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CHAPTER TWO

Microbiota in viral infection and disease in humans and farm animals

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Abstract

The influence of the microbiota on viral infection susceptibility and disease outcome is undisputable although varies among viruses. The purpose of understanding the interactions between microbiota, virus, and host is to identify practical, effective, and safe approaches that target microbiota for the prevention and treatment of viral diseases in humans and animals, as currently there are few effective and reliable antiviral therapies available. The initial step for achieving this goal is to gather clinical evidences, focusing on the viral pathogens—from human and animal studies—that have already been shown to interact with microbiota. The subsequent step is to identify mechanisms, through experimental evidences, to support the development of translational applications that target microbiota. In this chapter, we review evidences of virus infections altering microbiota and of microbiota enhancing or suppressing infectivity, altering host susceptibility to certain viral diseases, and influencing vaccine immunogenicity in humans and farm animals.

1. Introduction

The human and animal body surface and cavities are inhabited by a large number of commensal microorganisms, collectively referred to as the microbiota.[1] This network of microbial communities recently has been revealed to have significant impacts on host health and immunity against infectious diseases.[1] The microbiota has come to the forefront of infection and immunity research, as there are mounting evidence suggesting that microbiota plays an important role in the development of host immune system and immunity.[2] This discovery has provided many research opportunities regarding the role of microbiota in viral diseases, in which virologists can explore how the microbiota suppresses or enhances the infectivity of viruses, the host susceptibility, and how to harness this information for targeted antiviral therapeutics. This new field of research on microbiome-virus-host interaction is made possible by the massive advances in the nucleotide sequencing approaches, metagenomic analysis and data mining, and bioinformatics analytical software.[3,4]

Most available data on the microbiome describe the gut microbiota; however, microbiota is pertinent not only to enteric viral diseases, but also

to viral diseases that affect every organ and system in the body. Although the majority of the microbiota resides in the gut, there are large populations that also reside in the mouth, urinary tract and on the skin.[5–7] Most principles learned from the study of gut microbiome apply to microbial habitats throughout the body. The study of microbiota-virus-host interactions in each specific location of the body allows the understanding of localized immunity and susceptibility to invading viruses and how the local microbiota factor into these processes.

In addition to bacteria, developments in sequencing techniques have led to the expansion of knowledge in the viral aspect of the microbiome, deemed the virome. The virome is comprised of all prokaryotic and eukaryotic viruses found in and on humans and animals. The role of the virome in health and disease is poorly understood at this time. Studies of the role of microbiome in viral diseases have focused almost exclusively on bacteria instead of viruses.

In this chapter, we review and discuss how the microbiota (bacteria) enhances or suppresses infectivity and alters host susceptibility to certain viral diseases and also how virus infection alters microbiota in humans and farm animals, which include poultry, swine, and ruminant species. Because of the exceedingly fast-growing literature in this field, we only chose a few major viral diseases as examples for each species (Table 1). We also provide brief background information on virome in general health in each species.

2. Microbiota in virus infection and diseases in humans

2.1 Influenza virus

Influenza is a common viral infection responsible for the seasonal pandemics that sweep the globe each year. Influenza viruses possess a segmented negative sense, single-stranded RNA genome and belong to the family *Orthomyxoviridae*. Influenza virus infection causes acute respiratory inflammation in humans with symptoms such as high fever, body aches, fatigue, and can result in death. Up to one-half million deaths occur annually, with close to five million reported cases.[45] The best defense thus far is the yearly strain-specific vaccine. The efficacy of this vaccine is low due to several factors, including continual antigenic drift (minor mutational changes that occur over time) and annual vaccine mismatches to circulating strains.[45] The low efficacy of the vaccine coupled with the ease of transmission and

Table 1 Microbiota associated with exacerbation or amelioration of viral diseases.

Viral pathogen	Host species	Bacterial Taxa associated with exacerbated disease	Bacterial Taxa associated with reduced disease severity
Influenza virus	Human	*Streptococcus, Neisseria, Alloprevotella, Prevotella* (Lee et al.[2]), *Dolosigranulum, Staphylococcus, Moraxella* (Ding et al.[8])	*Bacteroidetes* (Lee et al.[2]), *Corynebacterium* (Bomar et al.[9]; Ding et al.[8]), *Bifidobacteriaceae* (Trompette et al.[10])
Human immunodeficiency virus (HIV)	Human	*Gardnerella, Mobiluncus* (Fredricks et al.[11]), *Prevotella amnii, Sneathia amnii, Sneathia sanguinegens, Fusobacterium, Aerococcus, Gemella, Mobiluncus mulieris* (Anahtar et al.[12])	*Lactobacillus* (Gosmann et al.[13]), *Lactobacillus crispatus* (Fredricks et al.[11])
Human norovirus (HuNoV)	Human	*Escherichia coli* (Nelson et al.[14])	*Ruminococcaceae, Faecalibacterium* (Rodriguez-Diaz et al.[15])
	Gn pig	*Bacteroides*, *Lactobacillus* (Lei et al.[16])	–
Human rotavirus (HRV)	Human	Bacteroidetes, *Bacteroides*, *Prevotella* (Harris et al.[17])	*Streptococcus bovis* (Harris et al.[17]), Clostridium cluster XI, *Proteobacteria, Serratia, Escherichia coli* (Harris et al.[18])
	Gn pig	*Proteobacteria* (Twitchell et al.[19])	*Bacteroidetes* (Twitchell et al.[19]), *Lactobacillus rhamnosus GG, Bifidobacterium lactis Bb12* (Vlasova et al.[20])
Human hepatitis B (HBV)	Human	*Proteobacteria, Enterobacteriaceae, Veillonellaceae, Streptococcaceae* (Wei et al.[21])	*Bacteroidetes* (Wei et al.[21])
Human hepatitis C (HCV)	Human	*Bacteroidetes, Prevotella, Acinetobacter, Veillonella, Phascolarctobacterium* (Aly et al.[22])	*Proteobacteria, Bifidobacterium* (Aly et al.[22]), *Ruminococcus* (Bajaj et al.[23])

Avian influenza virus	Chicken	*Proteobacteria* (Yitbarek et al.[24]), *Enterobacteriaceae, Escherichia coli, Clostridium, Veillonella* (Li et al.[25])	*Vampirovibrio, Pseudoflavonifractor, Ruminococcus, Clostridium cluster XIVb, Isobaculum* (Yitbarek et al.[24]) *Novosphingobium, Sphingomonas, Bradyrhizobium, Bifidobacterium, Lactobacillus, Enterococcus, Streptococcus, Candidatus Arthromitus, Bacteroides, Parabacteroides, SMB53* (Li et al.[25])
	Waterfowl	*Veillonella dispar, Rothia mucilaginosa* (Ganz et al.[26])	*Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria, Actinobacteria, Tenericutes* (Hird et al.[26])
Newcastle disease virus (NDV)	Chicken	*Sinobacteraceae, Rhodoplanes, Xanthomonadaceae, Cytophagaceae* (Cui et al.[27])	*Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria, Serratia, Escherichia, Chitinophagaceae, Saprospiraceae, Lactobacillus, Paenibacillus, Erwinia, Enterococcus faecium, Bifidobacterium bifidium* (Cui et al.[27]), *L. reuteri PIA16* (Gonmei et al.[28])
Infectious bursal disease virus (IBDV)	Chicken	*Escherichia coli, Shigella* (Li et al.[29]), *Campylobacter jejuni* (Li et al.[30]), *Salmonella enteritidis* (Phillips and Opitz[31], Arafat et al.[32])	–
Hemorrhagic enteritis virus (HEV)	Turkey	*Propionibacteriaceae, Clostridiaceae, Campylobacteriaceae* (D'Andreano et al.[33]), *Escherichia coli* (Fitzgerald et al.[34])	*Lactobacillaceae* (D'Andreano et al.[33])
African swine fever (ASFV)	Pig	–	*Plaudibacter, Anaeroplasma, Petrimonas, Moraxella* (Correa-Fiz et al.[35])

Continued

Table 1 Microbiota associated with exacerbation or amelioration of viral diseases.—cont'd

Viral pathogen	Host species	Bacterial Taxa associated with exacerbated disease	Bacterial Taxa associated with reduced disease severity
Porcine reproductive and respiratory syndrome virus (PRRSV)	Pig	*Methanobacteriaceae* spp. (Ober et al.[36])	*Ruminococcaceae*, Streptococcaceae (Ober et al.[36])
Porcine circovirus type 2 (PCV2)	Pig	*Prevotella* spp. (van Sambeek et al.[37]), *Methanobacteriaceae* spp. (Ober et al.[36])	Non-pathogenic *E. coli* (Niederwerder et al.[38]), *Ruminococcaceae, Streptococcaceae* (Ober et al.[36])
Porcine epidemic diarrhea virus (PEDV)	Pig	*Escherichia, Shigella, Enterococcus, Fusobacterium*, and *Veillonella* (Huang et al.[39])	*Rikenellaceae* (RC9 gut group), *Butyricimonas*, and *Alistipes* (Song et al.[40]), *Ruminococcaceae, Porphyromonadaceae* (Huang et al.[39])
Bovine rotavirus (BRV)	Cow	*Proteobacteria* (Singh et al.[41]), *Escherichia coli, Clostridium*, and *Streptococcus* (Margreiter et al.[42]; Jang et al.[43])	*Firmicutes, Bacteroidetes* (Jang et al.[43]), *Lactobacillus gasseri, Lactobacillus amylovorus* (Margreiter et al.[42]), *Gemmiger formicilis, Blautia glucerasei, Blautia coccoides, Blautia obeum* (Jang et al.[43])
Bovine leukemia virus (BLV)	Cow	*Sanguibacteroides* (Uchiyama et al.[44])	*Lachnospiraceae, Veillonellaceae* (Uchiyama et al.[44])
Lumpy skin disease virus (LSDV)	Cow	*Brucella* (de Macedo et al.[185]) and *Rickettsia genera* (Sazmand et al.[186]), *Bordetella* (Vordermeier et al.[187]) and *Neisseria* (Sneath and Barrett[188]), *Escherichia* (Wolf-Jackel et al.[189])	–

severity of disease among the very young, very old, and other immunocompromised individuals has made alternative therapies attractive.[46]

