Immunogenicity of a modified-live virus vaccine against bovine viral diarrhea virus types 1 and 2, infectious bovine rhinotracheitis virus, bovine parainfluenza-3 virus, and bovine respiratory syncytial virus when administered intranasally in young calves

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A B S T R A C T

The immunogenicity of an intranasally-administered modified-live virus (MLV) vaccine in 3–8 day old calves was evaluated against bovine viral diarrhea virus (BVDV) types 1 and 2, infectious bovine rhinotracheitis (IBR) virus, parainfluenza-3 (PI-3) virus and bovine respiratory syncytial virus (BRSV). Calves were intranasally vaccinated with a single dose of a multivalent MLV vaccine and were challenged with one of the respective viruses three to four weeks post-vaccination in five separate studies. There was significant sparing of diseases in calves intranasally vaccinated with the MLV vaccine, as indicated by significantly fewer clinical signs, lower rectal temperatures, reduced viral shedding, greater white blood cell and platelet counts, and less severe pulmonary lesions than control animals. This was the first MLV combination vaccine to demonstrate efficacy against BVDV types 1 and 2, IBR, PI-3 and BRSV in calves 3–8 days of age.

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1. Introduction

The bovine respiratory disease (BRD) complex is a significant problem to the cattle industry, especially in calves entering beef lots, often resulting in severe economic losses [1–4]. The major viral pathogens of the BRD complex include: bovine herpesvirus type-1, also known as infectious bovine rhinotracheitis (IBR) virus, bovine parainfluenza-3 (PI-3) virus, bovine respiratory syncytial virus (BRSV) and bovine viral diarrhea virus (BVDV) [5,6]. These pathogens, together with Mannheimia haemolytica and Pasteurella multocida, contribute to the development of shipping fever, with each pathogen having a differing role in disease development. Infection with IBR, PI-3 and BRSV results in the destruction of ciliated respiratory epithelium and each are considered primary respiratory pathogens [5,7]. Although BVDV is not a primary pathogen in the pathogenesis of BRD, it can suppress the host immune system and increase the risk of secondary bacterial infections, thus enhancing colonization of the lungs by other pathogens [5,8].

Modified-live virus (MLV) vaccines for BVDV, IBR, PI-3 and BRSV are commercially available for the prevention and control of infections associated with these viruses [9–12]. A majority of commercial MLV vaccines targeting these viruses are administered subcutaneously, and are capable of inducing protective immune responses against BVDV, IBR, PI-3 and BRSV, as well as effectively controlling respiratory diseases associated with these viruses. In the current immunization program for most cattle producers, these parenteral MLV vaccines are used in cattle 3 months of age or older, when maternal immunity has waned to the point that it may have no affect on immunization.

Attempts to use mucosal vaccination strategies for MLV vaccines against BRD antigens have been reported and were shown to be efficacious [13,14]. There are two distinct advantages to vaccinating young calves intranasally, instead of the subcutaneous route. First, intranasal (mucosal) immunization may be more likely to prime a young calf’s immune system successfully in the presence of ongoing maternal antibodies [13]. Second, antigens delivered mucosally are more likely to prevent infection rather than just reduce disease occurrence [13,15]. This is especially true for IBR, PI-3 and BRSV, since these viruses infect and replicate in mucosal epithelial cells [5,7]. There are currently no modified-live BVDV, IBR, PI-3 and BRSV combination vaccines licensed for intranasal use. The purpose of
these studies was to test the efficacy of a MLV vaccine, administered intranasally in 3–8 day old calves, against BVDV types 1 and 2, IBR, PI-3 and BRSV infections and the respiratory diseases caused by these pathogens.

2. Materials and methods

2.1. Calves

Colostrum-deprived (CD) calves (JR Livestock Inc, Lake Mills, IA) were used in BVDV, IBR and PI-3 studies. The calves were removed from dams at birth before taking colostrum and fed milkplacer. The CD calves were housed in individual hutches before vaccination and in separated pens after vaccination at 3–8 days of age. All calves were virus neutralizing (VN) antibody-free to BVDV, IBR and PI-3 at vaccination. The BRSV study was conducted at the Western College of Veterinary Medicine (Saskatoon, Saskatchewan, Canada). Neonatal Holstein calves, obtained from local dairies, were removed from dams at birth, and the calves were fed bovine colostrum that contained minimal antibody to BRSV, so that the calves had antibodies to BVDV, IBR and PI-3, but not to BRSV. These calves were kept outdoors in individual pens until the time of challenge.

