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Serological Survey of Bovine Coronavirus in Korea

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Bovine coronavirus (BCoV) is a causative agent of entero-pathogenic diarrhea in young calves and winter dysentery (WD) in adult cattle. In this study, we conducted a nationwide sero-epidemiological survey of BCoV infection in Korea. In total, 3,029 bovine sera collected between October and December 2005 were screened for the presence of antibodies against BCoV using a hemagglutination inhibition (HI) test. Half (50.0%) of individual cattle tested were positive for BCoV. The regional distribution of the seroprevalence of positive HI antibodies was 55.7% (234/420) in Gyeonggi, 53.0% (316/596) in Jeonra, 51.9% (374/720) in Chungcheong, 48.5% (401/827) in Gyeongsang, 43.9% (79/180) in Jeju, and 38.1% (109/286) in Gangwon Province. Analyzing the distribution of HI titer according to the age of the cattle showed the highest BCoV seropositive rate in 5-year-old cattle, and the incidence of cattle with an HI antibody titer of 1:160 or above was 12.1%.

Key Words: Bovine coronavirus, Seroepidemiology, Korea

INTRODUCTION

Bovine coronavirus (BCoV), a coronavirus, is a spherical, enveloped virus that ranges from 80 to 160 nm in diameter. The BCoV genome consists of a single linear molecule of positive sense RNA, approximately 31 kb long. It encodes the viral RNA-dependent RNA polymerase and the four major structural proteins (15). The peplomeric (S) and hemagglutinin (HE) proteins can cause hemagglutination (HA) with rat or mouse erythrocytes and are thought to be involved in protecting cattle from infection with BCoV (13, 17). BCoV is a common agent in neonatal calf diarrhea (CD) and is associated with winter dysentery (WD) in adult cattle, which causes large economic losses such as decreased milk production (2,21). BCoV also possesses a tissue tropism for the upper respiratory tract (14). Although there are minor antigenic and biological variations among BCoVs from CD and WD strains based on the clinical symptoms (4,12), it has been thought that there is only one BCoV serotype (9,14,21). BCoV infections in cattle have been reported in many countries throughout the world, including Korea (3,13) and have also been observed in other ruminants such as sheep, mules, and white-tailed deer (20,21). Various serological assays such as virus neutralization (VN) tests, hemagglutination inhibition (HI) tests, indirect immunofluorescence (IF) tests, and enzyme linked immunosorbent assays (ELISA) have been used to assess levels of antibody to BCoV (17,18). HI tests have been used to describe cow and calf serological status for BCoV in Canada (6) and have shown a high correlation with VN tests using sera obtained from cattle vaccinated with inactivated BCoV (17). Seroepidemiological surveys of BCoV are important, because the survey data could be used for setting up vaccination programs and initiating measures to prevent

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virus transmission. Although BCoV infections in the cattle industry have been reported in many countries throughout the world, nationwide sero-epidemiological surveillance of BCoV infection in Korea has not been performed. In this study, bovine sera collected nationwide were screened for the presence of antibodies against BCoV using an HI test.

MATERIAL AND METHODS

1. Virus and cells

BCoV strain KV0501 used in this study was isolated in Korea in 2005 from feces of naturally-infected calf. The BCoV had been passaged 5 times in HRT-18 cells that were derived from a human rectal adenocarcinoma. For the propagation of the BCoV, monolayered HRT-18 cells were rinsed twice with PBS and then inoculated with trypsin-treated BCoV. After adsorption at 37℃ for 1 h, the cultures were incubated in α-MEM containing 0.5 μg/ml of crystallized bovine trypsin (Sigma St. Louis, Mo, USA) until a BCoV specific cytopathic effect was seen. After three freeze-thaw cycles, the harvested virus was clarified by centrifugation for 30 min at 3,000 x *g* to remove cell debris. The crude BCoV was used as the antigen in the HI test.