Lee et al. conducted a longitudinal study on the human nose/throat microbiome and the potential influence it may have on the susceptibility of the human host to influenza infection.[2] They demonstrated that the level of host susceptibility to influenza is associated with microbial community makeup within the nose/throat.[2] Nose/throat samples were assigned nasal/oropharyngeal (NOP) community state types (CST) and samples from study participants were assigned to one of five groups based on bacterial taxa present. All five of these groups differed significantly in their relative abundance of 15 different bacterial genera.[2] When looking at the implications of certain taxa within the NOP CSTs on susceptibility to influenza, results showed a negative association between *Bacteroidetes* and NOP CST 4, of which the highest relative abundance was both *Streptococcus* and *Neisseria*, on host susceptibility to influenza infection. A positive correlation was noted between *Alloprevotella* and *Prevotella* and infection susceptibility.[43]

Lee et al. also demonstrated notable differences in the microbiome of people of different ages in regards to concurrent influenza infection. Of the NOP CSTs defined in the study, NOP CST 4, which was associated with a decreased vulnerability to influenza infection, was not as abundant or stable in children and showed a consistent increase in prevalence with increased age of a given participant.[2] This suggests the greater occurrence of influenza among young children can be attributed to immature and underdeveloped microbial networks in the nose/throat.[2]

The microbiome of the nasopharynx may also play a role in severity of disease and the development of secondary bacterial infections related to influenza. Ding et al. showed that both influenza A and B infected individuals had a nasopharynx microbiome containing taxa such as *Dolosigranulum* and *Staphylococcus*.[8] These genera have been correlated with the development of pneumonia, a common sequela of influenza in elderly patients, and *Dolosigranulum* also has been identified as a causative agent in septicemia.[47–49] This study classified nasopharyngeal (NP) swabs from infected and healthy subjects into four different NP types. NP type A was most prevalent in influenza-infected patients, and consisted of the two bacteria mentioned above.[8] NP type B was significantly enriched in the healthy control group and was dominated by *Corynebacterium,* NP type C was dominated by *Moraxella,* and NP type D had a high prevalence of *Staphylococcus*.[8] *Corynebacterium* has been shown to hinder *Streptococcus pneumoniae* growth in vitro and has been demonstrated to be abundant in children lacking

S. pneumoniae nasal colonization.[9] *Corynebacterium* has also been shown to have antimicrobial effects against *S. aureus*.[50] The discoveries made in the study conducted by Ding et al. support the previously mentioned study's findings, with NP type B being dominated by *Corynebacterium* with relatively low levels of *Staphylococcus*.[8] These results reiterate the need for more focused studies on the therapeutic potentials of certain bacteria among microbiota and how they can alter vulnerable patient outcomes.

A recent study using a mouse model of influenza virus infection has shown that fermentable dietary fiber increased survival of influenza-infected mice by dampening airway inflammation through several immune modulating mechanisms.[10] Dietary fiber inulin altered the intestinal microbiota in mice and exhibited butyrogenic properties. In inulin-treated mice, the microbiome structure was significantly improved, and beneficial bacteria that produce short-chain fatty acids (SCFAs), including Bifidobacteriaceae, became dominant. Inulin or SCFAs intake prevented excessive neutrophil influx into the airways by blunting the levels of CXCL1 produced by lung monocytes and macrophages. In addition, inulin and SCFAs increased antiviral immunity through $CD8^+$ T cell activation.[10] Another study in a mouse model of severe respiratory syncytial virus (RSV) reported similar findings that fermentable dietary fiber can reduce virus replication, protect the lungs against inflammation and pathological changes induced by RSV infection through microbiota-derived acetate in mice.[51] Since fermentable dietary fiber play a role in regulating immunity by promoting the growth of SCFA-producing bacteria, taking fermentable dietary fiber and reducing the excessive immune response and lung pathology caused by the viruses are a causal relationship, not just a correlation. To date, there are no human clinical studies on using fermentable dietary fiber to prevent influenza or other respiratory viral infection or to reduce the disease severity. This area of translational research warrants further investigation.

2.2 Human immunodeficiency virus (HIV)

According to the World Health Organization, 1.7 million new HIV cases occurred globally in 2018, and nearly 38 million people currently are living with the infection.[52] This disease typically is spread through sexual contact, and the disruption of cervicovaginal microbiota has been correlated with increased susceptibility to HIV infection.[53] Investigation of the cervicovaginal microbiota's influence on HIV infection susceptibility could lead to the development of therapeutic agents for preventing HIV in especially

at-risk communities, such as those in sub-Saharan Africa, where approximately 24 million people are infected, and young women are eight times more likely than men to acquire the virus.[13]

Though the majority of the human microbiota resides in the gut, the cervix and vagina maintain their own commensal microbial communities.[13] A cervicovaginal microbiota dominated by *Lactobacillus,* specifically *L. crispatus*, is associated with many health benefits, including reduced risk of contracting HIV.[52] When cervicovaginal dysbiosis occurs, *Lactobacillus* communities are disrupted and other resident microbes, such as *Gardnerella* or *Mobiluncus*, become the predominant species, resulting in bacterial vaginosis.[11] A study conducted by Fredricks et al. demonstrated increased bacterial diversity in women with bacterial vaginosis, and a relatively "normal" vaginal flora dominated by *L. crispatus* in women without the disease.[11] A HIV study by Gosmann et al. showed that none of the participants who contracted HIV had a cervicovaginal microbiota dominated by *Lactobacillus* prior to infection.[13] They also demonstrated that women who maintained the most diverse microbiome were significantly more likely to contract HIV than those with less diverse communities dominated by L. *crispatus*.[13] These results are consistent with the notion that a *Lactobacillus* dominated cervicovaginal microbiota for the prevention of HIV is critical. Several anaerobic bacteria also were implicated in the increased susceptibility to HIV infection in this study, including *Prevotella* and *Sneathia*.[13] These bacteria were shown to increase inflammation in the genital tract, further decreasing the participants' immune robustness against HIV infection.[13]

Bacterial vaginosis and high diversity/low *L. crispatus* presence in the vagina has been associated with increased susceptibility to HIV infection, partly due to resulting genital inflammation and the vulnerability of cell types that dominate the inflamed genital tract.[12,54] Genital inflammation has been shown to lead to disturbances in the vaginal epithelium, making women especially susceptible to HIV infection.[13,54] Anahtar et al. found that a diverse cervicovaginal microbiota is strongly linked to genital inflammation, including an increase in proinflammatory cytokines.[12] *Fusobacterium*, *Aerococcus, Sneathia*, *Gemella*, *Mobiluncus*, and *Prevotella* were all found to have a significant association with an increase in inflammatory cytokines.[12] These results were confirmed in vitro when human epithelial cells co-cultured with these bacteria, specifically *Prevotella amnii*, *Mobiluncus mulieris*, *Sneathia amnii*, and *Sneathia sanguinegens* were shown to have a marked increase in cytokine secretion than those co-cultured with *L. crispatus*, which showed no detectable levels of cytokine secretion.[12] Women with increased proinflammatory

cytokine levels attributed to highly diverse cervicovaginal microbiota also had higher levels of CD4 + T cells, one of HIV's main target cells, establishing a strong link between increased bacterial diversity and an increased risk of contracting HIV.[12]

Along with cervicovaginal microbiota diversity and the implications it has on HIV status and pathogenesis, gut microbiota diversity has also been identified as playing a significant role in these areas in regards to HIV. Because HIV is known to replicate in gut lymphoid tissues, it is relevant to discuss the implications the disruption of the gut microbiota and subsequent inflammation may have on HIV pathogenesis and vice versa.[55] HIV has been previously associated with gut dysbiosis, and again represents a potential tool that can be used to mitigate the impact of HIV infection.[56]

In a meta-analysis performed by Tuddenham et al., it was demonstrated that diversity of the microbiome in HIV-1 positive individuals was significantly decreased prior to controlling using demographic categories.[56] When controlling for sexual preferences and gender, this held true for men who have sex with women (MSW) as well as men.[56] Men who have sex with men (MSM) showed no significant association with microbiota diversity and HIV status; however, when controlling for MSM, HIV positive men were more likely to have a decreased diversity than HIV positive women.[56] This study highlights the complicated relationship between many factors involved in HIV infection, including diversity of the microbiota. In future studies, it will be beneficial to investigate the role the other factors play in diversity and how therapeutics can be tailored based on factors like gender and sexual preference.

In a study by Qing et al., researchers demonstrated how diversity of specific short-chain fatty acid (SCFA) producing bacteria can influence HIV progression through intestinal injury.[57] Because SCFAs are known to contribute to immune homeostasis and the expression of antimicrobial peptides by epithelial cells in the gut, they have been implicated in the progression of HIV infection.[57] This study found that certain species in the Firmicutes and Bacteroidetes phyla, who produce various SCFAs such as butyric and valeric acid, were much less abundant in the HIV positive group.[57] Butyric acid has been shown to reduce colonic inflammation in patients with inflammatory bowel disease, and the reduction of bacterial producers of this metabolite may contribute to increased intestinal injury and permeability, ultimately leading to bacterial translocation from the gut resulting in systemic inflammation.[58–62]

Several factors contribute to the enhancement of HIV infection, including cervicovaginal and gut dysbiosis. The resulting inflammatory and immune-related sequelae can have significant impact on the progression and outcome of the disease. The prevention and management of HIV infection in developed countries has improved dramatically since the first cases were reported in the 1980s; however, there are still millions of people in underdeveloped areas who would benefit from microbiota research in regards to HIV prevention.[52] The findings in the above studies show promise for the development of accessible and personalized medicine alternative to help these areas recover from a several decades long HIV epidemic.