2.2. Vaccination

A combination viral vaccine1, containing modified-live BVDV types 1 and 2, IBR, PI-3 and BRSV, as well as, avirulent M. haemolytica and P. multocida, was given intranasally as a single, 1 mL dose at 3–8 days of age. All antigens in the vaccine serial were batched at their minimum protective dose (MPD) level. In all studies, control animals received 1 mL of sterile water diluent, administered intranasally as well.

2.3. Challenge viruses

BVDV NY-1, a non-cytopathic (ncp) type 1b strain, was used as the BVDV type 1 challenge virus. BVDV 1373, an ncp type 2 strain, was used as challenge virus in the BVDV type 2 study. The Cooper strain of IBR was used to challenge calves in the IBR study. The BVDV and IBR challenge viruses were obtained from National Veterinary Services Laboratories (NVSL, Ames, IA). The challenge virus used in the PI-3 study, Reisinger SF-4, was originally provided by NVSL, from a newborn calf infected with BRSV, as previously described[16]. The PI-3 and BRSV lung lavage fluids were confirmed to be free of bacterial contamination, Mycoplasma spp., BVDV and IBR by standard 9 CFR tests.

2.4. Clinical assessment

Calves were observed for clinical signs on days −1 and 0, prior to challenge, and days 1 through 14 after challenge (days 1 through 8 for BRSV only). Clinical assessments were made at the same time each morning by investigators who were unaware of the treatment codes in each study. Clinical signs monitored included depression, nasal and ocular discharges, coughing and rectal temperatures. Clinical signs of diarrhea and mucosal hemorrhage for BVDV, nasal lesions and plaques for IBR, and respiratory rate for BRSV were also recorded in their respective studies.

For the BRSV study, calves were euthanized by a barbiturate overdose of Euthanyl Forte (MTC Pharmaceuticals, Cambridge, ON, Canada) 8 days after challenge. Moreover, in all studies, if calves were observed at any time with severe signs of respiratory distress, including pronounced open-mouthed, labored breathing (>100 breaths/min), severe signs of depression and/or recumbence with total reluctance to rise, or with PaO2 values <45 mm Hg (range 40–45 mm Hg), they were euthanized immediately according to USDA/CFIA Animal Care Committee guidelines.

2.5. Sample collection

Blood samples were collected at the day of vaccination, challenge and 14 days post-challenge, and SN antibody titers were determined for each virus. Deep nasal swab specimens were obtained from both nares at 1-day prior to challenge through 10 days post-challenge for BVDV, IBR and PI-3 studies. After collection, swabs were placed in 3 mL of cold transport medium consisting of Dulbecco’s Modified Eagle Medium (HyClone, Logan, UT) supplemented with 10% horse serum (Sigma, St. Louis, MO), 1% gelatin (Fisher Scientific, Fair Lawn, NJ), 1× Antibiotic Antimycotic Solution (Sigma, St. Louis, MO) and 30 μg/mL Gentamicin (Sigma, St. Louis, MO). Collection of nasal swabs in the BRSV study started 1-day prior to challenge and ended 8 days after challenge. Swab specimens for BRSV isolation were placed in 1 mL of transport medium as described previously[13]. All swab specimens were stored at −70 °C or below until they were cultured for quantitative virus isolation. For the BVDV studies, white blood cell (WBC) and platelet counts were conducted from 2 days pre-challenge through 8 days post-challenge by a clinical pathology laboratory (Physicians Reference Laboratory, Overland Park, KS). In the BRSV study, arterial blood samples were collected from the caudal thoracic aorta, and oxygen tension (mm Hg) measurements were performed by use of a gas analyzer (Ciba-Corning, Medfield, MA) [13].

2.6. Virus neutralizing antibody analysis

The virus neutralizing (VN) antibody titers to BVDV, IBR and PI-3 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 TCID50 (50% tissue culture infectious dose) of each respective virus was added to each serum dilution. After 3 days of incubation on bovine kidney cell monolayers at 37 °C, with 5% CO2, the plates were observed for cytopathic effect (CPE). The neutralizing antibody titer of each sample was determined using standard laboratory methods. Bovine respiratory syncytial virus-specific IgG ELISAs were performed as previously described to determine BRSV antibody endpoint titers [13].

