/13 (15%) of veterinary and human probiotics contained the specified organism at the label indicated concentration. Some products were missing organisms entirely or contained too little or too much of an active ingredient. Actual bacterial concentrations ranged from 0 to 215% of the claimed amounts. All veterinary products contained <2% of the listed concentration.[70]

When an adequate label was defined as containing specific (valid) names of bacteria with no spelling mistakes and expected bacterial content, only 8/25 (32%) products were labeled correctly. Only 21/25 (84%) listed the bacterial species, and of these, 7/21 (32%) were misspelled. Only 15/25 (60%) products disclosed the expected amount of active ingredient (bacteria) on the label and only 4 of these 15 (27%) met or exceeded the claimed amount.[71]

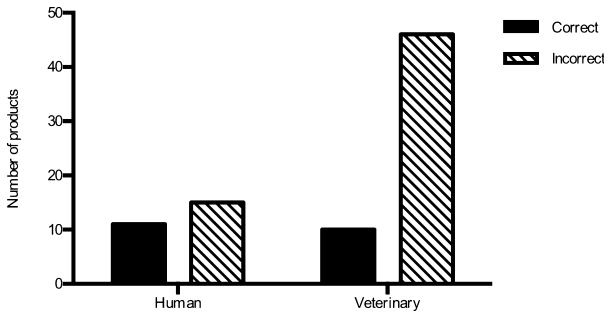

Fig. 2. Quality control of labeling and content of commercial probiotics, results based on 3 studies.[69–71] Incorrect labeling: Specified bacterial strain or dose or both was not present in the product.

Research studies can be affected by this poor quality control. As part of a clinical trial, the content of a commercial probiotic formulation was evaluated. Although the formulation claimed to contain 10 million viable lactobacilli (cfu/g), only 50,000 cfu/g were cultured. No positive effect of the probiotic was seen in this study.[72] It is uncertain whether there was a true lack of effect of the probiotic or whether the probiotic did not contain adequate numbers for a clinical effect.

Safety of Probiotics

Adverse Effects

Adverse effects of probiotic administration are rare in humans and animals. In humans, the few reports available usually describe extraintestinal infections, not enteric disease.[73] Although probiotics typically are used in individuals with enteric disease and adverse enteric consequences might be hard to discern, it is reasonable to assume that the incidence of adverse events is very low, something that is consistent with their GRAS classification.

In adult horses, there are no published reports of enteric disease after probiotic administration.[46,74] Doses generally are extrapolated from human recommendations and adjusted by weight.[47,75] Even administration of up to 3 times the manufacturer recommended doses to 18 healthy horses did not result in any adverse effects.[76] The effect of probiotics in horses with enteric disease might differ from the effect in healthy horses, but no adverse clinical effects were reported in horses with gastrointestinal disease.[47,76,77] Based on the above data, most authors consider probiotics, particularly *S. boulardii,* safe for use in healthy and diseased adult horses.[17,48,77]

Some adverse effects not related to enteric disease have been reported. One horses developed hives after administration of probiotics.[72] The association between the hives and the probiotics was unclear because resolution of hives coincided with the horse being switched from straw bedding to wood shavings. This indicates that the bedding could have been the reason for the allergic reaction.[72]

The effect of probiotics in foals is likely to be different from that in adult horses because of major difference in gastrointestinal microbiota composition.[78] Although several published studies demonstrate safety of commercially available and self-made probiotics in foals,[74,79] there are also reports on adverse enteric effects.[75] A self-made probiotic product containing *Lactobacillus pentosus* isolated from a healthy foal was evaluated for its ability to prevent neonatal diarrhea.[75] In a preliminary study, this probiotic was administered to 9 healthy foals and no adverse effects were seen.[74] When the probiotic was administered to healthy neonates in a clinical placebo-controlled field trial, the treatment group showed increased incidence of diarrhea and need for veterinary intervention.[75] Although it is unclear why these foals developed diarrhea, the immature microbiota of the foals could have allowed

overgrowth of LAB, resulting in osmotic imbalances and diarrhea. Alternatively, the probiotics could have changed the microbiota to allow for pathogen adhesion to the epithelial cells.

Fungaemia caused by treatment with *S. boulardii* has been reported in neonatal humans[80]. Whether this could be a problem in horses is unknown, because studies of horses have so far excluded neonates in the studies of *S. boulardii*.[77]

Evidence for Probiotic Efficacy in Treatment of Equine Gastrointestinal Disease

The scope of the current literature on equine probiotic use has focused mainly on gastrointestinal disease application[47,72,75–77,79,81,82] (Fig 3). Although some studies have shown beneficial effects of probiotics, other studies could not corroborate these results.

Overall, few studies are available, and these cannot be compared easily because of differences in study design and formulations used. Consequently, the overall evidence is weak.

The following sections summarize the various clinical applications for which probiotics have been tested. The exact strains, dosages, and length of treatment used in each of the studies cited in the text are outlined in Table 2.

Acute Enterocolitis

Acute colitis is a potentially devastating disorder than can be caused by several pathogens.[83] Because treatment is mainly supportive and an etiologic agent cannot be identified in up to 50% of cases, there is increasing interest in finding adjunctive treatment modalities.