2.3 Human norovirus (HuNoV)

2.3.1 HuNoV infection disrupts human microbiome

The majority of the world's acute gastroenteritis cases are caused by HuNoV, an enteric viral pathogen spread primarily via the fecal-oral route.[63,64] HuNoVs are non-enveloped RNA viruses with a positive-sense single-stranded genome in the family *Caliciviridae*.[65]. Due to the ease of transmission, cases of HuNoV tend to be concentrated and typically are more problematic in children, especially in low-income regions.[63] In more developed countries, HuNoV is known to cause outbreaks of gastroenteritis in all age groups.[63] HuNoV is of great concern in public health practice, but due to the lack of reliable and well-established in vitro cell culture systems, the development of therapeutics has been slow.[66]

Certain microbial populations can positively contribute to gut health and microbial balance, and one of the most intriguing benefits of these bacteria is their ability to influence outcomes of infectious diseases.[67] When the gut microbiota is disrupted, the protection potential is diminished and the host becomes susceptible to numerous infections.[14,67] Nelson et al. conducted a study whose results suggest that HuNoV infections are capable of causing significant alterations in human gut microbiota.[14] In this study, 20% of HuNoV-infected subjects showed significant changes in their gut microbiota, including an increase in Proteobacteria populations, especially *Escherichia coli*, and a decreased bacterial diversity.[14] Proteobacteria are associated with gut microbiota disruption, further suggesting the study participants had a disrupted microbiota at the time of sampling.[14] These patients were identified as being at risk for developing postinfection disease, such as irritable bowel syndrome.[68]

2.3.2 Microbiome and secretor status in the immunity to HuNoV infection

Along with establishing the need for a stable gut microbiota as a natural defense against HuNoV infection, other studies have identified a potential link between human genetic signatures, such as histo-blood group antigen (HBGA), specifically secretor status, the gut microbiome, and their combined influence on immunity to HuNoV infection. Secretor status of an individual is determined by the functionality of the *FUT2* gene, which is responsible for producing an enzyme critical to generating glycans present in saliva and mucus.[69] These glycans can serve as an infection initiating binding site of certain HuNoV strains.[69] When both alleles of this gene are altered and nonfunctional, a person is deemed a non-secretor.[69] Non-secretors have been shown to be highly resistant to symptomatic HuNoV infections due to the mutation in the *FUT2* gene, which diminishes the norovirus's ability to bind to receptors in the intestine, thus preventing HuNoV from entering cells and causing infection.[69] Several studies also have shown secretor status to be linked to microbiota composition.[15,70]

In a study by Rodriguez-Diaz et al., human patients were shown to have a correlation between their gut microbiota diversity, secretor status and susceptibility to HuNoV infection.[15] Host anti-HuNoV salivary IgA titers were obtained from the participants and were used as an indicator of previous infection susceptibility.[15] Though no significant differences in phyla composition of the gut (obtained through stool samples) and secretor status were found, significant family-level differences were present between non-secretors and secretors; with significantly higher levels of *Prevotellaceae* and *Paraprevotellaceae* found in non-secretors.[15] The study then involved separating the obvious groupings into operational taxonomic groups (OTUs) and were then analyzed for diversity levels. Sixteen of the more than 5000 groups were found to have significant differences, and consisted of Firmicutes and Bacteroidetes phyla, found in non-secretors. There were also significant differences in richness of the sampled microbiota, though they were not significant in regards to secretors versus non-secretors.[15] Firmicutes, Bacteroidetes, Actinobacteria, and Verrucomicrobia were the most abundant phyla categorized from both secretors and non-secretors. This study also found anti-HuNoV IgA levels were significantly correlated with secretor versus non-secretor status, with secretors maintaining higher levels of the two groups.[15] The viral infectivity and host susceptibility to HuNoV was negatively correlated to the level of *Ruminococcaceae* and *Faecalibacterium* present in host samples. When these insights are combined

with the negative correlation between anti-norovirus IgA titers and susceptibility to HuNoV infection, the notion that host microbiome, secretor status and susceptibility are all reliant on each other to some extent is apparent.[15] More thorough research is needed to evaluate other factors that simultaneously play a role in microbiome composition of all secretors and nonsecretors and how this influences host immunity.[15] This is especially important for the determination of the best strategies to protect those in low-income areas in which HuNoV is of greatest public health concern, and the microbiome is likely an effective target for future personalized therapies.[63]

2.3.3 Study of microbiota and HuNoV interaction in the gnotobiotic (Gn) pig model

To study the role of microbiota in response to virus infection and vaccines, animal models that are without a preexisting microbiota and can be colonized with well-defined microbiota, (i.e., Gn animals) are an indispensable tool. Gn animals have played a critical role in the understanding of interactions among microbiota, viral pathogen or vaccine antigen, and the host.[71] The neonatal Gn pig model is well suited for the study of HuNoV and microbiome interaction because Gn pig model of HuNoV infection reflects HuNoV biology in terms of supporting the natural oral route of infection and results in diarrhea, transient viremia, and fecal virus shedding.[72,73] Recently, we established a human gut microbiota (HGM) transplanted Gn pig model of HuNoV infection and disease, using a well-characterized stool sample from a healthy infant as HGM transplant and a HuNoV GII.4/2006b strain for virus inoculation.[16] Compared to germ-free Gn pigs, HuNoV inoculation in HGM transplanted Gn pigs resulted in increased HuNoV infection, characterized by significantly higher shedding titers and significantly longer mean duration of virus shedding. HuNoV infection also dramatically altered intestinal microbiota at the phylum and genus levels in HGM transplanted Gn pigs. At the phylum level, Proteobacteria (95.6% versus 56.5%) and Firmicutes (3.6% versus 0.5%) significantly decreased, while Bacteroidetes (0.1% versus 42.9%) significantly increased; at the genus level, *Enterococcus*, *Bifidobacterium*, *Clostridium*, *Ruminococcus*, and *Anaerococcus* significantly decreased, while *Bacteroides* and *Lactobacillus* significantly increased in HGM transplanted pigs infected with HuNoV compared to HGM transplanted pigs without HuNoV infection. In summary, enhanced GII.4 HuNoV infection was observed in the presence of HGM, and significant intestinal microbiota alterations were observed under HuNoV infection in HGM transplanted Gn pigs.[16] The mechanisms underlying the

enhancing effect of HGM on HuNoV infection are probed using transcriptome analysis of the ileum tissues of the Gn pigs. One of the most important impact of HGM is the down regulation of interferon (IFN)-λ gene IFN-1L expression. IFN-λ is known to play a critical role in the host defense against enteric viruses, including rotavirus, reovirus[74] and norovirus.[75]

2.4 Human rotavirus (HRV)

2.4.1 Microbiota and immunogenicity of HRV vaccines

Rotaviruses belong to the genus *Rotavirus* within the family *Reoviridae*, which are characterized by a segmented double-stranded RNA (dsRNA) genome. HRV is the leading cause of severe, dehydrating diarrhea among children under 5 years of age worldwide.[76] Annual mortality rates due to RV infections have declined from ~528,000 in 2000 to ~215,000 in 2013[76] owing to the implementation of HRV vaccines RotaTeq® and Rotarix® in the national immunization program in many countries. These two vaccine vaccines are highly efficacious (80–90%) in high income countries, but the efficacies are significantly reduced (40–60%) in low-income countries. Studies indicated that several factors, including microbiota dysbiosis,[77] concurrent uses of poliovirus oral vaccines,[78,79] enterovirus infections,[80] and malnutrition[81] contributed to the observed impaired efficacy of the oral RV vaccines,[79] likely by changing intestinal environments and homeostasis.[82,83] Harris et al.[17] analyzed the serum IgA antibody responses to Rotarix® in 78 Ghanaian infants and their fecal microbiome. Comparing between the 39 responder and non-responder pairs and to the Dutch infants, the overall microbiome composition was significantly different, with the responders more similar to healthy Dutch infants than non-responders. Responses to Rotarix® correlated with an increased abundance of *Streptococcus bovis* and the lack of response is correlated with the high abundance of Bacteroidetes phylum, especially several bacteria related to species from the *Bacteroides* and *Prevotella* genera.[17] Another study was reported for Pakistani infants.[18] IgA antibody responses to Rotarix® correlated with higher abundance of bacteria belonging to *Clostridium* cluster XI and Proteobacteria, including bacteria related to *Serratia* and *Escherichia coli*. Abundance of these Proteobacteria was also significantly higher in Dutch infants when compared to non-responders in Pakistan. These clinical studies showed that the intestinal microbiota composition correlated with seroconversion rate in infants after vaccination and identified microbiome signatures of vaccine responder versus non-responder infants. Identification of

key bacterial phylotypes that correlate with vaccine-induced responses could be used as indicator to predict which infants are at risk for vaccine failure and to design an intervention through modulating gut microbiota to improve rotavirus vaccine efficacy.[18,77] A study of microbiome diversity in RataTeq vaccinated versus unvaccinated children in Spain did not find any differences.[84]

