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Review Article

Rotavirus Infection Transiently Affects Intestinal Microbiome Composition in Newborn Calves

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- Intestinal microbiome
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Abstract

Bovine rotavirus is the causative agent of diarrheal disease in calf, which is characterized by atony of the small intestinal wall and growth impairment, resultingin largeeconomic losses. The diarrheal infection also changes the commensal intestinal microbiome. The intestinal Microbiome balance in calves partially affects the innate immune system and plays an important role in prevention of pathogenic microbe infection. In this study, we investigated the changes in intestinal Microbiome composition of rotavirus-infected calves using metagenomics analysis. We collected feces samples from 16 calves at 14, 28, and 42 days after birth. Four of these calves developed diarrhea after 28 days. These calves and four other calves were diagnosed with rotavirus infection after 28 days. Fecal metagenomic analysis at the onset of the illness showed high Firmicutes to Bacteroidetes ratio compared to the ratio in uninfected calves. The intestinal microbiomecomposition returned to normal levels 2 weeks after the onset of illness. The number of Prevotellaceae family members in Bacteroidetes increased in the healthy calves after 28 days; however, the rotavirus infection prevented similar increase in calves of the same age. These results indicate that rotavirus infection affects intestinal microbiome composition, which is important for the development of calf digestive organs.

ABBREVIATIONS

RA: Relative Abundance; PCR: Polymerase Chain Reaction; PED: Porcine Epidemic Diarrhea

INTRODUCTION

Rotavirus, a double-stranded RNA virus, is a member of the family Reoviridae and mainly causes diarrheal disease in newborn animals. The virus infects small-intestinal epithelial cells and causes thinning and atony of the small intestine. Based on its antigenicity, rotavirus is classified into types A to F. Types A, B, and C were detected in bovine species, and type A was the dominant strain detected in Japan [1-3]. Bovine rotavirus infects new born calves one or two weeks after birth. The virus infection inhibits calf growth, which results in huge economic loss [4]. Rotavirus infection often leads to secondary infection with *Escherichia coli*, coronavirus, bovine viral diarrhea-mucosal disease virus (BVDV), and others, which may result in lethality in certain cases of serious infection. The intestinal levels of lactate, are increased and those of volatile fatty acids (VFA) are decreased in diarrheic calves [5].

The intestinal microbiome was examined using microbial culture methods during the 1970s [6]. The detection methods have been improved ever since to include genomic analysis such as metagenomic analysis [6,7], and the intestinal Microbiome of bovine animals has been analyzed using metagenomics [8,9]. In these species, the intestinal microbiome changes significantly during the week after birth, especially in the neonatal period, when the individual-specific intestinal microbiome colonizes the gut and attains a stable combination. However, the intestinal Microbiome transition is often disturbed by pathogenic microbe infection, which disrupts the gut flora balance in the affected animal. Studies involving piglets showed that porcine epidemic diarrhea altered the intestinal microbiome, following an increase in Fusobacteria and decrease in Verruco microbia population [10]. In addition, the intestinal Microbiome changed in rotavirus-infected piglets [11].

In this study, we obtained the feces of rotavirus-infected calves before and after the infection andfecal intestinal bacterial microbiomes were examined using metagenomic analysis. Our results show that the intestinal microbiome changes after the onset of rotavirus infection in calves.

MATERIALS AND METHODS

Calves

We used 16 male Holstein calves for this study. Each calf was

separated from its mother after birth and housed in individual fence-partitioned rearing facilities at the Meiji Feed experimental farm. The calves were fed 2 L commercially available milk replacement twice a day. There were no clinical symptoms until the onset of diarrhea.

Fecal samples

Fecal samples were obtained 14, 28, and 42 days after birth. Four of 16fecal samples developed diarrhea at 28 days after birth. The diarrheal symptom persisted for 5-6 days after the onset of the illness without medical treatment. The fecal samples were tested using the multi-antigen detection kit (Test strips; Bio-X Diagnostics, Rochefort, Belgium) to detect pathogens such ascorona virus, rotavirus, *Escherichia coli* F5 (K99), and *Cryptosporidium parvum* in the feces. The collected samples were immediately tested using the kit and the rest of the samples were preserved at -30°C for DNA extraction. All the animal experiments were conducted in compliance with the protocol, which was reviewed by the Institutional Animal Care and Use Committee and approved by the committee of Meiji Feed Co., Ltd. (approval number #2011-01).

