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Research Article

Efficacy of a Modified-Live BVD1 and IBR Combination Vaccine against Virulent BVDV1 and IBRV when Administered Intramuscularly in Young Calves

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Abstract

The immunogenicity of a BVD and IBR combo live vaccine against bovine viral diarrhea and infectious bovine rhinotracheitis diseases was evaluated in the experimental study. Twenty claves aged 3 to 7 months old were selected and allocated into two groups, 10 of them were intramuscularly inoculated with 1ml vaccine per each calf, and 10 of them were injected with saline as control. Twenty-eight days post immunization, animals were challenged with virulent bovine viral diarrhea virus type 1 (strain JL) or infectious bovine rhinotracheitis virus (strain LN01/08). After challenge, the immunized calves showed significantly fewer clinical signs, lower rectal temperatures and less leukopenia and virus shedding than the mock immunized calves after challenge with the virulent strains. These data suggest that the BVD and IBR combo live vaccine may be used as an effective candidate vaccine in controlling BVDV and IBRV infections.

Keywords: Bovine Viral Diarrhea Virus; Infectious Rhinocbrontis Virus; Modified-Live Vaccine; Immunogenicity

Introduction

Bovine respiratory disease syndrome (BRDC) is a serious disease worldwide that causes serious economic losses to the cattle industry [1]. It is found that bovine viral diarrhea virus (BVDV) and infectious rhinotracheitis virus (IBRV) are the two most important pathogens causing BRDC. BVDV is a member of the genus Pestivirus of the family Flaviviridae. The virus can infect many livestock, such as cattle, sheep and pigs. Immunological study of bovine viral diarrhea virus and bovine infectious rhinotracheitis virus was been reported previously [2,3]. BVDV suppresses bovine immune response and induces serious clinical symptoms after secondary infection of other viruses and bacteria. Infectious bovine rhinotracheitis is a contagious disease mainly caused by bovine herpes virus type 1 (BoHV-1) with high fever, dyspnea and upper respiratory tract inflammation, invading a variety of tissues and organs, and resulting in reduced milk production, systemic infection and abortion [4,5]. Vaccination is one of the most important measures to prevent and control BRDC. Compared with inactivated vaccine, live vaccine can not only stimulate the production of humoral immunity, but also produce cellular immunity. It has been widely used worldwide and has good effect in preventing BRDC [6,7]. In recent years, with the intensive development of cattle industry in China, the harm of BRDC to cattle industry is increasingly enhanced [8-10]. At present, BVD and IBR combined inactivated vaccine have been developed in China, but it cannot provide good protective effect against BRDC. Based on this, we have first developed a BVD-IBR combo live vaccine, and the immunological efficacy of vaccine was investigated in this study.

Materials and Methods

Cells

MDBK (madin-darby bovine kidney) cell line was maintained in DMEM containing 8% fetal bovine serum (FBS, Hyclone Laboratories Inc, South Logan, UT, USA) at 37°C, with 5% CO_2.

Experimental animals

Twenty male dairy calves aged 3 - 7 months were used in this study. All calves were virus antigen-free and virus neutralizing (VN) antibody-free to BVDV and IBRV and obtained from Chuzhou dairy farm in Anhui province. The calves were housed in individual hutches before vaccination, and all the animal experiments were examined by the animal ethics committee.

Vaccination

A combination viral vaccine, containing modified-live BVDV type and IBRV (Type1, Strain NMM + Strain JSM) was given intramuscularly as a single, 1 mL/dose. Control animals received intramuscularly 1 mL of sterile saline as well.

Challenge viruses

BVDV JL strain F7, a non-cytopathic (ncp) type 1 strain, was used as the BVDV type 1 challenge virus. IBRV LN01/08 strain F3 was used as the IBRV challenge virus. The BVDV and IBRV challenge viruses were isolated and maintained in our laboratory as previously described. The virus content is 106.8FAID50/ml for each.

Experimental studies

Two separate immunogenicity studies were implemented in 3-7 months old calves for each viral antigen: BVDV1 and IBRV.