Saccharomyces boulardii was assessed in a randomized blinded placebo-controlled clinical trial.[47] Horses receiving *S. boulardii* had a shorter duration of diarrhea and watery diarrhea but not loose feces. Despite this positive effect, the results of this study must be interpreted with caution, because there was no difference between the groups in relation to outcome, duration of hospitalization, and recurrence of diarrhea. In addition to the confounding factor of additional treatments, the number of cases was low, with only 7 horses per group.[47]

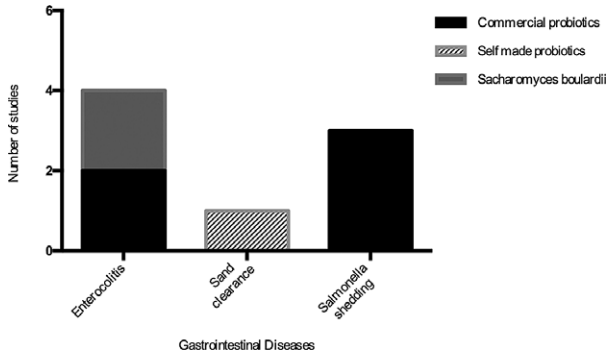

Fig. 3. Overview of probiotic studies in horses. The main objective of each study is presented on the *x*-axis and the number of studies on the *y*-axis. The shading indicate which probiotic strain was used in the studies.

Saccharomyces boulardii also was assessed as an adjunctive treatment in horses affected with antimicrobial-associated enterocolitis in a randomized placebo-controlled clinical trial involving 12 horses.[77] No significant differences were observed between groups for occurrence of normal fecal consistency or cessation of watery diarrhea. Also, days to improvement in attitude, resolution of leukopenia, appetite, normal heart rate, normal respiratory rate, normal temperature, duration of stay in hospital, survival to discharge, and occurrence of secondary complications was not different between groups. The authors postulated that the lack of efficacy could be attributable to a lack of colonization by *S. boulardii* because the fecal samples of some horses were negative for *S. boulardii*.[77] Although this study was influenced by fewer confounding factors and evaluated more clinical parameters compared to the previously described study,[47] the number of cases was low and adjunctive treatment was variable among horses, making interpretation of the results difficult.

Although both studies investigated the same probiotic agent, they are difficult to compare because of a heterogeneous horse population and different inclusion and outcome criteria. Overall, the evidence for an effect of *S. boulardii* as an adjunctive treatment for enterocolitis in horses is weak.

Given that drastic changes in the microbiota of horses with enterocolitis have been identified, it is questionable whether administration of the currently available probiotics containing a limited number of bacterial strains could be effective at all.

Diarrhea in Foals

Neonatal foal diarrhea is a common occurrence with >60% of foals developing diarrhea during their first 6 weeks of life.[84] Because etiologies are numerous and prevention measures limited, probiotic administration is an attractive option for the prevention of neonatal foal diarrhea.

In 1 study, an equine strain of *Lactobacillus pentosus* with *in vitro* antimicrobial properties was assessed in a randomized placebo-controlled clinical trial.[75] Probiotic administration in this study was associated with a significantly higher incidence of diarrhea, presence of clinical signs (lethargy, fever, and anorexia colic) and the need for veterinary examination and treatment.[75]

In another study, a multistrain probiotic product derived from equine gastrointestinal contents was studied in a randomized placebo-controlled double-blinded clinical trial.[79] Foals in the probiotic group showed statistically significant larger weight gain after treatment and a significantly lower incidence of diarrhea.[79] These effects, however, were only significant at 1 time point (2–3 weeks of age). It is unlikely that the diarrhea was clinically important as evidenced by a lack of

Table 2. Probiotic strains and doses used in clinical trials to evaluate effect of probiotics on gastrointestinal disease in horses.