Metagenomic analysis

DNA was extracted from the fecal samples on days 14, 28, and 42 using a Power Soil DNA extraction kit (Mobio Technologies Inc, Vancouver, Canada). To amplify the 16S rRNA gene, 10 ng extracted DNA was used as the template for subsequent polymerase chain reaction (PCR). PCR was performed using a primer set (784F: 5′-AGGATTAGATACCCTGGTA-3′ and 1061R: 5′-GGATTAGATACCCTGGTA-3′) targeting the V5-V6 region of the 16S rRNA gene. PCR conditions were 95°C for 3 min, 25 cycles at 98°C for 20 s, 61°C for 10 s, and 72°C for 10 s. Deep sequencing of the amplicon was performed on an Ion PGM sequencer using a 318 chip and Ion PGM sequencing 400 kit (Thermo Fisher Scientific, Waltham, MA, USA). The resulting sequences were analyzed using the QIIME pipeline [12].

Statistical analysis

Significant differences in the relative abundance of phyla and families were assayed using Tukey's Honestly Significant Difference (HSD) test of the statistical software R(R version 3.4.3, The R project for Statistical Computing).

RESULTS AND DISCUSSION

Fecal properties and detected pathogens

The multi-antigen detection kit detected rotavirus antigen from eight calves on day 28 after birth, whereas all the other pathogens (coronavirus, *E. coli*, *Cryptosporidium parvum*) were negative throughout the observation period. Four of the eightrotavirus-positive calves showed diarrheal symptoms for 5-6 days after the onset of the illness (unknown serotype), but the other rotavirus-positive calves did not show any clinical symptoms such as diarrhea during the observation period. The same examination was performed on faeces before the onset, but rotavirus was negative. The calves were classified into three groups based on fecal properties: rotavirus infected calves with diarrhea symptom (RD, n=4), rotavirus infected calves without diarrhea symptom (RN, n=4), and rotavirus-uninfected calves without clinical symptom (CN, n=8).None of the calves showed any symptom of secondary infection by other pathogens during the observation period.

Metagenomic analysis

We analyzed the metagenomic data of the three groups (RD, RN, and CN). The average total read of the samples were 215,000. Initially, we analyzed the relative abundance (RA) of different phyla in the feces of the three groups during the observation period (Figure 1). The RA of Actinobacteria in the RD and CN groups decreased from day 14 to 42; that of the RN group increased on day 28 and then decreased to the same level as that of the RD and CN group on day 42. Although none of the calves contracted any secondary bacterial infection during the observation period, the rotavirus-infected calves showed transient change in the RA of intestinal Bacteriodetes and Firmicutes. The RA value of Bacteroidetes in the CN group increased from day14 to day 28 (30.6 ± 4.9 to 51.3 ± 3.7), but that in the RD and RN group decreased (48.6 ± 4.5 to 26.3 ± 12.5 and 45.1 ± 10.1 to26.2 ± 4.3) although those values reached the same level as that of the CN group on day 42. The RA of Firmicutes in the CN group did not alter during the observation period, where as those of the RD and RN groups showed a transient increase on day 28. The RA of Proteobacteria in the RN and CN groups decreased during the observation period, but that of the RD group increased transiently on day 28, followed by a decrease. The RA of Proteobacteria in the RD group (6.8 ± 2.3) was slightly higher than those of the RN and CN groups (4.4 ± 0.6 and 5.1 ± 0.5, respectively) on day 42. The analysis of phyla showed that Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria were themain phyla in the intestinal microbiome of calves. The intestinal bacterial composition in healthy calves is similar to that of humans and other bovine animals [7,13,14]. The phyla composition was affected by rotavirus infection in calves; however, the changed microbiome of the infected calves reverted to a composition similar to those of uninfected calves 2 weeks after the infection when the calves recovered from the virus infection without any secondary bacterial infection. Similar reports show that porcine rotavirus-induced porcine epidemic diarrhea (PED) affected the intestinal microbiome of pigs. The numbers of Firmicutes and Bacteroidetes decreased in the PED piglets [10,15]. In human case, the numbers of intestinal Bacteroidetes and Firmicutes in intensive care unit patients in critical condition changed drastically at the onset of illness [16]. In our study, we observed a similar reduction in the number of Bacteoidetes in the rotavirus-infected calves (Figure 1). Thus, rotavirus infection might affect the balance of Bacteriodetes and Firmicutes in the intestinal tract regardless of diarrheal symptoms.

