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Detection of rotavirus and coronavirus from suckling Korean indigenous calves with acute diarrhea

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Abstract

In order to evaluation of prevalence of rotavirus and/or coronavirus, forty suckling Korean indigenous calves (between 2 and 98 days old) with acute diarrhea were investigated by the immunochromatographic rapid test in the field (veterinary practice) on October and on December 2006. Rotavirus and coronavirus were detected in 13 (32.5%) and 8 (20.0%) of the fecal samples from diarrheal calves, respectively. The highest mortality rate in diarrheal calves occurred in the neonatal period from 2 to 7 days old. Totally, 40.0% of diarrheal calves showing acute enteritis were not detected with rotavirus and/or coronavirus. The rotavirus infection rate was significantly difference ($p < 0.05$) between October and December, and the detection rate of rotavirus was bigger than that of coronavirus. These results suggested that rotavirus can be frequently associated with acute diarrhea of suckling calves and affected with changes of temperature.

Key words : Rotavirus, Coronavirus, Suckling calves, Immunochromatographic rapid test

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Introduction

Acute calf diarrhea is a disease associated with the presence of various agents. The known infectious agents include bacteria [enterotoxigenic *Escherichia coli* spp[1)], *Campylobacter* spp[2)] and *Salmonella* spp[3)]], viruses [rotavirus[4)], coronavirus[5)] and bovine viral diarrhea virus[6)]] and protozoa [*Cryptosporidium parvum*[7)]], *Giardia* spp[8)] and *Eimeria* spp[9)]. These microorganisms are also present in the intestinal tract of clinically normal calves.[10.11)] Most studies carried out in different countries have found rotavirus and coronavirus to be

the most commonly detected agents in calves with diarrhea. In microbiological surveys of diarrheal calves, the detection rates of mixed infections with two or more of the main enteropathogens range between 2.8% and 48.5%, and most of them were rotavirus or coronavirus.[5,12]

For diagnosis in large animal veterinary practises in field, difficulties in the clinical diagnosis of infectious enteritis arise from frequent nonspecific clinical signs and leisons, the presence of asymptomatic infection, the involvement of multiple agents, and the interactions of intrinsic and extrinsic factors that predispose the host to infection[13]. In addition, noninfectious agents, environmental, managemental and nutritional factors may influence the severity and outcome of the disease.[14-16]

Thus this study was conducted to examine the severity of the calf diarreal disease according to the temperature. That is, we compared the detection rate of viruses from diarrheal calves with two season (fall and winter)by using immnunochromatographic rapid tests.

Materials and Methods

Animals

Calves were investigated among those showing acute enteritis based on history of clients. They were between 2-98 days old after birth with clinical signs of acute enteritis. Appetite was shown normal or anorexia. Calves were presented to the Happy Veterinary Hospital from Gimje area (Gimje city, Mankyeong, Chuksan, Baeksan, Yongji, Baekgu, Buryang, Gongdeok, Cheongha, Seongdeok, Chinbong, Keumgu, Bongnam, Hwangsan, Keumsan and Kwanghwal).

Fecal samples

Diarrheal fecal samples were collected directly from calves on poly gloves within 1 or 2 days after onset of diarrhea. All samples were shipped to hospital. Among 40 calves, 20 were analyzed on October and others on December.

Detection of rotavirus and coronavirus

All the fecal samples were tested for the presence of rotavirus and coronavirus by immunochromatographic rapid test. Detection of rotavirus and coronavirus in all samples was performed using a commercially available kit (Bio-X diagnostics, Belgium), according to the manufacturer's instructions. Briefly, about 1g fecal samples were placed in individual tube with buffers and mixed inversely by hand. A precoated strip with antibody for anti-rotavirus or anti-coronavirus provided with the kit was dipped into the tubes mixed samples and left for 5 min. After 5 min, the dipped strip was withdrawn and regarded as the positive when sample showed two lines.