Probiotic strain	Dose	Fre-quency	Duration of probiotic use	Main variable investigated Type of study	Outcome	Ref.
S. cerevisiae, *L. acidophilus* *E. faecium*	40×10^9 2.25×10^9 1.55×10^9	q24h	35 days	Effect of probiotic/ psyllium on fecal sand clearance in healthy horses Clinical trial	Increased sand output after 4 weeks of treatment compared to baseline	Landes et al[82]
L. lactis *S. faecium* *S. cerevisiae*	5×10^9 5×10^9 1×10^8	q24h	Up to 5 days	Prevalence of Salmonella shedding, fever, diarrhea or leucopenia in horses hospitalized due to colic RPCCT	No differences between placebo and probiotic groups	Kim et al[81]
L. plantarum *L. casei* *L. acidophilus* *E. faecium*	3×10^8 total	q24h	10 days	Prevalence of Salmonella shedding, diarrhea, length of antimicrobial therapy and stay in hospital in colic surgery patients RPCCT	No differences between two probiotic and two placebo groups	Parraga et al[76]
L. acidophilus *E. faecium* *B. thermophilium* *B. longum*	8.25×10^9 total					
S. boulardii	1×10^{10}	q12h	14 days	Duration and recurrence of diarrhea, length of hospitalization, and outcome in horses with colitis RPCCT	Shorter duration of diarrhea in probiotic treated horses	Desrochers et al[47]
S. boulardii	1×10^{10}	q12h	2 days beyond passing normal feces, maximum of 14 days	Differences in duration of diarrhea, return to normal white blood cell count, heart and respiratory rate, improvement of attitude and appetite and survival to discharge RPCCT	No difference between the probiotic and placebo group	Boyle et al[77]
L. casei *L. acidophilus* *E. faecium*	1×10^7 total	q48h	3 doses	Incidence of salmonella shedding in horses with gastrointestinal disease RPCCT	Reduced incidence of Salmonella shedding by 65% in probiotic treated group; study power: 25%	Ward et al[72]
L. pentosus WE7	2×10^{11}	q12h	7 days	Diarrheic or soft feces, depression, anorexia, weakness, colic, need for veterinary intervention RPCCT	Increased incidence of diarrhea and need for veterinary intervention in the probiotic group	Weese et al[75]
L salivarius *L. reuteri* *L. crispatus* *L. johnsonii* *L. equi*	$1–4 \times 10^{10}$ total	q24h	7 days	Body weight, fecal characteristics, clinical findings RPCCT	Decreased incidence of diarrhea at one time point and more weight gain in probiotic treated foals	Yuyama et al[79]

RPCCT: randomized placebo controlled clinical trial.

difference in the need for medical intervention between the 2 groups.

These 2 studies cannot be compared directly because different products were used. However, the results show that each probiotic product must be evaluated separately to assess safety and efficacy in neonatal foals. Larger scale controlled studies of different strains and products are necessary before conclusions can be drawn on the clinical efficacy of probiotics for this specific application.

Salmonella Infection and Shedding

Probiotics have been used successfully to control infection by *Salmonella* spp in poultry[85] and calves.[86] In horses, the effect of probiotics on *Salmonella* shedding has been investigated by 2 studies, but results have been disappointing to date.[76,81]

In a double-blinded randomized placebo-controlled trial, the effect of 2 commercial multistrain probiotic formulations (Table 1) on fecal shedding of *Salmonella* were studied in the postoperative period after colic surgery.[76] A total of 186 horses were prospectively allocated to 4 treatment groups (2 probiotic and 2 placebo) and treated for 10 consecutive days once daily. Five clusters of gastrointestinal diseases were evenly distributed between treatment groups but no differences in *Salmonella* shedding rates were found between treatment groups. The overall shedding rate was 21%. Prevalence of postoperative diarrhea, duration of antimicrobial therapy, and duration of hospitalization were not statistically different between the groups.[76]

In a later placebo-controlled randomized trial, the effects of a multistrain commercial probiotic (Table 1) on *Salmonella* shedding, prevalence of diarrhea, leukopenia and fever were evaluated in colic cases with various underlying diseases.[81] Ninety-six horses were enrolled and received probiotics or placebo once daily for up to 5 days. The overall shedding rate for *Salmonella* was 9%, and no differences were observed in shedding rates or clinical parameters between probiotic- and placebo-treated groups.[81] Despite successful randomization in this study, there were limitations. The overall number of horses shedding *Salmonella* was small (n = 10). The numbers of horses that developed diarrhea, fever and leukopenia were not reported and neither was antimicrobial administration. These limitations could have greatly influenced the multivariate analysis performed in the study. Additionally, fecal samples were taken at irregular, arbitrary intervals, and given that *Salmonella* shedding can be intermittent, some cases might have been missed.[50,72]

Additionally, both studies used a commercial product that was not tested to assess the actual content claimed on the label.

The preventative effect of a multistrain commercial probiotic (Table 1) on *Salmonella* shedding in hospitalized horses without gastrointestinal disease was assessed in a randomized placebo-controlled double-blinded clinical trial involving 130 horses.[72] Horses were given the probiotic or placebo on admission before medical procedures commenced. There were no pretreatment differences between the groups. Although administration of a probiotic decreased the incidence of *Salmonella* shedding by 65%, this result was not significant ($P > .19$). Posthoc analysis showed that the study had a power of only 25% to show such a difference. Additionally, incidence numbers were low, only 5 and 2 cases were detected in the placebo and probiotic groups respectively throughout the study.[72]

In other species, it is known that the effect of probiotics depends on the agent studied and so far very few probiotics have been evaluated in horses.[85] Additional studies are necessary before excluding the beneficial effects of probiotics on *Salmonella* shedding in horses. There is currently very little evidence supporting the use of probiotics to decrease *Salmonella* shedding or salmonellosis in horses.