2.7. Quantitative virus isolation

For BVDV, IBR and PI-3 studies, virus isolation from nasal swabs was conducted using bovine kidney 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 3 days of incubation at 37 °C, with 5% CO2, the TCID50/mL was calculated for IBR and PI-3 viruses by CPE observation of cell monolayers. The titers of BVDV type 1 and type 2 were calculated by immunofluorescence of cell cultures stained by BVDV type-specific monoclonal antibodies. BRSV shedding was quantitatively determined by a plaque assay method using bovine embryonic lung (BEL) fibroblasts as previously described[17].

1. Vista Once SQ, Intervet Inc. Millsboro, Delaware, USA.
2.8. Postmortem analysis

At the end of the BRSV study, the respiratory tract of each calf was collected and analyzed to determine the percentage of pneumonia visible tissue as previously described [18]. Tracings were made of the dorsal surface, and the percentage area of pneumonia lung, including characteristic atelectatic, as well as, emphysematous areas for each tracing was determined by software imaging program (Universal Imaging Corp., West Chester, PA).

2.9. Experimental design

Five separate immunogenicity studies were implemented in 3–8 day old calf calves for each viral antigen: BVDV type 1, BVDV type 2, IBR, PI-3 and BRSV.

For the BVDV type 1 study, 15 calves were intranasally vaccinated with a single dose of the MLV vaccine as described, and 16 calves received sterile water diluent intranasally. Three weeks after vaccination, the calves were commingled and intranasally challenged with an aerosolized virulent type 1 BVDV strain NY-1. The challenge was performed by spraying 2 mL of virus into each nostril, using a Devilbiss Atomizer (Devilbiss, Somerset, PA). Each animal received approximately 1.3 × 10^5 TCID50 of challenge virus.

A total of 29 calves were enrolled in the BVDV type 2 study. Calves in the vaccinate group (n = 14) received a single 1 mL dose of the MLV vaccine administrated intranasally, and calves in the control group (n = 15) received a 1 mL dose of sterile water diluent. On day 21-post-vaccination, the calves were commingled and intranasally challenged with an aerosolized virulent type 2 BVDV strain 1373. The challenge was performed by spraying 2 mL of virus into each nostril, using a Devilbiss Atomizer. Each calf was challenged with approximately 1.6 × 10^5 TCID50 of challenge virus.

In the IBR study, 16 calves received a single 1 mL dose of the MLV vaccine administrated intranasally, and 14 control calves received a 1 mL dose of sterile water diluent. Four weeks post-vaccination, the calves were commingled and intranasally challenged with an aerosolized virulent IBR virus Cooper strain. The challenge was performed by spraying 2 mL of virus into each nostril, using a Devilbiss Atomizer. Each calf received approximately 1.3 × 10^5 TCID50 of IBR challenge virus.

For the PI-3 study, 31 calves were randomized into vaccinate (n = 15) and control groups (n = 16). Calves in the vaccinate group received a single 1 mL intranasal dose of the MLV vaccine, and calves in control group were given a 1 mL dose of sterile water diluent. Twenty-eight days after vaccination, the calves were mixed and intranasally challenged with an aerosolized virulent type 2 BVDV strain 1373. The challenge was performed by passing PI-3 lung wash using a Devilbiss Atomizer. The challenge was performed by spraying 2 mL of virus into each nostril, using a Devilbiss Atomizer. Each animal received approximately 1.6 × 10^7 TCID50 of PI-3 virus.

Finally, for the BRSV study, nine calves received a 1 mL single dose of the MLV vaccine intranasally, whereas seven control calves received 1 mL of sterile water diluent intranasally. The calves were commingled and challenged 21 days after vaccination via aerosol delivery using Ultra-Neb 99 ultrasonic nebulizers (Devilbiss, Somerset, PA) in the procedure described previously [13]. Approximately 10^6.2 PFUs of virulent BRSV were used to challenge calves.

2.10. Data analysis

In each study, the two treatment groups (vaccinate and control) were analyzed and compared with respect to the primary clinical signs including rectal temperature, nasal and ocular discharges, depression, leukopenia, or oral nasal mucosal lesions, lung lesions, pO2 values and virus shedding, using PROC NPAR1WAY of SAS® Version 9.1.3 to perform the Fisher Exact test. The prevented fraction and associated confidence interval were estimated.