Next, we analyzed the RA of different families of microbes in the feces obtained from the 3 groups during the observation period. Table 1 shows the RA of the top 10 families. The changes in the RA of families belonging to Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria of the 3 groups are shown in Figure 2. The predominant family in the CN group consisted of Bacteroidaceae (16.3 ± 5.2) on day 14, Prevotellaceae (36.9 ± 6.6) on day 28, and Lachnospiraceae (23.4 ± 2.1) on day 42. The predominant families, Bacteroidaceae in the RD and RN groups were identical to those in the CN group on day14; however, it

Table 1: The relative abundance of microorganisms of the top 10 families in rotavirus-infected calves.

	Day14		Day28		Day42	
Group	**families**	**% ± SE**	**families**	**% ± SE**	**families**	**% ± SE**
RD n=4	Bacteroidaceae	20.80 ± 10.38	Ruminococcaceae	21.60 ± 5.32	Lachnospiraceae	20.15 ± 2.93
	ratAN060301C	17.15 ± 10.81	Lachnospiraceae	17.45 ± 4.96	Ruminococcaceae	17.45 ± 2.91
	Ruminococcaceae	12.00 ± 4.01	Lactobacillaceae	14.53 ± 11.17	Prevotellaceae	15.23 ± 3.96
	Lactobacillaceae	11.65 ± 7.38	ratAN060301C	10.30 ± 6.78	S24-7	10.13 ± 2.87
	Lachnospiraceae	9.18 ± 1.86	Prevotellaceae	6.03 ± 3.69*	Bacteroidaceae	7.68 ± 2.21
	Prevotellaceae	8.65 ± 6.06	Enterobacteriaceae	5.95 ± 2.65	ratAN060301C	5.68 ± 1.69
	Coriobacteriaceae	5.53 ± 1.60	Veillonellaceae	4.58 ± 2.45	Porphyromonadaceae	3.40 ± 0.63
	Alcaligenaceae	4.35 ± 0.52	Bacteroidaceae	3.95 ± 2.71	Rhodospirillaceae	2.20 ± 0.89
	Bifidobacteriaceae	4.15 ± 0.36	Coriobacteriaceae	3.13 ± 1.67	Rikenellaceae	1.90 ± 0.31
	Porphyromonadaceae	1.025 ± 0.60	S24-7	2.78 ± 1.72	Enterobacteriaceae	1.88 ± 1.27
RN n=4	Bacteroidaceae	29.10 ± 15.61	Ruminococcaceae	21.40 ± 6.55	Prevotellaceae	25.30 ± 6.83
	Lactobacillaceae	17.58 ± 7.54	Lachnospiraceae	19.38 ± 1.02	Ruminococcaceae	17.73 ± 1.96
	Lachnospiraceae	9.35 ± 1.49	Prevotellaceae	13.08 ± 1.57*	Lachnospiraceae	16.68 ± 3.28
	Prevotellaceae	8.70 ± 5.45	Lactobacillaceae	9.83 ± 5.19	S24-7	11.53 ± 3.70
	Ruminococcaceae	8.68 ± 4.43	Bacteroidaceae	7.25 ± 2.81	ratAN060301C	4.45 ± 1.56
	ratAN060301C	7.08 ± 3.14	Coriobacteriaceae	7.25 ± 5.11	Bacteroidaceae	3.45 ± 1.47
	Alcaligenaceae	6.60 ± 2.24	ratAN060301C	3.88 ± 3.27	Rikenellaceae	3.15 ± 0.82
	Bifidobacteriaceae	3.63 ± 1.40	Alcaligenaceae	3.85 ± 2.23	Clostridiaceae	2.38 ± 0.68
	Coriobacteriaceae	2.60 ± 0.48	Veillonellaceae	3.40 ± 2.46	Alcaligenaceae	2.15 ± 0.26
	Enterobacteriaceae	2.45 ± 1.73	Bifidobacteriaceae	3.28 ± 2.43	Porphyromonadaceae	1.78 ± 0.31
CN n=8	Bacteroidaceae	16.29 ± 5.20	Prevotellaceae	36.86 ± 6.55	Lachnospiraceae	23.41 ± 2.09
	Lactobacillaceae	15.00 ± 4.56	Ruminococcaceae	16.48 ± 2.67	Prevotellaceae	21.25 ± 4.83
	Ruminococcaceae	11.83 ± 4.45	Lachnospiraceae	15.46 ± 2.69	Ruminococcaceae	14.50 ± 1.75
	Bifidobacteriaceae	8.63 ± 1.52	ratAN060301C	4.23 ± 1.92	ratAN060301C	8.91 ± 2.28
	Prevotellaceae	8.39 ± 3.48	Bacteroidaceae	4.10 ± 1.19	Bacteroidaceae	4.98 ± 1.16
	Enterobacteriaceae	7.20 ± 2.90	Lactobacillaceae	2.83 ± 1.27	Rikenellaceae	4.81 ± 1.47
	Alcaligenaceae	6.06 ± 2.16	Alcaligenaceae	2.65 ± 0.54	Porphyromonadaceae	3.25 ± 0.62
	Enterococcaceae	5.98 ± 3.22	Enterobacteriaceae	1.74 ± 0.58	S24-7	3.01 ± 1.37
	ratAN060301C	4.79 ± 3.85	Clostridiaceae	1.68 ± 1.15	Alcaligenaceae	2.09 ± 0.55
	Lachnospiraceae	3.95 ± 1.22	Veillonellaceae	1.58 ± 0.59	Rhodospirillaceae	1.96 ± 0.53

