THE COLONIZATION AND ESTABLISHMENT OF THE NEONATAL MAMMALIAN MICROBIOME

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MANUSCRIPT RECEIVED 13 APRIL 2017;
ACCEPTED 06 JULY 2017

ABSTRACT

In current agriculture practices, such as the dairy industry, the use of antibiotics is being discouraged due to the occurrence of antibiotic resistant bacteria. However, antibiotics are used commonly to treat calf diarrhea, which is a serious issue that negatively influences calf health, growth, and development. Recent research highlights the gut microbiota as a potential source to improve the gut health of a calf, which could minimize the antibiotic use. However, limited knowledge is available for the early life gut microbiota and its relationship with calf's performance. It is known that the microbiota has an influence on immune system development, as well as behavioral development, and metabolic development. Further, an atypical microbial population, or a microbial shift, has been linked to autoimmune, anxiety and metabolic disorders. The process of microbial and host interactions starts at birth, suggesting that mammals are initially colonized by microbes immediately following and during birth. Differing modes of delivery, caesarian or vaginal delivery, and possibly the length of time of the birthing process, may determine initial colonization of the infant. Further, the establishment of the microbiota can be influenced by host genetics, diet, and maternal environment. Therefore, this review aims to summarize the current understanding of the neonatal mammalian microbiota obtained from human and mice studies, and to outline future research directions on microbial colonization and possible manipulation strategies that can be used to manipulate the gut microbiota in dairy calves. By understanding the process of how mammals and microbes interact it is possible to better target future research in order to solve the problem of calf diarrhea.

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KEYWORDS

- Early-life Microbiome
- Calf
- Colonization
- Establishment

INTRODUCTION

Calf diarrhea can cause more of a financial loss than any other calf ailment (15). Diarrhea in the young animal prevents absorption of fluids and nutrients, inhibiting growth and health (1). Further, without immediate intervention, calf diarrhea can be transmitted between calves and can result in high mortality. Currently, the common practice to prevent calf diarrhea is the use of antibiotics. Yet, industry is encouraged to reduce the use of preventative antibiotics due to the increasing prevalence of antibiotic resistance. Therefore, an alternative to antibiotic prevention for calf diarrhea is needed. Recent research highlights the gut microbiota as an important factor in immune function development and maintaining neonate gut health, making the gut microbiota a potential source to improve the gut health of calves to reduce the prevalence of diarrhea. However, limited knowledge on the calf gut microbiota is available. Additionally, the mechanisms of how the microbiota influences calf gut development and health are still largely unknown and undefined. Therefore, this review aims to summarize the current understanding of the neonatal mammalian microbiota based on the findings from human and mice studies from three aspects including initial colonization of the neonatal mammals, the impact of the microbiota on gut immune function of the developing neonate mammal, and factors affecting microbial establishment during early life. Further, this review aims to identify the knowledge gap of the calf microbiota in order to direct future research towards identifying potential ways to reduce the incidence of, and possibly prevent, calf diarrhea through the manipulation of gut microbiota.

MAMMALIAN DEVELOPMENT AFFECTED BY MICROBIOTA

It was hypothesized by Louis Pasteur that the microbiota has an important and necessary influence on mammalian life (21). Mammals have a rich and diverse microbial population that interacts with, and influences the development of biological processes in mammals like immune function and metabolic systems (21). Additionally, the microbiota causes long-term impacts on emotional systems (4, 23). The following section of this review will outline specific changes in microbiota and how they can affect the immune system development, anxiety and depression, and digestive and metabolic functions.

IMPACT OF THE MICROBIOTA ON IMMUNE SYSTEM DEVELOPMENT

The interaction between host and microbes is essential for proper immune function development. Young animals enter a critical period soon after birth when exposure to antigens is imperative for immune development. If the exposure is delayed, immune development can be impacted. This can be explained by an experiment using germ free mice (21). Firstly, it is important to note that the authors demonstrated that germ free mice have an atypical cytokine response to orally treated lipopolysaccharide (LPS) when compared to conventional mice. It is important to note that LPS is a large molecule found on the outer membrane of *Gram-negative* bacteria, and the previously

