Fatal Winter Dysentery with Severe Anemia in An Adult Cow

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ABSTRACT. An adult dairy cow fatally affected with winter dysentery was investigated pathologically and virologically. The cow had severe anemia and diarrhea with massive blood. Pathologically, the loss of surface epithelial cells and necrosis of crypt epithelial cells in the large intestine were observed. Bovine coronavirus (BCV) antigen was observed in necrotic crypt epithelial cells of the large intestine. Virologically, BCV was isolated from the feces of the dead cow. The dead cow had no serum antibody against BCV although the co-habitants did. These suggest that severe infection of BCV in the cow without the BCV antibody accompanied by severe hemorrhagic anemia resulted in the cow’s death.

KEY WORDS: adult cow, fatal case, winter dysentery.

Virology

NOTE

Bovine coronavirus (BCV) is a RNA virus belonging to the group 2 member, of the Coronaviridae. BCV has a tropism to the small and large intestine and upper respiratory tract [11]. BCV is a causative agent of neonatal calf diarrhoea, winter dysentery of adult cattle, and respiratory disease in cattle [1]. Epidemic diarrhoea of adult cattle has occurred in Japan [6, 12]. The mortality of adult cattle due to winter dysentery is rare [7]. Not only adult cattle affected with dysentery but also normal adult cattle shed BCV in the feces [4]. Thus a pathological examination and virological examination are important to evaluate the role of BCV on the diarrhea in cattle. There are few reports on pathological changes in adult cattle naturally affected with winter dysentery, although pathological studies were reported in natural neonatal calf diarrhea [9, 11, 16]. This paper describes the pathology and virology of a dead adult cow affected naturally with winter dysentery.

Twenty-three adult dairy cows and four growing calves (Holstein) were kept in the barn of the farm. In November 2002, a 27-month-old cow (cattle No. 1) exhibited depression, anorexia, and diarrhea with blood (Fig. 1). The diarrhea with blood continued for two days until the cow died. The cow had delivered a calf three month earlier. The cow was treated with anti-hemorrhage medicine, obstipantia, and infusion solution. However diarrhea with blood continued in the cow. A hematological examination of the cow confirmed severe anemia (2,790,000 erythrocytes/μL, hematocrit; 16%) (Table 1). Two co-habitants (cattle Nos. 2 and 3) reared in the stall next to the dead cow revealed soft feces at the same time and three days later they recovered. Other co-habitants (cattle Nos. 4 to 6) had no clinical signs. The farm also experienced diarrhea probably caused by BCV at one year before the present onset. Only cattle No.1 had been kept on another farm at the onset prior to being introduced to the present farm. Other co-habitants (cattle Nos. 2–6) were born in the present farm and had been kept ever since. Then cattle No.1 had been introduced to the present farm. The farm’s cattle had not been vaccinated for BCV. All cattle except for cattle No. 5 were lactating.

Cattle No. 1 was autopsied on the day it died. After post-mortem examination, the liver, spleen, kidney, heart, omasum, abomasum, duodenum, small intestine, and large intestine, were fixed with 10% buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE).

The BCV antigens in the formalin-fixed, paraffin-embedded tissues of the intestine and other organs were discovered by the streptavidin-biotin complex immunoperoxidase method “Histofine SAB-PO(R) kit” (Nichirei Inc., Tokyo). Rabbit antiserum against BCV was used as the primary antibody at a dilution of 1:1,200.

Virus isolation from the feces of cattle Nos. 1 to 6 was attempted by cell cultures; HRT-18 cell for BCV [8] and bovine fetal aortal endothelial (BAT) cell for bovine viral diarrhea virus (BVDV) and infectious rhinotracheitis virus (IBRV). The samples were diluted 1:10 in Eagl’s minimal essential medium (MEM), and clarified by centrifugation (800 × g for 5 min). The supernatants were filtered with 450nm membrane filter. These samples were inoculated onto the HRT-18 cells and the BAT cells grown in roller tubes, and incubated in roller drum for 7 days at 37°C and observed for cytopathic effects (CPE). Confirmation of the isolates as BCV was done by using indirect immunofluorescence test [2].

