Passive Protection against Porcine Epidemic Diarrhea (PED) Virus in Piglets by Colostrum from Immunized Cows

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ABSTRACT. The effects of hyperimmune cow colostrum (HCC) on experimentally induced porcine epidemic diarrhea (PED) were inves-
tigated in piglets. In experiment 1, four 2-day-old piglets fed HCC containing an antibody titer of 1:512 and another four piglets fed unimmune cow colostrum (UCC) were orally inoculated with 10LD50 of PED virus. The piglets were given colostrum three times a day at 4 hr intervals. Half of the piglets fed HCC showed diarrhea and recovered, and all piglets survived. In contrast, all piglets fed UCC developed diarrhea and three of them died. In experiment 2, 2-day-old piglets fed HCC containing antibody titers of 1:512, 1:128 and 1:32, and UCC were inoculated with PED virus, and survival rates after challenge were 100, 75, 50 and 0 %, respectively. In experiment 3, 1-day-old piglets fed HCC with 1:512 antibody titer or UCC were inoculated and necropsied at 24, 48 and 72 hr after the inoculation for pathological examination. Piglets fed HCC remained healthy and PED virus antigen was not detected in the epithelial cells of the small intestine, and the length of the villi in small intestine was normal. On the other hand, in piglets fed UCC, villous atrophy and PED virus antigen were observed in epithelial cells of the jejunum and ileum from 24 hr. It was concluded that oral administration of HCC to piglets was effective in preventing PED virus infection and reduced their mortality.

KEY WORDS: cow colostrum, passive protection, porcine epidemic diarrhea virus, swine.

Porcine epidemic diarrhea (PED) causes acute and highly contagious viral enteritis in pigs. The principal features of PED are watery diarrhea, dehydration and high mortality in suckling pigs. In 1978, a coronavirus-like particle was first identified during episodes of epizootic diarrhea in pigs in Belgium [8] and the United Kingdom [2, 15]. In Japan, PED-like disease occurred from late 1982 to early 1983 [6, 13]. After that, the disease also occurred between late 1993 and 1996 [12, 14]. In 1996, acute outbreaks occurred and more than 39,000 suckling pigs died [14].

It is thought that protection from the clinical effects of PED virus infection can be achieved by the feeding of a specific antibody provided by colostrum from a naturally infected sow. Recently, Kweon et al. [7] showed that chicken egg yolk immunoglobulin (IgY) against PED virus reduced mortality in piglets after challenge exposure. Also, it has been reported that bovine immune-colostrum-protec-
ted piglets from clinical illness by a porcine rotavirus [1] and a transmissible gastroenteritis virus [11]. The present study was designed to test whether colostrum from immunized cows could protect piglets from clinical illness caused by a PED virus.

MATERIALS AND METHODS

Virus strains: The PED virus used for piglet inoculation was the Z94P5 strain passed serially three times in cesarean-derived colostrum-deprived pigs [10]. The 10% pooled homogenates of small intestines were centrifuged and the supernatants were passed through a 450 nm membrane filter. For use in the challenge exposure of pigs, the resulting virus fluid was diluted to 10 LD50/2 ml for a 3-day-old piglet. The tissue culture-adapted Z94P5 strain was used for cow immunization. For the serological test, the NK94P6 strain [14] passed in Vero cell culture was used.

Animals: Conventionally reared piglets that had suckled colostrum from sows were obtained from a herd which was seronegative for PED virus-neutralizing antibodies. They were weaned onto an artificial diet at the age of one or two days, and were reared in a barrier-maintained room.

Immunization of cows and preparation of hyperimmune cow colostrum: The cell cultures infected with the Z94P5 strain were centrifuged and the virus was precipitated from the suspension by 7% polyethlene glycol-6000 and inacti-vated with formaldehyde. The PEG-treated viral solution had a titer of 108.75 TCID50/ml before inactivation. For the first inoculation, inactivated virus fluid was mixed equally with complete Freund’s adjuvant. Three cows were immu-nized intramuscularly with the inactivated virus at a dose of 3 ml three times at 2-week intervals and third immunization was done two weeks before parturition.

Hyperimmune cow colostrums (HCC) was prepared from the colostrum collected within 24 hr of parturition from immunized cows. Fat was removed from the colostrum by centrifugation. The samples were stored at –20°C until use. Unimmune cow colostrum (UCC) which was seronegative for PED virus-neutralizing antibodies was prepared in the same manner as the control colostrum.

