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Comparison of probiotic lactobacilli and bifidobacteria effects, immune responses and rotavirus vaccines and infection in different host species

Anastasia N. Vlasova[*], **Sukumar Kandasamy**, **Kuldeep S. Chattha**, **Gireesh Rajashekara**, and **Linda J. Saif**[*]

Food Animal Health Research Program, The Ohio Agricultural Research and Development Center, Veterinary Preventive Medicine Department, The Ohio State University, Wooster, Ohio, USA

Abstract

Different probiotic strains of *Lactobacillus* and *Bifidobacterium* genera possess significant and widely acknowledged health-promoting and immunomodulatory properties. They also provide an affordable means for prevention and treatment of various infectious, allergic and inflammatory conditions as demonstrated in numerous human and animal studies. Despite the ample evidence of protective effects of these probiotics against rotavirus (RV) infection and disease, the precise immune mechanisms of this protection remain largely undefined, because of limited mechanistic research possible in humans and investigated in the majority of animal models. Additionally, while most human clinical probiotic trials are well-standardized using the same strains, uniform dosages, regimens of the probiotic treatments and similar host age, animal studies often lack standardization, have variable experimental designs, and non-uniform and sometime limited selection of experimental variables or observational parameters. This review presents selected data on different probiotic strains of lactobacilli and bifidobacteria and summarizes the knowledge of their immunomodulatory properties and the associated protection against RV disease in diverse host species including neonates.

Keywords

probiotics; lactobacilli; bifidobacteria; rotavirus; diarrhea; immunomodulation

[*]Corresponding authors: Drs. Anastasia N. Vlasova and Linda J. Saif, Food Animal Health Research Program (FAHRP). The Ohio Agricultural Research and Development Center, Veterinary Preventive Medicine Department, The Ohio State University, USA. 1680 Madison Avenue, Wooster, Ohio, 44691. Phone: (330) 263-3742/Fax: (330) 263-3677/3740. vlasova.1@osu.edu; saif.2@osu.edu.

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Introduction

Microbial colonization begins immediately after birth with facultative anaerobes, such as lactobacilli, enterococci and enterobacteria, being the first colonizers. Colonization by anaerobic microorganisms follows, including *Bifidobacterium*, *Bacteroides* and *Clostridium,* resulting in a gradual decrease of the ratio of facultative anaerobes to strict anaerobes over time (Arboleya et al., 2012). Bifidobacteria, along with lactobacilli, are an important part of normal intestinal microbiota of various mammalian species and are also the best characterized and widely commercialized probiotics. Both lactobacilli and bifidobacteria are non-spore-forming, gram-positive, lactic acid producing bacteria (LAPB). Lactobacilli have limited biosynthetic abilities and ferment refined sugars, generating lactic acid as the major end product (Wells, 2011), whereas Bifidobacteria are important producers of short chain fatty acids (SCFA) (Tojo et al., 2014). Despite some common properties, lactobacilli and bifidobacteria belong to two taxonomically distinct groups: the genus *Lactobacillus* in the phylum *Firmicutes* and the genus *Bifidobacterium* in the phylum *Actinobacteria*, respectively. In adults, *Firmicutes* and *Bacteroidetes* phyla usually dominate the intestinal microbiota, whereas *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* are considerably less abundant. However, in naturally delivered, breast-fed infants, bifidobacteria (*Actinobacteria*) appear between days 2 and 5 after birth and reach a maximum of up to 99% of all bacteria within one week becoming the predominant bacterial component of the infant fecal microbiota (Kurokawa et al., 2007; Mitsuoka and Kaneuchi, 1977; Turroni et al., 2012; Yatsunenko et al., 2012). Some studies report that *Bifidobacterium infantis* and *Bifidobacterium breve* were the most common species found in healthy infants (He et al., 2001).

Although not the most dominant in adulthood, Lactobacilli and Bifidobacteria remain stable elements of the normal intestinal microbiota, maintaining their important functions throughout life, and their dysbiosis is associated with a plethora of pathological conditions (Gerritsen et al., 2011). Numerous studies with different strains of *Lactobacillus* and *Bifidobacterium* have been performed in vitro and in vivo, in humans and animal models to investigate their immunomodulatory properties and probiotic potential to treat various infectious, allergic and inflammatory conditions (Grimm et al., 2014; Picard et al., 2005; Tojo et al., 2014; Wells, 2011) (Figure 1). While not always conclusive, most of them emphasized the beneficial effects of these probiotic bacteria, that appear to be pathogen/condition, bacteria and sometimes host species-specific. In most clinical trials, lactobacilli and bifidobacteria probiotics were demonstrated to be safe with the rare exception of probiotic-associated infections in immunosuppressed patients (Saarela et al., 2002). Historically, the most usual application of probiotics is to treat gastrointestinal disorders, including infectious diarrhea (de Vrese and Marteau, 2007).

Acute diarrhea due to viral or bacterial infections is still a frequent cause of death, especially in hospitalized children in developing countries. Group A Rotavirus (RVA) is the leading cause of acute viral gastroenteritis in children, accounting for ~440,000 deaths annually, mostly in developing countries (Parashar et al., 2006). Current licensed human RVA vaccines have low efficacy in impoverished countries (Armah et al., 2010; Widdowson et al., 2009; Zaman et al., 2010). Similarly, RVAs are a common cause of diarrhea in young

animals including nursing and weaned piglets. RVAs are responsible for 7-20% and 3-50% mortality in nursing and weaned piglets, respectively, resulting in economic losses to the pork industry (Yuan, 2006). Commercially available porcine RVA vaccines have low efficacy due to low immunogenicity, the presence of maternal antibodies in piglets, and genotypic variability of porcine RVs (Hoblet et al., 1986; Saif and Fernandez, 1996). This emphasizes the need for additional affordable host-targeted interventional strategies to alleviate the RV disease burden in children and young animals.

The initial evidence for protective effects of LAPB against RV diarrhea came from human clinical studies that in most cases do not allow evaluation of the precise biological mechanisms involved (Grandy et al., 2010). The therapeutic capacity of certain probiotic bacteria against RVA gastroenteritis has been suggested to be due to their ability to enhance and maintain mucosal integrity (Schiffrin and Blum, 2002), production of antimicrobial substances (including lactic acid, nitric oxide, H_2O_2, bacteriocins) (Ganzle et al., 2000) or stimulation of antimicrobial peptide and mucin production by intestinal epithelial cells (IECs), and stimulation of the local adaptive (increased production of BAFF and APRIL factors by IECs leading to an increase in secretory IgA Abs) and innate immune responses (Ganzle et al., 2000; Kaila et al., 1995) (Figure 1). Numerous cytokines produced by IECs (including IL25, IL33, TGFβ) and innate immune cells [including natural killer (IL22), antigen-presenting (APC) (IL12, IL25, IL10 TGFβ), innate lymphoid and γδ T cells (IL22)] are modulated by lactobacilli and bifidobacteria resulting in improved intestinal barrier function, reduced effector and increased regulatory immune responses (Figure 1). This review will compare the effects of lactobacilli and bifidobacteria probiotic supplementation/colonization on RVA disease and immune responses to RVAs in human clinical studies and those observed in animal experiments. We will also summarize common and distinct mechanisms observed in various studies to determine whether more guided and targeted use of these probiotics may improve the outcome in a variety of host species including livestock.