Statistical analysis

Significant differences between the values were statistically analyzed using a one-way analysis of variance (ANOVA), followed by a two pairs Student's t test. $p < 0.05$ or less was considered statistically significant.

Results

A total of 40 samples were collected from calves between 2 – 98 days old in Gimje area which showed acute enteritis. The presence of rotavirus and coronavirus in calves is shown in Table 1.

Rotavirus was detected with higher prevalence (40.0%, 16/40) in all samples than that of coronavirus (27.5%, 11/40). However, a statistically significant difference in the prevalence of both viruses was not observed ($p > 0.05$). The mixed infections with rotavirus and coronavirus were less commonly detected (7.5%, 3/40). Rotavirus and/or coronavirus were not detected in 40% (16/40) of fecal samples from diarrheal calves (Table 2).

Table 1. Detection of rotavirus and/or coronavirus in suckling calves with acute diarrhea

Days of age	October		December		No of infected samples (%)	
	Rotavirus	Corona-virus	Rotavirus	Coronavirus		
2-7		1	4	2	7	(17.5)
8-14			2	2	4	(10.0)
15-21			1	1	2	(5.0)
22-28			2		2	(5.0)
29-35	1				1	(2.5)
36-42	1			1	2	(5.0)
43-49				1	1	(2.5)
50-56	1	1			2	(5.0)
57-63	2	2			4	(10.0)
64-70			1		1	(2.5)
71-77					NS[a]	
78-84					None[b]	
85-91			1		1	(2.5)
92-98					None[b]	
No of infected samples (%)	5 (12.5)	4 (10.0)	11 (27.5)[c]	7 (17.5)	27[d]	(67.5)

[a] Not examined, [b]No detected of rotavirus and(or) coronavirus, [c]Detection number of rotavirus in December was significantly higher ($p<0.05$) than that of October, [d]This number was presented including 3 calves with mixed infection by rotavirus and coronavirus.

Analysis of the prevalence by monthly, rotavirus infection was detected in 18.5 % (5/27) of all positive samples in October and 40.7% (11/27) of all positive samples in December, respectively (Table 1). There was significant difference ($p < 0.05$) in the prevalence of rotavirus between October and December. On the other hand, coronavirus infection was detected in 14.8% (4/27) of all positive samples on October and 25.9% (7/27) of all positive samples on December, respectively. The detection rates of coronavirus were slightly increased in December, but there was no significant difference ($p > 0.05$) in the pre-

valence of coronavirus between October and December.

Of 40 calves, analysis of the prevalence by ages, rotavirus and/or coronavirus were detected in 25.9% (7/27), 14.8% (4/27) and 14.8% (4/27) of the positive samples in the age groups 2-7, 8-14 and 57-63 days old after birth, respectively. The prevalence of the both viruses was highest in the age group 2-7 days old, but there was no significant difference ($p > 0.05$) in the prevalence among these age groups when compared to the age group 8-14 and 57-63 days old. In contrast, there were significant differences ($p < 0.05$) in the prevalence between the age group 2-7 days old and the remaining age groups. In analysis of mortality among 40 calves, 4 calves died with the infection of rotavirus and coronavirus. However, 2 calves were not identified any infectious agents (Table 2).

The mortalities of calves were 15.3% for rotavirus (2/13), 9% for coronavirus (1/11), 33% (1/3) for mixed infection (rotavirus and coronavirus) in all positive samples, respectively. In mainly clinical symptoms, 2 calves that died with rotavirus infection were male showing yellowish-watery (2 days old calf) and bloody-watery diarrhea (25 days old calf). In the 1 female calf that died with coronavirus infection, 3 days old after birth, the clinical symptom was watery diarrhea. In the other 1 male calf that died with mixed infection at 7 days old, rotavirus and coronavirus, the clinical symptom was watery diarrhea.

