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Research Article

Evaluating an Inactivated Infectious Bovine Rhinotracheitis Vaccine for Safety and Efficacy

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Abstract

An inactivated infectious bovine rhinotracheitis (IBR) vaccine was developed using BoHV-1 LN01, a field strain recently isolated from Northeast China. The vaccine was evaluated at three dose levels for safety and efficacy in fifteen healthy 6-to 7-month-old calves. Calves were vaccinated intramuscularly and boosted with a second intramuscular vaccination 21 days later. No site or systemic reactions were observed after the vaccination. Following homologous challenge, only one of fifteen vaccinated calves showed mild clinical signs, whereas all (5/5) non-vaccinated, challenged calves showed severe clinical signs. Neutralizing antibody titers against BoHV-1 were induced in vaccinated calves (GMTs 1:199 - 1:221) but not in unvaccinated controls (GMT < 1:2). BoHV-1 was recovered post challenge from two of the five calves (2/5) in each of the three vaccinated groups (Groups 1- 3). However, virus was detected in all non-vaccinated control calves (5/5) and appeared earlier and persisted longer than in the vaccinated groups. Postmortem examination of vaccinated, challenged calves showed normal lungs and tracheas. All unvaccinated, challenged controls showed lung and tracheal lesions typical of IBR. Based on clinical signs and necropsy findings, protection rates were 100% in vaccinated Group 1 (5/5) and Group 2 (5/5), and 80% in vaccinated Group 3 (4/5). None of the unvaccinated animals was protected from challenge (0/5). These results demonstrate that the inactivated IBR vaccine provided effective protection against the wild type BoHV-1 challenge.

Keywords: Bovine Herpesvirus-1; Infectious Bovine Rhinotracheitis (IBR); Inactivated IBR Vaccine; Safety and Efficacy

Introduction

Bovine herpesvirus-1 (BoHV-1) is a major pathogen worldwide, causing infectious bovine rhinotracheitis (IBR), a respiratory and genital disease in cattle [1]. The disease initiates in the oro-respiratory mucosa with symptoms including fever, anorexia, coughing, excessive salivation and nasal discharge, conjunctivitis with lacrimal discharge and inflamed nares [2]. Like other alphaherpesviruses, BoHV-1 establishes lifelong latent infection, which can be periodically reactivated and transmitted. Vaccination is widely used both to protect cattle from clinical infection and to reduce the shedding of the virus [3]. However, vaccination cannot always prevent infection due to the endemic nature of BoHV-1 infection [4]. Vaccines that are currently available for IBR include modified-live-virus (MLV) vaccines and inactivated or killed-virus (KV) vaccines. It has been demonstrated that cattle vaccinated with the attenuated preparations can shed the virus from nasal secretions, urine, semen and milk, and that these secretions can serve as a source of infection to susceptible animals [5]. Abortion is another recognized post-vaccination problem of the attenuated IBR vaccines [6]. In contrast, the inactivated vaccines avoid the problem of shedding vaccine virus, reduce virus excretion after reactivation of latently infected calves, and also possess a better safety profile [7]. Currently, BoHV-1 is eradicated in several European countries [8]. However, BoHV-1 infection is still an economically important disease in China [9,10]. To address this, our strategy was to prepare a new inactivated IBR vaccine and evaluate a prime-boost combination for its ability to induce immune responses and protect calves against challenge. The inactivated IBR vaccine was derived from BoHV-1 LN01, a field strain recently isolated from nasal swabs collected from naturally infected cattle in Northeast China during an epidemiological study.

Aim of the Study

Our aim in this article is to report the assessment of the novel inactivated IBR vaccine for safety, immunogenicity and efficacy against challenge.