Lactobacilli and bifidobacteria probiotics and rotavirus diarrhea in human clinical studies

Probiotic efficacy in treatment of acute RV diarrhea was best exemplified for *Lactobacillus rhamnosus* GG (LGG), *L. reuteri* and some bifidobacteria in multiple randomized, double-blind, placebo-controlled trials (Table 1). In most of these trials, probiotic supplementation was combined with or preceded by oral rehydration therapy initiated within 24-48 hours after acute RV diarrhea confirmation or hospital admission (due to acute diarrhea). The statistically significant (in most cases) reduction in the duration of diarrhea was consistent among patients aged 1 month – 5 years. There were only a few trials that involved children from developing countries (China, India and Peru) with signs of clinical dehydration and generally low RV (vs other enteric pathogens) infection prevalence that did not show positive effects or only showed marginal effects of the probiotic supplementation (Mao et al., 2008; Misra et al., 2009; Salazar-Lindo et al., 2004). The authors suggested that lactose malabsorption, other underlying health conditions (unaccounted for) and dehydration might have contributed to the lack of therapeutic effects. Also, compromised nutritional status of most of the children reported in these studies could have contributed to the observed results,

because malnourishment itself may aggravate RV (Uhnoo et al., 1990; Zijlstra et al., 1999) and other enteric infections via direct or indirect mechanisms (by modulating intestinal microbiota). For instance, it was recently demonstrated that severe acute malnourishment was associated with commensal microbiota immaturity (relative microbiota maturity index was lower than it should be at a certain age) that was only partially ameliorated by widely used nutritional interventions [such as ready-to-use therapeutic food (RUTF; Plumpy'Nut)] (Subramanian et al., 2014). The microbiota immaturity, often implicated in various health disorders as well unresponsiveness to vaccines, was also evident in less severe forms of malnutrition (Subramanian et al., 2015; Subramanian et al., 2014). Interestingly, another study from Peru demonstrated a prophylactic effect of 15-month long LGG supplementation against diarrhea (due bacterial or RV infections) in non-breast-fed undernourished children, especially in the toddler age group (18-29 months) (Oberhelman et al., 1999). Thus, these trials provide sufficient evidence to recommend use of at least one probiotic strain, lactobacilli or bifidobacteria, in milk, water or rehydration solution, to treat acute RV diarrhea in children under 5 years of age (Reid et al., 2003). However, additional treatments or specifically designed probiotic/symbiotic therapies may be required when supplementing undernourished children (Oberhelman et al., 1999; Salazar-Lindo et al., 2004). Finally, some studies suggested that probiotics reduce RVA diarrhea and shedding in a dose dependent manner in children (Mao et al., 2008; Shornikova et al., 1997b). After these initial observations, several potential mechanisms of probiotic-associated reduction in RV diarrhea have been discussed (Figure 1), but most of them are based on in vitro studies using different pathogens/probiotic strains and so far none have been definitively proven. The first is receptor site blockage, in which probiotic bacteria bind to receptors, thereby preventing adhesion and invasion of the virus (Bernet et al., 1994). The second suggested mechanism refers to secretory IgA and cytokine response modulation that may lead to the observed clinical effect (Christensen et al., 2002; Kaila et al., 1992). However, the fact that diarrhea appears to cease within the first 3 days after the probiotic treatment initiation emphasizes that the observed therapeutic effect is unlikely to be mediated via enhancement of adaptive immune responses. Another mechanism might involve modulation of mucin (MUC2 and MUC3 gene mRNA) expression, ultimately affecting motility defences and removal of noxious substances (Mack et al., 1999; Xu and Verstraete, 2001) and thereby alleviating diarrhea. A final theory is that some lactobacilli species (such as *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14) produce unidentified substances that inactivate the viral particles (Cadieux et al., 2002).

There were limited efforts to evaluate the effects of RVA infection/diarrhea on probiotic lactobacilli and intestinal bifido- and enterobacteria in infants. These studies demonstrated that asymptomatic RVA infection did not affect colonization patterns of bifido- and enterobacteria in the gut of Indian neonates in the first month of life (Balamurugan et al., 2010), while RV diarrhea only negligibly altered the adherence properties of the evaluated probiotics (*L. rhamnosus* GG, *L. casei Shirota*, *L. paracasei* F19, *L. acidophilus* LA5, and *B. lactis* Bb12) or human intestinal mucus expression (Juntunen et al., 2001). The latter study also indicated that appropriate combinations of probiotics may increase their overall adhesion (possibly leading to improved immune responses), which may provide additional benefits in the treatment and prevention of RV diarrhea.

Overall, for all the reported pediatric clinical trials the exact protection mechanisms by probiotics remain unclear. Due to inability to conduct mechanistic studies in human subjects, especially in neonates, more research using animal models is critical to understand how different lactobacilli and bifidobacteria probiotic strains and various regimens of their administration modulate RV diarrhea in neonatal animals and children with variable nutritional and health status.

Lactobacilli and bifidobacteria interactions with the immune system and rotavirus in animal models

Animal models for biomedical (including probiotic) research allow for greater control of the environment, manipulations of multiple experimental variables and provide for careful monitoring of large numbers of testing parameters.

Studies in conventional or commensal microbiota transplanted animal models

So far, a few studies in conventional mouse models confirmed antagonistic effects of different lactobacilli and bifidobacteria strains against RVA diarrhea and attempted to define mechanisms of their action (Table 2). In one study, mouse pups of dams orally immunized against RVA and fed *Bifidobacterium breve* YIT4064 had higher levels of protection against subsequent RVA challenge than the pups born to dams immunized with RVA alone (Yasui et al., 1995). This correlated with higher levels of RV-specific Abs in the milk, feces and intestinal contents of the probiotic fed RV immunized dams. Further, in suckling rats infected with SA11 RVA and supplemented with milk fermented by *L. casei* DN-114 001 (known to increase small intestinal brush-border enzyme activity) (Thoreux et al., 1998), the cellular vacuolization in the small intestine was reduced, coinciding with decreased RVA load in all intestinal sections, and decreased diarrhea severity. This confirms that *L. casei* DN-114 001 reduced RV infection and the associated intestinal damage (Guerin-Danan et al., 2001). Another study demonstrated that *B. bifidum* and *B. infantis* supplementation mitigated rhesus RV (RRV) diarrhea and increased fecal and serum levels of RRV-specific IgA Abs in mice (Qiao et al., 2002). In agreement with previous findings in human neonates, superior results were demonstrated for *L. rhamnosus* GG (compared to 5 other species of lactobacilli) in reducing diarrhea severity and duration in BALB/c pups (Pant et al., 2007). Overall, these findings support major observations from human clinical trials emphasizing that RV diarrhea reduction is associated with increased local and systemic RV-specific IgA responses with effects varying for different probiotic strains. However, in these earlier (1995-2007) studies, mostly confirmatory in nature, the mechanism of the IgA increase and other related immunological modulations remained undefined. More studies on identifying novel probiotic strains (such as *B. longum subsp. infantis* CECT 7210) and evaluating their effects on RV diarrhea in animal preclinical experiments are underway (Munoz et al., 2011).