For BVDV1 study, 5 calves were enrolled in vaccinate group and received a single 1 mL dose of MLV vaccine administrated intramuscularly, and 5 calves in control group received a 1 mL dose of sterile saline. On day 28 post-vaccination, the calves were commingled and intranasally challenged with an aerosolized virulent BVDV1 strain JL. The challenge was performed by spraying 3 mL of virus into each nostril, using a Devilbiss Atomizer (Devilbiss, Somerset, PA). Each animal received approximately $6 \times 10^{6.5}$ $TCID_{50}$ of challenge virus.

A total of 10 calves were enrolled in the IBRV study, Calves in vaccinate group (n = 5) received a single 1 mL dose of MLV vaccine administrated intramuscularly, and calves in control group (n = 5) received a 1 mL dose of sterile saline. 28 day after vaccination, the calves were commingled and intranasally challenged with an aerosolized virulent IBRV strain LN01/08. The challenge was performed by spraying 2 mL of virus into each nostril, using a Devilbiss Atomizer (Devilbiss, Somerset, PA). Each animal received approximately $4 \times 10^{6.6}$ $TCID_{50}$ of challenge virus.

All experimental procedures have been reviewed and approved by local Animal Care and Use Committee of Taizhou agricultural commission.

Clinical assessment

Clinical observation was carried out on days -2, -1 and 0, prior to challenge, and days 1 through 8 d after challenge. For BVDV study, clinical observation mainly included mental malaise, diarrhea and mucosal hemorrhage, nasal and ocular discharges, loss of appetite, cough. For IBRV study, clinical observation mainly included the occurrence of increased intraocular secretion, large amount of tears, or multiple mucous rhinorrhea, or congestion of the nasal mucosa, and superficial ulceration. The body temperature was measured daily through the challenge course.

Sample collection

Blood sample were collected at the day of vaccination, 7, 14, 21and 28 days after vaccination for virus neutralizing antibody detection. After BVDV challenge, blood with EDTA anticoagulant was collected for white blood cell (WBC) count and virus isolation from 2 days pre-challenge through 8 days post challenge. After IBRV challenge, deep nasal swab specimens were obtained from both nares at 2-day prior to challenge through 8 days post challenge.

Virus neutralizing antibody detection

The virus neutralizing (VN) antibody titers to BVDV and IBRV were measured by use of a standard microplate VN procedure. Briefly, two-fold dilutions of each serum sample were made on 96-well tissue culture plates, and approximately 100 - 200 $TCID_{50}$ (50% tissue culture infectious dose) of each respective virus was added to each serum dilution. After 4 days of incubation on MDBK cell monolayers at 37°C, with 5% CO_2, the plates were observed for cytopathic effect (CPE). The neutralizing antibody titer of each sample was determined using Spearman-Karber method [11].

Virus isolation

For IBRV study, virus isolation from nasal swabs was conducted using MDBK cell monolayers in 96-well tissue culture plates. Briefly, following centrifugation of samples, the supernatants were used to infect cell monolayers for virus quantitation. After 4 days of incubation at 37°C, with 5% CO_2, virus isolation was determined as positive with CPE observation of cell monolayer. Negative control cell was detected with no CPE and positive control cell was detected with CPE [12].

For BVDV1 study, virus isolation from white blood cell was conducted using MDBK cell monolayers. Briefly, following two blind passage in 24-well tissue culture plates, samples freeze-thawed were transferred to 96-well tissue culture plates. Positive virus control and negative control were set for assay. After 3 days of incubation at 37°C, with 5% CO_2, indirect immunofluorescence assay was conducted and BVDV virus isolation was determined as positive with specific green fluorescence. Negative control cell was detected with no green fluorescence and positive control cell was detected with green fluorescence.

Data analysis

The significant differences of the results in individual group were analyzed using a one-way or two-way ANOVA in the GraphPad Prism (version 5.0) software. A t-test or F-test was used to

estimate the variability among the clinical signs, leukocyte count and neutralizing antibody level. Differences were considered statistically significant at a P value of < 0.05 and extremely significant at a value of P < 0.01 or P < 0.001.

Results and Discussion

Neutralizing antibody titer of BVDV

To investigate the immune response of the BVD and IBR combo live vaccine in young calves, neutralizing antibody levels of BVDV were detected after vaccination. As shown in figure 1, there is no BVDV neutralizing antibody detected on the day of vaccination and 7 day after vaccination. Two weeks after immunization, VN antibody level was first measured and increased gradually afterwards. At the end of immunization, the VN antibody titer peaked at 1:11585 on day 28 and the antibody titer range was from 1:5793 to 1:11585. On the contrary, no BVDV neutralizing antibody was detected throughout the experiment in the control group.