Materials and Methods

Cells, viruses and inactivated IBR vaccine stock preparations

To propagate the BoHV-1 LN01 field strain, Madin-Darby bovine kidney cells (MDBK, ATCC, CCL-22) were grown in Eagle's Minimum Essential Medium (DMEM; Gibco Laboratories), supplemented with 6% inactivated and γ-irradiated fetal bovine serum (Hyclone, New Zealand), penicillin (100 U/ml) and streptomycin (100 ug/ml). The cells and serum were BVDV virus free, mycoplasma free, and free of antibody against BVDV and BoHV-1. After the cell monolayer reached confluence, the growth medium was replaced with DMEM supplemented with 3.5% horse serum containing BoHV-1 LN01 virus at a multiplicity of infection (MOI) of 0.01. Infected cells were incubated at 37°C, 5% CO_2 for 48 hours. Cell culture supernatant was harvested when cytopathic effect (CPE) affected 90% of the cell monolayer. The supernatant was treated with binary ethyleneimine (BEI) to inactivate the virus, and an oil-based emulsion vaccine was formulated using Montanide™ ISA 206 VG (SEPPIC, China) as the adjuvant. Three batches of IBR vaccine were prepared, containing cell supernatants with pre-inactivation titers of $10^{8.25}$ $TCID_{50}$/ml, $10^{8.5}$ $TCID_{50}$/ml, and $10^{8.75}$ $TCID_{50}$/ml of BoHV-1 LN01, respectively, in the final vaccines.

Animal study

Twenty healthy calves at 6-7 months of age that were seronegative to BoHV-1 and BVDV were randomly allocated to four groups of five calves each. Groups 1 to 3 were vaccinated intramuscularly in the neck with 2 ml of one of the three inactivated IBR vaccine batches, respectively. Group 1 received vaccine S001, formulated with the lowest BoHV-1 titer, Group 2 received Vaccine S002, formulated with the mid-range titer, and Group 3 received Vaccine S003, formulated with the highest titer. The calves in Group 4 were inoculated intramuscularly with 2 ml of phosphate buffered saline (PBS). Twenty-one days following the initial vaccination, each group of vaccinated calves was boosted with the same amount of the respective vaccine, administered intramuscularly. Eighteen days after the

booster vaccination, the calves were transferred from the vaccination facility to a negative pressure animal facility. Three days later (21 days after the booster vaccination) calves were challenged intranasally with 4 mL of $10^{6.6}$ $TCID_{50}$/ml BoHV-1 LN 01 strain by administering 2 ml per nostril. Clinical signs and temperature were monitored and recorded daily for 14 days following challenge.

All experimental procedures were reviewed and approved by local Animal Care and Use Committee, Taizhou, China.

Serum neutralization and viral shedding assays

Blood samples taken from the calves before the initial vaccination, 7 days after the initial vaccination and 21 days after the booster vaccination were evaluated for the presence of neutralizing antibody to BoHV-1.

Virus isolation assays were performed to evaluate viral shedding post challenge. Nasal swabs were collected from the calves for 14 days post challenge. Swabs were mixed in a transport medium containing 2% horse serum, penicillin (2000 ug/ml), streptomycin (2000 ug/ml) and Amphotericin B (7.5 ug/ml). Samples were centrifuged at 1500g for 10 minutes and supernatants were filtered through 0.2 um filters. Monolayer cultures of MDBK cells in 96-well plates were inoculated with the supernatants, using 100 ul per well. After incubating for 1 hour in a 37°C incubator, the supernatants were removed and replaced with DMEM medium containing 2% horse serum. The plates were incubated at 37°C and 5% CO_2. Infected MDBK cells were examined microscopically for the presence of CPE daily for the next seven days.

Results and Discussion

Clinical evaluation

Following vaccinations, no site or systemic reactions were observed in any of the calves. Following challenge, no reactions were observed in the vaccinated calves of Groups 1 and 2. One of the five vaccinated calves in Group 3 showed mild reactions including depression, increased nasal secretions, mild conjunctivitis and slightly elevated (1°C) body temperature (Figure 1). All unvaccinated calves in Group 4 showed severe clinical signs including conjunctival redness, massive tearing, nasal mucosa redness, hyperemia, purulent nasal discharge, nasal mucosal ulcers and elevated body temperatures.

Figure 1: Body temperature in calves after BoHV-1 LN01 strain challenge. Body temperature were monitored and recorded daily for 14 days following challenge. Mean rectal temperature (MRT) of calves in inactivated IBR vaccines inoculation groups (Group 1, 2, 3, n = 5) and control group (Group 4, n = 5).

Viral loads following the challenge

Virus isolation rates post challenge in vaccinated groups were different from the control group. BoHV-1 was recovered from two of the five calves in each vaccinated Group (Groups 1- 3). However, virus was detected in all five non-vaccinated control calves (Group 4) and appeared earlier and persisted longer than in the vaccinated groups. In vaccinates, virus was isolated from day 3 to 7, whereas in the control group, virus was isolated from day 2 to 11.