In a recent study, 2 genotypically and phenotypically distinct strains of *L. reuteri*, DSM 17938 and ATCC PTA 6475, safe and effective in treating infantile colic (Savino et al., 2010; Savino et al., 2007), reduced RVA diarrhea duration in neonatal mouse pups and enhanced diversity of the intestinal microbiome (Preidis et al., 2012). Some observed probiotic effects were strain-specific and some were influenced by the mouse nutritional status. The

antidiarrheal effects of DSM 17938, but not of ATCC PTA 6475, correlated with the rate of intestinal epithelial cell proliferation. Also, both probiotic strains increased epithelial cell migration, decreased levels of proinflammatory cytokines and increased RV-specific Abs in all but undernourished mice (Preidis et al., 2012). This study also suggested that the IgA Ab increase was not essential for probiotic disease moderation, because strain 6475 ameliorated diarrhea in underweight mice without enhancing IgA Ab production. Enhancement of IgA responses by probiotics may be facilitated by simultaneous activation of multiple signaling pathways by RV (Blutt et al., 2004) and probiotic bacteria (Iyer et al., 2008). Beneficial bacteria stimulate enterocytes, dendritic cells, or macrophages expressing innate immune receptors to produce B-cell stimulatory factors (BAFF, APRIL or TGF-β1) (He et al., 2007; Massacand et al., 2008). They may also increase the activity of the polymeric Ig receptor resulting in more efficient transport of IgA Abs from the lamina propria into the intestinal lumen (Hooper and Gordon, 2001; Nakamura et al., 2012; Norderhaug et al., 1999). Similarly, cytokine suppression did not appear to lead to diarrhea attenuation by *L. reuteri* 17938 in underweight mice (Preidis et al., 2012). However, epithelial cell turnover rate - one of the host defence mechanisms of expelling pathogens from the epithelium (Boshuizen et al., 2003; Cliffe et al., 2005; Mulvey et al., 2000) - appears to be generally modulated by probiotics (if evaluated) and may ultimately lead to improved protection against RV.

Experiments using humanized piglets (i.e. piglets transplanted with intestinal microbiota from human infants) revealed that RV infection shifted bacterial abundance from *Firmicutes* to *Proteobacteria* phylum, whereas LGG supplementation prevented the human RVA infection-induced changes in the microbial community (Zhang et al., 2014). These findings suggest that probiotic bacteria influence the outcome of RV infections via protecting the stability of the intestinal microbiota and the associated host metabolic profiles (Zhao et al., 2013).

Studies in the gnotobiotic (Gn) pig animal model

In humans and conventional animals, the complex microbiome, diverse diets and various underlying conditions complicate understanding of the interactions among commensals, pathogens and the immune system. Therefore, Gn animals provide the additional benefit of modelling interactions exclusively between the target organisms (single or multiple probiotic bacteria and enteric pathogens) and the host immune system without confounding factors including commensal microbiota, maternal Abs, other pathogens.

Consistent with previous observations in various species, our recent study of neonatal Gn piglets, dual colonization with *Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium lactis* Bb12 (Bb12) resulted in less severe diarrhea and reduced virus shedding titers compared to uncolonized piglets and differentially modulated mucosal and systemic innate and adaptive immunity during human RVA infection of Gn pigs (Vlasova et al., 2013) (Table 2). These probiotics exerted inhibitory effects on dendritic cell (DC) populations at the systemic level as evident by lower frequencies of activated splenic DCs (conventional and plasmacytoid) in probiotic colonized versus non-colonized vaccinated piglets post-RVA challenge. However, probiotic colonization increased frequencies of activated DCs in ileum and blood suggestive of enhanced maturation of the intestinal (mucosal) immune

compartment and immune cell trafficking. We also observed a synergistic interaction between the attenuated human RVA (AttHRV) vaccine and LGG and Bb12 colonization as evident by increased frequencies of ileal TLR9+ mononuclear cells (MNCs) in intestinal tissues of probiotic colonized, RVA vaccinated piglets compared to uncolonized AttHRV vaccinated piglets pre-challenge. Further, the increased TLR9+ MNC frequencies pre-challenge coincided with a higher protective effect against virus shedding and diarrhea observed post-virulent human RVA challenge. In contrast, the LGG and Bb12 colonized, vaccinated piglets had decreased frequencies of ileal TLR2+ and TLR4+ MNCs compared to uncolonized vaccinated piglets. An earlier study of adult human subjects reported increased TLR2 and TLR4 expression in submucosal immune cells of inflamed intestinal mucosa compared to healthy mucosa (Hausmann et al., 2002) (Table 2). Thus, regulating the expression of specific TLRs by these probiotics in the small intestine might play a role in intestinal immune homeostasis and also prevent excessive inflammatory responses during viral infection.

Dual colonization of LGG and Bb12 probiotics had significant effects on human RVA vaccine induced B and T cell responses. B cell responses, including activation of intestinal B cells and RV specific IgA Ab titers were enhanced in vaccinated, probiotic colonized piglets compared to uncolonized, vaccinated piglets post-virulent human RVA challenge (Kandasamy et al., 2014). The latter effect coincided with increased TLR9 expression (see above), and according to the previous reports, TLR9 and BAFF up-regulation may be associated with increased IgA levels (Li et al., 2014), and may represent synergistic sensing of probiotics and RV by innate immune cells leading to increased IgA levels. Further, T cell responses, specifically ileal T regulatory cells, and systemic IFNγ producing T cell responses, were increased in probiotic colonized and vaccinated compared to uncolonized vaccinated piglets (Chattha et al., 2013b). Importantly, the probiotic induced immunomodulatory effects on adaptive immune responses coincided with decreased diarrhea severity and reduced fecal virus shedding.

Investigators have reported that immunomodulatory effects vary with strain (Medina et al., 2007) and composition of the probiotic bacteria (Gackowska et al., 2006). Thus, we also assessed the impact of two other lactic acid producing probiotic bacteria (LAB), *Lactobacillus acidophilus* (LA) and *Lactobacillus reuteri* (LR), on intestinal and systemic innate immune responses (Wen et al., 2009; Zhang et al., 2008a; Zhang et al., 2008b; Zhang et al., 2008c) (Table 2). Compared to uninfected negative control piglets, human RVA infection alone significantly increased monocytes/macrophages, but not the cDC population in ileum. However, LA+LR colonized human RVA infected piglets had lower frequencies of monocytes/macrophages compared to human RVA only infected piglets in ileum. Additionally, probiotic colonized piglets had lower frequency of activated macrophages post-human RVA infection. The APC populations in spleens were significantly reduced in LA+LR colonized, compared to uncolonzied piglets, post-virulent human RVA infection. Similarly, colonization of piglets with LA+LR significantly reduced TNF-α cytokine secreting cells in the ileum and spleens post-human RVA challenge (Azevedo et al., 2012) (Table 2). Thus, reduction in total, as well as activated intestinal monocyte/macrophage populations, and decreased inflammatory cytokine production in LAB colonized piglets

during human RVA infection indicates that these probiotics have a protective effect on inflammatory damage during human RVA infection.

Colonization of Gn piglets with LAPB alone resulted in significant modulation of innate immunity (Table 2). LA+LR dual colonization significantly increased both monocytes/macrophages and cDC populations in ileum in comparison to uncolonized Gn piglets (Zhang et al., 2008c). Further, in the absence of human RVA infection, probiotic colonization alone increased frequencies of TLR2 and TLR9 positive cDC in blood (Wen et al., 2009). These results indicate that LA and LR alone had significant stimulatory effects on the innate immune system.