Figure 1: The trend of BVDV neutralizing antibody titer detection in the sera from vaccinated and control group.

After vaccination, virus neutralizing (VN) antibody titers to BVDV were measured by use of a standard microplate VN procedure at day 7, 14, 21, 28 in vaccinated (n = 5) and control groups (n = 5). Data are presented as mean values ± SD.

Clinical signs observations after challenge with BVDV1 JL strain

After challenge with BVDV1 JL strain, all the control calves showed typical clinical symptoms of BVDV infection, including severe depression, nasal discharge, excessive lacrimation, coughing. All calves had a high body temperature over 40.5°C, with the highest body temperature (Calf # 135, 41.2°C) at day 7 post challenge. In contrast, calves immunized with combined vaccine remained healthy with normal body temperature throughout the experiment course. There is significant difference between vaccinated and control groups (p < 0.01, Figure 2).

Leukocyte (white blood cell) count

When the vaccinated and control animals were compared, statistical analyses showed significant differences (P < 0.05, Figure 3) in leukocyte counts (geometric means) from days 3 to 6 post-challenge. The average leukocyte counts in control group showed a sharp decrease (25%) on 3 days post challenge (dpc) and reached the lowest level at 4 dpc (45%). Then WBC developed a tendency to increase thereafter till 7 dpc and returned to normal level at 8 dpc. On the contrary, the decline percentage of WBC level in vaccinated group did not reach 30% during the course of the experiment (Figure 3). The results indicated that the MLV vaccination protected against leukopenia induced by challenge strain of BVDV1.

Figure 2: Body temperature of calves after BVDV JL strain challenge (°C).

After BVDV challenge, mean rectal temperatures of each animal were measured daily in vaccinated (n = 5) and control group (n = 5). Rectal temperatures equal or above 40°C (≥40°C) were defined as fever. Data are presented as mean values ± SD. Asterisks indicate significant differences (**p < 0.01) between vaccinated and control groups.

Figure 3: Changes in leukocyte count after BVDV JL strain challenge.

After BVDV challenge, blood with EDTA anticoagulant was collected for white blood cell (WBC) count from days 2 to 8 post-challenge in vaccinated (n = 5) and control group (n = 5). Data are presented as mean values ± SD. Asterisks indicate significant differences (*p < 0.05, **p < 0.01) between vaccinated and control groups from day 3 to day 6.

Virus isolation after BVDV challenge

White blood cells were obtained from anticoagulant blood for BVDV virus isolation. All calves were negative for virus isolation prior to challenge, the day of challenge and the first 2 days following challenge. As shown in table 1, for vaccinated group, one of five calves was positive for virus isolation from white blood cells only at 7 dpc. And all calves in the control group were positive for virus isolation. The results indicated that vaccination protected against viremia induced by BVDV1 challenge.

Neutralizing antibody titer of IBRV

Neutralizing antibody levels of IBRV were detected after vaccination in IBRV study. As shown in figure 4, there is no IBRV neutralizing antibody detected on the day of vaccination and 7 day after vaccination. Two weeks after vaccination, VN antibody level was first measured and increased gradually afterwards. At the end of challenge, the VN antibody titer peaked at 1:91 on day 28 and the antibody titer range was from 1:32 to 1:91. On the contrary, no IBRV neutralizing antibody was detected throughout the experiment in the control group (Figure 4).

Groups	No.	dpc														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Vaccinated	NO.26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	NO.40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	NO.49	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	NO.53	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	NO.70	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—
Control	NO.61	—	—	+	+	+	+	+	+	+	+	+	+	—	+	—
	NO.78	—	—	—	+	+	+	+	+	+	+	—	—	—	—	—
	NO.79	—	—	—	—	+	+	+	+	+	+	—	—	—	—	—
	NO.81	—	—	—	—	+	+	+	+	+	+	+	—	—	—	—
	NO.82	—	—	—	—	+	+	+	+	+	+	—	—	—	—	—

Table 1: Virus isolation in white blood cell from vaccinated and control group after BVDV challenge.

Note: "+": Virus isolation positive; "-": Virus isolation negative.