Groups/Vaccine batches	Animal number	Duration of higher than basal body temperature 1°C	Duration of clinical signs	Duration of virus isolation	Protection rates (%)
1 (S001)	423	-	-	-	100 (5/5)
	424	-	-	-	
	431	-	-	5 - 7	
	436	-	-	3 - 7	
	437	-	-	-	
2 (S002)	425	-	-	-	100 (5/5)
	426	-	-	-	
	427	-	-	4	
	432	-	-	-	
	653	-		3 - 6	
3 (S003)	428	-	-	-	80 (4/5)
	433	-	-	-	
	434	-	-	5 -7	
	439	-	-	-	
	440	3 - 7	4 - 8	3 - 6	
4 (Control)	429	4 - 7	4 - 11	2 - 10	0 (0/5)
	430	2 - 5	4 - 8	4 - 11	
	435	3 - *6*	2 - 10	3 - 8	
	438	3 - *6*	2 - 10	3 - 9	
	654	3 - 7	2 - 10	3 - 10	

Table 1: Summary of calf reactions after vaccination and challenge
Note: (-) normal temperature, no clinical signs or virus isolated.

Neutralizing antibody response

All calves were negative for neutralizing antibody to BoHV-1 before vaccination. Seven days following the initial vaccination, neutralizing antibody titers, expressed as geometric mean titers (GMTs), ranged from 1:15.4-1:17.2 in Groups 1-3. Twenty-one days following the booster vaccination, the neutralizing antibody titers exhibited a 4-fold or greater increase in all calves of the three vaccinated groups. The GMT increased to 1:221 in Groups 1 and 2 and to 1:199 in Group 3. None of the unvaccinated calves in Group 4 exhibited an antibody response to BoHV-1; the GMT was less than 1:2 (Figure 2). The serology results suggest that protection is correlated with level of neutralizing antibody.

Figure 2: Serum neutralizing antibody response in calves after vaccination with inactivated IBR vaccines

Blood samples taken from the calves before and after vaccination were evaluated for the presence of neutralizing antibody to BoHV-1. Geometric mean titer (GMT) of all calves in inactivated IBR vaccines inoculation groups (Group 1, 2, 3, n = 5) and control group (Group 4, n = 5).

Gross postmortem examination and clinical protection

At 14 days after the challenge, postmortem examination of vaccinated calves showed normal lungs and tracheas. However, gross postmortem examination of unvaccinated calves revealed lung congestion, necrosis and tracheal bleeding in all unvaccinated calves. Hyperemic inflamed necrotic tracheal mucosa with thick adherent mucopurulent exudate on the surface was also observed in all unvaccinated calves (data not shown).

Calves were evaluated clinically for protection on days 1-14 post-challenge. Protection rates were 100% in vaccinated Group 1 (5/5) and Group 2 (5/5), and 80% (4/5) in Group 3. None of the unvaccinated animals was protected from challenge (0/5) (Table 1). Results demonstrate that the oil emulsion vaccines formulated with inactivated BoHV-1 LN01 effectively protected calves from homologous BoHV-1 challenge. The observations in this study are supported by the findings of other researchers, which highlight the effects of administration of an established, inactivated IBR vaccine [11]. In a study of 234 newborn calves, Pospíšil., *et al.* demonstrated that immunization of their dams with an inactivated vaccine conferred full in utero protection against IBR-virus infection [12] and utilizing an inactivated IBR vaccine would be advantageous in preventing the spread of the virus [5].

Conclusion

In conclusion, utilizing an inactivated IBR vaccine would be advantageous in that the problem of abortions, latent or permanent infections, and spread of the virus by vaccination would be eliminated, and high humoral antibody titers could still achieved [5]. To our knowledge this the first developed inactivated IBR vaccine in China. The authors hope that the information provided here will be useful to the community of researchers and facilitate the use of inactivated IBR vaccines. Further studies to determine the impact of IBR immunization under large field conditions are required. Such studies will be necessary to gain a better understanding of inactivated IBR vaccine safety and efficacy.

Conflict of Interest

The author declares no conflicts of interest in this article

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