Correlation between Maternal Serum Antibodies and Protection against Bovine Rotavirus Diarrhea in Calves

Junko KOHARA and Hiroshi TSUNEMITSU

Shintoku Animal Husbandry Experiment Station, Shintoku, Hokkaido 081–0038 and 1) Shichinohe Research Unit, National Institute of Animal Health, Shichinohe, Aomori 039–2586, Japan

(Received 29 July 1999/Accepted 22 October 1999)

ABSTRACT. The correlation between maternal serum antibodies in beef calves at 2 days old and protection against diarrhea induced by natural bovine rotavirus (BRV) infection was examined. Virus neutralizing (VN) antibody titers against BRV in sera from calves that developed diarrhea by BRV infection within 14 days of age (BRV-diarrheal calves) were significantly lower than those from calves that had no diarrhea. In the BRV-diarrheal calves, a positive correlation was found between the VN antibody titers and age of the onset of diarrhea. There were negative correlations between the VN antibody titers and duration of the diarrhea, VN antibody titers and cumulative diarrhea scores, and the VN antibody titers and duration of virus shedding. These results suggest that the VN antibody titers against BRV in newborn calf serum could be an indicator of protection against BRV-induced diarrhea.—KEY WORDS: bovine rotavirus, calf, diarrhea.

Bovine rotavirus (BRV) is a major pathogen of newborn calf diarrhea [3, 5, 9, 13]. BRV infection usually occurs in newborn calves during the first few weeks of life before active immunity is developed. Therefore, passive maternal antibodies transferred to newborn calves via the colostrum are considered important for the disease prevention. However, the mechanisms for immunity to rotavirus infections and illness are not completely understood. It was observed that newborn calves frequently developed BRV-induced diarrhea despite a moderately high level of circulating antibodies against BRV derived from the colostrum [18]. In this study, we examined the correlation between maternal serum antibodies in natural suckling beef calves and protection against diarrhea by natural BRV infection. We evaluated that the virus neutralizing (VN) antibody titer in calf sera could be an indicator of protection against BRV.

From January to April 1997, we surveyed calf diarrhea in a beef cattle (Angus, Hereford and Japanese Black) farm, located in Hokkaido, Japan. One hundred sixty-eight calves were born and reared with their dams and suckled naturally. One hundred thirty-five calves (80.4%) developed diarrhea within 14 days after birth. Twenty-three calves had no diarrhea during the first month after birth (normal calves). There were significant differences in the VN antibody titers and IgG concentrations between the BRV-diarrheal calves and normal calves were determined by a Student’s t-test. The correlations of the VN antibody titers with age of onset of the diarrhea, duration of the diarrhea, cumulative clinical scores, and duration of the virus shedding in the BRV-diarrheal calves were analyzed by Spearman’s measure of correlation. A statistical significance was assessed at P<0.05.

Clinical signs and duration of the virus shedding in the BRV-diarrheal calves are summarized in Table 1. Diarrhea in the BRV-diarrheal calves started from day 5 to 13 after birth, and lasted for 1 to 9 days. Cumulative diarrhea scores were from 2 to 29. BRV was detected in 46 fecal samples (34.1%), and 30 of these samples were negative for other pathogenic agents. The remaining 16 samples were also positive for bovine coronavirus or Cryptosporidium spp.

Thirty calves from which BRV was only detected in the feces (BRV-diarrheal calves) were monitored daily for signs of diarrhea. Fecal score was determined as follows: 0=normal, 1=soft, 2=pasty, 3=semiliquid, and 4=watery feces. The daily fecal score was totaled as a cumulative diarrhea score to determine the severity of the diarrhea in each calf. Serum samples were collected from all calves on day 2 after birth to examine the transfer of maternal antibodies to the calves. The total IgG concentrations in the sera were measured by a single radial immunodiffusion assay (Saikin kagaku, Japan).

VN antibody titers against the Lincoln strain (G6 P[1]) of BRV in calf sera were determined by fluorescent focus neutralization (FFN) tests with MA104 cells [10]. The antibody titers were expressed as the reciprocal of the highest serum dilution that resulted in an 80% or greater reduction of fluorescent foci.

Significant differences in the VN antibody titers and IgG concentrations between the BRV-diarrheal calves and normal calves were determined by a Student’s t-test. The correlations of the VN antibody titers with age of onset of the diarrhea, duration of the diarrhea, cumulative clinical scores, and duration of the virus shedding in the BRV-diarrheal calves were analyzed by Spearman’s measure of correlation. A statistical significance was assessed at P<0.05.

Clinical signs and duration of the virus shedding in the BRV-diarrheal calves are summarized in Table 1. Diarrhea in the BRV-diarrheal calves started from day 5 to 13 after birth, and lasted for 1 to 9 days. Cumulative diarrhea scores were from 2 to 29. BRV was detected in 46 fecal samples (34.1%), and 30 of these samples were negative for other pathogenic agents. The remaining 16 samples were also positive for bovine coronavirus or Cryptosporidium spp.