Serological examination: The virus neutralization (VN) test was carried out using the microtiter method employing Vero cells as reported previously [14]. The antibody titer was expressed as the reciprocal of the highest serum dilution inhibiting cytopathic effect (CPE) in at least one of the 2 wells.

Experimental design: In experiment 1, 10 piglets were randomly divided into three groups at 2 days of age. Four
piglets were orally given HCC containing an antibody titer of 1:512 (group 1) and another four piglets were given UCC (group 2). They were fed 2 ml of cow colostrum three times a day at 4 hr intervals from 2 days of age until the end of the examination. These eight piglets were inoculated orally with 10 LD_{50} of the Z94P5 strain 3 hr after the first colos- 

trum administration. The remaining two piglets given UCC were left uninoculated to serve as controls (group 3). They were observed daily for signs of clinical disease and were bled weekly for serum. Surviving piglets were euthanatized and necropsied on post-inoculation day (PID) 14.

In experiment 2, 14 piglets were randomly divided into four groups: 2 piglets were orally given undiluted HCC containing an antibody titer of 1:512 (group 4); 4 were given HCC 4-fold diluted with UCC (antibody titer of 1:128) (group 5); 4 were fed 16-fold diluted HCC (antibody titer of 1:32) (group 6); and the remaining 4 were fed UCC (anti-

body titer of <1:2) (group 7). Other experimental conditions were the same as those of experiment 1. Piglets were inoculated orally with 10 LD_{50} of the Z94P5 strain in the same manner as in experiment 1.

In experiment 3, 6 one-day-old piglets were randomly divided into three groups: 3 piglets were orally given HCC containing an antibody titer of 1:512 (group 8) and necropsied at 24, 48 and 72 hr after inoculation; 2 piglets were fed UCC (group 9) and necropsied at 24 and 72 hr after inoculation; the remaining piglet was fed HCC and was not challenged (group 10). At the necropsy, small intestines were collected from these piglets for pathological examination.

Pathological examination: Histopathological examination was performed according to routine procedures. In brief, tissue samples were fixed in 20% neutral phosphate buffered formalin. Thin sections of paraffin-embedded samples were stained by hematoxylin and eosin. The avidin-biotin (AB) technique was used for detection of PED virus antigen in the small intestine as reported previously [10]. In experiment 3, the lengths of villi at the duodenum, jejunum and ileum were measured by an image analysis pro-

cessor (Nexus Qube 2: Nexus Inc. Japan).

RESULTS

Hyperimmune bovine colostrums: The neutralizing antibody titers of colostrums collected within 24 hr of parturi-

tion from three immunized cows with PED virus were 1:256, 1:256 and 1:512. HCC with 1:512 antibody titer was used for administration to the piglets. The antibody titer of the control colostrum from the unimmunized cow was <1:2.

Experiment 1: In group 1, two of the four piglets fed HCC showed diarrhea for three days and recovered, while the remaining piglets showed no clinical signs. All of them survived. In group 2, all piglets fed UCC developed diarrhea with watery faeces and vomiting after inoculation (Table 1). These piglets were depressed and anorectic. They showed dehydration and lost body weight, and three of them died on PIDs 3 and 5. Pig No.8 showed diarrhea for 8 days and recovered. In contrast, uninoculated piglets in group 3 remained healthy. The small intestine villi of the dead pig-

lets in group 2 showed marked atrophy, and PED virus antigens were detected in epithelial cells of the jejum and ileum by the immunohistochemical method. In the small intestines of the surviving piglets examined PID 14, PED virus antigen was not detected. VN antibody was not detected in any of the surviving piglets, except No.8 in group 2, on PID 14.

Experiment 2: The piglets fed HCC with 1:512 antibody titer in group 4 developed diarrhea for 2 days and recovered (Table 2). The piglets fed HCC with 1:128 antibody titer in group 5 developed diarrhea between PIDs 1 and 8 and one pig died on PID 8. In group 6, all piglets, except No.7, fed HCC with 1:32 antibody titer showed diarrhea and two pig-

lets died on PID 4 or 6. All piglets fed UCC in group 7 developed diarrhea with watery faeces within 24 hr after inoculation. These piglets were depressed and anorectic, but not detected in any of the surviving piglets, except No.8 in group 2, on PID 14.

Table 1. Effect of administration of hyperimmune cow colostrum on the course of PED in piglets (experiment 1)

<table>
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<tr>
<th>Group No.</th>
<th>Antibody titer in colostrum</th>
<th>Pig No.</th>
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and died between PIDs 4 and 7. VN antibodies were detected in the all surviving piglets on PID 14.