Similar to LGG and Bb12 effects on B cell responses, LA probiotic significantly enhanced the immunogenicity of AttHRV vaccine responses as indicated by higher numbers of ileal RVA specific IgA and IgG antibody secreting cells (ASCs) and increased intestinal IFNγ producing T cells compared to uncolonized piglets post RVA inoculation (Zhang et al., 2008b) (Table 2). Apart from individual effects of LA on adaptive vaccine-specific immunity, dual-colonization of LA and LR significantly modulated the types of γδ T cell (critical for early responses to infections at epithelial surfaces) responses during RVA infection of Gn piglets without vaccination (Wen et al., 2011). There were lower numbers of inflammatory type CD2+CD8− γδ T cells and higher regulatory type CD2+CD8+ γδ T cells in LA+LR probiotic colonized piglets in comparison to uncolonized piglets post-virulent human RVA infection. Additionally, higher systemic IFNγ and IL4 cytokine responses in LA+LR colonized compared to uncolonized RVA infected piglets suggest that LAPB modulated both Th1 and Th2 immunity, respectively (Wen et al., 2009). Thus, the probiotics tested had measurable beneficial effects on AttHRV vaccine protective efficacy and immunogenicity and they moderated the severity of RVA diarrhea, but only when given at least 21 days prior to human RVA challenge (Chattha et al., 2013b). However, whether these observed beneficial effects could be reproduced by these probiotics in the presence of complex microbiota remains to be determined.

Intestinal epithelial cells are the target cells for RV infection and their anatomic location facilitates interactions with probiotics and intestinal commensal bacteria. In a recent study, LGG colonization modulated human RVA effects on the levels of tight junction and adherent junction proteins (Liu et al., 2013) and down-regulated autophagy in Gn pig ileal epithelium after human RVA infection (Wu et al., 2013) (Table 2). Thus, it appears that probiotics can alleviate the RV induced pathological changes in intestinal epithelial cells and reduce diarrhea associated with the loss of mature enterocytes and subsequent malabsorption. Overall, the Gn pig represents a unique model allowing studies of the biological mechanisms of lactobacilli and bifidobacteria effects on RV and all compartments of the immune system with a high relevance to both swine and human health due to the significant immunological, digestive and anatomical similarities between the two species.

There is evidence from human pediatric trials that probiotic supplementation in the neonatal period may be affected by the breastfeeding status (Oberhelman et al., 1999). However, there are few studies on the impact of selected probiotics on responses to oral vaccines in neonates in the context of colostrum/milk (col/milk) feeding. We have recently examined how LGG

+Bb12 colonization with or without col/milk (to mimic breastfed versus formula-fed infants) affects development of B cell responses to an oral AttHRV vaccine in the relevant Gn pig model (Chattha et al., 2013a).

In agreement with previous findings that breast-milk promotes growth of Bifidobacteria and Lactobacilli, supplementation of col/milk (naturally containing TGF-β and other growth factors) increased fecal probiotic shedding. This increased in probiotic shedding suggested that milk containing regulatory cytokines (such as TGFβ) and other soluble factors such as glycans can promote establishment and extend colonization by probiotics (LGG+Bb12) (Ahrne et al., 2005; Rinne et al., 2005; Yoshioka et al., 1983) (Table 2). Breast milk is a major source of TGFβ for neonates when intrinsic production is limited (Nguyen et al., 2007; Penttila, 2010) promoting intestinal immune responses, including class-switch to IgA, induction of regulatory T lymphocytes, attenuation of pro-inflammatory responses and reducing immune mediated and allergic conditions (Kalliomaki et al., 1999).

Lower counts of probiotics detected in cecum/colon of col/milk fed pigs, irrespective of vaccination, suggested a differential impact of col/milk on fecal bacterial shedding vs intestinal distribution or mucosal adherence. Maternal to bacterial components Abs in sow col/milk including peptidoglycan may prevent mucosal adhesion of probiotics resulting in lower mucosa-associated bacterial counts as observed in suckling Gn mice previously (Kramer and Cebra, 1995) (Table 2).

Combined probiotic colonization and col/milk supplementation in vaccinated pigs enhanced serum RVA-specific IgA Ab titers and intestinal IgA RVA ASC levels, which were not observed in vaccinated pigs that did not receive col/milk, suggesting complex interactions between probiotics and col/milk components (Chattha et al., 2013a). Col/milk containing human RVA Abs transiently suppressed serum IgA Ab responses after two vaccine doses irrespective of probiotic colonization, but this effect was ameliorated after three doses of the vaccine (Chattha et al., 2013a). Thus, colonization with LGG+Bb12 in breast fed vaccinated infants (with pre-existing maternal human RVA Abs) may overcome the suppressive effects of maternal Abs, at least for IgA Ab responses. Similar to our study, Isolauri et al. (1995) showed enhanced RV IgA Ab responses in LGG fed infants of unknown breastfeeding status after oral immunization with live oral RV vaccine (Isolauri et al., 1995). Thus, our results using the Gn pig model suggested that feeding LGG+Bb12 to breastfed infants may be advantageous by enhancing human IgA RVA Abs and thus preventing adverse clinical effects of human RVA gastroenteritis.

Lactobacilli and bifidobacteria prevention of RV diarrhea in livestock and the associated immune mechanisms

Increased prevalence of bacterial strains resistant to antibiotics in humans has stimulated public and federal interest in eliminating the use of antibiotics in sub-therapeutic doses for growth promotion (antibiotic-growth promoters; AGP) in livestock. An alternative approach to improve health and productivity in livestock is the use of probiotics, prebiotic substrates that serve as nutrients to certain bacteria, or their combinations (synbiotics). A variety of microbial species (bacteria of *Bacillus*, *Escherichia*, *Lactobacillus*, *Bifidobacterium*,

Enterococcus, Lactococcus, Streptococcus, and *Pediococcus* genera, yeast and undefined mixed cultures) have been used as probiotics generally resulting in reduced mortality, enhanced immune responses, improved growth rates, feed intake and feed efficiency in poultry and livestock of different ages [reviewed in Cho et al. (2011) and Patterson et al. (2003)] (Cho, 2011; Patterson and Burkholder, 2003). While *Lactobacillus* and *Bifidobacterium* species have been used most extensively in humans; historically, various species of *Bacillus*, *Enterococcus*, and *Saccharomyces* yeast have been the most commonly used in livestock (Simon, 2001). Only during the past few decades, has there been an increase in research on supplementing *Lactobacillus* to livestock (Gusils et al., 1999; Jin et al., 2000; Pascual et al., 1999; Tellez et al., 2001) (Table 3). Further, while in some studies LAPB improved growth performance and post-weaning diarrhea (PWD) control in weanling pigs (Lessard, 1987; Shu et al., 2001), these effects were not observed in others (Walsh, 2007) (Table 3). As reviewed in Heo et al. (2013), this inconsistency in results of probiotic effects on PWD and performance in pigs may be attributed to differences in dosage and type of probiotic, management practices, diet, and age (Heo et al., 2013). One study evaluated the effects of bifidobacteria and LAPB (in place of AGPs) in newborn calves and piglets and demonstrated that these probiotics reduced mortality, improved weight gain, fecal condition and feed efficiency in both species (Abe et al., 1995). However, the effects of lactobacilli (including various strains of *L. reuteri*, as well as *L. gasseri*, *L. acidophilus* and *L. fermentum*) supplementation on infectious diarrhea occurrence, growth performance and feed conversion in neonatal and weanling piglets varied with age, feeding status (sow milk versus milk replacer) and lactobacilli strain (Chang et al., 2001; Chen et al., 2014; Huang, 2004; Liu et al., 2014; Wang et al., 2009a; Wang et al., 2013; Wang, 2011; Wang et al., 2012; Yu, 2008) (Table 3). Potential mechanisms of lactobacilli beneficial effects proposed in these studies included alleviation of oxidative stress (Wang et al., 2013; Wang et al., 2009b), protective modulation of gut microbiota (Chang et al., 2001; Huang, 2004; Liu et al., 2014) and associated metabolic profiles (Liu et al., 2014), enhancement of T-cell differentiation, ileal cytokine production (Wang et al., 2009a) and serum IgG Ab levels (Yu, 2008). Additionally, reduction in the levels of IL-1β mRNA expression in the ileum of neonatal piglets due to *L. reuteri* supplementation was reported (Hou, 2015; Liu et al., 2014).

