Vaccinating the young calf with a parenteral adjuvanted vaccine to develop a protective BRSV IgA nasal response

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Objective

The purpose of this study was to evaluate the efficacy of an adjuvanted modified live virus (MLV) vaccine in the presence of well-defined maternal passive immunity.

Study Description

Calves were vaccinated at approximately 1 month of age and challenged ~90 days later when BRSV systemic antibodies were less than 1:4. Clinical signs, nasal secretions and blood samples for virus measurement [polymerase chain reaction (PCR) and virus isolation (VI)] and to measure for mucosal BRSV IgA antibodies were collected and the animals were euthanized and necropsied 8 days post infection. Body temperature and other clinical signs were lower at 6 and 7 days post challenge in the vaccinates. Nasal viral shed was 3–4 times lower in the vaccinated animals as measured by VI and PCR compared to the controls. On day 8 following challenge, animals were necropsied, and lung lobes were scored and tested for virus by PCR and indirect fluorescent assay (IFA). There was a 25-fold reduction in PCR virus detection in vaccinates and two of the vaccinated calves’ lungs were PCR negative. Only 29.4% of vaccinated calves were BRSV positive on IFA testing at necropsy, while 87.5% of control calves were BRSV positive. Vaccinated calves developed a mucosal BRSV IgA response with over 50% of the vaccinated calves having IgA prior to challenge and all vaccinated calves were positive following challenge.

Take Home Points

This study established that an adjuvanted MLV vaccine could provide protection against BRSV as measured by clinical, virological, and pathological parameters while also activating both mucosal and systemic immunity.

Introduction

Bovine respiratory syncytial virus (BRSV) is major viral contributor to bovine respiratory disease (BRD). BRD is a major cause of morbidity and mortality in all classes of cattle but particularly young beef and dairy calves. Passive antibodies not only help protect the calf against infection, but may interfere with the immune responses following vaccination. The purpose of this study was to evaluate the efficacy of an adjuvanted modified live virus (MLV) vaccine in the presence of well-defined maternal passive immunity. The complete results were published in Vaccine (Kolb 2020).
**Experimental Procedures**

**Calves and Vaccination**

Thirty-three mixed sex newborn Holstein calves were obtained from a local dairy for use in the study. The newborn calves were born over a period of eight days. Cows were closely monitored at parturition to ensure that calves did not nurse. At birth, the calf was immediately removed from the calving pen, given post neonatal standard animal husbandry (navel treatment and prevention of hypothermia) and a uniquely numbered ear tag. Group 1 calves were assigned to the vaccine treatment group (17 calves) and Group 2 calves were assigned to the placebo control group (16 calves). Both treatment groups were fed characterized colostrum [colostrum with BRSV colostral SN antibody log2⁸ (64)] for the first three feedings, before being switched to commercial milk replacer. Calves were vaccinated at approximately 30 days old (30–38 days), Group 1 received 2.0 mL subcutaneously (SQ) of a multivalent, adjuvanted, MLV vaccine [Pyramid5; BHV-1, BVDV type 1 & 2, BRSV, PI3; Boehringer Ingelheim Animal Health USA, Inc. St. Joseph, MO]. The Pyramid5 vaccine utilizes the MetaStim lipid/surfactant based adjuvant system, designed to boost immune response by extending the duration of exposure to the vaccine antigens. Group 2 (control) received 2.0 mL of sterile diluent. Vaccine administered to Group 1 was stored and reconstituted according to label directions.

**Challenge and Necropsy**

When calf BRSV serum neutralization (SN) titers had declined to <1:4 at ~110 days of age, the calves were challenged with BRSV once a day for two consecutive days. The BRSV CA-1 strain was used for the challenge. Clinical disease parameters including attitude, body temperature, and general respiratory signs were monitored for 8 days following challenge by trained personnel blinded to treatment groups. Calves were considered to be pyrexic when body temperatures were 103.5ºF or greater. Each calf was visually examined and scored for signs of abnormal respiration, nasal and ocular discharge, and depression, using a scale of 0–3 except for nasal discharge which was on a scale of 0–4.