Fecal Consistency and Odor

The probiotic product ColiCure® contains *E. coli* and is licensed by EFSA to improve fecal consistency. There are no published studies in the peer-reviewed scientific literature on this product. The only information available on efficacy can be obtained from the EFSA scientific opinions (www.efsa.com). In the 4 studies submitted by the manufacturer, fecal consistency improved in treated animals by 1–2 scales. Only 3 of the 4 studies contained a control population, and fecal consistency improved in control animals as well, albeit at a slower rate. Inclusion criteria for cases cannot be determined from the provided information and the starting point of 'poor fecal consistency' as well as the endpoint 'improved' were not defined and were subjective. In 2 studies, 1 with healthy horses only and 1 with 'abnormal' horses, microbiological analysis was performed. An increase in the number of coliforms and a decrease in the number of *Clostridium* and *Bacillus* spp. in the treated horse were noted. Although this would indicate an effect of the probiotic on the microbiota of these horses, for the reasons described above, cultures have inherent technical limitations to assess the microbiota. It is unclear whether the changes that were noted actually represent beneficial effects on the intestinal microbiota. This is particularly true given recent studies that have indicated clostridia are important components of the intestinal microbiota, and decreases in this group actually may not be desirable.[4]

Fecal Sand Clearance

The effect of a combined probiotic and prebiotic (*Psyllium*) product on fecal sand clearance in a natural setting was evaluated. Eight adult healthy equids (horses and mules) were included in this trial and each animal served as its own control.[82] Baseline sand excretion was measured over 7 days then animals received the pre-/probiotic mixture once daily for 35 days and fecal sand clearance was measured. Fecal sand output increased significantly on day 4 after starting treatment and remained 2.5 times higher on average throughout the treatment period.[82] Whether this effect was caused by *Psyllium* or the probiotic or the combination thereof was not determined by this study. Several clinical case reports describing efficacy of this prebiotic in horses have been published.[87–89] There is little clinical evidence to substantiate the claim that feeding *Psyllium* helps prevent or treat sand accumulation.

Fecal Microbial Transplantation

The gastrointestinal microbiota of humans and animals consists of thousands of microbial species.[2] The interaction of probiotics with the gastrointestinal microbiota has been investigated and documented in many studies.[90] All existing probiotics consist of 1 or a few strains that compromise a very minor component of the intestinal microbiota. Therefore, they might have limited ability to influence and individual's entire gastrointestinal microbiota. With this in mind, scientists have looked into the other end of the 'probiotic' complexity spectrum, fecal transplants. Fecal transplants consist of an intact, highly complex microbial community composed by thousands of species.

Fecal microbial transplantation (FMT) constitutes the transfer a fecal suspension from a healthy donor into the bowel of the recipient. Prior antimicrobial administration to decrease the resident microbiota, by colonoscopy, retention enema, nasogastric tube or nasoduodenal tube[91] can be attempted.

Fecal microbial transplantation is not a novel therapeutic modality and its first reports in human medicine date back to the sixteenth century. However, it has only recently received increased attention after several published studies showed that feces is a biologically active mixture of living organisms with great potential for treatment of *C. difficile* infection,[92,93] and other gastrointestinal[94–96] and nongastrointestinal disorders.[97] In human medicine, the application of FMT for recurrent *C. difficile* infection is currently best researched. Two systematic reviews showed that this form of therapy was safe and effective in 83–92% patients to achieve sustained full resolution of clinical signs.[92,98]

Recently, FMT also has emerged in veterinary medicine. There are no peer-reviewed published studies about the use of FMT in domestic animals. In an ongoing clinical trial, fecal transplantation is used to treat dogs and cats with inflammatory bowel disease. Of the so far, 3 enrolled cats and 3 dogs, all have been treated successfully by transferring healthy donor feces by colonoscopy to the recipients[a] .

Although there are no published studies or abstracts in horses, anecdotal reports suggest that this form of therapy also might be effective in horses with acute colitis or chronic diarrhea.[83] Probiotic strains have limited ability to colonize the gastrointestinal tract for long periods of time.[46] Fecal transplantation might have lasting effects on colonization.[99] Fecal microbial transplantation studies in humans have shown that some index bacteria are still present in the feces of patients up to 24 weeks after treatment, indicating a permanent change in the microbiota associated with resolution of clinical signs.[99]

The safety of FMT has rarely been investigated. A few minor adverse effects were observed sporadically in a systematic review of approximately 300 patients treated with FMT for recurrent *C. difficile*-associated diarrhea.[92] Screening of potential FMT donors for the occurrence of pathogenic organisms is a major point of discussion. Ideally, the type and number of pathogenic species to be screened should be species dependent. In horses, screening for the presence of *Salmonella, C. difficile,* and Equine Infectious Anemia Virus is recommended. Whether other infectious agents such as coronavirus or rotavirus should be included in the screening needs to be evaluated. It is generally agreed that prior antibiotic treatment should exclude a horse from being a donor. However, what duration of time since the last antibiotic administration should elapse remains speculative.

Conclusions

Although probiotics have shown promise in the treatment of selected diseases in humans, the evidence that they can be used to control diseases in horses so far is weak. The aim of developing 1 probiotic to aid in prevention or treatment of all diseases is unrealistic. Each bacterial strain has different effects. The choice and combination of strains for a therapeutic formulation needs to be specific for each disease and should be based on the *in vitro* properties of the strains. Randomized placebo-controlled clinical trials under controlled conditions then are necessary to provide evidence for each probiotic formulation in horses.