3. Results

3.1. The intranasal administration of the MLV vaccine in 3–8 day old calves produced a measurable antibody response

All colostrum-deprived calves were free of VN antibodies to each of the five viruses at the time of vaccination (Table 1). At challenge, nearly all vaccinated calves exhibited minimal VN antibody titers to BVDV types 1 and 2, IBR and PI-3. ELISA antibody titers to BRSV were the same at challenge as they were at the time of vaccination. Upon conclusion of each challenge observation period, VN titers to each virus were significantly (p < 0.05) higher in the vaccinated calves when compared to control calves. These results demonstrated that calves were primed by intranasal vaccination with the MLV vaccine, even though there was an absence of significant antibody titers at the time of challenge. Similar antibody results were also obtained in the BRSV ELISA.

3.2. Efficacy against BVDV type 1 challenge

After challenge with BVDV type 1 strain NY-1, all control calves (16/16) developed clinical diseases associated with BVDV infection, including nasal discharge, coughing, diarrhea and depression after day 5 post-challenge (Fig. 1A). In contrast, only one-fifth (3/15) of vaccinated calves developed clinical signs, which were minimal in nature. The prevented fraction was 80% for the vaccinate group and the statistical analysis indicated that the total average daily clinical score in the control group was 16.8, which is significantly (p < 0.01) higher than that for the vaccinate group (daily average clinical score of 1.2). The rectal temperatures of calves were measured daily from days 1 to 14 post-challenge. All control calves had temperatures higher than 40 °C during the observation period, while only 3 of 15 vaccinated calves developed fever (Fig. 1B). Moreover, the vaccinated group had significantly (p < 0.05) lower temperatures at days 7–9 post-challenge than the control group, when the highest rectal temperatures were recorded.

BVDV infection usually results in leukopenia. Results of WBC counts demonstrated a decrease beginning on day 2 post-challenge. The WBC decrease in control calves (>45%) was more pronounced than in MLV-vaccinated calves (<20%) (Fig. 1C), and WBC counts in vaccinate group were significant (p < 0.05) than control group at days 3 and 4 post-challenge. In addition, the platelet count dropped beginning at day 2 post-challenge for all calves; however, the control calves displayed a greater decline in platelet counts when compared to the vaccinated calves (Fig. 1D). Specifically, the vaccinate group showed significantly less (p < 0.05) higher platelet counts at days 6, 7 and 8 post-challenge than the control calves.

Nasal swab samples were collected from challenged calves from day –1 to day 10 post-challenge for detection of virus shedding. All calves were negative for virus isolation prior to challenge, the day of challenge and for the first 2 days following challenge (Fig. 1E). Statistical analysis of virus isolation, in terms of positive or negative, and the total number of days positive, indicate that there were significantly (p < 0.01) less vaccines (4/15) that shed virus than controls (16/16). Additionally, the MLV-vaccinated calves were positive for significantly (p < 0.01) fewer days (mean = 0.4 days) compared to the controls (mean = 4.38 days). The titers of isolated BVDV1 from controls were significantly (p < 0.05) higher than vaccinated at days 5–10 post-challenge as well.

3.3. Efficacy against BVDV type 2 challenge

BVDV type 2 challenge strain 1373 is a virulent strain and causes severe clinical disease. All control calves (15/15) developed
Table 1
Induction of a virus neutralizing antibody or ELISA antibody response to each vaccine virus in 3–8 day old calves, intranasally vaccinated with a MLV vaccine.

<table>
<thead>
<tr>
<th>Antigens target</th>
<th>Treatment groups</th>
<th>Antibody titers* at different days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccinate</td>
<td>Challenge</td>
</tr>
<tr>
<td>BVDV1</td>
<td></td>
<td>0</td>
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<tr>
<td></td>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>BVDV2</td>
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<td></td>
<td>Control</td>
<td>0</td>
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<tr>
<td>IBR</td>
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<td></td>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>PI-3</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>BRSV</td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* The virus neutralizing antibody titers listed are the geometric mean titers (GMT) to BVDV types 1 and 2, IBR, and PI-3 viruses; and ELISA unit titers to BRSV.