2.4.2 Study of microbiota and immunogenicity of HRV vaccine in Gn pig model

To clearly identify the role of microbiota in the immunogenicity of HRV vaccine, a Gn pig model of enteric dysbiosis was developed to evaluate the effects of different HGM on immune responses to HRV vaccination and protective efficacy.[19] Fecal samples collected from infants from Nicaragua were analyzed and classified as healthy (HHGM) or unhealthy (UHGM) based on enteropathy score and seroconversion status after vaccination with RotaTeq®. Newborn Gn pigs were transplanted with the HHGM or UHGM, followed by oral vaccination with the live attenuated Wa HRV and challenged with virulent HRV. Prior to HRV challenge, HHGM in Gn pigs was predominantly composed of bacteria belonging to the phyla Firmicutes, Bacteroidetes, and Actinobacteria, whereas UHGM was composed of Proteobacteria and Firmicutes. When challenged, the severity of illness among vaccinated HHGM pigs was much lower compared to vaccinated UHGM pigs. Analysis of fecal microbiome post-challenge showed a significant increase in the abundance of Bacteroidetes in HHGM transplanted Gn pigs and a significant decrease in the abundance of Firmicutes, Proteobacteria, and Verrucomicrobia abundance. Stronger vaccine-induced immune responses (HRV-specific IgG, IgA and VN antibodies; and CD4 + IFN-γ + and CD8+ IFN-γ + T cells) were facilitated by HHGM, hence conferring a stronger protection against HRV disease among vaccinated HHGM pigs than UHGM pigs.[19] Similar results were also observed in Gn pigs colonized with *Lactobacillus rhamnosus GG* and *Bifidobacterium lactis* Bb12, commensal bacteria commonly found as part of the microbiota of breast-fed infants.[20] Findings in Gn pigs are highly translatable to humans and as suggested by Dr. Harris "Correlate microbiome composition with vaccine effectiveness in appropriate experimental platforms will lead to the identification of safe, vaccine-supporting microbiota targets that are relevant to populations in need of improvement in vaccine-induced immunity."[18]

2.5 Hepatitis viruses

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are important public health issues that can lead to chronic infections and together are the most common causes of liver cirrhosis and hepatocellular carcinoma (HCC). Due to the well-documented crosstalk between the gut and liver, the microbiota and its immunomodulatory effects on HBV and HCV infections have recently become a topic of interest in the research community.[85,85a]

As was demonstrated by the previously discussed studies, certain phyla and families of beneficial bacteria appear to be underrepresented in patients with certain viral diseases. In a study by Wei et al., it was shown that patients with chronic HBV infection that had progressed to cirrhosis were lacking in Bacteroidetes composition in the gut, as well as showing increased Proteobacteria concentration, a phylum known to contain several pathogenic bacteria.[21] There was also marked prevalence of *Enterobacteriaceae*, *Veillonellaceae*, and *Streptococcaceae*, which made up for less than 1% of the gut microbiota sampled from healthy patients.[21]

In two HCV studies by Aly et al.[22] and Bajaj[23] et al., low diversity of gut microbiome composition was associated with inflammation and disease. Interestingly, Aly et al.'s data showed chronic HCV patients had a significant increase in Bacteroidetes, as opposed to the previously discussed HBV patients, who had a marked decrease of this phylum in their composition.[21,22] In the HCV patients, *Prevotella* was significantly increased, perhaps explaining the increase in Bacteroidetes phylum composition in this group.[22] This study also demonstrated a higher abundance of Proteobacteria in the healthy control group, dissimilar to the HBV study, which showed a higher abundance in the HBV group.[21,22] In the HCV study, ill patients also displayed significant prevalence of *Acinetobacter*, *Veillonella*, and *Phascolarctobacterium*, as well, with no detection of *Bifidobacterium* genus, which was of relatively increased richness in two healthy patients.[22] *Ruminococcus* was also significantly enriched in healthy patients versus those with HCV, and this genus is considered to be largely beneficial in the gut.[22,23]

Though more research is needed in regards to the gut-liver axis and its role in immunity against HBV and HCV infections, the current literature has demonstrated a strong link between the gut microbiota and how the immune system functions in acute and chronic viral diseases. The disadvantage that remains is the microbiota's complexity, as is shown by the differences in compositions between patients with two different strains of the same virus. Fortunately, due to the development of vaccines, new hepatitis cases have dramatically decreased across the world, though chronic infections

continue to pose a threat to those who do contract the virus. The gut microbiota may be a means to harness new therapies to relieve the increased mortality that comes with progression of chronic hepatitis infection.

2.6 Virome in humans

The human virome is constituted of viruses that infect our cells, virus-derived elements in our genome, and viruses that infect the broad array of other types of microorganisms that inhabit us.[86] The integration of endogenous retroviral DNA into the human genome accounts for 8% of the total genome, and is thought to promote development of certain diseases such as cancers and autoimmune diseases.[87] These sequences are also passed from mother to child, perpetuating the effects to offspring.[88] Another aspect of the virome is made up of prokaryotic viruses, or bacteriophages. Bacteriophages are the most numerous beings on Earth and have been found to promote health in humans, mostly through the development of phage therapy. Phage therapy has been utilized to combat antibiotic-resistant bacterial infections in humans.[89]

Similar to the bacterial component of the microbiome, the virome contains certain makeup that varies between individuals.[90] Some common inhabitants of the human gut include *Phycodnaviridae*, *Herpesviridae*, *Poxviridae*, *Mimiviridae*, *Iridoviridae*, *Adenoviridae*, *Anelloviridae*, *Astroviridae*, *Parvoviridae*, *Picornaviridae*, *Picobirnaviridae*, and *Reoviridae* which have been identified in infant stool samples.[91–93] Viromes tend to stabilize over time as well, with humans establishing the individualized virome by around the age of 2 years, similar to the bacteriome.[94]

3. Microbiota in viral infection and diseases in avian species

3.1 Introduction

Poultry contain the most economically important avian species because they provide a great source of animal protein. Poultry eggs and meat are crucial components to maintaining food security all over the globe. Fast-growing domestic poultry species are exposed to many environmental factors that can affect their health, performance, and productivity. To protect poultry from factors that may render them vulnerable to disease, scientists continuously investigate internal and external factors that may affect their health and productivity. The gastrointestinal tract has been a central part of these investigations as the gut health, function, and integrity are essential for

overall wellbeing of birds. The gut microbiota, which contains tremendous numbers of bacterial species, has recently been extensively characterized in some avian species including chickens, turkeys, and ducks.[95–97] The influence of viral infections on the gut health and the homeostasis of commensal microbial populations of affected birds have been also investigated. Viral infections are also known risk factors for coinfection by other secondary, opportunistic pathogens as they cause damage to local and systemic immune defenses.[98] Here, we review the interplay between a number of viral pathogens and the gut microbiota of their respective natural avian hosts.

3.2 Avian influenza virus

Avian influenza viruses (AIV) belong to species Influenza A virus (IAV), genus *Influenza A*, family *Orthomyxoviridae*, order *Mononegavirales*, with a negative sense, single-stranded, segmented RNA genome (talk.ictvonline.org/ictv-reports). Based on their pathogenicity, AIV strains are classified into two major pathotypes: high pathogenicity avian influenza (HPAI) viruses, e.g., H5N1 and low pathogenicity avian influenza (LPAI) viruses, e.g., H9N2. However, AIV strains causing natural infections in different species can be categorized into four groups from highly virulent to avirulent. The morbidity, mortality, and lesions are highly dependent on the virulence of the strain, the bird's age, and the immune status. Highly virulent AIV infections cause morbidity and mortality nearing 100% and systemic, severe lesions. Mortality decreases substantially in mildly virulent AIV infections, which cause mild respiratory disease.[99]

3.2.1 Effect of IAV infection on microbiota composition

The effect of LPAI infection on the homeostasis of the gut microbiota of poultry has been investigated very recently. Experimental infection of young chickens with H9N2 virus caused alterations in the intestinal microbiota composition. In infected chickens, the bacterial numbers of the phylum Proteobacteria were increased and the bacteria belonging to the genera *Vampirovibrio*, *Pseudoflavonifractor*, *Ruminococcus*, *Clostridium* cluster XIVb, and *Isobaculum* were enriched differentially.[24] Proteobacteria contains important gram-negative pathogenic bacteria belonging to the genera *Escherichia*, *Shigella*, *Salmonella*, and *Helicobacter*.[100] In uninfected—apparently healthy—chickens, differential enrichment of the genera *Novosphingobium*, *Sphingomonas*, *Bradyrhizobium*, and *Bifidobacterium* was observed.[24] Comparing the same chicken group before and after infection, Yitbarek et al. observed notable changes in the relative abundance of bacteria at various taxonomic levels.[24] Pre-infection, chickens were

characterized by the increased abundance of two families and three genera of bacteria, while postinfection differential enrichment of members from one class, two orders, and one genus of bacteria were recorded. In a study by Li et al.[25] at the phylum level, the relative abundance of Proteobacteria was increased—as observed in the study above—while that of Firmicutes was decreased in H9N2 virus-infected young chickens. The relative abundance of endogenous bacterial genera within the family *Enterobacteriaceae* was increased; these genera included *Escherichia*—especially *E. coli* which colonized the ileum—, *Clostridium*, and *Veillonella*. On the other hand, a severe reduction in the probiotic organisms including the lactic acid-producing bacteria (i.e., *Lactobacillus*, *Enterococcus*, and *Streptococcus)*, *Candidatus* Arthromitus, *Bacteroides*, *Parabacteroides*, and SMB53 was observed.[25] These findings clearly indicate that an influenza virus infection can disrupt the commensal microbial population in affected chickens.