Blood samples for serum analysis was obtained from the calves via jugular venipuncture at 7–14 day intervals for approximately 8 weeks prior to challenge, the day before challenge (1DPC), and at necropsy (8DPC). The animal was restrained and 12.5 mL of blood was collected from the jugular vein. Blood samples were used for serum neutralization analysis to determine antibody levels against all major respiratory viruses, including BRSV. Nasal secretion samples were obtained from all calves weekly for the first three weeks following vaccination [0 days post vaccination (DPV), 7DPV, 14DPV and 21DPV]. Nasal secretion samples were also collected a total of 4 times in association with the challenge phase on -1, 3, 5 and 8DPC. Nasopharyngeal swabs were collected from all calves on days -1DPC and 3–8DPC for VI and BRSV polymerase chain reaction (PCR) assay.

Calves were euthanized and necropsied 8 DPC. The respiratory tract was removed from each calf, photographed, and scored. The lung scores for each animal were then added together to create a total lung lesion score for each group and these scores were compared between the two treatment groups. Two representative lung samples were collected from each calf for virus isolation (VI), BRSV immunofluorescence assay (IFA) and polymerase chain reaction (PCR) assays.

**Results and Discussion**

Body temperature and other clinical signs were lower at 6 and 7 days post challenge in the vaccinates (Figure 1). Nasal viral shed was 3–4 times lower in the vaccinated animals as measured by VI (Table 1) and PCR (Figure 2) and peaked 5 days post challenge compared to the controls (who peaked at days 6 and 7) (Table 1; Figure 2). On day 8 following challenge, animals were necropsied, and lung lobes were scored and tested for virus by PCR and indirect fluorescent assay (IFA). Respiratory tracts were evaluated at necropsy on a percent involvement basis of each lobe. The lung scores for each animal were then averaged together to create a total lung lesion score for each group and these scores were compared between the two treatment groups. A statistically significant reduction in total lung involvement was noted in the vaccinated group (Group 1) compared to the controls (Group 2) (P = 0.04) (Figure 3). Thus, 35% of the calves in the vaccinated group...
(Group 1) had ~20% total lung pathology, while 75% of the control group (Group 2) had more than 20% lung pathology (Figure 3). There was a 25-fold reduction in PCR virus detection in vaccinates (Figure 2) and two of the vaccinated calves’ lungs were PCR negative (Figure 4). Only 29.4% of vaccinated calves were BRSV positive on IFA testing at necropsy, while 87.5% of control calves were BRSV positive (Table 2). Vaccinated calves developed a mucosal BRSV IgA response with over 50% of the vaccinated calves having IgA prior to challenge and all vaccinated calves were positive following challenge (Figure 5).

This study established that calves vaccinated with an adjuvanted MLV vaccine IFOMA could be protected against BRSV. Clinical signs, lung lesions, and virus shed were reduced in the vaccinated calves as compared to control non-vaccinated calves. This is the first report of the use of a parenteral adjuvanted MLV vaccine to protect calves against a BRSV challenge IFOMA. Surprisingly, BRSV specific mucosal IgA immunity was also induced. Systemic CMI memory with a recall response following challenge also occurred following vaccination. Managing BRSV and the resulting secondary bacterial infection continues to challenge cattle producers nationwide to control bovine respiratory disease. The complications associated with both disease prevention IFOMA and the economics of high quality food production consistently create a strong demand for better vaccination strategies and products. The inhibition of BRSV vaccines administered both parenterally and intranasally by BRSV maternal antibody has been well documented [Ellis 2010; Ellis 2014; Ellis 2017; Kimman 1989] but the reality is that BRSV infection frequently occurs in young animals and vaccination needs to occur IFOMA to minimize disease risk in production systems. The purpose of this study was to evaluate the efficacy of an adjuvanted vaccine in the presence of well-defined maternal passive immunity. By controlling and defining the maternal transfer in the calves involved in the study, we hoped to evaluate the specific interactions between parenteral vaccination and passive acquired immune systems in young stock. In summary, the administration of parenteral adjuvanted BRSV MLV to calves of approximately one month of age fed colostrum containing BRSV antibodies decreased the severity and duration of clinical signs after direct BRSV challenge in animals fed maternal colostrum. Virological, immunological, and pathological findings also supported a significant advantage for vaccinated calves. While the protection conferred is not complete, up to a 50% reduction of severity of disease was noted in the vaccinated calves. Vaccinated calves mounted an immune response sufficient to reduce disease severity when challenged 3 months after vaccination in the face of maternal antibody. Further studies to characterize the mucosal response following the administration of an adjuvanted MLV needs to be done to elucidate the mechanism of mucosal immune induction.

