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Prevalence of coronavirus from diarrheic calves in the Republic of Korea

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ABSTRACT

Objective: To investigate the prevalence of bovine coronavirus (BCoV), bovine rotavirus, and bovine viral diarrhoea virus in the feces of normal and diarrheic Korean native calves aged 1-81 days between April and October of 2016 in the Republic of Korea. **Methods:** Samples were obtained from 50 normal and 93 diarrheic (56 semi-formed, 28 loose, and 9 watery feces) calves in six different regions of northern and southern Korea. These fecal samples were tested for BCoV, bovine rotavirus, and bovine viral diarrhoea virus by RT-PCR. **Results:** Among the three pathogens examined, infection with BCoV was especially prominent in relation to diarrhoea among calves aged 1-21 days [odds ratio (OR)=9.3, 95% confidence interval (CI): 1.1-78.9; P=0.02]. Infection with BCoV alone (OR=2.9; 95% CI: 1.1-7.6; P=0.03) or co-infection of BCoV with bovine viral diarrhoea virus (OR=3.6; 95% CI: 1.0-12.4; P=0.04) was significantly associated with the development of loose feces. Grazing and colostrum intake strongly reduced the occurrence of diarrhoea as compared to housed calves (OR=0.2; 95% CI: 0.1-0.4; P=0.00) and calves that had not been fed colostrum (OR=0.2; 95% CI: 0.1-0.7; P=0.02), respectively. **Conclusions:** The present study suggests that BCoV is involved in calf diarrhoea in the Republic of Korea. Therefore, grazing and colostrum intake is recommended for preventing and controlling calf diarrhoea caused by BCoV.

1. Introduction

Calf diarrhoea leads to severe economic losses worldwide in the livestock industry as a result of morbidity, poor growth rates, treatment costs, and mortality among affected young calves[1,2].

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Approximately half of all mortality among calves up to the first month after birth has been attributed to diarrhoea[3]. Calf diarrhoea has a complex and multifactorial etiology, because infectious diseases, environmental, nutritional, and herd management factors such as housing type, colostrum intake, and hygienic conditions may be

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associated with field outbreaks, indicating that these factors may influence the severity and outcome of the disease[2,4]. Calves are at the greatest risk of developing diarrhea during the first month of life and the incidence of diarrhea decreases with age[4,5].

Mostly, outbreaks of calf diarrhea are associated with various infectious agents that present either individually or as part of a co-infection. Among the numerous infectious agents that can cause calf diarrhea, bovine coronavirus (BCoV) and bovine rotavirus (BRV) are recognized as the most important viral pathogens and these viruses can concomitantly infect calves[6–8]. In addition, bovine viral diarrhea virus (BVDV) is frequently associated with acute diarrhea in calves[9], although further studies are needed to evaluate its role in calf diarrhea. In the Republic of Korea (ROK), BVDV1 and BVDV2 are predominant and widespread among cattle[10,11].

To date, there have been few reports on the detection of viral pathogens associated with calf diarrhea in the ROK. There is little information available for Korean native calves owing to the small numbers of animals studied[12,13]. The main objective of the present study was therefore to investigate the prevalence of BCoV, BRV, and BVDV in diarrheic calves aged 1–81 days with feces of various consistencies, and to report any associations between these viral pathogens and diarrhea in calves.

2. Materials and methods

2.1. Ethical statement

This manuscript does not report on any studies performed with animals.

2.2. Fecal samples

Between April and October of 2016, a total of 143 fecal samples were collected from farms in six different regions in northern and southern ROK, namely Gimje ($n=20$), Gochang ($n=20$), Hoengseong ($n=20$), Iksan ($n=7$), Sancheong ($n=14$), and Wanju ($n=62$). Fecal samples were collected from Korean native calves aged 1–84 days. Among 143 calves, 51, 54, and 38 calves were 1–21, 22–42, and 43–84 days old, respectively. Each animal's identification number, age, gender, and the number of animals in its herd were recorded. Fecal samples were scored for consistency based on accepted criteria (<https://www.vetmed.wisc.edu/dms/fapm/fapmtools/calves.htm>) and classified as normal, semi-formed (pasty), loose, or watery. All fecal samples were directly collected from the rectum, stored in plastic containers, photographed, and then categorized for consistency. A questionnaire was administered to farm managers on housing conditions, colostrum intake, and the vaccination of dams with the combined vaccine against coronavirus and rotavirus. The Korean native calves examined in this study were born to cows that were not vaccinated against BVDV. The collected samples were stored at -80°C until analysis.