Waterfowl species are natural reservoir of IAV and infections in these birds, unlike chickens, are asymptomatic. The composition and diversity of cloacal microbiome and their relationship with the active status of IAV infection and affected host have been studied in five waterfowl species: *A. acuta* (northern pintail), *A. americana* (American wigeon), *A. carolinensis* (green-winged teal), *A. clypeata* (northern shoveler), and *A. platyrhynchos* (mallard).[101] Six bacterial phyla, namely Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria, Actinobacteria, and Tenericutes (ordered based on proportions of abundance) were consistently represented in IAV-negative, i.e., healthy, ducks of the above-mentioned five species. These phyla composed an average of 97.1% of the ducks' microbiota and thus were further investigated in the study. The relative abundance of bacterial phyla was drastically different within the various host species, with no unique patterns across species. However, green-winged teals and mallards showed the same pattern of abundance for all phyla, while northern shovelers showed the opposite pattern. The relationship among the various groups of bacterial taxa was evaluated using a microbiome diversity measurement, bacterial operational taxonomic unit (OTU) co-occurrence patterns. The observed OTUs in IAV-positive and -negative birds showed no consistent patterns among host species. Moreover, the number of observed OTUs by IAV infection status was only significant in two out of five duck species.[101] Findings from this study indicates that host genetics (and possibly other environmental factors, e.g., feed and habitat) may play a crucial role in the response of waterfowl to influenza virus infections. Another study on juvenile wild mallards (*Anas platyrhynchos*) only analyzed the differences in the cloacal microbiome of IAV-infected birds as compared to

healthy ones.[26] Overall, the cloacal microbiome in healthy mallards was more rich, diverse, robust, and uniform than that in IAV-infected birds. Among 41 identified OTUs, IAV-infected mallards were found to have $\leq$12 versus 24 in healthy birds. Thus, IAV-negative mallards were richer in OTUs and had greater OTU diversity and evenness than those of IAV-positive group. The same six bacterial phyla listed above were also represented in both IAV-positive and -negative birds at similar levels of relative abundance. The higher differences were observed within the phyla Firmicutes, Proteobacteria, and Bacteroidetes. Members of the genus *Streptococcus* and species *Veillonella dispar* and *Rothia mucilaginosa* were major contributors to these differences.[26]

3.2.2 Effect of IAV infection on intestinal integrity and immunity

In chickens with compromised microbiota, intestinal damage was caused by H9N2 virus infection, including reduced villus height, increased crypt depth, and reduced villus height:crypt depth ratio of the ileum, as well as lymphocytic infiltration of ileal mucosa.[25,102] The breach of the intestinal wall integrity was consistent with downregulation in mRNA expression of the epithelial cell tight junction proteins (ZO-1, claudin 3, and occludin) and mucus layer proteins (trefoil factor 2 and mucin); the latter two have essential roles in preventing the inflammation and infection of the intestinal epithelium caused by invading bacteria.[25,103,104] Furthermore, the intestinal damage in H9N2 virus-infected chickens was associated by upregulation of mRNA expression of the proinflammatory cytokines IFN-γ, IL-22, IFN-α, and IL-17A by the intestinal epithelial cells.[25]

The importance of balanced gut microbiota in modulating innate immune responses and protecting chickens against influenza virus infections was demonstrated by several studies. In intestinal microbiota-depleted chickens, H9N2 virus infection caused significant increase in oropharyngeal and cloacal virus shedding. Also, the mRNA expression of type I IFNs (i.e., INF-α and IFN-β) and IL-22 was suppressed in the respiratory and gastrointestinal tracts.[102]

3.3 Newcastle disease virus

Newcastle disease virus (NDV, aka avian paramyxovirus 1) belongs to species *Avian orthoavulavirus 1*, genus *Orthoavulavirus*, family *Paramyxoviridae*, with a negative sense, single-stranded, monopartite RNA genome.[105] NDV affects a wide variety of avian species, among them the most susceptible and economically important are chickens and turkeys,[106] which can virtually be infected at all ages. Depending on the strain and tropism of

the virus, NDV can cause a variety of symptoms involving the gastrointestinal tract, the nervous system, the respiratory system, and the reproductive system of affected birds, leading to up to 100% morbidity and mortality.[107]

The adverse effect of NDV infection on the development of intestinal microbiota in chickens has been recently reported by Cui et al.[27] Although the study was performed in newly hatched chicks which were infected *in ovo* with NDV, it presented significant insight into the impact of such an infection on the intestinal microbial communities in chickens. In uninfected chicks, the duodenum had a richer (indicated by higher OTU counts and abundance indices) and more diverse microbiota than the cecum. In normal duodenum, the predominant bacterial phyla were Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria, with six more less-dominant phyla. At the genus level, bacteria from *Serratia*, *Escherichia*, *Chitinophagaceae* unclassified, *Saprospiraceae* unclassified, and *Lactobacillus* were represented the most in the normal duodenum among other genera. In the cecum of uninfected chicks, the prevailing bacterial phyla were only Firmicutes and Proteobacteria, with five more marginally-represented phyla detected. At the genus level, *Enterococcus*, *Paenibacillus*, *Erwinia*, *Escherichia*, *Lactobacillus*, and *Serratia* constituted the great majority of the normal cecal microbiota.[27] Comparing NDV-infected with uninfected chicks, the richness and diversity of duodenal microbiota were lower in the former. The cecal microbiota was almost unaffected by the NDV infection. As for the microbial composition, both duodenal and cecal bacterial communities were adversely affected in a similar manner. An increase in *Sinobacteraceae* and *Rhodoplanes* family members was observed. Also, at the genus level, a massive loss was observed in members of the following families in both duodenum and cecum of NDV-infected chicks: *Xanthomonadaceae*, *SC-I-84*, *Cytophagaceae*, *Bacteroidales*, *Chitinophagaceae*, *Gemm-1*, *Saprospiraceae*, *Ignavibacteriaceae*, *Rhizobiales*, *Sphingobacteriales*, *Ellin6067*, *Acidimicrobiales*, and *Pseudomonas*. Moreover, a dramatic reduction was found in the abundance of *Xanthomonadaceae* and *Cytophagaceae* family members.[27] A significant reduction in the relative abundance of *Serratia* and *Clostridium* genera residing in the duodenum was recorded as opposed to greatly increased numbers in the ceca. This implied a possible translocation of these genera from duodenum to cecum of NDV-infected chicks. Moreover, authors proposed a translocation of several bacterial genera from the cecum to the duodenum of infected birds according to changes in their relative abundance.[27]

Talebi et al. studied the effect of a mixture of probiotics (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium*, and *Bifidobacterium bifidium*), administered in water, on the antibody response and production

performance in NDV-vaccinated broiler chickens.[108] The study showed enhancement in birds' body weight and feed conversion rate; however, the humeral immune response to NDV vaccination was similar to that of untreated, vaccinated groups. Similar results were later revealed by Bautista-Garfias et al.,[109] who studied the effect of oral administration of *L. casei* (ATCC7469 strain) on the humeral immune response to NDV vaccination and production performance in fighting roosters. Blood anti-NDV antibody levels in *L. casei*-treated group were comparable to those of the vaccine control birds. On the other hand, the natural mortality rate and body weight gain were significantly improved in the probiotic-treated group, compared with the untreated one.[109] The use of two (cecal and jejunal) bacterial isolates of *L. reuteri* PIA16 strain in feed, either alone or with a prebiotic (mannan oligosaccharide), demonstrated substantially enhanced humoral immune response to NDV vaccine in broiler chickens. The cell-mediated immune response; however, remained largely unaffected throughout the course of the experimental period.[28] Results from the probiotics studies indicate that these beneficial bacteria can boost the performance and overall health status in poultry. Although these studies showed generic positive impact on NDV-vaccinated chickens, the associated changes in the intestinal microbiota have not been examined. Moreover, whether the supplementation of these probiotics/prebiotics has an enhanced protective effect for NDV vaccines against the consequences of an NDV challenge is yet to be investigated.

3.4 Infectious bursal disease virus

Infectious bursal disease virus (IBDV) belongs to the genus *Avibirnavirus*, family *Birnaviridae*, with a double-stranded, segmented RNA genome.[110] IBDV affects chickens at young ages and causes an acute, immunosuppressive disease, infectious bursal disease. IBDV infection causes depression of the local and humoral immune responses (due to a direct effect on B lymphocytes); and induces damages in the primary lymphoid organs, mainly the bursa of Fabricius, in addition to the spleen, thymus, and cecal tonsils.[111,112]

The role of IBDV in modulating the commensal microbiota in chickens has recently been studied.[29] Young chickens were infected with IBDV and the changes in the cecal bacterial communities were analyzed in comparison with uninfected, healthy birds. Regardless of the infection status, nine bacterial phyla were represented in the chicken cecal microbiota, with Firmicutes,

Proteobacteria, Actinobacteria, and Bacteroidetes members forming more than 95% of total bacterial population in the cecum. At the family level, *Lachnospiraceae* and *Ruminococcaceae* composed the majority of cecal microbiota, with the relative abundance of *Lachnospiraceae* decreasing over time and that of *Ruminococcaceae* increasing over time for up to 7 weeks of age. At the genus level, an increase in the abundance of *Faecalibacterium* was observed till ~4 weeks of age, and decreased thereafter. IBDV infection of young chickens caused immediate and obvious alteration in the composition of cecal microbiota. Changes were continuously seen over a three-week observation period postinfection,[29] though no certain pattern was observed. At the genus level, in comparison to uninfected group, IBDV-infected chickens had a lower abundance in the commensal *Clostridium XlVa* at day 3 postinfection, which became higher thereafter. On the other hand, after 7 days of IBDV infection chickens had a higher abundance of *Faecalibacterium* (Gram-positive bacteria) than uninfected ones, which became lower thereafter. A reduction in the abundance of *Escherichia/Shigella* (Proteobacteria) was observed in chickens until 3 weeks postinfection, which contributed to the overall decrease recorded for family *Enterobacteriaceae*.[29] These changes in the cecal microbiota were associated with IBDV-induced immunosuppression, the manifestations of which is described below. Another study showed that the infection of young chickens with IBDV or *Campylobacter jejuni*, either alone or combined, provoked significant modifications in the composition of gut microbiota, as compared with uninfected chickens.[30] Changes in the abundance of OTUs from the following bacterial genera were observed in the cecal contents of infected chickens: *Campylobacter*, *Clostridium XIVa*, *Eubacterium*, *Faecalibacterium*, *Lachnospiracea incertae sedis*, *Lactobacillus*, and *Roseburia*. The abundance of *Campylobacter* was increased in IBDV/*C. jejuni*-infected chickens and it was higher in birds inoculated with bacteria at 7 days versus 9 days postinfection. Obviously, the exposure time to a secondary pathogen following an immunosuppressive viral infection has a significant impact on its pathogenicity, colonization, and persistence, as is the case here with IBDV and *C. jejuni*. Similar observations have been reported for hemorrhagic enteritis of turkeys—a siadenovirus-based immunosuppressive disease—and secondary *E. coli* infections.[113]