Based on lack of regulation regarding quality control of commercial products, use of over-the-counter products is questionable, particularly in the absence of scientific information on safety and clinical efficacy. Efficacy trials should be conducted and published in peer-reviewed journals before recommending use. These products also should be carefully evaluated for their composition and concentration by the investigators of the clinical trials to ensure efficacy and reproducibility of results.

Despite all of these limitations, probiotics generally are regarded as safe, cost effective and easy to administer. Therefore, additional research is warranted to test possible applications in equine veterinary practice. Exploiting new knowledge of the composition of the equine microbiota, the focus of probiotic research should shift from currently used agents to species that are abundant in the intestinal microbiota of the horse. Particular emphasis should be given to bacterial species that are associated with the microbiota of healthy horses. The approach of administering 1 or few strains together should be rethought on the background on the vast microbiota. Given the promising results of FMT in humans, the clinical efficacy of this approach should be tested for prevention and treatment of enterocolitis in horses.

Footnotes

[a] Weese JS, Costa MC, Webb JA. Preliminary Clinical and Microbiota Assessment of Stool Transplantation in the Dog and Cat. Journal of Veterinary Internal Medicine 2013;27:705–705.

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References

1. Lederberg JM, McCray A. Ome Sweet. The Scientist 2001;15:8.

2. Group NHW, Peterson J, Garges S, et al. The NIH Human Microbiome Project. Genome Res 2009;19:2317–2323.

3. Al Jassim RA, Andrews FM. The bacterial community of the horse gastrointestinal tract and its relation to fermentative acidosis, laminitis, colic, and stomach ulcers. The Veterinary clinics of North America. Equine Pract 2009;25:199–215.

4. Costa MC, Arroyo LG, Allen-Vercoe E, et al. Comparison of the fecal microbiota of healthy horses and horses with colitis by high throughput sequencing of the V3-V5 region of the 16S rRNA gene. PLoS ONE 2012;7:e41484.

5. Costa MC, Weese JS. The equine intestinal microbiome. Animal health research reviews/Conference of Research Workers in Animal Diseases 2012: 1–8.

6. Friedrich MJ. Microbiome project seeks to understand human body's microscopic residents. J Am Med Assoc 2008;300:777–778.

7. Frank DN, St Amand AL, Feldman RA, et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A 2007;104:13780–13785.

8. Larsen N, Vogensen FK, van den Berg FW, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS ONE 2010;5:e9085.

9. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57–63.

10. Scher JU, Abramson SB. The microbiome and rheumatoid arthritis. Nat Rev Rheumatol 2011;7:569–578.

11. Baverud V, Franklin A, Gunnarsson A, et al. Clostridium difficile associated with acute colitis in mature horses treated with antibiotics. Equine Vet J 1997;29:279–284.

12. Baverud V, Gustafsson A, Gunnarsson A, et al. The association of erythromycin ethylsuccinate with acute colitis in horses in Sweden. Equine Vet J 1997;29:314–318.

13. Garrett LA, Brown R, Poxton IR. A comparative study of the intestinal microbiota of healthy horses and those suffering from equine grass sickness. Vet Microbiol 2002;87:81–88.

14. Milinovich GJ, Trott DJ, Burrell PC, et al. Fluorescence in situ hybridization analysis of hindgut bacteria associated with the development of equine laminitis. Environ Microbiol 2007;9:2090–2100.

15. Moreau MM, Eades SC, Reinemeyer CR, et al. Illumina sequencing of the V4 hypervariable region 16S rRNA gene reveals extensive changes in bacterial communities in the cecum following carbohydrate oral infusion and development of early-stage acute laminitis in the horse. Vet Microbiol 2014;168:436–441.

16. Bailey SR, Rycroft A, Elliott J. Production of amines in equine cecal contents in an in vitro model of carbohydrate overload. J Anim Sci 2002;80:2656–2662.

17. Medina B, Girard ID, Jacotot E, Julliand V. Effect of a preparation of Saccharomyces cerevisiae on microbial profiles and fermentation patterns in the large intestine of horses fed a high fiber or a high starch diet. J Anim Sci 2002;80:2600–2609.

18. Milinovich GJ, Burrell PC, Pollitt CC, et al. Microbial ecology of the equine hindgut during oligofructose-induced laminitis. ISME J 2008;2:1089–1100.

19. Milinovich GJ, Trott DJ, Burrell PC, et al. Changes in equine hindgut bacterial populations during oligofructose-induced laminitis. Environ Microbiol 2006;8:885–898.

20. Respondek F, Goachet AG, Julliand V. Effects of dietary short-chain fructooligosaccharides on the intestinal microflora of horses subjected to a sudden change in diet. J Anim Sci 2008;86:316–323.

21. Muhonen S, Connysson M, Lindberg JE, et al. Effects of crude protein intake from grass silage-only diets on the equine colon ecosystem after an abrupt feed change. J Anim Sci 2008;86:3465–3472.

22. Muhonen S, Julliand V, Lindberg JE, et al. Effects on the equine colon ecosystem of grass silage and haylage diets after an abrupt change from hay. J Anim Sci 2009;87:2291–2298.