2.3. Extraction of RNA and reverse transcription–polymerase chain reaction (RT–PCR) for pathogen detection

Total RNA was extracted from fecal suspensions using RNAiso Plus Reagent (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. RT-PCR was performed to amplify BVDV, BCoV, and BRV RNA using DiaStar™ 2× One-Step RT-PCR Smart Mix (Solgent, Daejeon, Korea). Briefly, reverse transcription was carried out at 50°C for 30 min, followed by 94°C for 5 min, and then 35 cycles at 94°C for 1 min and 72°C for 1 min, with a final extension step at 72°C for 10 min. Details regarding each primer and the PCR conditions used to detect BCoV, BRV, and BVDV have been described previously[14–16]. The amplicons were analyzed in a 1.5% agarose gel and visualized after ethidium bromide staining.

2.4. Data analysis

The PCR results for each fecal sample were recorded as either positive or negative for each pathogen and categorized according to the age (1–21, 22–42, or 43–84 days) and fecal consistency (normal, semi-formed, loose, or watery feces) of each calf. The data were analyzed using the SPSS 23.0 software package (SPSS, Chicago, IL, USA). Pathogens that were associated with liquid fecal samples (semi-solid, loose, and watery feces) more frequently than normal fecal samples were investigated using a multinomial logistic regression model. The associations between diarrhea and each pathogen were calculated for all ages together and for each age group separately using Pearson's *chi*-square test or Fisher's exact test, as appropriate. In addition, independent variables (infection, ages, colostrum intake, vaccination, and growth type) associated with diarrhea were predicted using binary univariable LogReg followed by binary multivariable LogReg only for variable with $P<0.1$ in univariable LogReg. Variables with $P<0.05$ were considered significant.

3. Results

3.1. Survey of enteric viral pathogens in 6 different regions

A total of 143 (50 normal and 93 diarrheal) fecal samples from Korean native calves aged 1–84 days (up to 12 weeks) were collected from six regions of the ROK, and these samples were tested for BCoV, BRV, and BVDV by RT-PCR. Among 143 fecal samples, the most prevalent pathogen was BVDV, which was detected in 86 animals (60.1%, 86/143) from five regions (Gimje, Gochang, Heongseong, Sancheong, and Wanju), while BVDV was not detected in fecal samples from the Iksan region (Table 1). As shown in Table 1, BCoV was identified in 51 samples (35.7%, 51/143) from all six regions surveyed. BRV was detected in only three calves (2.1%, 3/143) from two regions (Gimje and Gochang), and these calves were co-infected with BCoV. In addition, co-infections with two pathogens (BCoV+BVDV, 17.5%; BCoV+BRV, 0.7%) and three

Table 1

Summary of viral pathogens detected in fecal samples from 143 Korean native calves in six regions of the Republic of Korea.

Region	n	BCoV (Total)	BRV (Total)	BVDV (Total)	BCoV+BRV	BCoV+BVDV	BCoV+BRV+BVDV	No viral pathogens
Gimje	20	15 (75.0%)	2 (10.0%)	14 (70.0%)	0 (0.0%)	9 (45.0%)	2 (10.0%)	2 (10.0%)
Gochang	20	9 (45.0%)	1 (5.0%)	16 (80.0%)	1 (5.0%)	6 (30.0%)	0 (0.0%)	1 (5.0%)
Heongseong	20	7 (35.0%)	0 (0.0%)	8 (40.0%)	0 (0.0%)	2 (10.0%)	0 (0.0%)	7 (35.0%)
Iksan	7	2 (28.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (71.4%)
Sancheong	14	5 (35.7%)	0 (0.0%)	6 (42.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (21.4%)
Wanju	62	13 (21.0%)	0 (0.0%)	42 (67.7%)	0 (0.0%)	8 (12.9%)	0 (0.0%)	15 (24.2%)
Total	143	51 (35.7%)	3 (2.1%)	86 (60.1%)	1 (0.7%)	25 (17.5%)	2 (1.4%)	33 (23.1%)

