

Passive protection of neonatal calves against bovine coronavirus-induced diarrhea by administration of egg yolk or colostrum antibody powder

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Abstract

The protective effect of egg yolk and colostrum powders prepared from hens and cows vaccinated with inactivated bovine coronavirus (BCV) antigen was evaluated in a challenge model with a virulent BCV strain. Twenty three calves from BCV-free herds were randomly divided into control and several treatment groups. All calves were orally challenged with 1×10^9 TCID₅₀ of the virulent Kakegawa strain of BCV at 24 to 36 h after birth. Calves in treatment groups received either egg yolk powder or cow colostrum containing BCV specific antibodies. Daily treatment with these antibody preparations started 6 h until 7 days post-challenge. Control calves which received no antibody had severe diarrhea and all died within 6 days after infection. In contrast, calves fed milk containing egg yolk or colostrum with neutralization titers of 1:2560 or 1:10 240 respectively all survived and had positive weight gain unlike the other treatment groups. These results indicate that the orally administered egg yolk and colostrum powders protected against BCV-induced diarrhea in neonatal calves and that the egg yolk used provided a higher degree of protection compared to colostrum powder on a titer basis. Treatment with whole egg yolk from immunized hens therefore provides a more efficacious alternative to the existing methods of specific passive protection against BCV. © 1997 Elsevier Science B.V.

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1. Introduction

Bovine coronavirus (BCV) is an important agent of neonatal calf diarrhea and is associated with acute diarrhea of adult cattle referred to as winter dysentery. This virus is known to cause a more severe disease and higher mortalities than those caused by the bovine rotavirus because it multiplies in both the small intestine and the large intestine whereas the rotavirus infects only the small intestine (Kapil et al., 1990). To determine protective immune responses against BCV, there have been reports analyzing the titers of passive and active antibody isotypes to BCV in serum and mucosal surface (Heckert et al., 1991a,b). Coronavirus vaccines are available to control BCV diarrhea, but have not been found to be efficacious in protecting against infection (Heckert et al., 1991c; Kapil et al., 1990). In the case of passive immunization, there were few reports on oral administration of antibodies derived from serum or colostrum against BCV. Recently, chicken egg yolk antibodies have been used for prophylaxis and treatment of infectious intestinal diseases. We have been investigating the efficacy of the chicken egg yolk antibodies in calves against enterotoxigenic *Escherichia coli* (ETEC) (Ikemori et al., 1992) and bovine rotavirus (Kuroki et al., 1993). The objectives of the study reported here were (1) to evaluate the efficacy of chicken egg yolk antibodies and cow colostrum antibodies against BCV-induced diarrhea in calves where BCV causes a more severe disease than bovine rotavirus, and (2) to compare the therapeutic value of chicken egg yolk and cow colostrum antibody.

2. Material and methods

2.1. Virus strains

The strain NCDC of BCV was kindly provided by Dr. Murakami, the National Institute of Animal Health, Tsukuba, Japan. This strain was originally isolated by Mebus et al. (Mebus et al., 1973) in the U.S.A.. The Kakegawa strain of BCV was pathogenic to calves and was obtained from the Japanese Association of Veterinary Biologics. Both viruses were certified free from adventitious agents and grown in confluent human rectal tumour cell line (HRT-18) with Eagle's MEM as previously described (Tsunemitsu et al., 1991). The viruses were used for challenge exposure of calves and for production of antibody in chickens and cows.

2.2. Titration for infective viruses and neutralization test

Virus titration and neutralization test were performed in HRT-18 cell cultures grown in microdilution plates and conducted by the method previously described (Tsunemitsu et al., 1991). Briefly, for the titration, serial 10-fold dilutions of viruses were made and inoculated to 4 wells of HRT-18 cells with 0.1 ml of each dilution. The plates were incubated for 7 days at 37°C and the infectivity was expressed by median tissue culture infective dose (TCID₅₀) as determined by the appearance of CPE.

For the neutralization test, egg yolk and colostrum powders were prepared as 10% suspension in PBS. Serial 2-fold dilutions of these samples were mixed with the same

volume of virus suspensions containing 200 TCID₅₀/0.1 ml and incubated for 1 h at 37°C. Two wells of HRT-18 cells were inoculated with 0.1 ml of each mixture and incubated for 7 days at 37°C. The neutralizing antibody titers were expressed as the reciprocal of the highest dilution that inhibited 50% of CPE multiplied by the antibody powder dilution factor.