The ribonucleic acid (RNA) was extracted from the feces of cattle Nos. 1 to 6 with a commercial kit “RNasy Mini kit” (Qiagen GmbH, Tokyo). The BCV-specific primers that framed a region within the gene coding for BCV nucleocapsid designated upstream primer (5'-GGCGATCAGTC-
CGACCAATC-3') and P2 (5'-AGAATGTCAGCCGGGG TAT-3') were used [14]. The size of amplified RNA fragment using these two primers was expected to be 407 base pair (bp) long. Reverse transcriptase PCR (RT-PCR) was done with “One Step RT-PCR Kit" (Qiagen Gmbh, Tokyo). RT-PCR of the feces was also done for the detection of BVDV [15]. A commercial kit “Rapid Tester Rota-Adeno kit” (Orion Diagnostica, Espoo, Finland) for the detection of human rotavirus and adenovirus antigens in the feces were applied to detect BCV and bovine adenovirus, having the common antigens with human rotavirus and adenovirus, in the feces of cattle Nos. 1 to 6. Serum antibodies against BCV and bovine adenovirus type 7 (ADV-7) were measured by hemagglutination inhibition (HI) test [5]. Serum antibodies against BVDV (Nose strain of BVDV-1), IBRV (No. 758 strain) and bovine parainfluenza virus type 3 (PIV-3) (BN-1 strain) were detected using the neutralization test.

Bacteriologically, the heart, liver, kidney and spleen of cattle No. 1 were cultured on 5% sheep blood agar plates and DHL agar plates (Eiken Chemical Co., Ltd., Tokyo) for the isolation of Escherichia coli. The content of duodenum of cattle No. 1 was cultured anaerobically to isolate Clostridium by CW agar plate (“CW Agar Base with Kanamycin”, Nissui Pharmaceutical Co., Ltd.) containing 5% yolk and chocolate agar plates containing 5% sheep blood and aerobically to isolate Escherichia coli by 5% sheep blood agar plates and DHL agar plates. To isolate Salmonella, the feces of cattle Nos. 1 to 6 were enriched in Haina-trathionate broth (Eiken Chemical Co., Ltd.) and then grown in “ES Salmonella agar plates” (Eiken Chemical Co., Ltd.).

Parasitologically, the feces of cattle Nos. 1 to 6 were sampled to detect the eggs of parasites by the floating method using saturated saline solution.

Macroscopically, severe congestion and hemorrhages were observed on the mucous membrane of the small intestine. The lumen of the large intestine was filled with blood clots (Fig. 2).

Histologically, the small intestine had mild villous atrophy with occasional hemorrhages in the lamina propria. The loss of surface epithelial cells, and degeneration, necrosis and desquamation of crypt epithelial cells (Fig. 3) with mild hemorrhages of lamina propria were present in the large intestine. There were no significant histological lesions in the other organs.

Immunohistochemically, the crypt epithelial cells of the large intestine show strong positive reaction against BCV antigen (Fig. 4). There was no positive reaction against BCV in the duodenum, the small intestine, omasum, abomasums, liver, spleen, kidney or heart.

Ultrastructurally, the degenerative crypt epithelium had numerous aggregates of viral particles approximately 60 nm in diameter in the vesicles of the cytoplasm (Fig. 5).

The BCV-specific gene was detected by RT-PCR in the feces of cattle Nos. 1 to 3 that suffered diarrhea or soft feces, but not in cattle Nos. 4 to 6 without diarrhea. No BVDV-specific gene was detected by RT-PCR in the feces of any cattle. Rotavirus and adenovirus antigens were not detected by Rota-Adeno antigen detection kit in the feces of any cattle.

BCV was isolated from the feces of cattle No. 1. There was no viral isolation from the feces of cattle Nos. 2 to 6.

When the disease occurred, the HI antibody titer against BCV was less than 10 in cattle No.1, but was high in cattle Nos. 2 to 6 (Table 2). Three weeks after onset, the BCV HI antibody titers of cattle Nos. 2 to 6 increased. There were no significant elevation of antibodies against ADV-7 and PIV-3 of cattle Nos. 1 to 6. HT antibodies against BVDV and IBRV were negative in all cattle.

No bacteria were isolated from the organs of Cattle No. 1. The Clostridium was less than 10² CFU/g, and E. coli less than 10⁶ CFU/g in the rectal contents of cattle No. 1. No Salmonella was isolated from the feces of cattle Nos. 1–6.

The numbers of the oocysts of Coccidium in the feces of cattle Nos. 1 to 6 were less than 10⁶ oocysts/g.