described atypical response differs from a normal response in that the atypical response is delayed and exaggerated. Further, these authors noted that the juvenile mice can resume a normal cytokine response, and behave normally, normal being immediate and moderate, when treated with probiotic *Bifidobacterium infantis*. However, when adult germ free mice were treated with the same probiotics they were unable to be converted back to the normal response. Additionally, these effects on the immune system can have long-term consequences, which are still being investigated (10, 19). It has been found that antibiotic use in early life influenced fecal bacterial composition and can be linked with development of intestinal diseases later in life (17). However, there is convincing research that interruptions in the establishment of the microbiome during early life can result in allergies, asthma and other autoimmune diseases in adults (10). Additionally, it was found that the development of eczema can be minimized if infants were treated with probiotics (10).

After birth, the gut microbiota may contribute to mammalian immune system development through interactions between hosts. The interaction between microbe and host immune development is important, which has been demonstrated by germ free animals as described above. Germ free animals do have immunities; however, they cannot cope with pathogens (22). This reveals that without the proper microbial exposure in early life, the immune system will not properly develop and can increase risk of disease in later life. Further, a more diverse intestinal microbiota during the first week of life is associated with a reduced risk of subsequent eczema in infants (10). Additionally, interventions that enhance microbial diversity in early life may provide an effective means for the prevention of eczema in high risk infants (10).

IMPACT OF MICROBIOTA ON ANXIETY AND DEPRESSION

Initially, the evidence of microbial impact on host behavior was gained from comparisons of germ free and bacterial colonized mice. In order to test the idea that postnatal microbial colonization may affect the development of brain plasticity, researchers compared the hypothalamic-pituitary response to differing levels of stress restraint using genetically identical germ free and specific pathogen free mice (23). In this study, the individual mice were placed into a 50 ml conical tube for 1 hour, or into a glass container lined with ether soaked absorbent paper for 2.5 minutes. The authors found exaggerated stress response in germ free mice when compared to pathogen free mice (23). Further, the same authors observed a reduction in stress response in germ free mice after administration of probiotic *Bifidobacterium infantis*. These results have demonstrated the differences in brain function between germ free and colonized mice, suggesting the potential relationship between gut microbiota and animal behavior. However, other researchers found that administration of antimicrobials to colonized mice reduced the microbial population, and increased exploratory behavior in mice (4). They also found that the same antimicrobials given to germ free mice had no effect on their behavior. Furthermore, they reported that administering the microbial population from colonized mice to germ free mice reduced exploratory behavior in the germ free mice. Such change of exploratory behavior suggests a higher activity of mice when treated with antimicrobials. Though increased exploratory behavior proposes a contrast to a greater fear response, they may actually be complementary depending on the motivation of the exploration (23). However, it is clear that more research needs to be done to determine the impact of postnatal microbial colonization on the development of neural systems that induce stress in animals.