Very few mechanistic studies addressing interactions among LAPB, immunity and RV were conducted in livestock species, and primarily in pigs. In 3-week old piglets, the administration of *B. lactis* HN019 led to lower concentrations of fecal RVA and reduced severity of weanling diarrhea (Shu et al., 2001) (Table 3). Indicative of immune enhancement, higher blood leukocyte phagocytic and T-lymphocyte proliferative responses, and higher intestinal RV-specific Ab (IgM, IgG and IgA) titers were detected in *B. lactis* HN019 fed piglets. Interestingly, another study using suckling piglets demonstrated reduced RVA shedding due to *Enterococcus faecium* NCIMB 10415 supplementation that was not associated with increased RV-specific Ab titers (Kreuzer et al., 2012). However, the probiotic supplementation resulted in significant differences in effector and regulatory T cell responses. These data suggest, once again that reduction in RV diarrhea/infection may be achieved via different mechanisms by different probiotic bacteria, while the increase of

RVA-specific Ab levels (often found due to probiotic supplementation) is not essential for the disease attenuation.

Future research should be focused on more detailed characterization of probiotic properties of various lactobacilli and bifidobacteria, optimal regimens and doses of their supplementation, as well as immune mechanisms of the probiotic mediated protection against RV disease.

Parallels between mucosal transcriptome responses and immunomodulatory effects of lactobacilli

Molecular mechanisms of probiotic action on neonatal intestinal mucosal immunity remain largely undefined. The remarkable study by van Baarlen et al. (2011) elucidated the mucosal transcriptome responses of healthy adults to three lactobacilli strains (van Baarlen et al., 2011). Expectedly, different expression profiles were observed in response to consumption of *L. acidophilus* Lafti L10, *L. casei* CRL-431 and *L. rhamnosus* GG. Further, the in vivo expression profiles of distal duodenum were similar statistically to expression profiles from high-throughput pharmaceutical experiments in vitro (van Baarlen et al., 2011). The authors demonstrated that *L. acidophilus* Lafti L10 regulated IL-23 signaling, consistent with a role in immune tolerance, and likely to promote Th1 immune responses (important for protection against RV) as reflected by the up-regulation of expression of Th1-specific IFN-induced chemokines (such as CXCL10 and CXCL11) and IFN-responsive genes. Contrastingly, transcriptome responses to consumption of *L. casei* CRL-431 suggested a possible shift in the Th1/Th2 balance to a Th2 type and/or Th17 type and up-regulation of surface marker expression on antigen presenting cells. The latter mirrored an earlier study in a mouse model using *L. casei* CRL-431 (Galdeano and Perdigon, 2006). Finally, consumption of *L. rhamnosus* GG in this study was associated with induction of the cytokine-encoding genes CCL24, CCL2, and CXCL3 that are especially effective in stimulating Th1 responses (Yang et al., 1997). The observed up-regulation of several IFN-induced genes and STAT4 further emphasizes that consumption of *L. rhamnosus* may have promoted expression of genes that stimulate Th1 effector-cell development (Korman et al., 2008; Steinman, 2007). In two previous microarray studies (using a mouse cell line and profiling intestinal responses of humans suffering from esophagitis) the common major modulated pathways for *L. rhamnosus* GG were related to the regulation of the immune response, apoptosis, and cell growth and differentiation (Di Caro et al., 2005; Tao et al., 2006), suggesting that different host species exhibit at least a few similar responses to the same bacterial strain.

Recently, using a transcriptomic approach, we assessed mucosal tissue responses to LGG or LA monocolonization of neonatal Gn piglets (Kumar et al., 2014). Results suggest that transcriptomic responses vary with the strain of probiotic, duration of probiotic colonization, and region of the intestinal tract. Immediately after probiotic colonization (day 1), both LA and LGG induced higher transcriptional responses in ileum, whereas at later stages (7 days), LGG, but not LA, induced profound changes in expression of transcripts in duodenum. In agreement with previous results by van Baarlen et al. (2011), both of these probiotics seem to polarize mucosal immunity towards Th1 type (van Baarlen et al., 2011) as indicated by higher expression of chemokine (C-C motif) ligand 9 [CCL9; macrophage inflammatory

protein-1 gamma (MIP-1γ)] in LA and LGG piglets and higher granzyme (serine proteases involved in apoptosis) expression in LA piglets.

Compared to LA, LGG significantly modulated genes associated with the following pathways: inflammatory response, immune cell trafficking and hematological system development in the duodenum. Pathways associated with immune modulation and carbohydrate metabolism were highly altered by LGG, whereas LA predominantly induced changes in energy and lipid metabolism-related trancriptomic responses. Further LA, but not LGG, induced prominent changes in transcription of vitamin A related genes in duodenum. Thus, LA and LGG differentially modulated major pathways in intestinal tissues. Further, both LGG and LA colonization resulted in higher expression of glucagon-like peptide 2 receptor (GLP2R) which regulates villus height and crypt depth in the small intestine (Jeppesen et al., 2001). LGG colonization also increased expression of claudin-8, a tight junction protein that regulates paracellular permeability (Ulluwishewa et al., 2011). Collectively, our intestinal tissue transcriptomic study revealed that lactobacilli have prominent impacts on the host immune and metabolic functions and that different strains have significantly varying biological effects as reported before in various in vivo and in vitro studies. These studies may illuminate the precise molecular mechanisms of probiotic action on mucosal immunity and emphasize that some mechanisms of probiotic actions may be conserved across different mammalian host species of different ages. This provides an avenue to develop optimal strategies to tailor preventive and therapeutic probiotic therapy for RV infections.