Mebus et al. [9] reported pathological findings of experimental cases of calf diarrhea. The small intestine had BCV antigens in the villous epithelial cells with histological lesions at the onset of diarrhea. The lesions in natural cases of neonatal calf diarrhea and winter dysentery were compared [16]. The lesion of winter dysentery was mainly localized in the colon. It consisted of pyknosis, karyorrhexis, granular degeneration, hydropic degeneration, and hyaline droplet degeneration of crypt epithelial cells. In neonatal calf diarrhea, the lesions were confined to the small intestine, colon and regional lymph nodes. In particular, the colon was severely affected and had extensive crypt epithelial cell pyknosis, karyorrhexis, lysis and regenerative hyperplasia. Thus, Van Kruiningen et al. [16] named neonatal calf diarrhea and winter dysentery as “virus-induced

<table>
<thead>
<tr>
<th>Cattle No.</th>
<th>Clinical signs</th>
<th>Erythrocytes (10⁶/µL)</th>
<th>Hematocrit (%)</th>
<th>tMCH (fL)</th>
<th>Hemoglobin (g/dL)</th>
<th>WBC (10⁶/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>dysentery, dead</td>
<td>279</td>
<td>16</td>
<td>47.3</td>
<td>4.5</td>
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<tr>
<td>2</td>
<td>soft feces</td>
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<td>29</td>
<td>51.7</td>
<td>8.1</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>soft feces</td>
<td>432</td>
<td>30</td>
<td>53.7</td>
<td>8.4</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
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<td>31</td>
<td>48.0</td>
<td>8.9</td>
<td>87</td>
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<tr>
<td>5</td>
<td>normal</td>
<td>416</td>
<td>30</td>
<td>48.8</td>
<td>7.5</td>
<td>72</td>
</tr>
<tr>
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<td>normal</td>
<td>515</td>
<td>31</td>
<td>44.9</td>
<td>8.2</td>
<td>92</td>
</tr>
</tbody>
</table>

MCH: Mean corpuscular hemoglobin. WBC: White red cells.
enterocolitis”.

In experimental cases of neonatal calf diarrhea [9], BCV antigens are demonstrated in the surface epithelium of small intestine and colon with villous atrophy at the onset of diarrhea, and in the surface and crypt epithelium of colon. BCV antigens were present in the crypt epithelial cells of the colon in natural cases of neonatal calf diarrhea and winter dysentery [16]. The distribution of BCV antigen in the present case was similar to these reports.

Other viral infections and bacterial and parasitic infections may contribute to the pathological conditions in coroaviral diarrhea [7, 10]. However, there was no evidence for the involvement of other pathogens in the present case.

Usually the calves affected with neonatal calf diarrhea exhibit weakness, depression, and lethargy. Some of them die from severe hydration and hypovolemic shock [7], while winter dysentery of adult cattle is rare. Experimental studies of BCV in calves and lactating adult cows without BCV
antibody suggested that lactating adult cows were affected severely with BCV than calves [3, 13]. The cattle No. 1 of the present case had no BCV antibody and three months after delivery was most lactating stage. The cattle No.1 had severe anemia probably due to the loss of massive blood by dysentery. Generally, the number of erythrocytes maintains by the homeostasis of the host even when the erythrocytes disappear in the peripheral blood. There are no reports on anemia in the adult cattle affected with winter dysentery. Therefore, severe anemia of cattle No. 1 may have been caused by acute and massive hemorrhage in the intestinal tract. In addition, the lactation may have been a stress factor that induced the mortality.

ACKNOWLEDGEMENTS. We thank Mr. Masaru Kobayashi and Ms. Megumi Shimada for excellent technical assistance.

REFERENCES


Table 2. Serum antibodies against bovine viruses in the dead cow and co-habitant

<table>
<thead>
<tr>
<th>Cattle No.</th>
<th>Clinical signs</th>
<th>Hemagglutination inhibition antibody titers</th>
<th>Neutralization antibodies titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BCV</td>
<td>BADV-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>At onset</td>
<td>Post</td>
</tr>
<tr>
<td>1</td>
<td>dysentery</td>
<td>&lt;10</td>
<td>NT</td>
</tr>
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<td>2</td>
<td>soft feces</td>
<td>160</td>
<td>640</td>
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<td>80</td>
<td>1280</td>
</tr>
<tr>
<td>6</td>
<td>normal</td>
<td>160</td>
<td>640</td>
</tr>
</tbody>
</table>

BCV: Bovine coronavirus; BADV-7: Bovine adenovirus type 7; BVDV: Bovine viral diarrhea virus; IBRV: Infectious bovine rhinotracheitis virus; PI-3: Bovine parainfluenza virus type 3. Post, at 21 days after the onset of winter dysentery. NT: Not tested.