The calves were classified into three groups based on fecal properties at the onset of the disease:rotavirus infected calves with diarrhea symptom (RD), rotavirus infected calves without diarrhea symptom (RN), and rotavirus-uninfected calves without clinical symptom (CN). *p<0.05 versus CN

changed on day 28 to Ruminococcaceae (21.6 ± 5.3 and 21.4 ± 6.6, respectively). Furthermore, the diversity of the predominant families in the RD and RN groups was evident on day 42; Lachnospiraceae was present in the RD group (20.2 ± 2.9) and Prevotellaceae in the RN group (25.3 ± 6.8).

The dominant bacterial families of phylum Actinobacteria observed in the three groups during the observation period were Bifidobacteriaceae and Coriobacteriaceae. The RA of these families did not differ significantly among the 3 groups on days 28 and 42. The dominant bacterial family in Bacteroidetes among the 3 groups changed from Prevotellaceae to Bacteroidaceae during the observation period (day14 to 42). Prevotellaceae was dominant in the CN group on day 28, but its numbers were significantly lower in the rotavirus-infected groups (RD: p=0.009, RN: p=0.04).Among Firmicutes, the dominant bacterial families in the three groups changed from Lactobacillaceae, Lachnospiraceae, and Ruminococcaceae to Clostridiales Family XIIIIncertaeSedis, and Ruminococcaceae. The three groups did not differ significantly with respect to the diversity of families of phylum Firmicutes during the observation period. The RA of certain bacterial families, especially Prevotellaceae, of phylum Bacteroidetes decreased in the calves infected with rotavirus. On the contrary, there was no significant change in the RA of specific families of Firmicutes. In healthy calves; the rate of Prevotellaceae was increased during two to four weeks old calves. The results was the same as compared to previous study that investigate in the calf and growing up stage (3.0-11.1%) [14,17]. Bacteria of the Prevotellaceae family are detected in the bovine rumen as commensal microbes, where they produce enzymes such as xylanase, which promotes fiber degradation. Reports show that the presence of Prevotellaceae is an indicator of good cattle health [17,18]. Our results indicated intestinal Prevotellaceae is observed to increase in healthy calves but the suppression occurs in the calves infected with rotavirus. The increase inhibition was accompanied with diarrheal symptoms. Furthermore, we found the rate of Prevotellaceae in RD calves were lower than RN calves. It suggested that viral infection with diarrhea influence the growth of Prevotellaceae strongly, but the details remain to be elucidated. Bovine rotavirus is able to induce a potent inflammatory response mediated by IFN and IFN-induced genes as well as inflammatory cytokines and accumulation of fluid and visible histological alterations in the gut of infected animals [19].