2.3. Preparation of specific antibody against BCV

Two kinds of specific antibody against BCV were prepared: egg yolk powder and bovine colostrum powder. NCDC antigen containing about 10^{8.5} TCID₅₀/ml was added with 0.3% formalin and inactivated for 1 week at 4°C. The antigen was mixed with an equal volume of oil adjuvant with 5% mannide monooleate (Maine Biological Laboratories, Maine) and 1.0 ml of the mixture was injected intramuscularly into 10 white Leghorn hens. Six weeks after the initial injection, booster inoculation were administered in a similar manner and eggs were harvested 2 weeks later. The yolks were carefully separated from the egg white and was then applied to a spray-dry machine operated at air-inlet temperature of 140°C. The dried egg powder was stored in a desiccator at room temperature until use.

The cows used were 3 to 5 years old and had no history of disease or vaccination against BCV. One ml formalin-inactivated NCDC vaccine was administered intramuscularly in two cows 6 weeks before the anticipated calving followed by a similar dose spaced 3 weeks apart. Early colostrum was collected from cows after parturition and applied to the spray-dry machine operated under the same conditions. The dried colostrum was stored in a desiccator at room temperature until use.

2.4. Challenge exposure and clinical observations

Twenty-three colostrum-deprived, newborn Holstein calves from farms that were free from neonatal diarrhea due to bovine coronavirus, rotavirus, viral diarrhea virus and ETEC were used in this study and were fed a commercially available milk formula. Calves were randomly distributed as non-treated control group (group 1), egg powder treatment groups (groups 2 and 3) and colostrum powder treatment groups (groups 4, 5 and 6). At 24 to 36 h after birth, calves were orally challenge-exposed with 1 × 10^{9.0} TCID₅₀ of the virulent Kakegawa strain (Fig. 1). At 6 h after BCV challenge, the treatment groups were given between 1.5 and 2 l of milk containing the egg yolk or colostrum powder. Subsequently, this was given 2 times per day for 7 days after challenge exposure. Particularly, the egg powder treatment groups 2 and 3 were given milk containing antibody titers of 1:1280 and 1:2560 (0.25 and 0.5 g of egg powder) respectively. On the other hand, colostrum powder treatment groups 4, 5 and 6 were given milk containing antibody titers of 1:2560, 1:5120 and 1:10 240 (0.5, 1.0 and 2.0 g of colostrum powder) respectively. The control group received no antibody. The clinical response of each calf was recorded throughout the experiment and evaluated in terms of fecal consistency score, weight gain, and mortality. Fecal scoring was done by persons who did not know the treatment condition for each calf. Scoring was done 2 times per day with numerical scores as follows: 0 = normal, 1 = soft consistency, 2 = mild

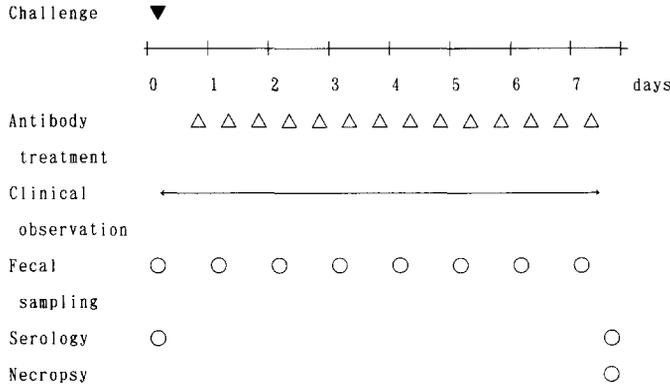


Fig. 1. Summary of the experimental protocol.

diarrhea, 3 = severe watery diarrhea and death. The cumulative fecal score of each calf was expressed as total score for 7 days. Examination of feces for infectious BCV was done daily for 7 days by culturing the homogenates of the specimens in HRT-18 cells. On day 7 post-challenge, samples of the middle part of jejunum and colon were tested for infectious BCV by the same method. Body weight gain of calves was expressed as percentage weight gain at day 7 over initial body weight on day 0. The serum of calves collected at the start and at the end of the experiment were checked for anti-BCV antibodies by neutralization test.

