Preliminary investigation of the probiotic potential of *Lactobacillus rhamnosus* strain GG in horses: fecal recovery following oral administration and safety

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Abstract — This study was designed to evaluate whether *Lactobacillus rhamnosus* strain GG (LGG), an extensively studied probiotic organism in humans, can colonize the intestines of adult horses and foals. *Lactobacillus rhamnosus* strain GG was administered to adult horses at doses of 1×10^9 CFU/50kg bodyweight (BW)/day (group 1, 7 horses), 1×10^{10} colony forming units/50kg BW/day (group 2, 7 horses) and 5×10^{10} colony forming units/50kg BW/day (group 3, 7 horses) for 5 d. Foals received 2×10^{10} colony forming units/50kg BW/day (group 1, 7 foals) or 1×10^{11} colony forming units/50kg BW/day (group 2, 7 foals) for 5 d. Fecal levels of *L. rhamnosus* strain GG in adult horses were low and variable in the 2 lower dose groups. Even in the high dose group, colonization was relatively low. In contrast, more consistent intestinal colonization was present in foals, and colonization persisted for up to 9 d following cessation of administration. No adverse effects were observed in any animal. Clinical studies evaluating this probiotic are indicated in foals. The presence of this organism in the feces of adult horses may only represent passive movement through the intestinal tract, not actual colonization. Consistent intestinal colonization in adults was only achieved with a prohibitively high dose.

Résumé — Étude préliminaire sur le potentiel probiotique de *Lactobacillus rhamnosus,* souche GG, chez les chevaux : récupération fécale à la suite de l'administration orale, et innocuité. Cette étude a été conçue afin de vérifier si *L. rhamnosus,* souche GG, un organisme probiotique très étudié chez l'homme, pouvait coloniser l'intestin des chevaux adultes et des poulains. *L. rhamnosus,* souche GG, a été administré à des chevaux adultes aux doses de 1×10^9 unités formatrices de colonie (UFC)/50 kg de poids corporel (PC)/jour (1er groupe, 7 chevaux), de 1×10^{10} UFC/50 kg PC/jour (2e groupe, 7 chevaux) et de 5×10^{10} UFC/50 kg PC/jour (3e groupe, 7 chevaux) pendant 5 jours. Les poulains ont reçu 2×10^{10} UFC/50 kg PC/jour (1er groupe, 7 poulains) ou 1×10^{11} UFC/50 kg PC/jour (2e groupe, 7 poulains) pendant 5 jours. Chez les chevaux adultes, les concentrations fécales de *L. rhamnosus,* souche GG, étaient basses et variables chez les 2 groupes ayant reçu les doses les plus faibles. Même dans le groupe ayant reçu la dose la plus élevée, la colonisation était relativement faible. Cependant, une colonisation intestinale plus substantielle était présente chez les poulains, et la colonisation a persisté jusqu'à 9 jours après la fin du traitement. Aucun animal n'a présenté d'effets indésirables. Des études cliniques pour évaluer ce probiotique sont souhaitables chez le poulain. La présence de cet organisme dans les fèces de chevaux adultes pourrait n'être attribuable qu'à son transit passif dans le tractus intestinal, sans qu'il y ait de colonisation véritable. La colonisation intestinale n'était régulièrement obtenue chez les adultes qu'au prix d'une dose excessivement élevée.

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Introduction

A probiotic can be defined as a living microorganism that upon ingestion in certain numbers exerts health effects beyond inherent basic nutrition (1). Elie Metchnikoff first discussed the concept of probiotics almost 100 y ago (2). He suggested that the longevity of certain ethnic groups was related to their ingestion of fermented milk products and that these products manipulated the intestinal microflora to maintain the normal balance between pathogenic and nonpathogenic bacteria.(2) Since this initial observation, it has been recognized that a number of factors beyond simple manipulation of the intestinal microflora can play a role in the success of probiotic therapy. Despite a relative paucity of research, probiotic therapy is generating increasing attention in veterinary medicine.

Appealing properties of probiotics include the ability to reduce antibiotic use, the apparently high index of

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safety, and the public's positive perception about "natural" or "alternative" therapies. Probiotics are "generally regarded as safe," as opposed to antibiotics, which have a number of recognized adverse effects (3). Very little research has been performed in the field of equine probiotic therapy. Parraga et al (4) were unable to demonstrate any influence on the shedding of *Salmonella* spp., prevalence of postoperative diarrhea, length of antimicrobial therapy, and duration of hospitalization in horses at a teaching hospital as a result of the administration of 2 commercial probiotics. A second study reported no effect on *Salmonella* shedding in hospitalized horses with colic (5). However, there was no indication that the strains used in this study possessed any of the probiotic properties listed above or that an adequate dose was administered.