The particular effect of immunosuppression-inducing IBDV infection on the colonization and shedding of *C. jejuni* and *Salmonella* in the gastrointestinal tract of chickens has been investigated. Unlike *C. jejuni*, *Salmonella* is pathogenic in poultry; however, both are important pathogens in human

and can cause severe food-borne gastroenteritis.[114,115] Apart from *C. jejuni* and *Salmonella* infections, inoculation of young chickens with various strains of IBDV resulted in atrophy and pronounced lesions in the bursa of Fabricius, bursal B cell depletion, reduction or depletion in circulating B lymphocytes as well as those localized in the cecal lamina propria and cecal tonsils, and a reduced thickness of cecal mucosa.[27,29,32,116,117] Virus replication in the bursa, cecal tonsils, and cecum was detected for up to several weeks postinfection.[27,29,116] In IBDV-infected chickens with immunosuppression, unlike *C. jejuni* only-infected birds, the bacteria colonized the small intestines, and was detected at an earlier time point and at much higher titers in the cecum and cloaca (i.e., virus shedding).[27,116] These results were consistent with the reduced B lymphocytes and the lack of *C. jejuni*-specific IgG antibodies in the cecal lamina propria.[27] Likewise, IBDV infection of chickens exacerbated *Salmonella* multi-organ colonization, pathogenicity, fecal shedding, and persistence in chickens exposed to infections at early ages.[31,32,117] The highest *Salmonella*-mediated pathogenicity and mortality rates were recorded in chickens coinfected with IBDV and *S. enteritidis,*[31,32] but not *S. typhimurium*.[117] Both intestinal mucosal[41] and systemic (*Salmonella*-specific IgG) antibody responses were lower in chickens coinfected with IBDV and *Salmonella*, which coincided with the immunosuppressive effects described above. However, unlike *C. jejuni*, *Salmonella* clearance appeared not to be related to the antibody response. Numbers of localized T lymphocytes and cell-mediated immune responses did not seem to be affected by IBDV infections.[27,32]

3.5 Hemorrhagic enteritis virus/turkey adenovirus 3

Turkey adenovirus 3, commonly known as hemorrhagic enteritis virus (HEV), belongs to genus *Siadenovirus*, family *Adenoviridae*, with a double-stranded, linear DNA genome.[118] HEV is present naturally in two strains; virulent, which causes an immune-mediated, acute disease characterized by bloody diarrhea, enteric hemorrhage, and death; and avirulent, which is widely used as a vaccine strain. Both strains cause transient period of immunosuppression and splenomegaly and target the IgM^+ B cells in the spleen and peripheral blood. In addition to turkeys, HEV causes immune-mediated diseases in pheasants and chickens characterized by pulmonary edema and splenomegaly. Lesions in several organs have been also reported in affected birds.[113]

Changes in the abundance of microbial populations between HEV-infected and uninfected turkeys have recently been investigated. Analysis of OTUs detected in the gut of healthy turkeys revealed the presence of four abundant phyla: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. In healthy turkeys, Firmicutes was the predominant phylum (80–100%) in the three small intestinal sections, while in the cecum a more heterogeneous population containing representatives from Firmicutes, Bacteroidetes, and Proteobacteria was observed.[33] The diversity of cecal microbial population was also observed throughout the developmental stages of turkeys up to 16 weeks of age. The bacterial populations were also dissected at the genus level in the different gastrointestinal tract sections and *Lactobacillus*, *Streptococcus*, and *Clostridium XI* were the prevailing genera forming nearly 80% of the total microbiota. An interesting finding was that *Campylobacter* spp. was most abundant in the cecum of turkey poults at 10 weeks of age.[96] Interestingly, similar to turkeys, both chickens and ducks have a greater microbial diversity in the cecum as compared to the rest of the small intestine.[96,119,120] The abundance of jejunal bacterial populations at the family level was altered by natural infections of HEV in turkeys. Compared to uninfected turkeys, infected birds with clinical signs and HEV detected in the intestines—indicating an early-phase infection—had a lower abundance in *Micrococcaceae* and a higher abundance of *Propionibacteriaceae,* while those turkeys with no clinical signs and virus detected in the spleen—indicating a late-phase infection—had a much lower abundance in both families. The abundance of family *Bacteroidaceae* was extremely increased in HEV-infected turkeys as compared to uninfected controls. For the phyla Firmicutes, a substantial reduction in *Lactobacillaceae* and an increase in *Clostridiaceae* was observed in intestinally-positive turkeys with clinical signs. An opposite trend was recorded for turkeys with positive spleens and no clinical signs. However, both groups of infected turkeys showed an increased abundance of three other families belonging to Firmicutes. Family *Campylobacteriaceae* increased in abundance by 18- to 26-folds in HEV-infected turkeys.[33] HEV infection of turkeys has been associated with secondary *E. coli* infections during the transient immunosuppression period.[34] HEV is transmitted through fecal-oral route and eventually reaches the blood stream. In the spleen, the virus replicates rapidly in B lymphocytes and to a much lower extent in macrophages. Hyperplasia of the splenic white pulp occurs as a result of infiltration with high numbers of macrophages and lymphocytes, including CD4+ T cells. The interplay among these various types of immune cells within the site of

viral replication leads to the production of very high levels of pro-inflammatory cytokines, e.g., interleukin 6 (IL-6) and tumor necrosis factor (TNF) as well as the anti-inflammatory cytokines of type I and II IFNs. The latter activates macrophages, inducing the production of the antiviral and immunosuppressive nitric oxide (NO). Most likely, IFN-I and NO play a role in clearing the virus later from the spleen.[121–123] Moreover, it was suggested that the intestinal hemorrhage in the jejunum area occurs through inflammation-induced "diapedesis."[124] This might be associated with a compromised intestinal epithelial cell barrier of the gut as noticed with other enteric viral infections.

3.6 Virome in avian species

Viruses which can colonize a healthy poultry gut have been recently identified in chickens. Specific pathogen-free chickens were placed for a few days with age-matched peers in commercial and back yard farms with previous history of enteric and respiratory problems. Intestinal samples were then collected and the enteric virome and microbiome were analyzed.[125] The analysis revealed that the new viruses which colonized the gut of SPF birds belong to the families *Picornaviridae*, *Picobirnaviridae*, *Reoviridae*, and *Astroviridae*. This implies that members of these families are associated with enteric diseases in chickens. In these birds, alterations of the gut microbiome were also observed, specifically in the *Lachnospiracea* family and the *Clostridium* and *Lactobacillus* genera. Other virus families that were represented in the enteric virome of chickens included *Adenoviridae*, *Birnaviridae*, *Caliciviridae*, *Coronaviridae*, *Leviviridae*, *Siphoviridae*, and *Retroviridae*.[125] In another study, the fecal virome in healthy commercial chickens was analyzed by viral nucleic acid purification, illumina sequencing, and de novo assembly.[126] Analysis of the assembled viral genome sequences indicated the presence of viruses belonging to the families *Adenoviridae*, *Caliciviridae*, *Circoviridae*, *Parvoviridae*, *Picobirnaviridae*, *Picornaviridae*, and *Reoviridae*.[126] Additionally, novel, unclassified, viruses with circular single-stranded DNA genomes were identified. The fecal virome of wild waterfowls has been also recently analyzed in various species of migratory wild ducks.[127] The DNA and RNA viral genomes were isolated from purified viral particles, their nucleotide sequences obtained, and analyzed. The most abundant viral familied recognized in the duck's virome were *Herpesviridae*, *Alloherpesviridae*, *Adenoviridae*, *Retroviridae*, and *Myoviridae*.[127] In a more recent study on healthy waterfowl and shorebirds, the presence of 27 viral

species was reported. These species represent the families *Picobirnaviridae*, *Reoviridae*, *Astroviridae*, *Caliciviridae*, *Picornaviridae*, *Hepadnaviridae*, and *Parvoviridae*. Variations in virus abundance and diversity were detected among the various species studied.[128] The results signify the potential of wild birds as reservoir of disease-causing poultry pathogens.

4. Microbiota in viral infection and diseases in swine

4.1 Introduction

Over 35% of agriculturally produced protein for consumption is produced in pigs globally.[129] China is the leading producer of pork generating over 54 million metric tons annually. The majority of the pork exported to the rest of the world comes from the European Union.[130] A constantly increasing human population demands for the availability of more animal-based protein for consumption around the world. This demand for more meat has led to the intensification of farming practices often causing overcrowding of animals allowing for rapid pathogen transmission.[129] Pork production and trade are primarily impacted by infectious diseases that affect swine. The domestication of pigs for the purpose of livestock and husbandry has increased their exposure to emerging and re-emerging viral diseases causing a significant impact on the worldwide pig populations.