23. Shepherd ML, Swecker WS Jr, Jensen RV, Ponder MA. Characterization of the fecal bacteria communities of forage-fed horses by pyrosequencing of 16S rRNA V4 gene amplicons. FEMS Microbiol Lett 2012;326:62–68.

24. Dougal K, Harris PA, Edwards A, et al. A comparison of the microbiome and the metabolome of different regions of the equine hindgut. FEMS Microbiol Ecol 2012;82:642–652.

25. Dougal K, de la Fuente G, Harris PA, et al. Characterisation of the faecal bacterial community in adult and elderly horses fed a high fibre, high oil or high starch diet using 454 pyrosequencing. PLoS ONE 2014;9:e87424.

26. O' Donnell MM, Harris HM, Jeffery IB, et al. The core faecal bacterial microbiome of Irish Thoroughbred racehorses. Lett Appl Microbiol 2013;57:492–501.

27. Dougal K, de la Fuente G, Harris PA, et al. Identification of a core bacterial community within the large intestine of the horse. PLoS ONE 2013;8:e77660.

28. Schoster A, Arroyo LG, Staempfli HR, Weese JS. Comparison of microbial populations in the small intestine, large intestine and feces of healthy horses using terminal restriction fragment length polymorphism. BMC Res Notes 2013;6:91.

29. Metchnikoff E. Optimistic studies. New York: Putman''s Sons. 1908:22.

30. FAO/WHO. Working group for drafting guidelines for the evaluation of probiotics in food. ftp://ftpfaoorg/es/esn/food/wgreport2pdf 2002.

31. Kailasapathy K, Chin J. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and Bifidobacterium spp. Immunol Cell Biol 2000; 78:80–88.

32. Gibson GR, Fuller R. Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and prebiotics for human use. J Nutr 2000;130:391S–395S.

33. Yuki N, Shimazaki T, Kushiro A, et al. Colonization of the stratified squamous epithelium of the nonsecreting area of horse stomach by lactobacilli. Appl Environ Microbiol 2000;66:5030–5034.

34. Laukova A, Simonova M, Strompfova V, et al. Potential of enterococci isolated from horses. Anaerobe 2008;14:234–236.

35. Laukova A, Strompfova V, Ouwehand A. Adhesion properties of enterococci to intestinal mucus of different hosts. Vet Res Commun 2004;28:647–655.

36. Rinkinen M, Westermarck E, Salminen S, Ouwehand AC. Absence of host specificity for in vitro adhesion of probiotic lactic acid bacteria to intestinal mucus. Vet Microbiol 2003;97: 55–61.

37. Schoster A, Kokotovic B, Permin A, et al. In vitro inhibition of Clostridium difficile and *Clostridium perfringens* by commercial probiotic strains. Anaerobe 2013;20:36–41.

38. Gueimonde M, Sanchez B, de los Reyes-Gavilan CG, Margolles A. Antibiotic resistance in probiotic bacteria. Front Microbiol 2013;4:202.

39. Bennedsen M, Stuer-Lauridsen B, Danielsen M, Johansen E. Screening for antimicrobial resistance genes and virulence factors via genome sequencing. Appl Environ Microbiol 2011;77:2785–2787.

40. Cebrian R, Banos A, Valdivia E, et al. Characterization of functional, safety, and probiotic properties of *Enterococcus faecalis* UGRA10, a new AS-48-producer strain. Food Microbiol 2012;30:59–67.

41. Banwo K, Sanni A, Tan H. Technological properties and probiotic potential of *Enterococcus faecium* strains isolated from cow milk. J Appl Microbiol 2013;114:229–241.

42. Munoz-Atienza E, Gomez-Sala B, Araujo C, et al. Antimicrobial activity, antibiotic susceptibility and virulence factors of Lactic Acid Bacteria of aquatic origin intended for use as probiotics in aquaculture. BMC Microbiol 2013;13:15.

43. Mater DD, Langella P, Corthier G, Flores MJ. A probiotic Lactobacillus strain can acquire vancomycin resistance during digestive transit in mice. J Mol Microbiol Biotechnol 2008;14:123–127.

44. Tannock GW, Luchansky JB, Miller L, et al. Molecular characterization of a plasmid-borne (pGT633) erythromycin resistance determinant (ermGT) from Lactobacillus reuteri 100-63. Plasmid 1994;31:60–71.

45. Vescovo M, Morelli L, Bottazzi V, Gasson MJ. Conjugal transfer of broad-host-range plasmid pAMbeta1 into enteric species of lactic acid bacteria. Appl Environ Microbiol 1983;46:753–755.

46. Weese JS, Anderson ME, Lowe A, Monteith GJ. Preliminary investigation of the probiotic potential of Lactobacillus rhamnosus strain GG in horses: Fecal recovery following oral administration and safety. Can Vet J 2003;44:299–302.

47. Desrochers AM, Dolente BA, Roy MF, et al. Efficacy of *Saccharomyces boulardii* for treatment of horses with acute enterocolitis. J Am Vet Med Assoc 2005;227:954–959.