Thirty calves from which BRV was only detected in the feces (BRV-diarrheal calves) were monitored daily for signs of diarrhea. Fecal score was determined as follows: 0=normal, 1=soft, 2=pasty, 3=semiliquid, and 4=watery feces. The daily fecal score was totaled as a cumulative diarrhea score to determine the severity of the diarrhea in each calf. Serum samples were collected from all calves on day 2 after birth to examine the transfer of maternal antibodies to the calves. The total IgG concentrations in the sera were measured by a single radial immunodiffusion assay (Saikin kagaku, Japan).

VN antibody titers against the Lincoln strain (G6 P[1]) of BRV in calf sera were determined by fluorescent focus neutralization (FFN) tests with MA104 cells [10]. The antibody titers were expressed as the reciprocal of the highest serum dilution that resulted in an 80% or greater reduction of fluorescent foci.

Significant differences in the VN antibody titers and IgG concentrations between the BRV-diarrheal calves and normal calves were determined by a Student’s t-test. The correlations of the VN antibody titers with age of onset of the diarrhea, duration of the diarrhea, cumulative clinical scores, and duration of the virus shedding in the BRV-diarrheal calves were analyzed by Spearman’s measure of correlation. A statistical significance was assessed at P<0.05.

Clinical signs and duration of the virus shedding in the BRV-diarrheal calves are summarized in Table 1. Diarrhea in the BRV-diarrheal calves started from day 5 to 13 after birth, and lasted for 1 to 9 days. Cumulative diarrhea scores were from 2 to 29. BRV was detected in 46 fecal samples (34.1%), and 30 of these samples were negative for other pathogenic agents. The remaining 16 samples were also positive for bovine coronavirus or Cryptosporidium spp.

Thirty calves from which BRV was only detected in the feces (BRV-diarrheal calves) were monitored daily for signs of diarrhea. Fecal score was determined as follows: 0=normal, 1=soft, 2=pasty, 3=semiliquid, and 4=watery feces. The daily fecal score was totaled as a cumulative diarrhea score to determine the severity of the diarrhea in each calf. Serum samples were collected from all calves on day 2 after birth to examine the transfer of maternal antibodies to the calves. The total IgG concentrations in the sera were measured by a single radial immunodiffusion assay (Saikin kagaku, Japan).

VN antibody titers against the Lincoln strain (G6 P[1]) of BRV in calf sera were determined by fluorescent focus neutralization (FFN) tests with MA104 cells [10]. The antibody titers were expressed as the reciprocal of the highest serum dilution that resulted in an 80% or greater reduction of fluorescent foci.

Significant differences in the VN antibody titers and IgG concentrations between the BRV-diarrheal calves and normal calves were determined by a Student’s t-test. The correlations of the VN antibody titers with age of onset of the diarrhea, duration of the diarrhea, cumulative clinical scores, and duration of the virus shedding in the BRV-diarrheal calves were analyzed by Spearman’s measure of correlation. A statistical significance was assessed at P<0.05.

Clinical signs and duration of the virus shedding in the BRV-diarrheal calves are summarized in Table 1. Diarrhea in the BRV-diarrheal calves started from day 5 to 13 after birth, and lasted for 1 to 9 days. Cumulative diarrhea scores were from 2 to 29. BRV was detected in 46 fecal samples (34.1%), and 30 of these samples were negative for other pathogenic agents. The remaining 16 samples were also positive for bovine coronavirus or Cryptosporidium spp.

Thirty calves from which BRV was only detected in the feces (BRV-diarrheal calves) were monitored daily for signs of diarrhea. Fecal score was determined as follows: 0=normal, 1=soft, 2=pasty, 3=semiliquid, and 4=watery feces. The daily fecal score was totaled as a cumulative diarrhea score to determine the severity of the diarrhea in each calf. Serum samples were collected from all calves on day 2 after birth to examine the transfer of maternal antibodies to the calves. The total IgG concentrations in the sera were measured by a single radial immunodiffusion assay (Saikin kagaku, Japan).

VN antibody titers against the Lincoln strain (G6 P[1]) of BRV in calf sera were determined by fluorescent focus neutralization (FFN) tests with MA104 cells [10]. The antibody titers were expressed as the reciprocal of the highest serum dilution that resulted in an 80% or greater reduction of fluorescent foci.

Significant differences in the VN antibody titers and IgG concentrations between the BRV-diarrheal calves and normal calves were determined by a Student’s t-test. The correlations of the VN antibody titers with age of onset of the diarrhea, duration of the diarrhea, cumulative clinical scores, and duration of the virus shedding in the BRV-diarrheal calves were analyzed by Spearman’s measure of correlation. A statistical significance was assessed at P<0.05.

