Efficacy of Bovine Viral Diarrhea Vaccine Used in Japan against Bovine Viral Diarrhea Virus Type 2 Strain 890

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ABSTRACT. Bovine viral diarrhea virus (BVDV) has been segregated into two genotypes, type 1 and type 2. To determine the efficacy of the commercially available bovine viral diarrhea type 1 vaccine used in Japan against BVDV type 2, calves were infected with BVDV type 2 strain 890 4 weeks after administration of the vaccine. The vaccinated calves did not develop any clinical signs and hematological changes such as observed in unvaccinated calves after the challenge. Furthermore, the challenge virus was not recovered from the vaccinated calves throughout the duration of the experiment, whereas it was recovered from all unvaccinated calves. The bovine viral diarrhea vaccine used in Japan is efficacious against infection with BVDV type 2 strain 890.

KEY WORDS: antigenicity, BVDV, vaccine.

Bovine viral diarrhea is an economically important disease of cattle having been reported throughout the world and is caused by infection with bovine viral diarrhea virus (BVDV) [1, 5]. BVDV is a member of the genus Pestivirus within the family Flaviviridae [14]. There are uniquely two biotypes, the cytopathogenic (CP) biotype and the non-cytopathogenic (NCP) biotype, in BVDV.

Recently, BVDV has been segregated into two genotypes, type 1 and type 2, by phylogenetic analysis based on comparison of sequences from the 5'-untranslated region of the genome [21]. Originally, the new genotype, BVDV type 2, was identified in severe outbreaks of acute hemorrhagic disease in the United States and Canada [7, 8] and subsequently identified in Europe, South America and Japan [6, 16, 22, 24]. In addition to the classical clinical signs, some virulent strains of BVDV type 2 cause severe thrombocytopenia with hemorrhage [8, 20]. Regrettably, the pathogenesis of the clinical outcome is still unclear.

Antigenic heterogeneity in BVDV has been demonstrated by some investigators [3, 4, 10, 12, 19, 21, 23], but their serotypes have not been established yet. Especially, BVDV type 2 is antigenically distinct from BVDV type 1 [20, 21]. In our previous study, we reported that three strains of BVDV type 2 isolated in Japan antigenically differed from BVDV type 1 strain No. 12–43, which is used to prevent these infectious diseases in Japan. The other group (Calves D and E) served as the unvaccinated control group. Four weeks after the administration of the vaccine, both groups were infected intravenously with BVDV type 2 strain 890 (10⁴ TCID₅₀/head). General clinical observation was performed daily during the whole experiment. Special attention was paid to depression, anorexia and diarrhea. The clinical parameters were recorded daily as described previously [15]. The total daily clinical scores were calculated. The rectal temperature of each calf was recorded every morning from 4 days before the challenge to 2 weeks after the challenge.

For isolation of the challenge virus, buffy coats were collected from all calves after the challenge. The virus isolation procedure was a modified method based on the interference phenomenon as described previously [17, 18], because strain 890 is a NCP biotype. Additionally, the existence of strain 890 was checked by RT-PCR and detection of the PstI site on the products was performed as described previously [22].

Blood samples were collected from all the calves 2 and 4 days prior to the challenge, on the day of the challenge, and on every day for 2 weeks after the challenge. The number of total red blood cells (RBC), total white blood cells (WBC) and total platelets (PLT) were counted immediately with an automatic cytometer machine, PCE-170 (ERMA Inc.).

Sera collected from all the calves every week after the vaccination were examined for neutralizing antibody to BVDV type 2 strain 890 and type 1 strain No. 12–43 by the
A pronounced increase in rectal temperature was measured in both unvaccinated calves. Seven days after the challenge, the average rectal temperature of the unvaccinated calves had risen to 41.15°C (Calf D: 40.9°C, Calf E: 41.4°C) (Fig. 1A, filled symbols). On the following days, however, they had rectal temperatures within the reference range. On the other hand, the average rectal temperatures of the vaccinated calves continued within the reference range throughout the duration of the experiment (Fig. 1, empty symbols).

The clinical signs were validated using an established scoring system. The clinical scores were recorded from 4 to 8 days after the challenge in the unvaccinated calves (Fig. 1B). They showed clear peaks at 6 and 7 days after the challenge. In contrast, all the total daily scores were “0” in the vaccinated calves.

BVDV was not isolated from buffy coats collected from the unvaccinated calves at 1 to 3 and 7 to 14 days after the challenge. But, BVDV was isolated from buffy coats collected from the unvaccinated calves at 4, 5 and 6 days after the challenge (Fig. 1B, cross symbols). Furthermore, specific fragments of expected size (256 bps) were observed in electrophoresis as a result of RT-PCR. The fragments were still 256 bp after digestion of the restriction enzyme PstI. Thus it was confirmed the isolation of BVDV type 2.