48. Jouany JP, Gobert J, Medina B, et al. Effect of live yeast culture supplementation on apparent digestibility and rate of passage in horses fed a high-fiber or high-starch diet. J Anim Sci 2008;86:339–347.

49. Jouany JP, Medina B, Bertin G, Julliand V. Effect of live yeast culture supplementation on hindgut microbial communities and their polysaccharidase and glycoside hydrolase activities in horses fed a high-fiber or high-starch diet. J Anim Sci 2009;87:2844–2852.

50. van Duijkeren E, Flemming C, Sloet van Oldruitenborgh-Oosterbaan M, et al. Diagnosing salmonellosis in horses. Culturing of multiple versus single faecal samples. Vet Q 1995;17:63–66.

51. Oelschlaeger TA. Mechanisms of probiotic actions – A review. Int J Med Microbiol 2010;300:57–62.

52. Watanabe T, Nishio H, Tanigawa T, et al. Probiotic Lactobacillus casei strain Shirota prevents indomethacin-induced small intestinal injury: Involvement of lactic acid. Am J Physiol Gastrointest Liver Physiol 2009;297:G506–513.

53. de Roock S, van Elk M, van Dijk ME, et al. Lactic acid bacteria differ in their ability to induce functional regulatory T cells in humans. Clin Exp Allergy 2010;40:103–110.

54. Thomas CM, Versalovic J. Probiotics-host communication: Modulation of signaling pathways in the intestine. Gut Microbes 2010;1:148–163.

55. Rinkinen M, Jalava K, Westermarck E, et al. Interaction between probiotic lactic acid bacteria and canine enteric pathogens: A risk factor for intestinal *Enterococcus faecium* colonization? Vet Microbiol 2003;92:111–119.

56. He B, Xu W, Santini PA, et al. Intestinal bacteria trigger T cell-independent immunoglobulin A(2) class switching by inducing epithelial-cell secretion of the cytokine APRIL. Immunity 2007;26:812–826.

57. Fukushima Y, Kawata Y, Hara H, et al. Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children. Int J Food Microbiol 1998;42:39–44.

58. Park JH, Um JI, Lee BJ, et al. Encapsulated *Bifidobacterium bifidum* potentiates intestinal IgA production. Cell Immunol 2002;219:22–27.

59. Saarela M, Mogensen G, Fonden R, et al. Probiotic bacteria: Safety, functional and technological properties. J Biotechnol 2000;84:197–215.

60. Collado MC, Grzeskowiak L, Salminen S. Probiotic strains and their combination inhibit in vitro adhesion of pathogens to pig intestinal mucosa. Curr Microbiol 2007;55:260–265.

61. Collado MC, Meriluoto J, Salminen S. Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. Lett Appl Microbiol 2007;45:454–460.

62. Chen X, Kokkotou EG, Mustafa N, et al. *Saccharomyces boulardii* inhibits ERK1/2 mitogen-activated protein kinase activation both in vitro and in vivo and protects against Clostridium difficile toxin A-induced enteritis. J Biol Chem 2006;281:24449–24454.

63. Bayoumi MA, Griffiths MW. In vitro inhibition of expression of virulence genes responsible for colonization and systemic spread of enteric pathogens using *Bifidobacterium bifidum* secreted molecules. Int J Food Microbiol 2012;156:255–263.

64. Allaart JG, van Asten AJ, Vernooij JC, Grone A. Effect of *Lactobacillus fermentum* on beta2 toxin production by *Clostridium perfringens*. Appl Environ Microbiol 2011;77:4406–4411.

65. Medellin-Pena MJ, Wang H, Johnson R, et al. Probiotics affect virulence-related gene expression in *Escherichia coli* O157:H7. Appl Environ Microbiol 2007;73:4259–4267.

66. Castagliuolo I, Riegler MF, Valenick L, et al. *Saccharomyces boulardii* protease inhibits the effects of Clostridium difficile toxins A and B in human colonic mucosa. Infect Immun 1999;67:302–307.

67. Gratz S, Taubel M, Juvonen RO, et al. *Lactobacillus rhamnosus* strain GG modulates intestinal absorption, fecal excretion, and toxicity of aflatoxin B(1) in rats. Appl Environ Microbiol 2006;72:7398–7400.

68. Turner PC, Wu QK, Piekkola S, et al. *Lactobacillus rhamnosus* strain GG restores alkaline phosphatase activity in differentiating Caco-2 cells dosed with the potent mycotoxin deoxynivalenol. Food Chem Toxicol 2008;46:2118–2123.

69. Weese JS. Evaluation of deficiencies in labeling of commercial probiotics. Can Vet J 2003;44:982–983.

70. Weese JS. Microbiologic evaluation of commercial probiotics. J Am Vet Med Assoc 2002;220:794–797.

71. Weese JS, Martin H. Assessment of commercial probiotic bacterial contents and label accuracy. Can Vet J 2011;52:43–46.

72. Ward MP, Alinovi CA, Couetil LL, et al. A randomized clinical trial using probiotics to prevent Salmonella fecal shedding in hospitalized horses. J Equine Vet Sci 2004;24:242–247.