A variety of microorganisms, typically lactic acid bacteria, such as lactobacilli, bifidobacteria, and enterococci, have been evaluated as potential probiotics (6). A small number of yeasts have also been evaluated (7,8). Commercial probiotic preparations are available for human and animal use; however, there is little to no objective research available for many of them, particularly those intended for companion animals. Based on the definition (1) stated above, it is clear that adequate numbers of viable probiotic organisms must reach the intestinal tract. To do this, they must be able to survive transit through the acidic environment of the stomach and resist bile digestion. Organisms that survive acid and bile must possess a variety of other properties, including the ability to adhere to intestinal epithelial cells, colonize the intestinal tract, produce an antimicrobial factor, and inhibit enteric pathogens (9–12). Other properties, such as immunomodulation, modulation of metabolic activities, and inactivation of procarcinogens, are also desirable (13,14). An organism can only be considered to be a probiotic after these properties have been identified and a positive health effect has been documented.

One of the best-studied probiotics in human medicine is *Lactobacillus rhamnosus* strain GG (LGG). *Lactobacillus rhamnosus* GG has been shown to survive acid and bile digestion and to colonize the gastrointestinal tracts of humans (15–18). It also possesses powerful adhesive properties, suppresses bacterial enzyme activity, can displace or eliminate certain components of the normal intestinal flora, and produces an antimicrobial substance active against a variety of bacteria, including *Escerichia coli*¸ *Salmonella* spp., *Clostridium* spp., *Streptococcus* spp., and *Bacteroides* spp. (12). In humans, LGG has been shown to be effective in the treatment of several forms of diarrhea, including rotaviral diarrhea in children, acute nonrotaviral diarrhea in children, antibiotic-associated diarrhea in children and adults, "travellers" diarrhea, and relapsing *Clostridium difficile* diarrhea in placebo-controlled studies (12,19–26). Gastrointestinal disease is of serious concern in equine medicine and these results in humans suggest that probiotics, particularly LGG, might be treatment options.

Some authors believe that probiotic organisms should be naturally occurring in their target species to be effective (10). However, cross-species efficacy has been demonstrated for some probiotic strains, including LGG (27). Prior to evaluating the efficacy of any probiotic, it should be demonstrated that the organism has the ability to survive transit through the gastrointestinal tract of the intended host. This does not indicate that an organism will have probiotic properties in the given species; however, demonstration of intestinal survival and fecal presence are a prerequisite for studies evaluating efficacy. This study was designed to evaluate whether LGG can colonize the gastrointestinal tract of adult horses and foals, and do so without causing adverse effects.

Materials and methods

The study was approved by the University of Guelph Animal Care Committee. Twenty-one, clinically healthy, adult standardbred horses weighing between 450 and 500 kg were enrolled in the study. Fourteen, clinically healthy, pony foals, ranging from 1 to 3 d of age and weighing 18 to 25 kg were also used. Diet and management were not altered. Horses were individually housed and randomly allocated to treatment groups receiving *L. rhamnosus* GG at dosages of 1×10^9 colony forming units (CFU)/50kg bodyweight (BW) (group 1, $n = 7$), 1×10^{10} CFU/50kg BW (group 2, $n = 7$), or 5×10^{10} CFU/50kg BW (group 3, $n = 7$), once daily for 5 d (days 0 through 4). *Lactobacillus rhamnosus* GG was administered by opening capsules and mixing the contents with a small amount of moistened pelleted feed. Foals were housed as a group on pasture with their dams. Foals were divided into 2 groups. Group 1 ($n = 7$) received 2×10^{10} CFU/50kg BW and group 2 received 1×10^{11} CFU/50kg BW ($n = 7$), once daily for 5 d. *Lactobacillus rhamnosus* GG was administered either by opening the capsule and sprinkling contents directly into the mouth, or via a dosing syringe. Horses and foals were monitored daily for changes in clinical condition, appetite, and fecal consistency. Fecal samples were collected on days 0, 1, 3, 5, 6, 7, and 9, and every 48 h thereafter until day 15, or until 2 consecutive samples that were negative for LGG were obtained, whichever occurred first. Freshly passed fecal samples were obtained from adult horses. Fecal sampling from foals was performed directly per rectum. Samples were refrigerated and processed within 2 h or stored at −80°C until processed. Serial 10-fold dilutions of feces were performed in phosphate buffered saline (pH 7.2). Aliquots of the serial dilutions, from 10^2 to 10^8, were inoculated onto deMan, Rogosa, Sharp (MRS) agar and incubated in an anaerobic chamber at 37°C for 72 h. Colonies were identified as LGG, based on morphology (large, round, white, creamy colonies), Gram stain appearance (gram-positive uniform rods), and the inability to ferment lactose (28). Randomly selected isolates were confirmed as LGG by using the API 50 CHL (BioMerieux, St. Laurent, Quebec) biochemical identification assay.