Fig. 1. Intranasal vaccination protected young calves from clinical diseases caused by type 1 BVDV challenge. The vaccinated calves were protected from development of severe clinical signs (A); elevated rectal temperatures (B); leukopenia (C); decrease of platelet counts (D); and virus shedding (E). Statistical values are *p < 0.05 and **p < 0.01.
Intranasal vaccination protected young calves from clinical diseases caused by type 2 BVDV challenge. The vaccinated calves were protected from development of severe clinical diseases (A); elevated rectal temperatures (B); leukopenia (C); decrease of platelet counts (D); and virus shedding (E). Fatality of calves caused by the virulent type 2 BVDV challenge was significantly decreased by the vaccination (F). Statistical values are *p* < 0.05 and **p* < 0.01.

Severe clinical disease associated with BVDV2 infection, including nasal discharge, coughing, diarrhea and depression post-challenge. In comparison, fewer vaccinated calves (6/14) developed clinical signs from the challenge, which were moderate in nature (Fig. 2A). Statistical analysis of depression, diarrhea and nasal discharge demonstrated prevented fractions of 70–100% for the MLV-vaccinates, indicating the immunogenicity of the vaccine in reducing clinical disease caused by BVDV2 challenge. Post-challenge, all control calves developed temperatures higher than 40°C for 4–10 days. However, only 3 of 14 vaccinated calves showed temperatures higher than 40°C for only 1–3 days (Fig. 2B). As a result, the proportion of calves with fever (>40°C) was significantly higher (*p* < 0.01) in the control group versus the vaccinated group, with a prevented fraction of 79% for the MLV-vaccinates.

Results of WBC counts showed that the counts of control calves began decreasing on day 2 post-challenge, with a greater reduction from days 3 through 8, when compared to MLV-vaccinates (Fig. 2C). The vaccinate group had a slight overall decrease in WBC counts (about 20% drop) from days 3 to 6, then recovered to normal levels. However, the control group did not fully recover its WBC count to normal levels during the challenge observation period. The difference between control and MLV-vaccinated calves in WBC counts was significant (*p* < 0.05), with control calves developing leukopenia from the challenge. Platelet counts started to decrease on day 2 post-challenge for all calves (Fig. 2D). However, the decrease was significantly different (*p* < 0.05) between the control and vaccinate calves. The mean platelet count of control calves decreased from 820 K/mm³ at challenge day to <256 K/mm³ at day 8 post-challenge. In contrast, vaccinated calves had platelet counts decrease from 700 K/mm³ at challenge day to 531 K/mm³ at day 8 post-challenge.

Nasal swab samples were collected from challenged calves from day –1 to day 10 post-challenge for virus shedding detection. All control calves shed virus at multiple days during the challenge (Fig. 2C).
period, with high titers of shed virus compared to MLV-vaccinated calves, which shed virus for a much shorter period after challenge. Statistical analysis of the virus isolation results demonstrated that there were significantly \( p < 0.01 \) less vaccinates that shed virus than controls. Furthermore, the BVDV titers from controls, were significantly \( p < 0.05 \) higher than vaccinates at days 5 through 9 post-challenge (Fig. 2E), with peak viral shedding occurring at days 7 and 8 post-challenge.

Due to the BVDV2 challenge, 9 of 15 control animals died, or were euthanized for humane reasons, on days 10–14 post-challenge (Fig. 2F). In the vaccinate group, one calf died on day 11 post-challenge, but necropsy results could not confirm the cause of death to be associated to the BVDV2 challenge. Statistical analysis showed a prevented fraction of 88% for the MLV vaccination group.

3.4. Efficacy against IBR challenge

After challenge with IBR Cooper strain, 14 of 14 control calves developed clinical signs associated with IBR infection, including nasal lesions, nasal discharge, ocular discharge, coughing and depression. Total clinical scores were significantly \( p < 0.05 \) higher in the control calves than the vaccinated calves (Fig. 3A). Specifically, control calves (14/14) showed significantly more nasal lesions \( p < 0.01 \) than vaccinated calves (4/16). Eleven of 14 control calves showed nasal discharge on various days, but only 3 of 16 MLV-vaccinated calves displayed nasal discharge. This difference in nasal discharge between control and vaccinates was significant \( p < 0.01 \).

All control calves showed elevated rectal temperatures >40 °C, and 13 of 14 controls had rectal temperatures of 40–41.2 °C for 4–11 days after challenge, with temperatures peaking at days 5 and 6 post-challenge (Fig. 3B). In contrast, 4 of 16 vaccinated calves developed elevated temperatures of >40 °C, each for only 1 day. The proportion of calves with fever (>40 °C) was significantly \( p < 0.01 \) different between vaccinate and control groups.