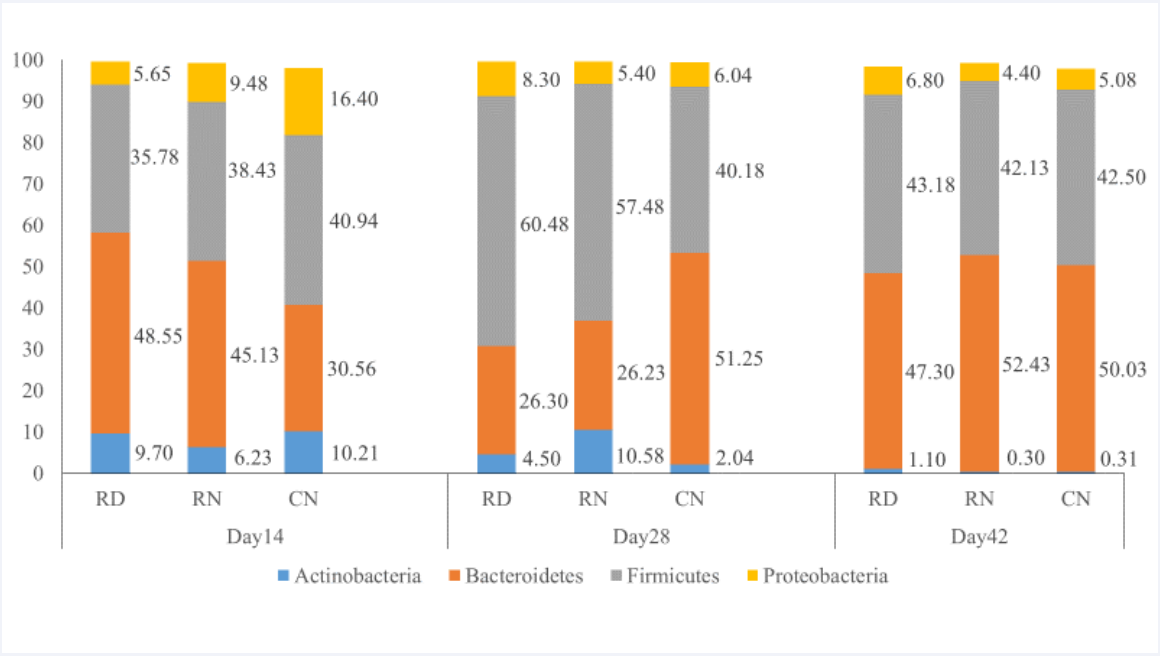

Figure 1 The relative abundance (RA) of various phyla of the intestinal microbiome in rotavirus-infected calves. The RA of phyla, namely, Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria in the intestinal microbiome of calves on days 14, 28, and 42 are shown. The calves were classified into three groups depending on the condition of fecal properties after 28 days (onset of illness): rotavirus infected calves with diarrhea symptom (RD, n=4), rotavirus infected calves without diarrhea symptom (RN, n=4) and rotavirus-uninfected calves without clinical symptom (CN, n=8).