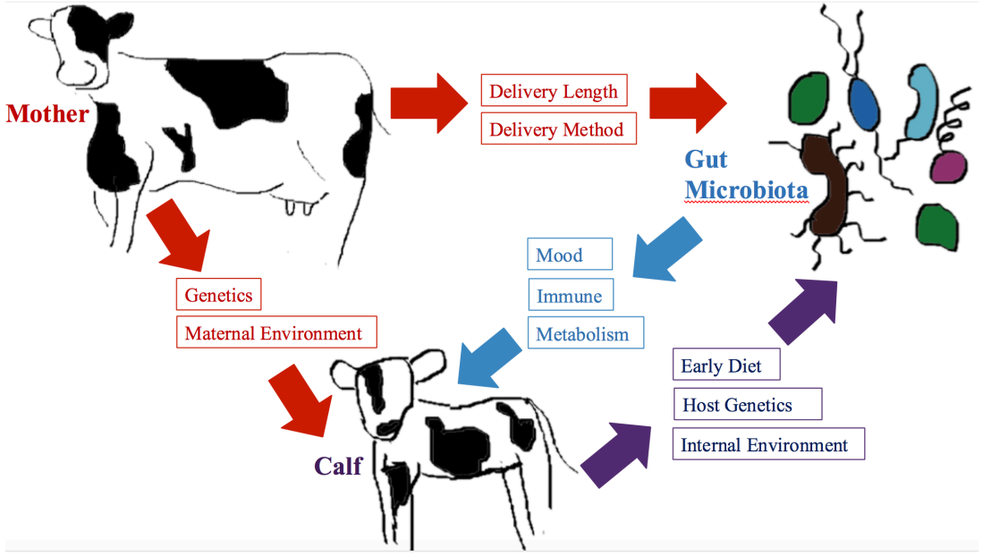

Figure 1. The relationship between the colonization and establishment of the gut microbiota on the early-life calf's immune system, mood and metabolism, shown in blue, and the impact of the calf's early diet, genetics and internal environment on the gut microbiota, shown in purple. Also shown is the maternal impact on the calf by genetics and maternal environment, and the maternal impacts on the microbial colonization of the calf, shown in red.

IMPACT OF THE GUT MICROBIOTA ON DIGESTION AND METABOLIC FUNCTIONS

Recent studies have found that a shift in microbiota during early life can cause obesity, as well as other metabolic diseases like diabetes in later life (19). The study on fecal microbiome of 298 stool samples from 22 children with type 1 diabetes, and 22 normal children of the same age found a significant difference in host-microbial interactions between two groups (6). There was a higher interaction between host and *Enterococcus, Sarcina, Prevotella*, and *Corynebacterium* in the children with type 1 diabetes (7). Relatedly, the authors also found less interaction between *Enterococcus, Sarcina, Prevotella,* and *Corynebacterium* and host in children without type 1 diabetes (7). This correlation of host and bacterial interaction, may suggest that metabolism could be affected by the gut microbiota. To further identify the causal effect of microbiota on host metabolic dysfunction, genetically deficient Toll-like receptor 5 (TLR5) mice were used to induce obesity, and then the obese TLR5 mice microbiota was transplanted to wild type germ free mice (24). Found in the gut mucosa, Toll-Like receptor 5 is a class of receptor involved in innate immune responses, which plays a role in satiety. Without TLR5, the mice exhibited significant hyperphagia, and developed metabolic disease (24). Next, the germ free wild type mice treated with the gut microbiota derived from the TLR5 deficient mice, immediately showing distinct microbial changes, and later becoming obese (24). The change in body composition of germ free mice

from normal to obese after colonized with a specific microbial population, suggests that the gut microbiota may impact body composition through affecting the host metabolic functions.

INITIAL COLONIZATION

Mammals are currently believed to be sterile when *in utero* and microbial colonization can be observed minutes after birth (18). Initial colonization can be defined as the period during, and immediately following birth when microbes first interact with and colonize the sterile infant. Because microbial colonization of the mammalian neonate happens so quickly it is assumed that initial colonization happens from the birth canal or from the first exposure to the environment in neonates delivered by caesarean section (18).

MODE OF DELIVERY: VAGINAL OR CAESAREAN SECTION

Since the mode of initial colonization is thought to happen during the birthing process, it can be assumed that the two different modes of birth, caesarean section or vaginal birth, would result in different infant microbial populations. Indeed, mode of delivery impacts microbial population initially, but the differences minimize with aging (18). Specifically, caesarean section was associated with both lower abundance and diversity of the phyla Actinobacteria and Bacteroidetes, and higher abundance and diversity of Firmicutes from birth to three months of life. It was also found that *Bifidobacterium* and *Bacteroides* genera seems to be more frequent in vaginally delivered infants compared with caesarean section delivered ones (18). However, after six months of age, the microbial differences minimized. Furthermore, it was found that delivery mode was not significantly associated with childhood development of obesity through a longitudinal study from infancy to 7 years of age (2). Though they noted initial differences in microbial population with the different mode of delivery, the differences did not make a lasting impact and by 7 years of age the mode of delivery no longer had an impact on microbial population.