The area under the curve of fecal LGG level versus day for each horse was analyzed using a Kruskal-Wallis 5 sample rank test. Multiple comparisons were based on a Tukey adjustment to control the overall experimentwise error rate. Shapiro Wilk test on the residuals of the areas confirmed that the data were normally distributed ($P < 0.1301$). Analysis was performed by using statistical analysis software (29).

Results

All horses but 1 consumed LGG readily in feed, and it appeared to have been completely consumed. The exception was in group 3, and this horse was administered the LGG via a dosing syringe after mixing it with water and corn syrup. No problems were encountered in the administration of LGG to foals, and it is believed that all was consumed. No adverse effects were identified in any horses or foals throughout the study period.

Lactobacillus rhamnosus strain GG was not detected in the feces of any adult horse prior to administration (Figure 1). Intestinal colonization was identified in 5/7 (71%) of horses in group 1, 2/7 (29%) of horses in group 2, and 6/7 (86%) of horses in group 3. The mean number of positive samples was 1.0 in group 1 (s = 1.0, range 0 to 3), 0.28 in group 2 (s = 0.49, range 0 to 1), and 1.7 in group 3 (s = 0.95, range 0 to 3). Twenty-four hours after cessation of administration, LGG was still present in the feces of 1/7 (14%) horses in group 1, 1/7 (14%) in group 2, and 4/7 (57%) in group 3. By 48 h after cessation of administration, LGG was present in the feces of 1/7 (14%) horses in each of groups 1 and 2, and no horses in group 3. In adult horses, overall growth ranged from $\log_{10}$ 2.6 to 6 CFU/g of feces. Among horses that were colonized with LGG, mean $\log_{10}$ levels detected during the administration period (days 0 to 4) were 3.9 CFU/g in group 1, 3.0 CFU/g in group 2, and 4.7 CFU/g in group 3. Based on area under the curve calculation, there was not a significant difference in intestinal level of LGG between the adult horse groups ($P > 0.05$).

Lactobacillus rhamnosus GG was not present in any fecal samples from foals prior to administration. All foals in both groups were colonized with LGG on at least 1 d. The mean number of positive samples was 3.0 in group 1 (s = 1.73, range 1 to 5) and 2.7 in group 2 (s = 0.95, range 1 to 4). Intestinal colonization was somewhat intermittent, so not all samples were positive on all days. Among foals, fecal levels of LGG ranged from $\log_{10}$ 3.7 to 7.5 CFU/g. Persistence of intestinal colonization was greater than for adults. The median day on which LGG was detected in feces after cessation of treatment was day 3, while LGG was not present in the feces of any adult horses on this day. One foal maintained detectable fecal levels of LGG for 9 d following cessation of administration. Among foals that were colonized, mean $\log_{10}$ fecal levels during the administration period were 5.3 CFU/g in group 1 and 5.4 CFU/g in group 2. The peak mean level of colonization was achieved at day 7 for group 1 ($\log_{10}$ 4.2 CFU/g) and day 3 for group 2 ($\log_{10}$ 3.9 CFU/g). There was not a significant difference in intestinal LGG colonization between foal groups. Intestinal LGG levels were, however, significantly higher in both foal groups compared with adult horse groups 1 and 2.