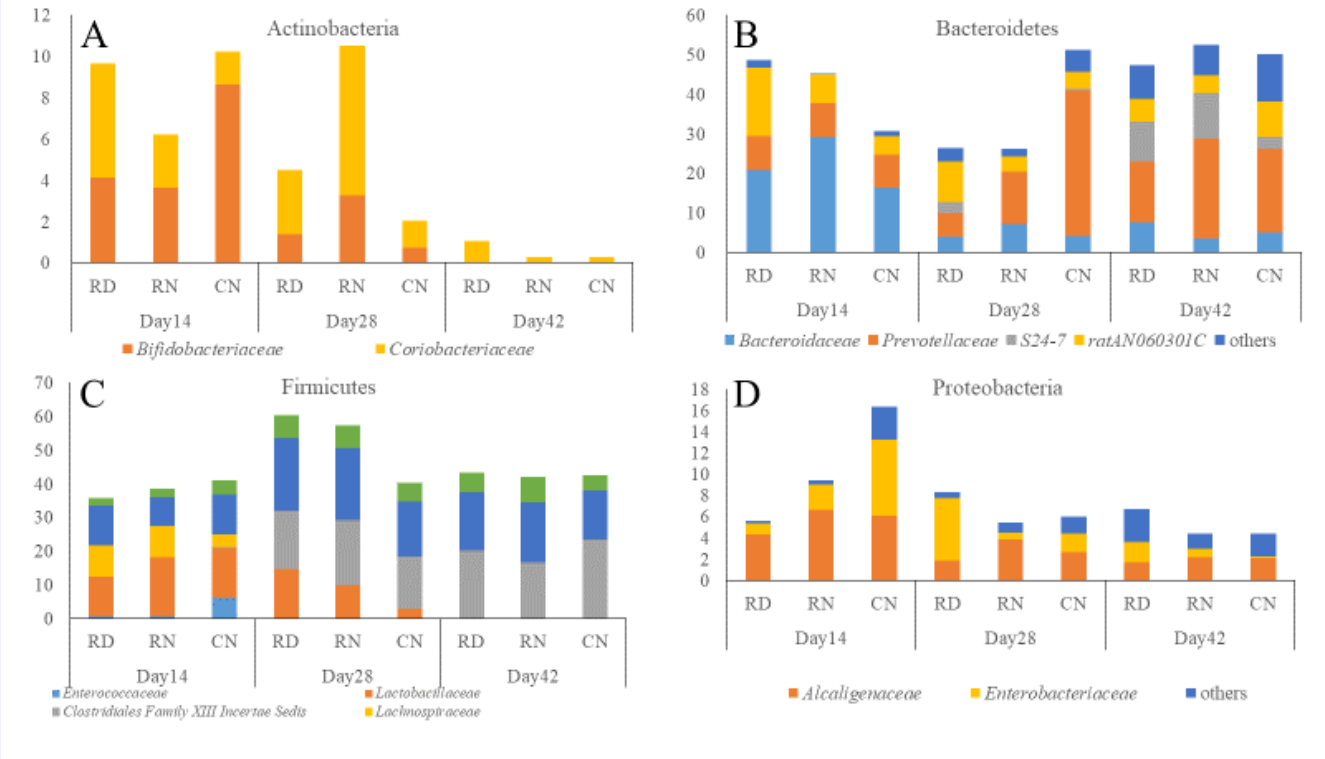

Figure 2 The relative abundance of families belonging to Bacteroidetes and Proteobacteria phyla in the intestinal microbiome of rotavirus infected calves. The relative abundance of families in Actinobacteria (A), Bacteroidetes (B), Firmicutes (C) and Proteobacteria (B) in the intestinal microbiome of calves at days 14, 28, and 42.Data for ≥ 5% families in the samples from each group are shown; RD, rotavirus-infected calves with diarrhea symptom; RN, rotavirus infected calves without diarrhea symptom; CN, rotavirus-uninfected calves without clinical symptom.

The degree of inflammatory response and intestinal structural change by bovine rotavirus infection probably differ according to the calves with diarrhea or not. One theory posits that rotavirus produces nonstructural protein 4 (NSP4), which exerts a toxin-like effect on the tight junctions of intestinal mucosa and collapses the balance of moisture and ions in the intestine [10,20]. This micro environmental change in the intestine might influence the intestinal microbiome composition. Bovine rotavirus infection inhibits calf growth and causes economic loss. Thus, the virus-induced alterations in the intestinal microflora identified in this study might explain the growth inhibition of calves reported previously.

In summary, this study indicates that rotavirus infection affects intestinal microbiome composition, which returns to normal levels upon recovery from illness. Thus, there is no net difference in the composition of the intestinal microbial population after a single episode of rotavirus infection accompanied by diarrhea. However, the mechanism of microbiome composition alteration during the course of rotavirus infection is still unclear. In this study, it was possible to show the change of the intestinal microbiome composition of calf changes after a single episode or rotavirus infection. We suggested that this may trigger in the Prevotellaceae population following infection in calf.

CONCLUSIONS

The intestinal microbiome in the rotavirus-infected calves showed compositional changes after the viral infection. Rotavirus infection affects intestinal microbiome composition, which is important for lumen development. The infection of this virus suggested the possibility of affecting the microflora, which is important for the growth of calf digestive organs. Also supporting this effect will be of importance in infected calves.