2. Hemagglutination inhibition (HI) tests

For the seroprevalence survey, serum samples were obtained from 3,029 cattle from 1,118 farms in six provinces of Korea between October and December 2005. The HI test was carried out according to standard microtiter procedures using mouse red blood cells (11,16). Briefly, for removing non-specific inhibitors, 100 μl of serum and 500 μl of PBS were mixed with 400 μl of 25% kaolin (Sigma). After shaking for 1 h, kaolin was removed by centrifugation of 12,000 rpm for 5 min in a microfuge. Clear supernatant was mixed with 50 μl of packed normal mouse erythrocytes to remove natural agglutinins. After incubation for 1 h at 37℃, the treated serum was separated from mouse erythrocytes by centrifugation. For HI test, four to eight HA units of BCoV (in 25 μl) were added to 25 μl of the treated sera. After incubation for 1 h at 37℃, 50 μl of 1% mouse erythrocytes were added. The plates were incubated at 4℃ for 90 min. The HI titer was expressed as the reciprocal of the highest dilution of serum showing complete inhibition of hemagglutination. The serum samples showing HI titer equal to or greater than 1:20 were considered positive (6).

3. Statistical analysis

Chi-square tests were used to analyze the differences in seroprevalence between the breed, genders, age, and regions, respectively. A p-value less than 0.05 was considered to be statistically significant.

RESULTS

The average prevalence of antibodies against BCoV was 50.0% in 3,029 serum samples (Table 1). The regional

Table 1. Cattle population and regional distribution of BCoV antibodies from cattle in Korea

Breed	Province						Total
	GG*	GW	CC	GS	JR	JJ	
Holstein	108/156	4/7	50/106	63/137	70/131	30/70	325/607
%	69.2	57.1	47.2	46.0	53.4	42.9	53.5
Population (%)†	38.9	4.0	21.5	16.9	15.3	1.1	97.7
Korean native	126/264	105/279	324/614	338/690	246/465	49/110	1,188/2,422
%	47.7	37.6	52.8	49.0	52.9	44.6	49.1
Population (%)	6.9	8.3	19.6	32.5	26.5	1.0	94.8
Total	234/420	109/286	374/720	401/827	316/596	79/180	1,513/3,029
%	55.7	38.1	51.9	48.5	53.0	43.9	50.0

* GG; Gyeonggi, GW; Gangwon, CC; Chungcheong, GS; Gyeungsang, JR; Jeonra, and JJ; Jeju, respectively.
† Data was obtained from Agricultural and forestry statistical year book, National Agricultural Product Quality Management Service (March 1, 2006), Korea.

Table 2. Age distribution of HI titer against BCoV

Age of cattle	No. of positive (%)	HI titer	
		1:20 ~ ≤1:80 (%)	≥1:160 (%)
≤1	68/144 (47.2)	53 (38.6)	15 (10.4)
2	381/847 (45.0)	293 (34.7)	87 (10.3)
3	587/1,137 (51.6)	446 (39.2)	141 (12.4)
4	296/554 (53.4)	223 (40.3)	73 (13.2)
5	100/184 (54.3)	77 (41.8)	23 (12.5)
≥6	81/163 (49.7)	55 (33.7)	26 (16.1)
Total	1,513/3,029 (50.0)	1,148/3,029 (37.9)	365/3,029 (12.1)

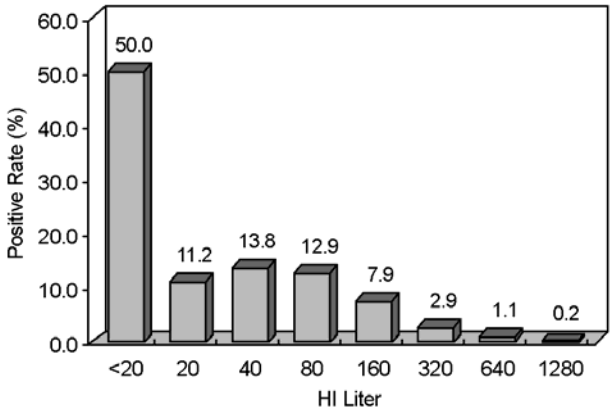

Figure 1. Frequency of distribution of HI titer in 3,029 serum samples collected from Korean cattle.

distribution of the seroprevalence of a positive HI titer was 55.7% (234/420) in Gyeonggi, 53.0% (316/596) in Jeonra, 51.9% (374/720) in Chungcheong, 48.5% (401/827) in Gyeongsang, 43.9% (793/180) in Jeju, and 38.1% (109/286) in Gangwon Province. We found no significant differences of the seropositive rate between native Korean (49.1%) and Holstein dairy (53.5%) cattle in all regions except for Gyeonggi where significant difference ($p<0.05$) was observed.