Abbreviations: BCoV, bovine coronavirus; BRV, bovine rotavirus; BVDV, bovine viral diarrhea virus.

pathogens (BCoV+BRV+BVDV, 1.4%) were detected in 26 and two calves, respectively (Table 1). Co-infections with BCoV and BVDV were found in four regions (Table 1). In 33 fecal samples (23.1%), none of the tested viral pathogens were found.

3.2. Prevalence of viral pathogens in feces from normal and diarrheic calves

As summarized in Table 2, the prevalence of each viral pathogen was determined in normal and diarrheic fecal samples. BVDV (62.4%) and BCoV (39.8%) were commonly detected in feces from diarrheic calves, whereas BRV was found at a lower frequency (3.2%). BVDV (56.0%) and BCoV (28.0%) were also detected in normal feces at lower frequencies than in diarrheic feces. BRV was not found in normal feces. Co-infections of BCoV and BVDV were detected more than twice as often in diarrheic feces as compared to normal feces. Multiple infections with three pathogens (BCoV+BRV+BVDV) were found only in diarrheic samples. Among the pathogens identified, co-infection of BCoV and BVDV increased the odds ratio (OR) for diarrhea by 2.5-fold [95% confidence interval (CI): 0.9-7.0; $P=0.08$], but there were no significant associations between the individual pathogens and diarrhea.

3.3. Relationship between the presence of viral pathogens and fecal consistency

The associations between the viral pathogens and fecal consistency (semi-formed, loose, and watery feces) were investigated. Based on the multinomial regression analysis, BCoV detection was significantly correlated with the development of loose feces (Table 3). BCoV was detected in loose feces 2.9-fold more frequently

than in normal feces (95% CI: 1.1-7.6; $P=0.03$). Furthermore, co-infection with BCoV and BVDV was significantly associated with a loose fecal consistency (OR=3.6; 95% CI: 1.0-12.4; $P=0.04$) (Table 3). There was no statistically significant association between the pathogens detected and semi-formed or watery feces.

Table 2

Detection frequency of viral pathogens in feces from normal and diarrheic calves among 143 Korean native calves.

Pathogen(s)	Positives among diarrheic feces	Positives among normal feces	P-value	OR (95% CI)
BCoV (Total)	37/93 (39.8%)	14/50 (28.0%)	0.17	1.7 (0.8-3.5)
BRV (Total)	3/93 (3.2%)	0/50 (0.0%)	0.55	-
BVDV (Total)	58/93 (62.4%)	28/50 (56.0%)	0.46	1.3 (0.6-2.6)
BCoV+BRV	1/93 (1.1%)	0/50 (0.0%)	1.00	-
BCoV+BVDV	20/93 (21.5%)	5/50 (10%)	0.08	2.5 (0.9-7.0)
BCoV+BRV+BVDV	2/93 (2.2%)	0/50 (0.0%)	0.54	-

Abbreviations: BCoV, bovine coronavirus; BRV, bovine rotavirus; BVDV, bovine viral diarrhea virus.

3.4. Prevalence of viral pathogens according to the age of the calves

To analyze which pathogens are associated with diarrhea by age group among the calves, infections with BCoV, BRV, and BVDV in fecal samples from diarrheic calves aged 1-81 days were examined. In calves below 21 days of age, diarrhea was significantly associated with BCoV infection (Table 4). Among the three pathogens detected, the prevalence of BCoV was detected 9.3-fold more frequently in diarrheic feces than in normal feces among calves aged 1-21 days (OR=9.3; 95% CI: 1.1-78.9; $P=0.02$), while BCoV infection showed

Table 3

Multinomial logistic regression model to assess relationship between viral pathogens and three categories of fecal consistency among 143 Korean native calves.