Discussion

Compared with other species, intestinal colonization of LGG in adult horses is sporadic and poor. The lack of a statistically significant difference between the adult groups was surprising. An explanation for the lower intestinal level of LGG in the intermediate dose group is not apparent. Even with a high level of supplementation

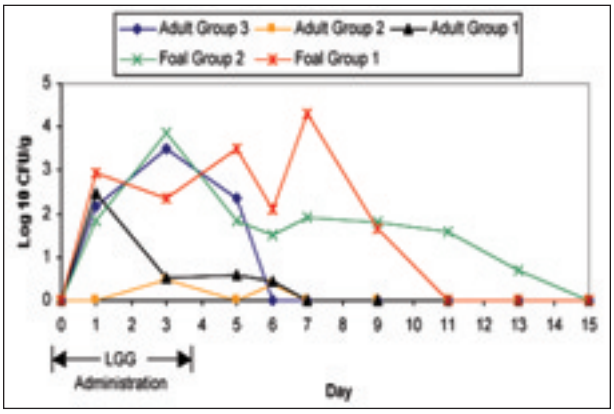

Figure 1. Mean fecal counts of *Lactobacillus rhamnosus* strain GG following oral administration in horses and foals. (Adult group 1: 1×10^9 CFU/50kg BW/d, adult group 2: 1×10^{10} CFU/50kg BW/d, adult group 3: 5×10^{10} CFU/50kg BW/d, foal group 1: 2×10^{10} CFU/50kg BW/d, foal group 2: 1×10^{11} CFU/50kg BW/d).

(5×10^{10} CFU/50kg BW/d), the peak intestinal colonization was on day 3 when only 5/7 horses were colonized with a mean level of 3.5 $\log_{10}$ CFU/g of feces. In humans, levels of 5 to 7 $\log_{10}$ CFU/g have been reported following administration of 1×10^{10} CFU/d (18,28). Different dosage groups complicate direct comparison between adult horses and foals. However, it appears that LGG is better able to colonize the intestinal tract of foals. Being of human origin, it is possible that LGG is less adapted to successfully compete with the intestinal microflora of adult horses. Presumably, the immature nature of the gastrointestinal microflora in foals facilitated colonization. It is possible that LGG is not able to adhere well to equine intestinal epithelial cells. This would not explain the differences between adults and foals, unless age-related differences in enterocytes adhesion properties were present. This warrants further study.

Persistence of colonization following cessation of administration in adult horses was limited. Even transient colonization of LGG can have an effect on the resident microflora in humans; however, the inconsistent and generally low levels present in adult horses in this study may not be adequate for such an effect. Further, because LGG did not persist in adult fecal samples for more than 48 h after cessation of administration, even in the high dose group, it is not clear whether fecal levels were due to actual transient colonization of the intestinal tract or simply due to passive movement through the gastrointestinal tract. In foals, however, LGG persisted for a median of 3 d and a maximum of 9 d following cessation of administration. Campbell et al (30) reported complete intestinal clearance of barium within 36 h in 2 young foals, suggesting that LGG colonized the intestinal tract of foals, although it may not be reasonable to equate movement of barium to that of bacteria. In humans, LGG is reported to persist for longer periods of time. One study reported that 87% of humans shed LGG in feces 4 d following cessation of administration, while 33% shed LGG after 7 d (15).

The lack of a dose response in foals was interesting and unexpected. It was suspected that higher fecal

levels would be present with the higher oral dose; however, this was not the case. Perhaps, the intestinal microflora in foals is quite susceptible to colonization and the dose used in group 1 was optimal.

Based on the sporadic colonization of LGG in adult horses, it is unlikely that this organism has significant probiotic potential in healthy horses. It is possible that colonization would be better in diarrheic horses or in those undergoing antimicrobial therapy because of disruption of the normal protective intestinal microflora. This should be evaluated. In contrast, LGG may have potential as a probiotic in foals. While peak intestinal levels were lower than those encountered in humans, LGG was able to colonize the majority of foals and persisted longer than in adults. Neonatal foals were used in this study. It is unclear at this point whether these results can be extrapolated to older foals, as maturation of the intestinal microflora in foals is poorly understood.

Lactobacillus rhamnosus GG cannot be considered an equine probiotic at this point. The poor intestinal colonization in adult horses is not encouraging. Unless improved colonization by LGG can be demonstrated in diarrheic or antibiotic-treated horses, further efficacy studies are likely not warranted. This situation may be different in foals. Adequate colonization of LGG in foals without any adverse effects was identified in this study. Efficacy studies should be performed to evaluate this organism in the prevention or treatment of disease in foals. It has been reported that LGG is able to affect antigen transport in the intestinal tract via closure of large molecular transport pores (12). It is unclear whether this property could interfere with passive transfer of maternal antibodies. As a result, it would be prudent to avoid administering LGG to foals less than 24 h of age. CVJ

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