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Short communication

Immunogenicity of an inactivated Chinese bovine viral diarrhea virus 1a (BVDV 1a) vaccine cross protects from BVDV 1b infection in young calves

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ABSTRACT

Bovine viral diarrhea virus (BVDV) 1a and 1b strains are the predominant subgenotypes in China. Because of the genetic and antigenic variability among different BVDV strains, a vaccine effective in one region may fail to protect against infections caused by different virus strains in another region. No BVDV vaccine developed with the predominant strains in China are available. In this study, the immunogenicity of an inactivated Chinese BVDV 1a NM01 vaccine strain was evaluated by challenging with a Chinese BVDV 1b JL strain. Ten 2–4-month-old calves were intramuscularly vaccinated with a single dose of the vaccine strain and boosted with same dose three weeks after the first vaccination, with five mock immunized calves serving as a control group. The average titer of neutralization antibody to BVDV 1a and BVDV 1b of immunized calves reached 1:410 and 1:96, respectively, at 21 days post the second vaccination. Twenty-one days post the second vaccination, all calves were challenged with strain JL. The clinical signs, such as the temperature and leukopenia of the immunized calves and viral shedding, were significantly less than the mock immunized calves after challenging with the virulent BVDV 1b strain, indicating that the BVDV 1a vaccine strain elicited efficacious protection against the endemic BVDV 1b strain in China. To the best of our knowledge, this is the first report of an inactivated BVDV vaccine which demonstrated effective cross-protection against BVDV type 1b infection in China.

1. Introduction

Bovine viral diarrhea virus (BVDV), a member of the pestivirus genus of the flaviviridae, is a major pathogen causing bovine respiratory disease complex (BRDC) of ruminants worldwide, resulting in severe economic losses (Alkan et al., 2000). Two genotypes, BVDV1 and BVDV2, are identified based on comparison of the conserved genes of 5′ UTR, N^{pro} or E2. Each genotype is further divided into different genetic subgroups, and at least 15 genetic subgroups of BVDV1 and four genetic subgroups of BVDV2 are identified (Xue et al., 2010a), as well as two biotypes cytopathic (cp) and noncytopathic (ncp) according to their cytopathic effects. BVDV related-diseases range from subclinical to clinical diseases, depending on the virulence of different virus strains, and are complicated with fever, leukopenia,

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diarrhea, lacrimation, nasal discharge, persistent infections (PI), abortions, hemorrhagic and systemic diseases such as mucosal disease (Laureyns et al., 2011).

Although measures, including identification and removal of PI cattle and biosecurity procedures, are taken to control and prevent BVDV exposure to a herd (Borsberry, 2012), vaccination is still a basic and important strategy to control and prevent BVDV exposure (Xue et al., 2011). Inactivated BVDV vaccines used worldwide to protect against BVDV are generally safer in pregnant cattle; thus, it was advocated that inactivated instead of live BVDV vaccines be used during cattle pregnancy in some vaccination programs (Beer et al., 2000).

It is very important to consider that the presence of multiple genotypes and antigenic diversity of BVDV isolates may affect protective efficacy of BVDV vaccines (Xue et al., 2010b). A vaccination may fail to protect against virus infection due to the genetic and antigenic variability among different strains (Ridpath et al., 2010). Previous studies revealed that the cross protection of a BVDV vaccine from different strains with different genotypes or subgroups is significantly different (Fairbanks et al., 2003; Makoschey et al., 2001; Xue et al., 2009, 2010b). Although the cross-protection between the BVDV 1a Singer strain and the BVDV 1b NY1 and T1186a strains in modified live virus (MLV) vaccines has been confirmed (Ho et al., 2013), the cross-protection of the inactivated BVDV 1a vaccine against BVDV 1b infection, especially among the different regional strains, still remains unknown. BVDV 1a and BVDV 1b are the predominant subgenotypes in China (Wang et al., 2014; Xue et al., 2010a). Unfortunately, no commercial vaccine or immunogenicity data of the BVDV isolates has been available in China.

In this study, we developed a new inactivated vaccine candidate containing Chinese strain NM01 of cpBVDV 1a. Young calves were immunized with the vaccine strain through intramuscular injection, and the efficacy of the vaccine was evaluated by challenging with the predominant ncpBVDV 1b JL strain in China.

2. Materials and methods

2.1. Experimental animals

Fifteen 2–4-month-old healthy calves free of persistent infection of non-cytopathic BVDV were purchased from a calf farm in Inner Mongolia Autonomous Region, China. All calves were seronegative to BVDV antibody. All animal experiments were approved by the ethics committee of the Chinese Academy of Agricultural Science.

2.2. Vaccine and administration

BVDV1 NM01 strain, used as the vaccine strain, was isolated from Inner Mongolia Autonomous Region in China. The virus was infected into MDBK cells and harvested, frozen and thawed three times. Viruses were inactivated with binary ethyleneimine (BEI; Sigma, USA), mixed with Montanide™ ISA 206 VG (Seppic, France) adjuvant and then emulsified. Antigen in the vaccine was batched at a minimum immunization level of $10^{7.0}$ $TCID_{50}$/dose.

2.3. Animal immunizations and challenging

Ten 2–4-month-old calves were intramuscularly immunized with a single 2 ml dose of the inactivated vaccine and calves in the control group ($n = 5$) were mock immunized with 2 ml of PBS diluent. The immunization, including the mock-immunization, was repeated once three weeks later. Blood samples were collected on day 0 of vaccination and 7, 14, 21, 28, 37 and 42 days post-immunization. The sera were used to test neutralization antibody against BVDV by the virus neutralization (VN) method. Twenty-one days post the second vaccination, all calves were intranasally challenged with 3 ml of aerosolized BVDV 1b JL strain virus, a non-cytopathic (ncp) strain isolated from Jilin province of China, into each nostril using a Devilbiss Atomizer (Devilbiss, Somerset, PA, USA). Each animal received approximately $10^{7.5}$ $TCID_{50}$ (50% tissue culture infectious dose) of the challenge virus.

2.4. Clinical assessment

The calves were observed for clinical signs, including depression, cough, asthma, excessive lacrimation, and rectal temperature. Rectal temperature was taken at the same time each morning at days −2, −1 and 0 prior to the challenging and at days 1 through 14 after the challenging.

2.5. Sample collection

Blood samples were collected from each calf using EDTA-coated tubes from day 2 pre-challenge through day 8 post-challenge and white blood cell (WBC) counts were conducted by a Vetscan HM5 veterinary hematology system (Abaxis, USA). Deep nasal swab specimens were collected at 1 day prior to challenge through 8 days post-challenge and used for virus isolation.

2.6. Virus neutralizing antibody analysis

The neutralization antibody titers to BVDV were measured using a standard microplate VN procedure with BVDV 1a strain NM01 or BVDV 1b strain JL as the neutralizing viruses in the assay. Briefly, 56 °C-heat inactivated serum samples were two-fold serially diluted in 96-well plates. Approximately 200 $TCID_{50}$ of the VN challenge virus was added to each diluted serum sample and incubated at 37 °C for 1 h. The serum-virus mixture was then inoculated onto MDBK cells. At 4–5 days post the incubation, the plates were evaluated for CPE or immunofluorescence assay. The neutralizing antibody titer each sample was calculated using the Spearman-Kärber method.

2.7. Virus isolation

Viruses were isolated from nasal swabs and blood leukocytes on MDBK cell monolayers in 24-well tissue culture plates for two passages and finally were placed in