2.5. Statistical analysis

The Student's *t*-test was used to assess the statistical significance of differences in fecal consistency scores, total positive days of virus detection, percentage of body weight change and virus titers in intestines, whereas the Fischer exact test was used to assess differences in mortality between treated and control calves.

3. Results

3.1. Production of antibody powders from immunized egg yolk and colostrums

The *in vitro* neutralizing antibody titers of egg yolk powder from chickens and colostrum powder from cows immunized with the strain NCDC were almost the same (1:5120) against the homologous strain. Apparently, there was not much difference in the magnitude of antibody response between chickens and cows. The antibody titers (1:5120) against the strain Kakegawa were the same as those against the homologous strain in both powders. A cross-reactivity between the two strains in the neutralizing antibody test revealed a close serological relationship between the NCDC and Kakegawa strains (titer of 1:5120 using egg yolk or colostrum against each strain).

3.2. Clinical response of calves after challenge exposure

All calves of the control group (group 1), when exposed to coronavirus, developed severe watery diarrhea including mucus and blood from postchallenge exposure (PE) day 1 to day 2 and died on PE day 6 (Table 1). Severe diarrhea was observed with a mean change in body weight of $-7.4 \pm 2.3\%$ at the time of death. Calves of group 2 which was treated with 1:1600 antibody titer of the egg yolk antibody were not protected against severe diarrhea and mortality. Three calves in this group developed severe diarrhea of which two calves died on PE days 4 and 7 respectively. Although the other calves of group 2 survived the infection, cumulative fecal score was 20.0 ± 17.4 and dehydration resulted in weight loss of 5.1%.

All calves of group 3 recovered from the disease (Table 1). Diarrhea was temporary and was not accompanied by weight loss in these calves. The difference in cumulative fecal score and weight gain between groups 1 and 3 was significant ($P < 0.01$). Based on isolation of coronavirus from feces and intestinal specimens, the total number of days of positive virus detection in the feces in group 3 was 1.8 ± 1.5 days which was of shorter duration than that of group 1 (difference not significant). The titers of coronavirus in the intestines of this group were very low. The virus was not detected in the small intestine of group 3 calves.

On the other hand, all calves of the colostrum-antibody (1:2560) treated group 4 developed severe watery diarrhea and later died on PE day 4 (Table 1). Two calves in group 5 (1:5120) also died with severe diarrhea and the cumulative fecal score and weight loss in this group were as high as those in the control group. In group 6, all calves recovered from disease but 3 calves in this group developed watery diarrhea which continued for 4 to 5 days. The cumulative fecal score in this group was a high 14.8 ± 11.3 together with a low weight gain. The mean total positive days of virus detection in feces among groups 4,5 and 6 were shorter than that of group 1 but this was not statistically significant (Table 2). Titers of coronavirus in the intestine of groups 4, 5 and 6 decreased with increasing colostrum dose and virus titers in the small intestine were generally higher (not statistically significant) than in the large intestine in these 3 groups. The neutralizing antibody titers of the serum of all calves were < 10 against

Table 1
Clinical response of calves to challenge exposure with BCV and subsequent treatment with egg yolk or colostrum antibody powder of different titers

Group	Antibody powder	Antibody titer	No. diarrhea/ total no. (%)	No. dead/ total no. (%)	Cumulative fecal score	Weight gain (%)
1	—	—	4/4 (100)	4/4 (100)	30.5 ± 6.1	-7.4 ± 2.3
2	Egg	1280	3/4 (75)	2/4 (50)	20.0 ± 7.4	-5.1 ± 10.4
3	Egg	2560	2/4 (50)	0/4 (0)*	$3.8 \pm 3.8^{**}$	$6.3 \pm 2.4^{**}$
4	Colostrum	2560	3/3 (100)	3/3 (100)	32.3 ± 0.6	-9.6 ± 1.5
5	Colostrum	5120	4/4 (100)	2/4 (50)	28.8 ± 3.4	-8.2 ± 5.3
6	Colostrum	10 240	3/4 (75)	0/4 (0)*	$14.8 \pm 11.3^*$	$1.2 \pm 3.2^{**}$

* $P < 0.05$, ** $P < 0.01$.