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REFERENCES

1. Kohara J, Hirai T, Mori K, Ishizaki H, Tsunemitsu H. Enhancement of passive immunity with maternal vaccine against newborn calf diarrhea. J Vet Med Sci. 1997; 59: 1023-1025.
2. Minami-Fukuda F, Oba M, Nishiura N, Sassa Y, Omatsu T, Furuya T, et al. Detection of bovine group a rotavirus using rapid antigen detection kits, rt-PCR and next-generation DNA sequencing. J Vet Med Sci. 2013; 75: 1651-1655.
3. Mawatari T, Hirano K, Ikeda H, Tsunemitsu H, Suzuki T. Surveillance of diarrhea-causing pathogens in dairy and beef cows in Yamagata Prefecture, Japan from 2002 to 2011. Microbiol Immunol. 2014; 58: 530-535.
4. Chinsangaram J, Akita GY, Castro AE, Osburn BI. PCR detection of group A bovine rotaviruses in feces. J Vet Diagnostic Investig. 1993; 5: 516-521.
5. Sato H. Increased fecal lactate and decreased volatile fatty acid (VFA), particularly n-butyrate concentrations in diarrheic young calves. J Vet Med Sci. 2009; 71: 117-119.
6. Mitsuoka T. Establishment of intestinal bacteriology. Biosci Microbiota Food Health. 2014; 33: 99-116.
7. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA. 2007; 104: 13780-13785.
8. Monira S, Nakamura S, Gotoh K, Izutsu K, Watanabe H, Alam NH, et al. Metagenomic profile of gut microbiota in children during cholera and recovery. Gut Pathog. 2013; 5: 1.
9. Tafazoli F, Zeng CQ, Estes MK, Magnusson K. NSP4 Enterotoxin of Rotavirus Induces Paracellular Leakage in Polarized Epithelial Cells. J Viol. 2001; 75: 1540-1546.
10. Liu S, Zhao L, Zhai Z, Zhao W, Ding J, Dai R, et al. Porcine epidemic diarrhea virus infection induced the unbalance of gut microbiota in piglets. Curr Microbiol. 2015; 71: 643-649.
11. Mao X, Gu C, Hu H, Tang J, Chen D, Yu B, et al. Dietary *Lactobacillus rhamnosus* GG supplementation improves the mucosal barrier function in the intestine of weaned piglets challenged by porcine rotavirus. PLoS One. 2016; 11: 1-14.
12. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7: 335-336.
13. Rudi K, Moen B, Sekelja M, Frisli T, Lee MR. An eight-year investigation of bovine livestock fecal microbiota. Vet Microbiol. 2012; 160: 369-377.
14. Takino T, Kato-mori Y, Motooka D, Nakamura S, Iida T. Hagiwara K. Postnatal changes in the relative abundance of intestinal Lactobacillus spp . in newborn calves. J Vet Med Sci. 2017; 79: 452-455.
15. Koh HW, Kim MS, Lee JS, Kim H, Park SJ. Changes in the swine gut microbiota in response to porcine epidemic diarrhea infection. Microbes Environ. 2015; 30: 284-287.
16. Ojima M, Motooka D, Shimizu K, Gotoh K, Shintani A, Yoshiya K, et al. Metagenomic analysis reveals dynamic changes of whole gut microbiota in the acute phase of intensive care unit patients. Dig Dis Sci. 2016; 61: 1628-1634.
17. Myer PR, Wells JE, Smith TPL, Kuehn LA Freetly HC. Microbial community profiles of the colon from steers differing in feed efficiency. Springerplus. 2015; 4: 454.
18. Laguardia-Nascimento M, Branco KMGR, Gasparini MR, Giannattasio-Ferraz S, Leite LR, Araujo FMG, et al. Vaginal microbiome characterization of Nellore cattle using metagenomic analysis. PLoS One. 2015; 10: 1-19.
19. Villena J, Aso H, Rutten VPMG, Takahashi H, van Eden W, Kitazawa H. Immunobiotics for the Bovine host: Their interaction with intestinal epithelial cells and their effect on antiviral immunity. Front Immunol. 2018; 9: 326.
20. Guttman JA, Finlay BB. Tight junctions as targets of infectious agents. Biochim Biophys Acta. 2009; 1788: 832-841.

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