Concluding remarks

Lactobacilli and bifidobacteria provide significant health benefits to various host species, improving feed conversion and growth performance, modulating immune responses and intestinal crypt dynamics, and ultimately protecting the host from pathogens including RV. Human clinical randomized placebo controlled trials provide the strongest evidence of lactobacilli/bifidobacteria mediated protection against RV diarrhea and infection in pediatric patients, but lack mechanistic explanations for the observed protection. Animal studies confirm findings in humans and contribute substantial knowledge on the mechanisms of the probiotic mediated immune enhancement and increased protection against RV. These include modulation of effector and regulatory T cell responses resulting in Th1 or Th2 polarization of the immune response, increased activation of APCs, modulation of innate immune signaling via interactions with multiple TLRs, decrease in the levels of pro-inflammatory cytokines (Figure 1) and differential increase in enterocyte proliferation and/or migration resulting in more efficient flushing of RV from the intestinal epithelium or more rapid replacement of the epithelial layer after the necrotic RV infection. Interestingly, in different animal models as well as in human studies, the increase in systemic and mucosal IgA Ab levels was commonly observed due to lactobacilli and bifidobacteria supplementation/colonization but was not essential for reduction of RV diarrhea and infection. Recently increased interest in the use of these probiotic bacteria in livestock confirms previous results observed in humans and animal models and demonstrates that these bacteria can be universally beneficial in a variety of mammalian and avian species and provide an alternative to AGPs. Studies in animals and human subjects confirm that the probiotic action may vary

with different dosages, regimens, bacterial strains, host age, health condition, and nutritional status. The presence of maternal lactogenic immune factors (in breast-fed infants or suckling animals) is of particular importance and may play a dualistic role in promoting immune maturation or interfering with probiotic actions and persistence in the gut. Finally, transcriptome research in various host species may provide additional knowledge regarding diverse effects of the probiotics and aid in designing of optimal preventative and interventional health promoting strategies.

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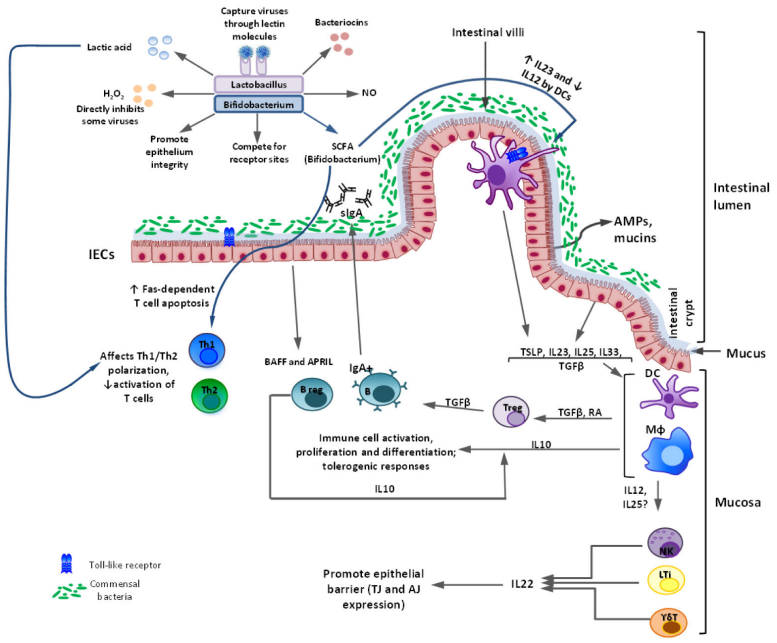

Figure 1. Interactions between probiotics of Lactobacillus and Bifidobacterium genera and the immune system modelled in vitro and in vivo (in mice and Gn pigs)

In the intestinal lumen, *Lactobacillus/Bifidobacterium* strains (Lacto/Bifido) inhibit some viruses directly by producing lactic acid, H_2O_2, bacteriocins, and other inhibitory agents; (2) Lacto/Bifido also preserve the integrity of the epithelium and compete with pathogens for Intestinal epithelial cell (IEC) receptors; (3) Lacto capture viruses by lectin-mediated binding to viral glycoproteins to prevent infection; (4) Lacto-derived nitric oxide (NO) has microbicidal and tumoricidal activities; (5) short chain fatty acids (SCFA) produced by Bifido block dendritic cell (DC) development; induce Fas-mediated T cell apoptosis; decrease IL-12 expression, but increase IL-23 production by DCs.

Intestinal epithelial cells (IECs) secrete mucins and antimicrobial peptides (AMPs) in response to the commensal microbiota/probiotics, regulating microbial replication and interaction with intestinal mucosa. IECs produce BAFF and APRIL factors, stimulating activated B (plasma) cells to produce secretory IgA (sIgA) that further limits microbial interaction with the epithelium. Under homeostatic conditions, commensal microbiota/ probiotics stimulate the secretion of cytokines [including thymus stimulating lymphoprotein (TSLP), IL-33, IL-23, IL-25, and TGFβ] by IECs that promote development of antigen presenting cells [macrophages (Mϕ) and DCs]. Antigen presenting cells (APCs) induce regulatory T (Treg) cell generation through TGFβ- and retinoic acid (RA)-dependent mechanisms. APC and Treg derived TGFβ and IL-10, maintain the anti-inflammatory nature of the intestine by inhibiting/reducing effector responses. Intestinal innate lymphoid cells (ILCs), including natural killer (NK) cells, lymphoid tissue inducer (LTi) cells, and γδ T cells, produce IL-22 that regulates expression of tight and adherent junction (TJ and AJ) proteins by IECs, regulating intestinal barrier function.

Table 1

Human pediatric clinical trials (randomized, double-blind, placebo-controlled) demonstrating lactobacilli and bifidobacteria effects on RV diarrhea and disease.

Probiotic bacteria species/dose	Patient age	Number of patients	Treatment regimen	Treatment outcome	Study location	Reference
Intervention studies						
Lactobacillus casei sp rhamnosus strain GG (10^{10}-10^{11}CFU/dose)	4-45 months (≥80% RV)	n=71	Orally twice a day during acute diarrhea	Treatment significantly decreased duration of diarrhea	Finland	(Isolauri et al., 1991)
Bifidobacterium bifidum and *Streptococcus thermophilus* with formula (10^{8}-35 × 10*8* CFU/g of formula powder)	5-24 months	n=55	Orally, every feeding during diarrhea	Treatment decreased incidence of diarrhea and RV shedding	USA	(Saavedra et al., 1994)
L. rhamnosus GG (10^{10} CFU/ml)	5-28 months	n=42	Twice daily for 5 days	Treatment diarrhea duration was shortened	Finland	(Isolauri et al., 1994)
L. rhamnosus GG (10^{10}-10^{11}CFU/dose)	13 months (mean)	n=40	Twice daily for 2 days	Treatment decreased incidence of diarrhea and voimiting	Pakistan	(Raza et al., 1995)
L. rhamnosus GG (Lactophilus), or *S. thermophilus* and *L. delbruckii* subsp. *bulgaricus* (Yalacta)	6-35 months	n=49	Orally twice a day for 5 days, after RV diarrhea was confirmed	LGG improved Ab and ASC responses to RV and significantly decreased mean duration of diarrhea compared to other treatments	Finland	(Majamaa et al., 1995)
L. rhamnosus GG (10^{10}-10^{11}CFU/dose)	8 months (mean)	n=39	Twice daily for 2 days	Treatment decreased duration of diarrhea	Thailand	(Pant et al., 1996)
L. rhamnosus GG (3×10^{9} CFU/dose)	3-36 months (61% RV)	n=100	Orally twice a day during acute RV diarrhea	Treatment decreased duration of diarrhea	Italy	(Guarino et al., 1997)
L. rhamnosus GG (5×10^{9} CFU/ml)	1-36 months (27% RV)	n=123	Twice daily for 5 days with ORS	Treatment significantly shortened the duration of RV diarrhea and decreased frequency of stools (but not diarrhoea with confirmed bacterial etiology)	Russia	(Shornikova et al., 1997c)
L. reuteri (10^{7} or 10^{10}/10^{11} CFU/dose)	6-36 months (89% RV)	n=97	Orally for up to 5 days	Treatment decreased duration of diarrhea in a dose-dependent manner	Finland	(Shornikova et al., 1997b)
L. reuteri DSM 17938 (10^{10}-10^{11} CFU/ml)	6-36 months (75% RV)	n=40	Daily with formula for up 5 days	Treatment decreased duration of acute diarrhea	Finland	(Shornikova et al., 1997a)