Experiment 3: Piglets fed HCC and challenged in group 8 and the uninoculated control piglet in group 10 remained healthy. Piglets fed UCC in group 9 developed diarrhea with watery faeces and vomiting within 24 hr after inoculation. The villi in the jejunums and ileums of group 9 were atrophied and apparently shorter than those in groups 8 and 10 (Table 3), and PED virus antigens were detected in high concentrations in epithelial cells of the jejunum and ileum of these piglets by the immunohistochemical method. The villi of the piglets in group 8 showed no changes at 24, 48 and 72 hr after inoculation and no virus antigen was detected in the small intestines of these piglets.

DISCUSSION

In this study, we evaluated the efficacy of HCC against experimentally induced PED. The administration of HCC had a marked effect on the clinical course of PED. The protective effect of HCC was manifested mainly by milder clinical symptoms and markedly reduced mortality, especially in the piglets in groups 1 and 4 treated with undiluted HCC.Survival rates after PED virus challenge were 100, 75 and 50% when HCCs with 1:512, 1:256 and 1:64 antibody titers, respectively, were orally administered to piglets. All of the control piglets fed UCC in group 7 had died by PID 7.

The piglets in groups 1, 4 and 8 were fed HCC with high antibody titer (1:512). Virus multiplication in the piglets in group 4 was confirmed by the presence of VN antibody against PED virus in the convalescent sera. On the other
hand, antibody response was not recognized until PID 14 in piglets of group 1. In group 1, the viral multiplication in the intestine would have been almost completely prevented, but not in group 4. Furthermore, PED virus antigen was not detected in epithelial cells of the small intestines of piglets in group 8 between 24 and 72 hr after inoculation. These results indicate that the multiplication of the virus in the small intestine is possibly prevented or reduced by feeding with HCC with high antibody titer.

It has been reported that villous atrophy was observed in the jejunum at the onset of clinical signs in two- to three-day-old piglets experimentally infected with PED virus [3, 4, 12], and the length of the villi in these pigs was about 200 to 300 μm [5]. Our results regarding the infected piglets fed UCC in experiment 3 closely resembled these findings. On the other hand, the length of the small intestinal villi of the piglets fed HCC varied between 500 and 1,050 μm after PED virus inoculation, which was almost the same as that of the uninoculated control piglet. This indicated that the administration of HCC protected villous shortening by PED virus infection.

In a preliminary examination, the protective effect of HCC with 1:512 antibody titer against PED virus infection in piglets was evaluated using inoculated virus titer of 10^{3.3} LD_{50}. Although the development of clinical signs in the piglets fed HCC were delayed compared with those fed UCC, all piglets fed HCC or UCC died on PID 4. Consequently, we used a challenge exposure dose of 10 LD_{50} of the virulent Z94PS strain for the evaluation of HCC in the present study. The level of field exposure in herds with PED should vary. To protect piglets from a great amount of PED virus exposure, HCC with higher antibody titer against PED virus than that used in present study would be necessary. Stepánek et al. [11] reported that a higher degree of protection against TGE virus infection was found in piglets that were given HCC at 2 hr intervals than at 4 hr intervals. Cow colostrum immunoglobulins persist in the intestinal tract in active form only for a limited length of time as reported previously [9]. In this study, piglets were administered HCC only three times a day at 4 hr intervals. The administration of HCC would be more effective when pigs were fed at more frequent intervals (eg., at 2 hr intervals).

Kweon et al. [7] showed that IgY against PED virus with 1:32 to 64 VN antibody titers reduced mortality in piglets after challenge exposures. In that report, the mortality rates were 58.8% and 26.3% in control and treatment groups, respectively, after challenge with a 5 LD_{50} dose of PED virus. The efficacy of IgY administration against PED virus infection is similar to that of HCC shown in the present study. These results show that oral administration of immune substances is effective against PED virus infection in piglets.

From the results of our study, we concluded that administration of HCC protected piglets from the clinical effects of PED virus infection and considerably reduced their mortality. Our previous study indicated that piglets less than 7 days old developed severe clinical signs and died, while 2-week-old pigs developed diarrhea and recovered after PED virus exposure [10]. In pig herds with serious problems due to diarrhea caused by PED virus, HCC should be administered at least for 2 weeks after birth to protect piglets from clinical illness.

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REFERENCES