In contrast, BVDV was not isolated from any vaccinated calves throughout the duration of the experiment, and no specific fragment was amplified, either.

There was no remarkable change of RBC count in all the calves after the challenge (data not shown). The unvaccinated calves had markedly decreased WBC count starting 2 days after the challenge (Fig. 1C, filled symbols). The average WBC count was lowest at 2 days after the challenge, then recovered gradually and reached the baseline value by the end of the experiment. On the other hand, the average WBC count in the vaccinated calves did not decrease as drastically as in the unvaccinated calves (Fig. 1C, empty symbols). The PLT count of one unvaccinated calf (Calf D) decreased temporarily from 2 days to 4 days after the challenge, but there was no marked change of average PLT count (data not shown).

As expected, neutralizing antibody titers against BVDV type 1 strain No. 12–43 (component of the vaccine) in sera of the vaccinated calves began to increase from 2 weeks after the vaccination, and reached their maximum titers 5 weeks or 6 weeks after the vaccination (Fig. 2A, empty symbols). On the other hand, neutralizing antibodies were only detected in low titers in the sera collected from the unvaccinated calves 3 weeks after the challenge (Fig. 2A, filled symbols). As shown in Fig. 2B, all calves had negative results for neutralizing antibody against BVDV type 2 strain. 

![Fig. 1. Average rectal temperatures (A), Total clinical scores (B) and Average white blood cells (C) of calves after challenge with bovine viral diarrhea virus type 2 strain 890. Cross symbol in B: Recovery and RT-PCR amplification of BVDV](image-url)
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890 until 5 weeks after the vaccination, but they had high neutralizing antibody titers from 2 weeks after the challenge (6 weeks after the vaccination). The first detectable titers of the vaccinated calves (Fig. 2B, empty symbols) were just a little higher than those of the unvaccinated calves (Fig. 2B, filled symbols).

It was reported by Bolin and Ridpath [3] that BVDV type 2 strain 890 infection in vivo induced marked thrombocytopenia. In the present experiment, however, marked thrombocytopenia was not observed. In another study, thrombocytopenia was not observed in calves following BVDV type 2 strain 890 [11]. Concerning the cause of it, two predisposing factors may be considered. One is the age of the calves used in the experiments. Though most calves used in the study by Bolin and Ridpath were 3- to 4-weeks-old, we used 3-month-old calves. Generally, the older an animal is, the more resistant it is to infectious diseases. The other factor is virulence attenuation of the BVDV type 2 strain 890 by passage in cell culture [2].

The above results indicate that vaccination with Japanese modified live vaccine containing BVDV type 1 strain No. 12–43 can protect young calves from infection with BVDV type 2 strain 890. Such a heterotypic protection by BVDV type 1 vaccine has been already reported by some investigators [9, 11, 15]. It seems that these vaccine strains are antigenically closely related to the challenge viruses in spite of the difference of genotype. In the present study, however, antibody against BVDV type 2 strain 890 (the challenge virus) was not detected in any sera from the vaccinated calves at the time of the challenge, though their antibody titers against BVDV type 1 strain No. 12–43 were not less than 64. This result suggests that BVDV type 1 strain No. 12–43 (the vaccine strain) is antigenically far from BVDV type 2 strain 890 (the challenge virus); nevertheless protection was observed in all the vaccinated calves.

Though a difference between BVDV type 1 and type 2 in antigenicity has been already indicated, they have cross-reacted in neutralizing antibody tests to greater or lesser degrees [13, 22]. Therefore, it could be conjectured that the challenge virus does not multiply adequately for development of the clinical signs because of the antibodies induced by the vaccination.

The antibodies against the challenge virus were detected in the sera of all calves from 2 weeks after the challenge (6 weeks after the vaccination) (Fig. 2B). It is generally acknowledged that increase of antibody titer is induced by the immune response against the increase of pathogen. Although calves that received the vaccine did not develop detectable viremia and were spared the clinical signs, as observed in the unvaccinated calves, the antibody against BVDV type 2 strain 890 was detected in the sera of them after the challenge. We conjecture that the antibody response might be induced by a “secondary immune response” based on the vaccination. The vaccinated calves would be able to respond well to the challenge virus, BVDV type 2 strain 890, because of an “immunological memory” condition induced by the vaccination. Secondary immune response is stronger than primary immune response. This theory is supported by the fact that the antibody titers against BVDV type 2 strain 890 in the vaccinated calves were higher than those in the unvaccinated calves from two to three weeks after the challenge in this experiment (Fig. 2B).

In the present experiment, calves administrated the vaccine containing BVDV type 1 were not affected by the challenge of BVDV type 2. However, it is known that BVDV has antigenically and pathogenically much variation. BVDV type 2 isolated in Japan may differ from BVDV type 2 strain 890 antigenically. Therefore, it is suggested that it’s necessary to confirm the effectiveness of the vaccine on several strains of BVDV type 2 isolated in Japan.

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References