Probiotic bacteria species/dose	Patient age	Number of patients	Treatment regimen	Treatment outcome	Study location	Reference
L. rhamnosus GG (5×10^9 CFU/ml)	6-36 months (92% RV)	n=123	Twice daily with formulafor 5 days	Treatment decreased duration of acute diarrhea, improved weight gain, corrected acidosis	Finland	(Rautanen et al., 1998)
L. acidophilus LB (10^{10} CFU/dose)	3-24 months (50% RV)	n=73	Five doses every 12 hrs with ORS	Treatment decreased duration of diarrhea	Thailand	(Simakachorn et al., 2000)
L. rhamnosus GG (at least 10^{10} CFU/250 ml), given ad libitum	1-36 months	n=287	Orally until diarrhea stopped	Treatment significantly decreased mean duration of diarrhea	Italy	(Guandalini et al., 2000)
L. rhamnosus GG at 6×10^9 CFU	1-36 months	n=81	Orally twice daily for the duration of their hospital stay	Treatment significantly reduced the risk of rotavirus gastroenteritis (1 of 45 [2.2%] vs. 6 of 36 [16.7%] in placebo group, respectively	Poland	(Szajewska et al., 2001)
L. rhamnosus 19070-2 and *L. reuteri* DSM 12246 (each species 10^{10}CFU/dose)	6-36 months	n=71	Orally twice a day for 5 days during acute diarrhea (≥80% RV)	Treatment ameliorated acute and reduced the period of rotavirus excretion	Denmark	(Rosenfeldt et al., 2002)
L. rhamnosus GG (10^9CFU/ml)	3-36 months (with signs of mild-moderate dehydration; 24.4% RV in LGG group and 39.3% RV in placebo group)	n=179 (all males)	Daily with formula for diarrhea duration	No significant differences in duration of diarrhea, rate of treatment failure, and proportion of unresolved diarrhea	Peru	(Salazar-Lindo et al., 2004)
B. lactis Bb12 and *S. thermophilus* TH4 (10^8 - 10^9 CFU/dose)	3-36 months (87% RV)	n=212	Orally during acute RV diarrhea until 24 hrs after diarrhe subsided (~3 days?)	Treatment only slightly decreased RV shedding in a dose dependent manner	China	(Mao et al., 2008)
L. rhamnosus GG (10^9CFU/dose)	<36 months (moderately malnourished, 25.6% RV)	n=229	For 10 days	No difference in duration of diarrhea or number of stools on days 3, 6 and 10	India	(Misra et al., 2009)
L. rhamnosus GG (10^9CFU/dose)	4 months - 2 years (11% RV)	n=64	Three times a day for 3 days	No differences in duration or severity of diarrhea. However, the number of diarrheic stool was significantly lower on day 2 in the treatment group	Australia, Aboroginal children	(Ritchie et al., 2010)
L. acidophilus, *L. rhamnosus*, *B. longum* and *Saccharomyces boulardii* (each species 9×10^6-7×10^7CFU/dose)	1-23 months	n=64	Orally for 5 days with ORS, after RV diarrhea was confirmed	Treatment decreased duration of diarrhea and incidence of voimiting	Bolivia	(Grandy et al., 2010)
B. lactis (30mg/day)	5 months - 5 years	n=75 (38 females,	Orally for 5 days, after RV diarrhea was	Treatment decreased duration of diarrhea	Turkey	(Erdogan et al., 2012)

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Probiotic bacteria species/dose	Patient age	Number of patients	Treatment regimen	Treatment outcome	Study location	Reference
		37 males)	confirmed			
L. reuteri DSM 17938 (4×10^8 CFU/ml)	6-36 months (with clinical signs of dehydration; 62% RV)	n=74	Daily with formula for 7 days	Treatment reduced the frequency, duration and recrudescence rate of acute watery diarrhea	Italy	(Francavilla et al., 2012)
L. rhamnosus GG (10^9CFU/capsule)	6 months - 5 years (21% RV)	n=200	One capsule/day for 7 days	Treatment reduced the duration of acute watery diarrhea	India	(Aggarwal et al., 2014)
L. rhamnosus GG (10^{10}CFU/capsule)	6 months - 5 years (66% RV)	n=124	One capsule/day in milk for 4 weeks	Treatment reduced repeated diarrheal episodes and significantly increased RV-specific IgG levels	India	(Sindhu et al., 2014)
Prophylactic studies						
L. rhamnosus GG (3.7×10^{10} CFU/ml)	6-24 months (undernourished)	n=204	Daily for 6 days/wk for 15 months	Prophylactic effect of LGG supplementation on diarrhea incidence in non-breast-fed children	Peru	(Oberhelman et al., 1999)

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Table 2

Probiotic lactobacilli and bifidobacteria effects on RVA immune responses and disease studied in animal models (rodents and pigs).

Probiotic bacteria species/dose	Animal/Age	Treatment regimen	Treatment outcome	Reference
Rodent models				
Bifidobacterium breve YIT4064 (heat-killed, 0.05% of diet)	Neonatal suckling BALB/C mice	Dams were treated	Treatment increased lactogenic and intestinal RV-specific Abs and passive protection of mouse pups against RV challenge	(Yasui et al., 1995)
Lactobacillus casei DN-114 001 fermented milk	Neonatal suckling rats	Supplemented daily starting from 2 days of life	Treatment reduced RVA infection and the associated intestinal pathology	(Guerin-Danan et al., 2001)
B. bifidum (0.75×10^8 CFU/mL) and *B. infantis* (0.75×10^8 CFU/mL)	Neonatal suckling BALB/C mice	Orally, pups received 10 μl daily, days of life 1-21, 20 μl once a week (weeks 3-5) and 40 μl once a week (weeks 6-7) ± prebiotic compounds	Treatment reduced RVA diarrhea and increased serum and intestinal IgA Abs	(Qiao et al., 2002)
L. rhamnosus GG (ATCC 53103), *L. paracasei* NCC 2461 (ST11), *L. johnsonii* NCC 533 (La-1), *L. rhamnosus* NCC 596 and *Streptococcus thermophilus* NCC 2496 (10^8/dose)	Neonatal suckling BALB/C mice	Orally, once daily, days of age 1-3, with or without anti-RVA immunoglobulins	*L. rhamnosus* GG treatment reduced RVA diarrhea severity and duration	(Pant et al., 2007)
B. longum subsp. *infantis* CECT 7210 (1×10^9 CFU/dose)	8 week-old BALB/C mice	Orally, once	Treatment reduced RVA infection	(Munoz et al., 2011)
L. reuteri DSM 17938 and ATCC PTA 6475	Neonatal suckling CD-1 mice, overweight, underweight and normal weight	Orally daily from days 5 to 14 of life	Treatment reduced RVA diarrhea, increased epithelial migration and increased diversity of intestinal microbiome (correlated with diarrhea reduction for both strains); also increased RVA IgA Abs, decreased pro-inflammatory cytokines, increased epithelial cell proliferation (strain-specific, did not correlate with diarrhea reduction or did not have equal effects in mice of different nutritional status)	(Preidis et al., 2012)
Pig models				
L. rhamnosus GG (10-fold incremental LGG dose increase every day from 10^3 to 10^9 CFU/dose)	Neonatal Gn pigs colonized with human infant intestinal microbiota	Orally, daily, 3 - 16 days of age	Treatment prevented RVA-induced shift of relative microbiota abundance from *Firmicutes* to *Proteobacteria*	(Zhang et al., 2014)
L. rhamnosus GG and *B. lactis* Bb12 (10^5 CFU/dose)	Neonatal Gn pigs	Orally once at 3-5 days of age (colonization 28 days prior to RVA challenge)	Treatment decreased severity of RVA infection and disease; decreased systemic, but promoted intestinal innate immune responses and immune trafficking, differentially affected TLR	(Chattha et al., 2013a; Chattha et al., 2013b; Kandasamy et al., 2014; Vlasova et al., 2013)