Pathogen(s)	Semi-formed		Loose		Watery	
	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
BCoV (Total)	0.51	1.3 (0.6-3.0)	0.03*	2.9 (1.1-7.6)	0.77	1.3 (0.3-5.7)
BRV (Total)	1.00	-	1.00	-	-	-
BVDV (Total)	0.50	1.3 (0.6-2.9)	0.48	0.48, 1.4 (0.5-3.7)	0.98	1.0 (0.2-4.1)
BCoV+BRV	1.00	-	1.00	-	-	-
BCoV+BVDV	0.17	2.2 (0.7-6.8)	0.04*	3.6 (1.0-12.4)	0.91	1.1 (0.1-10.9)
BCoV+BRV+BVDV	1.00	-	1.00	-	-	-

Abbreviations: BCoV, bovine coronavirus; BRV, bovine rotavirus; BVDV, bovine viral diarrhea virus. * $P<0.05$ compared with normal feces.

Table 4

Relationship between viral pathogens and diarrhea among 143 Korean native calves (1-84 days).

Pathogen(s)	1 to 21-day-old calves (n=51)		22 to 42-day-old calves (n=54)		43 to 84-day-old calves (n=38)	
	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
BCoV (Total)	0.02*	9.3 (1.1-78.9)	0.93	1.0 (0.3-2.8)	0.36	2.2 (0.4-12.6)
BRV (Total)	0.56	-	-	-	-	-
BVDV (Total)	0.71	0.8 (0.2-2.8)	0.56	1.4 (0.5-4.2)	0.25	2.5 (0.6-9.0)
BCoV+BRV	1.00	-	-	-	-	-
BCoV+BVDV	0.17	-	0.73	1.3 (0.3-4.5)	0.15	-
BCoV+BRV+BVDV	1.00	-	-	-	-	-

Result is expressed as odds ratio from binary logistic regression model. Abbreviations: BCoV, bovine coronavirus; BRV, bovine rotavirus; BVDV, bovine viral diarrhea virus. * $P < 0.05$ showing a significant difference.

Table 5

Variables associated with diarrhea in fecal samples from 143 Korean native calves on 6 different regions of the Republic of Korea.

Variables	Categories	No. of calves	No. of diarrheic calves (%)	P value	OR (95% CI)
Univariable LogReg					
Infection	No (Reference)	33	20 (60.6%)	-	-
	Yes (any agents)	110	73 (66.4%)	0.54	1.3 (0.6-2.9)
	Single infection	82	50 (61.0%)	0.97	1.0 (0.4-2.3)
	Co-infection (any combination of agents)	28	23 (82.1%)	0.07	3.0 (0.9-9.9)
Ages (days) of calves	1-21 (Reference)	51	36 (70.6%)	-	-
	22-42	54	31 (57.4%)	0.16	0.6 (0.3-1.3)
	43-84	38	26 (68.4%)	0.83	0.9 (0.4-2.5)
Colostrum intake	No (Reference)	28	22 (78.6%)	-	-
	Yes	115	71 (61.7%)	0.09	0.4 (0.2-1.7)
Vaccination	No (Reference)	61	38 (62.3%)	-	-
	Yes	82	55 (67.1%)	0.55	1.2 (0.6-2.5)
Growth type	Housing (Reference)	122	87 (71.3%)	-	-
	Grazing	21	6 (28.6%)	0.00*	0.2 (0.1-0.4)
Multivariable LogReg					
Infection	No (Reference)	-	-	-	-
	Single infection	-	-	0.72	1.2 (0.5-3.0)
	Co-infection (any combination of agents)	-	-	0.11	2.9 (0.8-10.5)
Colostrum intake	No (Reference)	-	-	-	-
	Yes	-	-	0.02*	0.2 (0.1-0.7)
Growth type	Housing (Reference)	-	-	-	-
	Grazing	-	-	0.00*	0.1 (0.0-0.4)

LogReg, logistic regression analysis; * $P < 0.05$ showing a significant difference.

no association with diarrhea in the other age groups (Table 4). BRV was detected only in the diarrheic feces of calves aged 1-21 days (Table 4). However, no statistically significant association between BRV and diarrhea was observed and the OR for BRV was not calculated because very few BRV infections were detected. These results revealed that BCoV infection was strongly associated with diarrhea in calves aged 1-21 days, whereas BRV and BVDV infections were not significantly associated with diarrhea in any age group.