73. Shanahan F. A commentary on the safety of probiotics. Gastroenterol Clin North Am 2012;41:869–876.

74. Weese JS, Anderson ME, Lowe A, et al. Screening of the equine intestinal microflora for potential probiotic organisms. Equine Vet J 2004;36:351–355.

75. Weese JS, Rousseau J. Evaluation of *Lactobacillus pentosus* WE7 for prevention of diarrhea in neonatal foals. J Am Vet Med Assoc 2005;226:2031–2034.

76. Parraga ME, Spier SJ, Thurmond M, Hirsh D. A clinical trial of probiotic administration for prevention of Salmonella shedding in the postoperative period in horses with colic. J Vet Intern Med 1997;11:36–41.

77. Boyle AG, Magdesian KG, Durando MM, et al. *Saccharomyces boulardii* viability and efficacy in horses with antimicrobial-induced diarrhoea. Vet Rec 2013;172:128.

78. Earing E, Bacterial J. Colonization of the equine gut; comparison of mare and foal pairs by PCR-DGGE. Adv Microbiol 2012;02:79–86.

79. Yuyama T. Evaluation of a host-specific Lactobacillus probiotic in neonatal foals. J Appl Res Vet Med 2004;2:26–32. (Yusa S, ed.)

80. Chioukh FZ, Ben Hmida H, Ben Ameur K, et al. [*Saccharomyces cerevisiae* fungemia in a premature neonate treated receiving probiotics.]. Med Mal Infect 2013;43:359–360.

81. Kim LM, Morley PS, Traub-Dargatz JL, et al. Factors associated with Salmonella shedding among equine colic patients at a veterinary teaching hospital. J Am Vet Med Assoc 2001;218:740–748.

82. Landes AD, Hassel DM, Funk JD, Hill A. Fecal sand clearance is enhanced with a product combining probiotics, prebiotics, and psyllium in clinically normal horses. J Equine Vet Sci 2008;28:79–84.

83. Feary DJ, Hassel DM. Enteritis and colitis in horses. Vet Clin North Am Equine Pract 2006;22:437–479, ix.

84. Frederick J, Giguere S, Sanchez LC. Infectious agents detected in the feces of diarrheic foals: A retrospective study of 233 cases (2003–2008). J Vet Intern Med 2009;23:1254–1260.

85. Vandeplas S, Dubois Dauphin R, Beckers Y, et al. Salmonella in chicken: Current and developing strategies to reduce contamination at farm level. J Food Prot 2010;73:774–785.

86. Frizzo LS, Zbrun MV, Soto LP, et al. Pathogen translocation and histopathological lesions in an experimental model of Salmonella Dublin infection in calves receiving lactic acid bacteria and lactose supplements. J Vet Sci 2012;13:261–270.

87. Ramey DW, Reinertson EL. Sand-induced diarrhea in a foal. J Am Vet Med Assoc 1984;185:537–538.

88. Bertone JJ, Traub-Dargatz JL, Wrigley RW, et al. Diarrhea associated with sand in the gastrointestinal tract of horses. J Am Vet Med Assoc 1988;193:1409–1412.

89. Hart KA, Linnenkohl W, Mayer JR, et al. Medical management of sand enteropathy in 62 horses. Equine Vet J 2012;45:465–469.

90. Arora T, Singh S, Sharma RK. Probiotics: Interaction with gut microbiome and antiobesity potential. Nutrition 2013;29:591–596.

91. Borody TJ, Campbell J. Fecal microbiota transplantation: Current status and future directions. Expert Rev Gastroenterol Hepatol 2011;5:653–655.

92. Gough E, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent *Clostridium difficile* infection. Clin Infect Dis 2011;53:994–1002.

93. Khoruts A, Dicksved J, Jansson JK, Sadowsky MJ. Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent *Clostridium difficile*-associated diarrhea. J Clin Gastroenterol 2010;44:354–360.

94. Kunde S, Pham A, Bonczyk S, et al. Safety, tolerability, and clinical response after fecal transplantation in children and young adults with ulcerative colitis. J Pediatr Gastroenterol Nutr 2013;56:597–601.

95. Bazzocchi G, Gionchetti P, Almerigi PF, et al. Intestinal microflora and oral bacteriotherapy in irritable bowel syndrome. Dig Liver Dis 2002;34(Suppl 2):S48–53.

96. Borody TJ, Warren EF, Leis S, et al. Treatment of ulcerative colitis using fecal bacteriotherapy. J Clin Gastroenterol 2003;37:42–47.

97. Vrieze A, Van Nood E, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 2012;143(913–916):e917.

98. Guo B, Harstall C, Louie T, et al. Systematic review: Faecal transplantation for the treatment of *Clostridium difficile*-associated disease. Aliment Pharmacol Ther 2012;35:865–875.

99. Grehan MJ, Borody TJ, Leis SM, et al. Durable alteration of the colonic microbiota by the administration of donor fecal flora. J Clin Gastroenterol 2010;44:551–561.