Virus isolated from nasal swabs at day 1 post-challenge was probably residual challenge virus, while the virus isolated at day 2 post-challenge and thereafter was likely replicating virus. Although all animals, both controls and vaccinates, shed viruses at various days after challenge, results demonstrated that control calves shed higher amounts of the IBR virus and for a longer time (Fig. 3C). The maximum post-challenge titers of isolated viruses were significantly higher \( p < 0.01 \) from control calves (8.2 \( \log_{10} \text{TCID}_{50}/\text{mL} \)) than from the vaccinated calves (6.4 \( \log_{10} \text{TCID}_{50}/\text{mL} \)).

3.5. Efficacy against PI-3 virus challenge

PI-3 typically does not cause severe disease in cattle. After challenge, the majority of the control animals (14/16) developed clinical signs of respiratory disease, including dyspnea, coughing and nasal discharge (Fig. 4A). In contrast, clinical signs were observed in only 2 of 15 vaccinated calves during the observation period. The percentage of calves developing clinical disease associated with PI-3 challenge was significantly lower \( p < 0.01 \) in the vaccinate group versus the control group, with an estimated prevented fraction of 85% for the MLV vaccination group.

In addition, the control calves developed higher rectal temperatures after PI-3 challenge, compared to MLV-vaccinated calves. Elevated rectal temperatures were observed in control animals from day 2 post-challenge, returning to levels comparable to the vaccinated group after day 6 post-challenge (Fig. 4B). Statistical analysis demonstrated that the maximum elevated temperature was significantly higher \( p < 0.01 \) in the control group than in the vaccinated group.

![Fig. 3. Intranasal vaccination protected young calves from development of severe clinical signs (A); elevated rectal temperatures (B); and virus shedding (C) caused by IBR challenge. Statistical values are *\( p < 0.05 \) and **\( p < 0.01 \).](image-url)
Fig. 4. Intranasal vaccination protected young calves from development of clinical signs (A); elevated rectal temperatures (B); and virus shedding (C) caused by PI-3 challenge. Statistical values are *p < 0.05 and **p < 0.01.

Nasal swab samples for virus isolation were collected from each calf prior to challenge (days −1 and 0) and days 1–10 post-challenge. The results demonstrated that the control calves shed significantly (p < 0.01) more virus than the vaccinated calves. The median PI-3 titer isolated from the vaccinated group was less than that in the control group. Statistical analysis of the duration of virus shedding showed that the vaccinated group had a significantly (p < 0.01) shorter duration of virus shedding (2 days) than the control group (6 days). Fig. 4C presents the virus titers shed by calves from both vaccinate and control groups.

3.6. Efficacy against BRSV challenge

After challenge with virulent BRSV, the control animals developed clinical signs of severe respiratory disease, including high fever, high respiratory rates, dyspnea, depression, anorexia, coughing and nasal discharge beginning at approximately day 3 post-challenge and continuing through to the end of the study on day 8 post-challenge. Vaccinated calves developed milder clinical disease and showed significantly lower (p < 0.05) total clinical scores than the controls following challenge. Respiratory rates increased in both groups of challenged calves (Fig. 5A). Although the difference in maximum respiratory rates was not significant (p = 0.14) between vaccinated and control groups, the vaccinated group displayed a lower respiratory rate at days 5–8 post-challenge.

Arterial blood oxygen concentration (PO2) is a key indicator of pulmonary function. Arterial blood was collected from all calves at day 6 post-challenge, and the (PO2) in the vaccinated calves (80.7 mm Hg) was significantly (p < 0.05) higher than in the control group (65.2 mm Hg) (Fig. 5B).

Nasal swab samples for virus isolation were collected from each animal prior to challenge and on days 2–8 days post-challenge. The samples were titrated on BEL cells and titers of shed virus were expressed as plaque-forming units per mL (PFU/mL). Titration results demonstrated that the control animals shed significantly (p < 0.05) more BRSV than the vaccinated animals, and the median BRSV titer for the vaccinated group was less than the control group. Furthermore, virus shedding was significantly (p < 0.05) higher in control calves on days 4–7 post-challenge, compared to the vaccinated calves (Fig. 5C).