3.5. Variables associated with diarrhea

Information such as colostrum intake, vaccination, and herd management (grazing or housing) was obtained from the farms in the regions where fecal samples were collected. Initially, univariable binary LogReg identified 3 variables associated at $P < 0.01$ with diarrhea (Table 5). Interestingly, grazing calves had a 5-fold lower OR for the incidence of diarrhea as compared to housed calves ($OR = 0.2$; 95% CI: 0.1-0.4; $P = 0.00$). As shown in Table 5, vaccination

of pregnant dams against BCoV and BRV did not prevent diarrhea among the calves. Although there were no significant associations, diarrhea may be slightly influenced by co-infection and colostrum intake. Co-infection increased the OR for the incidence of diarrhea by 3-fold ($P = 0.07$) as compared to mono-infection. Next, 3 variables closely associated with diarrhea were analyzed with the multivariable LogReg model. The final model showed that colostrum intake decreased the OR for the incidence of diarrhea by 5-fold as compared to calves that had not been fed colostrum ($P = 0.02$; Table 5). Grazing had a strong effect on the decrease in diarrhea ($OR = 0.1$; 95% CI: 0.0-0.4; $P = 0.00$). However, there was no statistical significance between diarrhea and co-infection.

4. Discussion

We investigated the prevalence of BCoV, BRV, and BVDV in normal and diarrheal (semi-formed, loose, or watery) feces of Korean native calves from six different regions in the ROK. In the present

study, BVDV was the most frequently detected pathogen, followed by BCoV and then BRV; however, no association between diarrhea and the presence of BVDV was found. Co-infections with two or three pathogens were detected in 28 Korean native calves. BCoV was found in fecal samples from all six regions surveyed, reflecting the broad circulation of BCoV among Korean native calves. Our results revealed that the prevalence of BCoV was significantly higher in diarrheal calves aged 1-21 days than in non-diarrheal calves of the same age group. Infection with BCoV either alone or together with BVDV was associated with a significantly higher incidence of loose feces. Among the various factors evaluated, grazing and colostrum intake were associated with a significant reduction in the incidence of diarrhea. Interestingly, no viral pathogens were found in 33 diarrheal feces. It is possible that these cases were associated with other pathogens, including bacteria (*e.g.*, *Escherichia coli* and *Salmonella*), parasites (*e.g.*, *Cryptosporidium* and *Giardia*), or other viruses (*e.g.*, torovirus and norovirus). Further studies are necessary to identify additional infectious agents in the feces of diarrheic calves.

In this study, BCoV was the second most prevalent pathogen and was detected in 35.7% (51 out of 143) of all fecal samples. This pathogen was present alone or as part of a co-infection with other viral pathogens (BVDV and/or BRV). According to our results, the prevalence of BCoV was higher than that of BRV in the ROK. Several studies have reported the lack of an association between BCoV infection and calf diarrhea[17,18]. However, our results showed that BCoV infection was more commonly detected in loose feces ($OR=2.9$; $P=0.03$) and the incidence of loose feces was significantly higher among calves that were co-infected with BCoV and BVDV ($OR=3.6$; $P=0.04$) as compared with those that were infected with BCoV alone. Consequently, we demonstrated a statistically significant relationship between the detection of BCoV and the occurrence of loose feces. In addition, cases of diarrhea caused by BCoV were most prevalent among diarrheic calves aged 1-21 days in the ROK ($OR=9.3$; $P=0.02$). Consequently, it seems that BCoV infection may contribute to calf diarrhea either alone or as a co-infection with other viral pathogens, or in conjunction with other factors.