Table 2. Comparison of outbreaks, symptoms and mortalities according to viral infected patterns in 40 acute diarrhea of suckling Korean indigenous calves

	Calves infected with			Without rotavirus and/or coronavirus
	Rotavirus	Coronavirus	Rotavirus and Coronavirus	
Number	13	8	3	16
%	32.5	20.0	7.5	40.0
Patterns of feces	Yellowish-watery Bloodish-watery	Creamy Watery	Watery	Mucous Watery
Mortality	2	1	1	2
%	5.0	2.5	2.5	5.0
Age group with days	2-7 22-28	2-7	2-7	2-7

Additionally, the 2 calves that died with unknown agents were female and male, 2 days old, with mucous and watery diarrhea, respectively. Out of 6 died calves, 4(66.6%)were died less than 25 days old. However, a significant difference ($p > 0.05$) in mortality associated with ages by rotavirus and coronavirus infection was not found.

Discussion

Rotavirus and coronavirus are well known as causative agents in neonatal calf diarrhea,[17,18] and mixed infection

was reported in most studies all over the world. The detection rates of rotavirus and coronavirus were variable because that can be affected with samples of calves with diarrhea or not. This study showed that the detection rates of rotavirus and coronavirus were 32.5% and 20%, respectively (Table 2). This results were little different to other reports,[19.20] namely, rota virus was less prevalent than others but coronavirus was higher. However, these results in this studies are generally similar to the pattern which were high prevalence for rotavirus infection and low prevalence for coronavirus infection with slightly differences of values.[21-23]

The prevalence of rotavirus and coronavirus infection in the different age groups is generally similar to the pattern of rotavirus and coronavirus detection reported for calves.[1.24] In this present study, the prevalence of diarrheal calves infected with rotavirus and coronavirus in the first and second week of life was 25.9% and 14.8%, respectively. As expected, the prevalence of first two weeks of life was higher than that of remaining age groups (Table 1). A relatively high prevalence was shown in 57-63 days old after birth, but we could not explain exactly the reason. The rotavirus detection rate by age groups was similar to those reported by Kim et al[25], but the pattern of the coronavirus infection had different results. However, it was unclear that the infection occurred in different age groups in detection of rotavirus and coronavirus. One of the reasons might be due to low morbidity for diarrheal calf associated with the coronavirus.

On the other hand, the rotavirus prevalence on October and on December were 12.5% and 27.5%, and coronavirus were 10.0 % and 17.5%, respectively. In comparison by monthly, 18 of 27 positive samples for rotavirus and/or coronavirus significantly occurred on December, but 9 of 27 positive samples were on October. This result indicates that infections of rotavirus and coronavirus causing acute diarrhea in neonatal calves may be affected by temperature. The season of the year is another variable causing the highest occurrence in the winter, which is consistent with other studies.[26.27]

The mortality of diarrheal calf was evaluated with 2 different parameters in this present study, which were analyzed by age and monthly. Mortality rates for diarrheal calves were 15% (6/40). However, substantial mortality was 10.0% because 4 of 6 dead calves died by rotavirus and/or coronavirus infections.

Neonatal calves are susceptible to many environmental pathogens, consequently, morbidity and mortality can be high. The morbidity of diarrhea from calves most commonly occurred in April and December during the year, but the highest mortality occurred in December. Further, 1.5-8% of mortalities were reported in acute diarrhea from calves by Bendali et al[28]. 83.3% of total mortality rates within 7 days after birth were related either to immune response or environmental factors. Because neonatal calves are often exposed to significant environmental and immunological stressors, provision of additional nutrients may

exacerbate health problems and cause anorexia. Johnson[29] suggested that anorexia associated with immunological challenge may be an adaptive response and force-feeding of anorexic animals may increase mortality. On the other hand, Cho et al[30] reported that the disease of digestive and respiratory system occurred frequently in winter season. One of this reason, relatively high occurrences of diarrhea in neonatal calves, was caused by overeating.

Conclusively, acute enteritis can be affected with other infectious agents as well as rotavirus and corona virus, and this study had not many samples. For the more effective control of the diarrheal disease in the neonatal calves, we would like to suggest that further studies with many samples should be conducted.

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