LENGTH OF DELIVERY

Currently, there is no research on the impact of length of delivery on microbial population. The length of delivery can be hypothesized to impact initial microbial colonization because a longer birth would mean the infant spends longer in the birth canal, meaning more exposure to birth canal microbiota. This could be the case, especially in the birthing process of calves. For example, heifers generally have a longer delivery than cows because it is their first time through the birthing process. It is possible that having calves born from heifer or cow may impact the exposure to birth canal microbiota thus impact initial microbial colonization. However, it can be assumed that length of delivery would be similar to type of delivery in that though it may be initially significant, the differences in microbial population may not last into adulthood. However, if there is a difference in length of delivery, it is important to determine if those differences last into adulthood.

FACTORS AFFECTING MICROBIAL ESTABLISHMENT

The establishment of the microbiota is a dynamic process following initial colonization, which depends on factors such as host genetics, diet, maternal environment, early antibiotic exposure, and highly sterile environments (9, 12, 13).

HOST GENETICS

The genetics of the host determine the microenvironment of the gut thus affecting the suitability of the internal environment for microbial colonization. An early study in mice found host genetics had a 12% impact on microbial variation in the gut (25). Additionally, different researchers found a significant link between a mutation in human genes and a corresponding shift in gut microbiota characterized by a depletion of total numbers of bacteria, loss of diversity, and major shifts in bacterial populations within the Bacteroidetes, Firmicutes and Proteobacteria phyla (11). Moreover, another different research group was able to identify a core measurable microbiota of 64 conserved taxonomic groups using quantitative pyrosequencing of the microbiota and individual host genotype had a measurable contribution to microbial variation which can be explained by litter and cohort effects (3). Through further statistical analysis they found suggestive genome-wide linkage with relative abundances of specific microbial taxa, providing clear evidence for the importance of host genetic control in shaping individual microbiome diversity in mammals (3). Although there have been recent linkages between host genetics and microbial population, and microbial population and body composition (2, 3, 17), there has not yet been a genetic connection made between gut microbiota and long-term growth performance such as weight gain.

DIET DURING EARLY LIFE

Differences in microbial population have been found with different diets during early life (9, 12). Breast-fed infants have a gut microbiota dominated by Bifidobacteria, whereas formula fed infants have a more heterogeneous composition and less Bifidobacteria (9). Furthermore, formula with the more closely resembles maternal milk results in a microbial population that is dominated by Bifidobacteria, like breast-fed babies (9, 12, 14, 20). These results suggest that the diet of infants can change the gut microbiota population through the establishment process.

MATERNAL ENVIRONMENT

Like diet, prenatal maternal stress can influence the composition of the infant's gut microbiota. Maternal stress is hypothesized to change the maternal physiological environment, including increased heart rate and introduction of stress hormones (2). Additionally, stress can also change the maternal environment behaviorally by increasing or decreasing the mother's appetite, activity level, and potentially changing the mother's diet. Such change in maternal environment can affect the mother's microbial population, and can potentially influence initial colonization of offspring at birth (8, 26). Maternal stress can cause increase in cytokine concentration and inflammation, possibly influencing the developing fetus. This maternal immune response creates a different environment for the developing fetus potentially influencing, or changing their immune function development. In addition to maternal stress, other maternal states, like maternal fitness, can impact the infant's microbial population. Additionally, researchers have found a high body mass index (BMI) of the pre-pregnancy mother had a significant impact on the development of obesity in the offspring (2). Further, the same study reported that if those children of a high BMI mother were treated with antibiotics early in life, it reduced obesity prevalence in these children (2). Contrastingly, children born to normal

BMI mothers and treated to early antibiotics were seen to have an increased risk of becoming obese later in life (2). Such influence of the maternal body composition on the fetal microbiome can potentially be due to a change in the maternal microbial population influencing initial colonization of the fetus. Further, because when children born from a mother with a high BMI had a decreased chance of developing obesity when they were treated with antibiotics early in life, suggests a potential cause for microbial influence (2). Understanding, and acknowledging the impact of the maternal microbiota on the offspring's microbiota is important when it comes to management and treatment because it is clear that maternal environment has an effect on the infant's microbiota.