The recent advancement of technology and overcoming the requirement of culture-based species identification has allowed for extensive research initiatives that have investigated the role of microbiome and its influence on host immunity.[131–134] This section will focus on different microbial (bacterial) communities that constitute the microbiome and their effects on viral infections in pigs that have a significant impact on domestic pig rearing.

4.2 African swine fever

African swine fever (ASF) is a severe hemorrhagic disease, with a near 100% mortality rate. The disease is characterized by high fever, loss of appetite, ataxia and depression.[135] The causative agent African swine fever virus (ASFV) is a double-stranded DNA virus belonging to the *Asfaviridae* family. ASF is a reportable disease to the World Health Organization (WHO), and is often accompanied with regional, national and international trade restrictions with the capacity to impact the global pig meat economy.[136] Pigs of all ages are susceptible to virulent strains of ASF with the exception of African wild pigs. No vaccines or therapeutics are available, with the

control of disease spread often carried out by culling large numbers of domestic pigs.

Transmission between traditional ASFV hosts, warthogs (*Phacochoerus aethiopicus*) and bushpigs (*Potamchoerus porcus*), occurs through the bite of soft ticks belonging to the genus *Ornithodoros,* which are endemic to sub-Saharan Africa.[137] Ingestion of feed contaminated with biological fluid from wild boars and pigs, and fomites have been identified to be efficient modes of virus transmission in the absence of the arthropod vector.[135,138] The oral route of infection is considered to be a major portal of entry for ASFV among pigs outside the natural territory of its arthropod host. Little work has been carried out in order to characterize the bacterial composition among different pigs, based on their susceptibility status to ASFV.

Specific pathogen-free (SPF) pigs have been demonstrated to be highly susceptible to lethal infection by an attenuated strain of ASFV (E75CV1) that was previously tested to be safe for administering to domestic pigs as a vaccine.[139] Diversity analysis of bacterial communities belonging to SPF, and domestic pigs from the same breed, domestic pigs indigenous to Africa, and warthogs from Africa showed that SPF pigs had the lowest diversity among the different species of pigs analyzed. Firmicutes were found to be the most abundant phylum among all the animals. Bacteria belonging to the genera *Plaudibacter*, *Anaeroplasma*, *Petrimonas*, and *Moraxella* were found to be among the core communities in pig species that were naturally resistant to ASFV.[35] Naturally, pigs reared under strict SPF-conditions were observed to be the least diverse based on microbial composition which has been correlated with an impaired immune system development and the ASFV-resistant warthogs were identified to contain OTUs belonging to previously-uncharacterized bacterial genera.

4.3 Porcine circovirus type 2

Porcine circovirus type 2 (PCV2) is a single-stranded circular DNA virus and is the primary causative agent of porcine circovirus associated disease. PCV2 belongs to the genus *Circovirus* within the family *Circoviridae*.[140] PCV2 targets the lymphoid organs leading to lymphoid depletion and tissue damage.[141] Direct contact with an infected pig has been known to be the most efficient route of virus transmission along with other routes such as contaminated vectors and fomites.[142]

Barrows (pigs aged 27–40 days) infected with PCV2 were observed to show an increase in abundance of *Prevotella* spp. in their microbiota composition.[37] The presence of a more diverse microbiota and the presence

of non-pathogenic *E. coli* in pigs have been associated with a reduced in clinical signs of PCV associated disease as well as improved growth in sub clinically infected pigs. Fecal microbiota transplant in 3-week old barrows reduced the number of pigs affected by PCV infection, including a reduction in viral load and increased viral-antigen specific antibodies.[38]

Coinfections with porcine reproductive and respiratory syndrome virus (PRRSV) and PCV2 occur regularly among overcrowded pig farms.[36] Bacterial colonization dynamics at the time of viral infection, that have been associated with a reduced PCV2/PRRSV burden include the presence of low abundance of *Methanobacteriaceae* spp., and an increased abundance of the species of *Ruminococcaceae*, and *Streptococcaceae*.[36] PRRSV is a single-stranded, positive-sense RNA virus belonging to the family *Arteriviridae* of the order Nidovirales.[143] Characteristics of PRRSV infection in pigs include reproductive failure in sows, and respiratory disease in growing and finishing pigs.[144] Each year, the pig industry in the US loses $664 million due to the respiratory disease and reduced weight gain among growing pigs infected with PRRSV and coinfected with other pathogens, including PCV2.[38]

4.4 Porcine epidemic diarrhea virus

Porcine epidemic diarrhea (PED) is a highly contagious and lethal enteric disease and is caused by PED virus (PEDV) which belongs to the *Alphacoronavirus* genus in the *Coronaviridae* family. Transmission occurs through the fecal-oral route with disease characterized by severe vomiting, diarrhea and dehydration.[145] Genomic sequencing of different PEDV isolates have shown the emergence of PEDV strains with insertion/deletions or multiple mutations in the spike (S) protein sequence allowing for the circulation PEDV among the global pig population.[146] The age of infected pigs, immune status and the virulence of the virus strain have all been attributed to the development of clinical signs of disease.[147] Pigs of all ages are susceptible to PEDV, with the highest mortality rate occurring among neonatal and suckling pigs.[148] A vaccine is available in the form of an adjuvanted attenuated virus that is administered to healthy pregnant sow. Antibodies generated by the sow during and after gestation have been successful to an extent but fail to induce sufficient protection in neonatal piglets farrowed from PEDV naïve sows.[149]

Bacterial composition of the neonatal gastrointestinal tracts[35] often begins with the colonization of aerobic or facultatively aerobic bacteria belonging to *Escherichia coli*, *Shigella flexneri*, and *Streptococcus* spp. The consumption of oxygen within the GIT of the newborn piglets allows for

the subsequent colonization of anaerobes such as *Bacteroides*, *Clostridium*, *Oscillibacter*, *Bifidobacterium*, *Lactobacillus*, and *Ruminococcaceae* spp.[150,151] Analysis of the microbiota composition in the small and large intestines of PEDV infected piglets showed an increase in abundance of *Escherichia-Shigella*, *Enterococcus*, *Fusobacterium*, and *Veillonella*.[39] In parallel to this, a decrease in SCFA-producing bacteria such as *Rikenellaceae* (RC9 gut group), *Butyricimonas*, and *Alistipes* were also observed among infected piglets.[40] A reduction in SCFA production within the GIT has been linked with an increased state of dysbiosis in piglets implicating reasons for severity of PEDV infection.[152,153] Huang et al. showed that bacteria belonging to *Ruminococcaceae, Rikenellaceae, and Porphyromonadaceae,* were associated with healthy piglets as compared to PEDV infected piglets.[39]

4.5 Virome in swine

Investigative studies on viruses outside their pathogenic interaction with their swine hosts have been limited. With an increased access to advanced sequencing technology, the influences of virome on swine health can be investigated in greater detail. Liver biopsies taken from healthy pigs revealed a diverse range of small ssDNA viral genome sequences that were closely related to viruses belonging to *Anelloviridae*, *Circoviridae* and *Parvoviridae* families.[154] Another study also reported a rich viral abundance and diversity among blood-fed mosquitoes collected from pig farms. Apart from the presence of viruses belonging to families that were known to cause infection among pigs, humans, and mosquitoes, a considerable number of non-classified virus reads were also detected that were presumed to belong to unexplored novel virus species.[155]

4.6 Summary

Microbiota diversity is an integral part of pig health. As identified by Ober and colleagues, an increased microbial diversity together with shifts in the presence of several bacterial families prior to infection allowed pigs to recover faster after the infection fully resolved.[36] The use of antimicrobials in regular farming practices have been attributed to the reduction in microbiota diversity increasing their susceptibility to infections. Considering alternative therapy such as fecal microbiome transplantation for enhancing swine health would enhance the overall economic benefits associated with pig farming.

5. Microbiome and virus disease in ruminants

5.1 Introduction

Rumen microbes play a critical role in the development of digestive system in neonatal, nursing calves. They also play important roles in the nutritional acquisition (feed digestion), and physiological and immunological functions of the host. Generally, they support each other to ferment plant structural and nonstructural carbohydrates and proteins. Along with the large-scale application of high throughout sequences and metagenome techniques, literatures in characterization and variation of microbiota in ruminant animals increased explosively.[156–160] The rumen microbiota is very complex, and the diversity of ruminal microorganisms can be affected by diet composition, genetics and environmental factors. There are approximately 7000 bacterial species and 1500 archaeal species in the rumen.[161] Rumen protozoa is present when animals are fed high-grain diets, and rumen fungi represents approximately 10% of the total rumen microbiota at any given time.[162] Plenty of studies have found differences of micro-biological development in the digestive system of calves in relation to variations in management practices, such as housing, feeding, and antimicrobial administration during the neonatal period.[163,164] The following review focuses on the microbiota and how it changes and acts during virus infection in ruminants.

5.2 Virus infection changes microbiota of infected animals

5.2.1 Bovine rotavirus

Bovine rotavirus is the main pathogen associated with neonatal calf diarrhea. It infects young calves orally, replicates in the cytoplasm of intestinal epithelial cells, and destroys mature intestinal cells.[165] It has been reported that the incidence of rotavirus diarrhea was associated with a decrease in bacterial diversity in the gut, which is a well-known hallmark of dysbiosis in the early stages of life in the calves.[43] Alterations were found in the gut microbiota of neonatal diarrhea samples as a result of rotavirus infection. The relative abundances of Firmicutes and Bacteroidetes increased in healthy calves, while Proteobacteria was abundant in rotavirus diarrhea samples.[43] This was consistent with human rotavirus diarrhea cases that the phylum Proteobacteria is associated with gut dysbiosis and inflammation.[41] At the genus level, in rotavirus-infected calves, there was significant increase of the genera *Escherichia, Clostridium, and Streptococcus*. However,

Subdoligranulum, Blautia, Bacteroides, and Coprococcus were decreased. Notably, *Lactobacillus* was significantly decreased in the rotavirus diarrhea calves. At the species level, *Lactobacillus* species such as *L. gasseri* and *L. amylovorus* were found to be relatively abundant in healthy calves. *L. gasseri* is known to prevent the severity of acute self-limiting diarrhea in adults.[42] In rotavirus-infected calves, the relative abundance of *G. formicillis, B. glucerasea, B. coccoides*, and *B. obeum* were significantly higher in the healthy group. Two species of *C. perfringens* and *E. coli* associated with the diarrheic calves were identified.[43] This study demonstrated that rotavirus infection changed the structure of the gut microbiota, which would affect the follow up diagnosis and administration.