Analyzing the distribution of HI titer against BCoV according to the age of the cattle showed the highest seropositive rate in 5-year-old cattle (54.3%). The incidence of cattle showing an HI antibody titer above 1:160 was 12.1% (Table 2). Female cattle showed significantly higher seroprevalence (50.8%; 1355/2665) than males (43.4%; 158/364). Seroprevalence was significantly higher ($p<0.05$) in females (50.8%; 1355/2665) than males (43.4%; 158/364). Of the cattle that had an HI antibody titer of 1:20 or more, the most frequent HI titer was 1:40 (13.8%; Fig. 1).

DISCUSSION

BCoV infection has been reported in most cattle-raising countries, and its seroprevalence has been estimated to range from 61% in Sweden to 90% in Canada (2,13,20). Antibodies to BCoV have also been detected in several other species, such as sheep, white-tailed deer, and pigs, with the prevalence ranging from 6.6 to 44.2% (8,20,21). BCoV is antigenically related to murine hepatitis virus, hemagglutinating encephalomyelitis virus, rat virus, and human coronavirus OC43. But the virus does not cross-react the other bovine viruses (19). Several studies have reported BCoVs isolated from the feces of calves and cows in Korea (3,10). Jeong *et al.* (10) reported consistent detection of BCoV from all herds with winter dysentery in Korea during 2002-2004. By using BCoV antigen-capture ELISA, 34 of 97 (35.1%) fecal samples were found to be positive. Of the 32 herds tested, 17 herds (53.1%) showed BCoV-positive fecal samples (10). Since calf diarrhea and winter dysentery cause enormous economic losses in the cattle industry in Korea (3,10), we wanted to estimate the nationwide infection rate of cattle with BCoV by performing an HI test on bovine serum from Korean cattle. Although BCoV has been classified as a single serotype (14), some reports described antigenic variations among BCoV strains (5,7,22). BCoV strains hemagglutinated both mouse and chicken erythrocytes at 4℃. All strains agglutinated mouse erythrocytes at 4℃ with similar HA titers (7,22), but the HA titers were different according to BCoV strains used when chicken erythrocytes were used. Therefore in this study, recently isolated BCoV KV0501 strain and mouse erythrocytes were used for HI test. The regional prevalence ranged from 38.1% to 55.7%, depending on the province. As shown in Table 1, seroprevalence of BCoV in Holstein was the highest in Gyeonggi province, which showing the highest Holstein cattle population. Since a BCoV vaccine has been used to prevent wild infection in the Korean cattle population, this seroprevalence result may not represent the true prevalence of BCoV infection. Although the exact BCoV vaccination status of cattle in Korea is not known,

the use of a BCoV vaccine seems to be very limited. About 130,000~170,000 doses of BCoV live vaccine are produced annually in domestic veterinary biological companies (1). The serological survey results indicate that cattle seropositive for BCoV are evenly distributed throughout the country. Takamura *et al*. (17) reported that cattle with an HI antibody titer ≤1:80 and an SN titer ≤1:160 developed severe clinical signs such as watery diarrhea and fever after challenge. Therefore, mucosal infection with BCoV can be prevented if the HI titer of ≥1:160 or the SN titer of ≥ 1: 640 is present in the blood (17). In this study, only 12.1% of cattle were found to have an HI antibody titer of 1:160 or above, indicating that a large number of cattle (about 88%) are faced with the danger of wild BCoV infection associated diarrhea or respiratory syndrome; therefore, effective vaccination should be practiced to elevate cattle immunity to BCoV.

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