BRV is a primary cause of acute diarrhea in neonatal calves[19,20]. In this study, the prevalence of BRV among diarrheic calves was very low and no correlation between the BRV infection and diarrhea was found. However, we cannot rule out the possibility that other BRV infections, such as group B and group C rotaviruses, might be circulating in Korean native calves. A previous study showed that group C rotavirus caused diarrhea in Korean native calves[12]. Although the present results do not show the importance of BRV as a major enteric pathogen, BRV should be continuously surveyed to monitor its circulation among Korean native calves.

BVDV was widespread among both normal and diarrheal fecal samples in the ROK. In the present study, we could not demonstrate any significant relationship between BVDV infection and diarrhea, despite a high positive rate of BVDV among all age groups. We cannot rule out the possibility of BVDV transmission to calves through colostrum feeding or the presence of persistently

infected animals on these farms. Although there was no statistical significance, it is estimated that co-infection with BVDV and BCoV may increase the occurrence of diarrhea ($P=0.08$). The current study did not demonstrate the role of BVDV in calf diarrhea; however, the clinical significance of BVDV infections, especially persistently infected, should not be overlooked. Future studies are therefore necessary to clarify the association between BVDV and the occurrence of diarrhea.

To date, no studies have estimated the relationship between viral pathogens and fecal consistency (semi-formed, loose, or watery feces). According to our findings, BRV, which has previously been shown to be highly associated with acute watery diarrhea, was not detected in watery feces, whereas BCoV and BVDV were detected in watery feces. BCoV and BVDV were also detected in normal feces. Our results indicate that the consistency of feces did not necessarily correlate with the viral pathogens detected. The present study demonstrated that only the incidence of loose feces was significantly related with BCoV infection. Such a difference in fecal consistency may be a useful criterion for determining the clinical significance of BCoV detection during diagnostic investigations. Larger epidemiological surveys are needed to identify any associations between fecal consistency and the presence of infectious pathogens. Given such large scale data, it should be possible to estimate which pathogens cause diarrhea according to the consistency of the fecal samples.

We investigated the associations between viral pathogens and diarrhea among calves by age group. Interestingly, the detection rate of the three viral pathogens examined in this study gradually decreased as the age of calves increased (data not shown). Our results showed that BCoV was strongly associated with diarrhea among calves aged 1-21 days. This observation was quite different from the results of previous studies[18,21,22]. Bartels *et al.* mentioned that because BCoV is an opportunistic infection, its prevalence may be affected by calf immunity and environmental factors such as overcrowding, housing of calves in age-mixed rather than age-specific groups, and poor hygiene[17]. Moreover, it is speculated that the prevalence of BCoV at this age may be due to the presence of infected animals that were shedding virus. The results highlight the importance of farm management strategy, *i.e.*, good hygienic procedures, as a protective factor in calf-rearing.

Diarrhea in calves is closely associated with infectious agents, nutritional quality, environmental stress, and other management factors. The present study indicated that vaccination was not associated with the occurrence of diarrhea, whereas grazing ($P=0.00$) and colostrum intake ($P=0.02$) may significantly reduce the incidence of diarrhea. Several studies have shown that delayed colostrum intake, feeding with infected or purchased colostrum, calving season, and parity (*i.e.*, primiparous dams) are related to the incidence of calf diarrhea because of the low levels of maternal antibodies in calves[23,24]. The results of the present study emphasized the importance of the first colostrum intake as soon as possible after birth. Grazing may not only reduce the incidence of diarrhea, but also should be encouraged in terms of animal welfare in the ROK. Taken together, the occurrence of diarrhea may be more

commonly associated with other factors such as passive immunity, cattle management systems, herd size, and access to grazing. Further studies are necessary to determine the effects of these variables in preventing calf diarrhea.

In conclusions, this study showed that BCoV was strongly associated with diarrhea in Korean native calves aged 1-21 days. However, further investigations to identify the presence of other enteropathogens such as *Escherichia coli* and *Cryptosporidium* are needed. The prevalence of BCoV described in this paper highlights the need for in-depth epidemiological studies. Therefore, grazing and colostrum intake is recommended for preventing and controlling calf diarrhea caused by BCoV.

Conflict of interest statement

The authors declare no conflict of interests.

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