Figure 4: IBRV neutralizing antibody titer detection in the sera from vaccinated and control group.

After vaccination, virus neutralizing (VN) antibody titers to IBRV were measured by use of a standard microplate VN procedure at day 7, 14, 21, 28 in vaccinated (n = 5) and control groups (n = 5). Data are presented as mean values ± SD.

Clinical signs after challenge with IBRV LN01/08 strain

After challenge with IBRV LN01/08 strain, all the control calves showed typical clinical symptoms of IBRV infection, including severe depression, purulent nasal discharge, excessive lacrimation. Conversely, no clinical signs, except one calf with mild nasal secretion, were observed in the vaccinated group. All control calves had a high body temperature over 40.5°C for two and more times, with the highest body temperature at day 5 post challenge (Figure 5). In contrast, calves immunized with combined vaccine remained healthy with normal body temperature throughout the experiment course. There is significant difference between vaccinated and control groups ($p < 0.01$, Figure 5).

Figure 5: Body temperature record of calves after IBRV LN01/08 strain challenge (°C).

After IBRV challenge, mean rectal temperatures of each animal were measured daily in vaccinated (n = 5) and control group (n = 5). Rectal temperatures equal or above 40°C (≥40°C) were defined as fever. Data are presented as mean values ± SD. Asterisks indicate significant differences (**$p < 0.01$) between vaccinated and control groups.

Virus shedding after IBRV challenge

Nasal swab samples were collected from all calves for virus isolation after IBRV challenge. All calves were negative for virus isolation prior to challenge, the day of challenge and for the first day following challenge. As shown in table 2, in vaccinated group, 2 of 5 calves were positive for virus isolation from nasal swab samples for one or two days. And all calves in the control group were positive for virus isolation lasting for more than 6 days. Statistical analysis showed that the vaccinated group had a significantly shorter duration of virus shedding than the control group.

Groups	No.	dpc														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Vaccinated	NO.56	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—
	NO.63	—	—	—	—	+	+	—	—	—	—	—	—	—	—	—
	NO.71	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	NO.58	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	NO.60	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Control	NO.62	—	—	—	+	+	+	+	+	+	+	+	—	—	—	—
	NO.66	—	—	+	—	+	+	+	+	—	+	—	—	—	—	—
	NO.64	—	—	+	+	+	+	+	+	+	+	—	—	—	—	—
	NO.77	—	—	+	+	+	+	+	+	+	+	+	—	—	—	—
	NO.83	—	—	+	+	+	+	+	+	+	—	—	—	—	—	—

Table 2: Virus shedding in Nasal swab from vaccinated and control group after IBRV challenge.

Note: "+": Virus isolation positive; "-": Virus isolation negative.

Conclusion

In 2013, Julian Ruiz-Sáenz., *et al* had evaluated the inactivated BoHV-1 vaccine. The results demonstrated that the vaccine has good immunogenicity, and the level of neutralization antibody is more than 1:16 after immunization, and optimal protection against challenge with the reference strain with decreased clinical signs of infection, protection against the onset of fever and decrease of virus excretion post challenge [13]. The efficacy of an attenuated marker BoHV-1 based vaccine against BoHV-1 challenge was evaluated and showed that after vaccination, BoHV-1 replication was significantly reduced by approximately three titer points compared to the controls [14]. After vaccinated with a pentavalent modified live vaccine containing bovine herpesvirus-1, bovine respiratory syncytial virus, BVDV1a, BVDV2a, and bovine parainfluenza-3, vaccinated animals had higher levels of circulating white blood cells and fewer animals were viremia than sham-vaccinated animals, indicating that the vaccine has good immunogenicity (Stevens., *et al* 2011). In this study, the efficacy of a modified-live BVD and IBR combination vaccine was evaluated. After vaccination, the titers of neutralizing antibody against BVDV and IBRV were not lower than the international standard. The results showed that there is significant difference between vaccinated and control groups from the temperature change, virus shedding and clinical symptoms. It is indicated that the modified-live BVD and IBR combo vaccine had good immunogenicity against virulent BVDV1 and IBRV when administered intramuscularly in young calves, suggesting that the combined vaccine may be an effective candidate vaccine to protect against both BVDV1 and IBRV infections in China.

Conflict of Interest Statement

The authors have agreed that there is no conflict of interest between them.

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