Table 2

Detection of BCV in feces from daily fecal samples after challenge exposure and from intestines at the time of necropsy among calves treated with egg yolk or colostrum powder of different titers

Group	Total positive days of virus detection (mean \pm S.D.)	Virus titers (TCID ₅₀ /g)	
		Small intestine (mean \pm S.D.)	Large intestine (mean \pm S.D.)
1	6.0 \pm 0.8	4.63 \pm 0.14	5.00 \pm 1.00
2	5.5 \pm 1.9	2.38 \pm 2.74	4.00 \pm 1.70
3	1.8 \pm 1.5	0.00 \pm 0.00*	1.25 \pm 1.44*
4	6.3 \pm 0.6	5.41 \pm 0.14	5.08 \pm 0.72
5	5.5 \pm 1.0	2.81 \pm 2.08	2.75 \pm 2.02
6	3.5 \pm 2.4	1.25 \pm 1.40*	0.63 \pm 1.25*

All mean values had $n = 4$ except for group 4 where $n = 3$, * $P < 0.05$.

BCV before challenge. Similar titers were obtained 7 days later indicating that antibodies from administered egg yolk and colostrum did not enter the systemic circulation of calves.

4. Discussion

In this study, we evaluated the efficacy of the antibody preparations derived from egg yolks of immunized laying hens and colostrums of immunized cows against experimentally induced bovine coronavirus calf diarrhea. This is to fill the gap in the literature concerning passive protection against BCV using specific antibody. Our data showed that significant protection was achieved in calves treated with high titers of the egg yolk and colostrum antibodies against the challenge strain (Table 1). Survival against coronavirus-induced mortality was 100% when 1:2560 and 1:10 240 antibody titers were used for treatment of calves with the egg yolk or colostrum powder, respectively. Particularly, the calves treated with the egg yolk antibody did not have severe diarrhea and had higher weight gains. About 8 g of egg yolk powder were obtained from one chicken egg and only one egg yolk was needed to protect one calf from diarrhea over a 7-day course of treatment. The present data corroborate an earlier protection trial using egg antibody specific for ETEC in ETEC-infected calves (Ikemori et al., 1992).

The minimal antibody titers against fatal diarrhea were found to be 1:2560 for the egg yolk and 1:10 240 for the colostrum. Although diarrhea was observed in calves treated with these antibody powders, the cumulative fecal scores were higher in the calves treated with colostrum than those treated with egg yolk. It appears that greater amount of colostrum powder than egg yolk powder is necessary to prevent diarrhea. The virus titers in the intestines of groups 4 to 6 calves were high irrespective of the dose of antibody given; virus titers in the small intestines tended to be higher than in the large intestines in the above groups (Table 2). It was speculated that continued viral proliferation in the small intestine was the reason for the persistent diarrhea observed in these groups of calves.

The difference in the minimal protective titers of the antibody powders between the egg yolk and the colostrum may have two possible explanations. Firstly, the avidity of

antibodies derived from colostrum is lower than that of antibodies obtained from egg yolk (Ikemori et al., 1993). Compared to colostral antibodies, the BCV specific antibody from egg yolk may have reacted more strongly and stably with coronavirus epitopes *in vivo* in the neutralization reaction. Secondly, less chicken antibody may have been digested and inactivated by the gastric juice. The antibodies that escaped digestion in the stomach are still functional in the small intestine. The antibody of the egg yolk is thought to be of almost the same stability or slightly more susceptible to gastric juice than mammalian antibody (Shimizu et al., 1992). However, the present data demonstrated that the antibody from egg yolk was more effective than colostral antibody suggesting that yolk components in the egg yolk powder such as proteins and fats may have protected the immunoglobulin fraction from digestive enzymes and allowed safe passage of yolk immunoglobulins through the stomach enough to confer protection in the target areas of the small intestine of calves. On the other hand, colostral proteins and fats may be more susceptible to digestion by gastric juice or quantitatively less than those of the egg yolk.

In conclusion, the egg yolk powder from chickens immunized with bovine coronavirus has a greater therapeutic potential against BCV-induced diarrhea than colostrum powder from immunized cows. It took about four times more colostral antibody than egg yolk antibody to prevent mortality in calves. Oral egg yolk powder containing specific antibodies may therefore provide an alternative approach to passive prevention of BCV infections in the field.

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