Probiotic bacteria species/dose	Animal/Age	Treatment regimen	Treatment outcome	Reference
			responses (decreased pro-inflammatory, increased B cell promoting); promoted adaptive immune (including B, effector and regulatory T cell) responses	
L. reuteri ATCC 23272 and *L. acidophilus* NCFM (1:1 mixture, with 10-fold incremental dose increase every other day from 10^3 to 10^6 CFU/dose)	Neonatal Gn pigs	Orally dosed at 3, 5, 7, 9, 11 days of age (first dose 2 days prior to RVA challenge, others subsequent)	Treatment differentially affected APC frequencies in RVA infected and non-infected piglets, increased TLR expression by blood cDCs; promoted T cell responses and decreased pro-inflammatory cytokine production; did not reduce RVA diarrhea severity	(Azevedo et al., 2012; Wen et al., 2009; Wen et al., 2011; Zhang et al., 2008a; Zhang et al., 2008c)
L. acidophilus NCFM (10-fold incremental dose increase every other day from 10^3 to 10^6 CFU/dose)	Neonatal Gn pigs	Orally dosed at 3, 5, 7, 9, 11 days of age (first dose 2 days prior to RVA vaccine 1st dose, others subsequent)	Treatment enhanced immunogenicity of RVA vaccine	(Zhang et al., 2008b)
L. rhamnosus GG (10-fold incremental dose increase every other day from 10^3 to 10^{12} CFU/dose)	Neonatal Gn pigs	Orally dosed daily (3-19 days of age), (colonization 9 days prior to RVA challenge)	Treatment decreased severity of RVA infection and disease; decreased intestinal epithelial damage and other effects of HRV infection	(Liu et al., 2013; Wu et al., 2013)
B. lactis HN019 (10^9 CFU/dose)	3 week old conventional pigs	Orally dosed, daily until the end of experiment	Treatment decreased severity of RVA infection and disease; promoted blood lymphocyte phagocytic and T cell proliferative responses and intestinal B cell (IgM, IgA and IgG) responses	(Shu et al., 2001)

Table 3

Probiotic lactobacilli and bifidobacteria (or their derivatives) in livestock.

Probiotic bacteria species/dose	Animal/Age	Probiotic effects	Reference
L. acidophilus or a mixture of 12 Lactobacillus strains (2 strains of *L. acidophilus*, 3 strains of *L. fermentum*, 1 strain of *L. crispatus*, and 6 strains of *L. brevis*)	Arbor Acres broiler chicks/1-day-old	Significantly increased the levels of amylase in the small intestine, but significantly reduced the intestinal and fecal beta-glucuronidase and fecal beta-glucosidase.	(Jin et al., 2000)
Lactobacillus salivarius strain, CTC2197	Leghorn chickens/1-day-old	Prevented *Salmonella enteritidis* C-114 colonization in chickens	(Pascual et al., 1999)
L. acidophilus and S. faecium, given with *Salmonella Enteritidis*, *Salmonella Typhimurium*, and *Salmonella Heidelberg*-Specific antibodies	Broiler chicks/3-day-old	Reduced *Salmonella enteritidis* intestinal colonization	(Tellez et al., 2001)
Lactobacillus fermentation product	Cross-bred piglets/4-5-week-old	Stimulated growth, increased feed intake and slightly increased serum concentration of IgG.	(Lessard, 1987)
B. lactis HN019	Cross-bred piglets/4-5-week-old	Reduced the severity of RV and *E. coli* associated weanling diarrhea, improved feed conversion index and immune responses	(Shu et al., 2001)
Direct fed microbials	Cross-bred piglets/4-5-week-old	No effect on growth performance and gut health	(Walsh, 2007)
Bifidobacterium pseudolongum or *Lactobacillus acidophilus*	Newborn calves and piglets	Improved body weight gain, feed conversion, reduced mortality, and decreased frequency of diarrhea	(Abe et al., 1995)
L. reuteri BSA131	Landrace piglets/1-month-old	Enhanced weight gain and feed conversion; modulated intestinal microbiota (increased lactobacilli and decreased enterobacteria fecal counts)	(Chang et al., 2001)
Reuteran from *L. reuteri* TMW1.656 and levan from *L. reuteri* LTH5794	Crossbred gilts/4-week-old	Decreased levels of adherent ETEC K88 resulting in less outflow liquid in intestinal loops	(Chen et al., 2014)
Lactobacillus gasseri, *L. reuteri*, *L. acidophilus* and *L. fermentum*	Crossbred pigs (Duroc×Landrace×Yorkshire)/4-week-old	Significantly improved average daily feed intake, feed conversion, average daily weight gain and improved microbial balance	(Huang, 2004)
Lactobacillus fermentum I5007	Piglets/4-day-old	Affected microbial composition (decreased numbers of Clostridium spp.), promoted intestinal development (increased villous height), and modulated immune function (reduced IL-1β in ileum of non-challenged piglets)	(Liu et al., 2014)
Lactobacillus fermentum I5007	Barrows/28-day-old	Increased CD4+ T cell frequencies, TNF-α and IFN-γ levels in ileum of *E. coli* K88ac challenged pigs; increased the anti-oxidative responses (increased catalase, superoxide dismutase and glutathione peroxidase levels, inhibited superoxide anion production in liver and muscle; decreased levels of malondialdehyde)	(Wang et al., 2009a; Wang et al., 2013)

Probiotic bacteria species/dose	Animal/Age	Probiotic effects	Reference
Bacillus subtilis M-1 and *Lactobacillus reuteri* X-1	Piglets/21-day-old	Increased feed intake. average daily weight gain, but decreased immune function (serum IgG and IgM, TNF-α, IL-6 and NO levels)	(Wang, 2011)
L. fermentum I5007	Piglets/28-day-old	Alleviated weaning stress syndrome by enhancing the levels of proteins involved in energy metabolism, lipid metabolism, cell structure and mobility, protein synthesis, and immune response, thereby facilitating cellular proliferation and depressing apoptosis.	(Wang et al., 2012)
Lactobacillus fermentum	Large White×Landrace barrows/28-day-old	Improved average weight gain, feed conversion and anti-OVA serum IgG levels	(Yu, 2008)