KNOWLEDGE GAPS IN STUDYING EARLY LIFE: MICROBIOTA IN CALVES

The microbial colonization in cattle, especially in neonates, has profound impacts on nutrition, health, animal physiology and productivity (6). Recent research outlining the importance of the microbiome in maintaining neonate health makes the microbiota a potential source of treatment for calf diarrhea (1). Nevertheless, information on the calf microbiota is still limited and more research is necessary before the microbiota can be used to limit instances, and possibly even prevent, calf diarrhea. Additionally, food safety (*E. coli* O157 colonizes the young claves) may be another area of possible research into the importance of the microbiome of beef cattle (6). By understanding the process of how neonatal mammals and microbes interact, it is possible to better target future research in order to solve the problem of calf diarrhea, or other related concerns. Two interesting effects of the microbiota on dairy calves worth noting are the effects of antibiotic treatment on calf behaviour, and the maternal control on the calf`s microbiota. Also, another important area of investigation is the long term effects of microbial on weight gain and body composition of cattle.

The effect of the microbiota on behaviour is important to highlight in calves because when calves are treated with antibiotics for calf diarrhea, the antibiotics may reduce their microbial population, which may lead to exaggerated stress response. Therefore, it is important to investigate if these results can be replicated in calves, and potentially investigate a probiotic for calves to reduce their stress response. Especially a link between stress and immune efficiency has also been noted (5). The implications of antimicrobials and probiotics on calf behavior may be important for calf management. Based on these findings, producers may have the options to give calves the probiotics after antibiotic treatment of calf diarrhea.

Another important aspect is the effect of the mother on the calf microbiota colonization, including maternal genetics and maternal environment. The relationship of host genetics to microbial population is important for calves because it suggests that potentially genetic selection, together with a probiotic, could be the solution to treat calf diarrhea. Also, maternal environment is important to note when investigating potential treatments to calf diarrhea because potentially a change in management of pregnant animals may reduce instances of calf diarrhea. However, more research needs to be done to see the influence of the mother's environment, and body composition, on the microbial population of the calf. In addition, it is a common practice to remove dairy calves from their mothers at a very young age, often increasing their risk of microbial infection because of this high stress period. There are many gaps in the knowledge of the calf microbiota that need to be addressed through research before microbial manipulation can be a potential treatment of calf diarrhea. To date, the understanding of the early life colonization in dairy claves is very limited and it is therefore important to research the role of calf gut microbiome in immune system development.

Since beef cattle are raised in less intensive production systems than dairy, there have been different areas of focus on each of them (15). While microbial investigation of dairy cattle has focused mostly on calf diarrhea, and mastitis, or bacterial colonization of the utter, the microbial focus of beef cattle has been on weight gain and feed efficiency. Though dairy and beef cattle are different in production systems, and physiology, each bovine sector may benefit from collaboration. Therefore, it is important to outline some recent advances in the beef industry. Firstly, rumen variation between beef cattle has been noted between animals housed in the same environment and fed the same diet (6). Thus, the genetic component, or possibly another undiscovered factor, influence the establishment of the gut microbiota. However, more research is needed to explain the observed individualized microbial variation. Further, a significant relationship between weight gain and rumen microbiome was discovered when comparing the most and least efficient animals (17). Regardless, more research needs to be done to investigate long term impacts of microbial manipulation on gut health to prevent calf diarrhea, and growth for better weight gain.

CONCLUSION

Due to the financial impacts of calf diarrhea and the increased occurrence of antibiotic resistance, it is important to find way to treat calf diarrhea that does not include the use of preventative antimicrobials (14). It is common for calves in the dairy industry to be given preventative antimicrobial during early life. Recent research has identified the gut microbiota as a potential tool in reducing the incidence of calf diarrhea. However, research on the calf microbiome is limited. Consequently, this review summarized the current understanding of neonatal mice and human microbiota to outline the importance of the microbiota, as well as microbial colonization and establishment. In addition, the knowledge gaps in calves have been identified. However, it was determined that future research is needed to investigate the relationship between early life microbiota and calf immune system, behavior, and metabolism (Figure 1). Additionally, further investigation is needed to identify the response of the calf microbiome to a change in host genetics, birth length, diet, and maternal environment. Though the understanding of mammalian microbiota has had many recent advances, specific information on the calf microbiome is needed before microbial manipulation can be a potential treatment to calf diarrhea. More research is needed in order to understand how the calf gut microbiota may respond to manipulation.

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