Clinical signs and duration of the virus shedding in the BRV-diarrheal calves are summarized in Table 1. Diarrhea in the BRV-diarrheal calves started from day 5 to 13 after birth, and lasted for 1 to 9 days. Cumulative diarrhea scores were from 2 to 29. BRV was detected in 46 fecal samples (34.1%), and 30 of these samples were negative for other pathogenic agents. The remaining 16 samples were also positive for bovine coronavirus or Cryptosporidium spp.

Thirty calves from which BRV was only detected in the feces (BRV-diarrheal calves) were monitored daily for signs of diarrhea. Fecal score was determined as follows: 0=normal, 1=soft, 2=pasty, 3=semiliquid, and 4=watery feces. The daily fecal score was totaled as a cumulative diarrhea score to determine the severity of the diarrhea in each calf. Serum samples were collected from all calves on day 2 after birth to examine the transfer of maternal antibodies to the calves. The total IgG concentrations in the sera were measured by a single radial immunodiffusion assay (Saikin kagaku, Japan).

VN antibody titers against the Lincoln strain (G6 P[1]) of BRV in calf sera were determined by fluorescent focus neutralization (FFN) tests with MA104 cells [10]. The antibody titers were expressed as the reciprocal of the highest serum dilution that resulted in an 80% or greater reduction of fluorescent foci.
these calves by the latex agglutination test. There were no differences in the electrophoretic migration patterns of dsRNA among all the BRV in 46 feces which were collected at the first day of diarrhea. These BRVs in feces belonged to serotype G6 and genotype P[5], which were determined by RT-PCR. These results suggested that BRV circulating among these calves was a single strain. There was no significant difference in the serum IgG concentrations between the BRV-diarrheal calves (21.1 mg/mL) and normal calves (22.6 mg/mL). In contrast, the serum VN antibody titers against BRV in the BRV-diarrheal calves (geometric mean antibody titers [GMT]=442) were significantly lower than those in normal calves (GMT=947).

In the BRV-diarrheal calves, a positive correlation was found between the VN antibody titers and the age of onset of diarrhea (r=0.69). There were negative correlations between the VN antibody titers and duration of the diarrhea (r = -0.43), the VN antibody titers and the cumulative diarrhea scores (r = -0.44), and the VN antibody titers and the duration of virus shedding (r = -0.57) (Fig. 1).

In this study, there were significant differences in the serum VN antibody titers against BRV between the BRV-diarrheal calves and normal calves. Additionally, in the BRV-diarrheal calves, the serum VN antibody titers against BRV correlated with the age at onset of the diarrhea, duration of the diarrhea, cumulative diarrhea scores, and duration of the virus shedding. These results suggested that the serum VN antibody titer against BRV in a newborn calf could be an indicator of protection against the BRV-induced diarrhea and severity of the disease. VP7 serotypes of the detected-BRV and the Lincoln strain used for the virus neutralization test were the same G6, but the VP4 genotype of detected-BRV (P[5]) was different from that of the Lincoln (P[1]). The immunogenicity of the VP7 protein is much higher than that of the VP4 protein [4]. Therefore, it is presumed that the serum VN antibody titers against BRV G6 P[5] in the calves are similar to those against BRV G6 P[1].

Studies on passive immunity have demonstrated that continuous feeding of colostrum or milk containing rotavirus-specific antibodies prevented the BRV disease in calves.

### Table 1. Clinical signs and duration of virus shedding in diarrheal calves infected with bovine rotavirus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset of diarrhea (days)</td>
<td>5.0–13.0</td>
<td>8.2 ± 2.32</td>
</tr>
<tr>
<td>Duration of diarrhea (days)</td>
<td>1.0–9.0</td>
<td>4.0 ± 2.98</td>
</tr>
<tr>
<td>Cumulative diarrhea score</td>
<td>2.0–29.0</td>
<td>10.1 ± 7.67</td>
</tr>
<tr>
<td>Duration of virus shedding (days)</td>
<td>1.0–6.0</td>
<td>3.2 ± 1.42</td>
</tr>
</tbody>
</table>

*a) n=30.*
calves, which is called lactogenic immunity [8, 11, 12, 14, 16, 17]. Additionally, circulating maternal BRV-specific antibodies in newborn calves appeared in the gastrointestinal tract, and these antibodies could prevent infection and diarrhea after BRV challenge [1, 2]. The present study suggested that protection against BRV in calves with a high titer of maternal VN antibodies is likely due to both lactogenic immunity and the intestinal serum-derived antibody.

ACKNOWLEDGMENTS. We thank Miss E. Matsumoto and Mr. A. Fujikawa for their assistance.

REFERENCES