5.2.2 Bovine leukemia virus (BLV)

Bovine leukemia virus (BLV) is a retrovirus which causes enzootic bovine leukosis in cattle, and it is closely related to the human T-lymphotropic virus type 1.[166] BLV infection of domestic cattle is highly prevalent in several geographic regions.[167] Once BLV infects a cow, it cannot be eliminated.[168] Because the immune system of host cattle can be impaired during infection, BLV infection consistently results in the inability of cattle to maintain normal health.[169] Comparing to BLV infected dairy cows, *Lachnospiraceae* and *Veillonellaceae* families associated with ruminal fermentation were more abundant in the fecal microbiota of uninfected cows.[44] Higher titer BLV infection was associated with less diversity of microbiota in infected cows. Meanwhile, the virus propagation ability of BLV strains was negatively correlated with one taxon of *Sanguibacteroides*. Besides causing lymphoproliferation and leukemia, BLV infection may decrease energy production efficiency in the infected cows through modification of rumen and hindgut microbiota.[44] According to function speculation of the bacteria detected in the differential abundance analysis, energy production loss in the rumen and hindgut was assumed, which may explain the secondary negative effects such as increased susceptibility to other infections and decreased lifetime milk production and reproductive efficiency.[170–173] Further studies need to systematically investigated rumen microbiota with rumen nutrition status, such as the level of volatile fatty acids together with BLV strain genetics.

5.3 Opportunistic pathogen activities during virus infection

5.3.1 Escherichia coli *and rotavirus and coronavirus infection*

Escherichia coli strains comprise a group of bacteria with a huge genetic diversity that make them able to colonize different niches and to adapt as a

component of intestinal commensal microbiota of animals and human beings.[174] *E. coli* are classified into eight different phylogenetic groups (A, B1, B2, C, D, E, F and Clado1).[175] Changes in compositions of commensal *E. coli* were observed during rotavirus and coronavirus intestinal infection in calves with diarrhea.[176] From this study with 30 calves each in the infected and control group, *E. coli* isolates identified in virus-infected calves were phylogenetically classified as B1 (70%), B2 (3.33%), C (3.33%), D (3.33%), E (13.33%) and unknown (6.7%), whereas *E. coli* isolates from the control group were classified only as B1 (83.3%), E (10%) and unknown (6.7%). B2, C and D groups were found only in samples from animals with diarrhea due to rotavirus and coronavirus infection.[176] According to Escobar-Paramo[177] and Clermont,[175] B2 and D are *E. coli* phylogenetic groups that comprise strains with pathogenic potential, being more commonly observed in extra-intestinal infections.

In *E. coli* isolated from diarrheic calves, F5 and F18 Fimbriae were found.[176] Fimbriae are virulence factors that allow *E. coli* colonization mainly in the small intestine, avoiding bacteria elimination along with the feces.[178] F5 is one of the most frequent factors in enterotoxigenic *E. coli* pathotype isolated from calves with diarrhea.[179] F18 is more commonly associated with post-weaning diarrhea and edema disease in swine.[178,180,181] In summary, rotavirus and coronavirus infection changes the homeostasis of intestinal microbiota which involves the participation of *E. coli* in the pathological progress. *E. coli* was the only species that positively correlated with the number of neutrophils, which may explain the inflammation during diarrhea.[43]

5.3.2 Lumpy skin disease virus and secondary bacterial infection

Lumpy skin disease (LSD) is a viral disease of cattle caused by lumpy skin disease virus (LSDV). LSDV is one of the most important animal poxviruses because of the serious economic consequences in cattle. The World Organization for Animal Health[182] categorizes LSD as a notifiable disease.[182] It is characterized by fever, reduced milk production and skin nodules. Mastitis, swelling of peripheral lymph nodes, loss of appetite, increased nasal discharge and watery eyes are also common. Temporary or permanent infertility occur among infected cows and bulls. The disease can cause high morbidity and low mortality.[183,184] Secondary bacterial infection in the affected skin lesions can increase the severity and prolong the course of the disease. The analysis of prevalent bacterial communities in affected lesion were carried out using 16s rRNA gene sequencing. Up to 98 species were found, most of them belonging to the phyla of *Proteobacteria*, followed by

Firmicutes, *Actinobacteria*, and *Bacteroidetes*. Many common mammalian pathogens are found in the *Proteobacteria* phylum. For example, the *Brucella*[185] and *Rickettsia*[186] genera belong to the *Alphaproteobacteria* class; *Bordetella*[187] and *Neisseria*[188] belong to the *Betaproteobacteria* class; and *Escherichia,*[189] *Shigella* and *Salmonella* belong to the *Gammaproteobacteria* class. All bacterial species found are known as opportunistic pathogens, but can withstand the inflammatory reaction.

5.4 Virome in ruminants

Ruminant virome is important in the nutrient and energy cycling,[190] development of immunity,[191] and a major source of genes through lysogenic conversion.[192] As metagenomic next-generation sequencing (mNGS) has been employed to identify uncommon and novel infectious etiologies, massive virus species have been identified in ruminants.[193]

Besides the common diarrhea-causing viral pathogens rotavirus and coronavirus, torovirus, norovirus, nebovirus, astrovirus, kobuvirus and enterovirus have been detected from calves' feces with diarrhea by mNGS.[194] Viral genomes of pestivirus A, Ungulate erythroparvovirus 1, bosavirus, and hypothetical circular Rep-encoding single-stranded DNA viruses were also identified from calf serum with mucosal disease.[195] Most studies have involved epidemiologic investigations seeking to show association with diarrhea for each virus alone or in combination with potential pathogens.

In bovine respiratory disease, 16 viruses were identified, of which bovine parvovirus 2 was the most prevalent (11.5%, 15/130) followed by ungulate tetraparvovirus 1 (UTPV1, 8.5%, 11/130) and bovine respiratory syncytial virus (BRSV, 8.5%, 11/130).[7] Unconventional viruses such as influenza D virus, bovine rhinitis A virus, bovine coronavirus and bovine rhinitis B virus had also been detected.[196,197]

Non-suppurative encephalitis is one of the most frequent pathological diagnosis in cattle with neurological disease. Six virus candidates: parainfluenza virus 5, bovine astrovirus CH13/NeuroS1 (BoAstV-CH13/NeuroS1), bovine polyomavirus 2 (BoPV-2 SF), bovine herpesvirus 2, bovine herpesvirus 6 (BoHV-6) and a novel bovine betaretrovirus termed BoRV-CH15 had been detected in neurologically-diseased cows. BoAstV-CH13, BoPV-2 SF and BoHV-6 were significantly associated with the disease. These data expanded our knowledge on encephalitis-associated pathogens in cattle and point to the value of NGS in resolving complex infection scenarios in a clinical disease setting.[198]

5.5 Summary

Most of the studies have focused on the variety of microbiome in ruminants. Limited work has been done to study the interaction between microbiota and ruminant viruses or the microbiota changes before and after different virus infection. Diarrhea due to rotavirus and coronavirus infection was associated with a decrease in bacterial diversity in the gut.[43,176] Diarrhea of any etiology may result in a variety of complications as acid-base and electrolyte imbalance and increased lumen-fluid volume with consequent dehydration.[199,200] These poor health condition encourages mass propagation of conditional pathogenic bacteria, which leads to the secondary bacterial infection. Meanwhile, the immunosuppression caused by BLV infection may decrease energy production efficiency in the cows through modification of rumen and hindgut microbiota. The mechanisms of interactions between microbiota and viruses in ruminant host in regard to pathological progress in viral diseases should be better explored in future research.

Like the bacterial microbiome studies, the metagenome sequencing opens a door for us to peek into the virome structure in ruminant diseases. The diarrhea, respiratory diseases, and encephalitis are the main health threats to ruminants. The detection of virome components will fill in the knowledge gap in identifying the disease-associated pathogens. However, determining the contribution of these viruses to health and diseases in ruminants and other animal species would be challenging and much uncertainty still remains concerning their roles as primary pathogens, coinfection agents, or commensals.

6. Concluding remarks

Microbiota is capable of modulating host immune systems and determining the susceptibility to various viral pathogens. The studies reviewed in this chapter have shown such significant implications of the microbiota on host immunity. The influence of the microbiota on viral infection susceptibility and viral disease outcome is undisputable although varies among viruses. These data are very important in terms of viral infection, as currently there are few effective and reliable antiviral therapies available. Further understanding of the interactions between viral pathogens, host species and their microbiota will eventually allow us to tailor the contents of the microorganisms to the extent that it is able to protect the host from certain life-threatening viral infections. More research is needed to establish

concrete cause and effect relationships between the microbiota composition and viral infection, as many of these studies did not address other factors that also are known to influence microbiota composition, such as genetics, diet, and environment. It should also be noted that some of these studies focused on small cohorts of participants, and more work is needed to establish trends among larger populations. Despite the limitations of some of the presented works, the microbiota still prevails as a hopeful means of immunotherapy for prevention of viral infection in the future in both humans and farm animals.

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