Lungs were collected six days after challenge from the two euthanized calves (1 each from vaccinated and control groups), and eight days after challenge from all of the remaining calves, and pneumonic lesions were measured in the lungs. Although the lung lesion scores of the vaccinated group (28.1%) was not significantly different (p = 0.07) than the control group, it was still measurably lower compared to the score of control calves (40%) (Fig. 5D).

4. Discussion

Currently available parenteral combination MLV vaccines against BVDV types 1 and 2, IBR, PI-3 and BRSV can clearly induce protective responses against these viral pathogens [9,11,12]; however, no comparable combination vaccine is commercially available for intranasal administration. The development of a multivalent MLV vaccine for mucosal immunization has potential advantages compared to traditional parenteral vaccination. First, given the variability in passive transfer of maternal immunoglobulin, it is difficult to predict when parenteral vaccination of young calves will confer immune protection from these viruses. Mucosal vaccination may be more likely to immunologically prime young calves in the face of maternal antibodies. In that case, whenever the maternal antibodies decay to nonprotective concentrations, intranasally vaccinated calves would have active immunity, and avoid the “window of susceptibility” to virus infection [15]. A second advantage is that mucosal vaccination is more likely to prevent viral infection,
Fig. 5. Intranasal vaccination protected young calves from clinical respiratory diseases caused by BRSV challenge. Vaccinated calves had significantly lower respiratory rates (A), titers of shed virus (C), and percentages of lung lesions (D). After challenge, blood oxygen tension values in control calves significantly decreased (B). Statistical values are \( *p<0.05 \) and \( **p<0.01 \).

rather than simply reducing disease, by preferentially stimulating local (IgA) responses [15,19].

Many commercial MLV vaccines are available for control of the BRD complex, but nearly all of the vaccines are administered by either subcutaneous or intramuscular routes. Combination modified-live intranasal IBR and PI-3 vaccines have documented efficacy [20–22] and are the only vaccines currently licensed for intranasal administration in cattle [23,24]. In fact, intranasal vaccination using IBR and PI-3 vaccines has been accepted as a good practice for many years within the cattle industry [25,26]. Immunization via mucosal administration with BRSV vaccines, either monovalent or multivalent, has been reported [13,15]. Results of the studies reported in this paper indicate that the vaccine used for immunization was completely efficacious in intranasally immunizing against all viruses contained in the product. To our knowledge, at the time of preparation of this manuscript, the vaccine used in this paper is the only one currently approved by the regulatory authorities to be used intranasally to protect calves from virulent BVDV types 1 and 2 (unpublished data).

A feature of BVDV infection resulting from both natural exposure, and, potentially, vaccination, is immunosuppression [27–29]. Therefore, a concern was that intranasal inoculation using a MLV vaccine containing both BVDV types 1 and 2 might create a risk of immunosuppression. In this study, vaccinated calves did not show any clinical illness post-vaccination, and, importantly, as well, had no decrease in WBC counts. These findings indicate that immunosuppression is not likely to be a safety concern with this MLV vaccine.

The MLV vaccine studied here was a multivalent vaccine, including avirulent cultures of \( P. \) multocida and \( M. \) haemolytica bacterial antigens. These two antigens also induced strong immunity in young calves and protected the calves from challenges with virulent \( P. \) multocida and \( M. \) haemolytica strains (unpublished data).

In previous studies with BRSV, mucosal immunization provided clinical protection associated with an anamnestic mucosal IgA response, without a notable systemic IgG response at challenge [13,18]. Similar results were demonstrated herein, in that no significant VN antibodies were detected in the serum to any of the viruses in vaccinated calves at the time of challenge; however, serological VN antibody titers to BVDV types 1 and 2, IBR and PI-3 viruses all increased anemnestically following challenge. Since mucosal IgA and cell-mediated immune responses, including IFN-\( \gamma \), were not monitored in the current studies, no conclusions can be reached regarding which immune effector responses were associated with the observed disease-sparing. It is possible that more than one vaccine-stimulated immunologic mechanism can confer clinical protection from different virulent virus challenges. Nevertheless, the vaccine used in this study has previously been shown to induce antigen-specific T-cell subset activation to each of the five viruses in parenterally vaccinated calves [30]. Together, these findings clearly demonstrate that intranasal administration of this vaccine to young calves primes immune responses, including memory responses that reduce disease resulting from challenge with each of the viruses. Practically, cattle producers like to physically
References