**Implications**

This study established that an adjuvanted MLV vaccine could provide protection against BRSV as measured by virological, and pathological parameters while also activating both mucosal and systemic immunity.
References


## Table 1. BRSV Nasal Virus Isolations

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days after Challenge</th>
<th>-1</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 incidence (#Pos / #animals)</td>
<td></td>
<td>0/17 (0%)</td>
<td>1/17 (5.9%)</td>
<td>3/17 (17.6%)</td>
<td>6/17 (35.3%)</td>
<td>3/17 (17.6%)</td>
<td>5/17 (29.4%)</td>
<td>0/17 (0%)</td>
</tr>
<tr>
<td>Group 1 Average BRSV virus titer (TCID&lt;sub&gt;50&lt;/sub&gt;/ ml)</td>
<td></td>
<td>0 +/-7.39</td>
<td>2.31 +/-7.39</td>
<td>6.77 +/-7.39</td>
<td>8.65 +/-7.39</td>
<td>2.26 +/-7.39</td>
<td>3.76 +/-7.39</td>
<td>0 +/-7.39</td>
</tr>
<tr>
<td>Group 2 incidence (#Pos / #animals)</td>
<td></td>
<td>0/16 (0%)</td>
<td>2/16 (12.5%)</td>
<td>1/16 (6.3%)</td>
<td>8/16 (50%)</td>
<td>9/16 (56.3%)</td>
<td>9/16 (56.3%)</td>
<td>2/16 (12.5%)</td>
</tr>
<tr>
<td>Group 2 Average BRSV virus titer (TCID&lt;sub&gt;50&lt;/sub&gt;/ ml)</td>
<td></td>
<td>0 +/-7.62</td>
<td>1.96 +/-7.62</td>
<td>2.46 +/-7.62</td>
<td>32.57 +/-7.62</td>
<td>33.73 +/-7.62</td>
<td>29.61 +/-7.62</td>
<td>1.96 +/-7.62</td>
</tr>
</tbody>
</table>

1. Group 1: Five (5) of the 17 calves remained VI negative during the challenge phase. Five (5) of the calves were positive (low titer) on VI for more than one consecutive day post challenge (only 2 days). Seven (7) of calves were positive for VI one day.

2. Group 2: Five (5) of the 16 calves remained VI negative during the challenge phase. Eight (8) of the calves were VI positive for more than one day post challenge (5 were ≥3 days). Three (3) of the calves were positive for VI one day. An overall trend was noted for each treatment (P=0.06), but there was a statistically significant effect on each treatment over time (P<0.0001).

## Table 2. BRSV Antigen Positive Lungs<sup>1</sup>

<table>
<thead>
<tr>
<th>Group #</th>
<th>BRSV FA Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>5/17 (29%)</td>
</tr>
<tr>
<td>Group 2</td>
<td>14/16 (88%)</td>
</tr>
</tbody>
</table>

1. Frozen lung sections were stained with BRSV Ab and examined for fluorescence. There was significant reduction (p<0.05) in BRSV infected lungs in Group 1 (Vaccinates) as compared to Group 2 (Controls).
Figure 1. Average Daily Rectal Temperatures. Group 1 (Vaccinates) and Group 2 (Control). Body temperatures were recorded from all calves daily beginning one day prior to challenge, and daily after challenge for the remainder of the study (-1DPC to 8DPC). Calves with a rectal temperature of 103.5°F or higher were considered pyrexic (error bars indicate the standard error). The body temperatures for each group were averaged and graphed to evaluate the difference in disease severity between Group 1 and Group 2. There were statistically significant lower temperatures in Group 1 (Vaccinates) at day 6 and 7 (indicated with the presence of an *) (P ≤ 0.01) and tendency for lower temperature in Group 1 (Vaccinates) at day 8 (P < 0.1) (noted with a presence of a #).
Figure 2. BRSV RNA in Nasal Samples Post Challenge. Group 1 (Vaccinates) and Group 2 (Controls). BRSV RNA levels were similar at day 5 (error bars indicate the standard error). The Group 1 animals peaked at day 5 and declined quickly to undetectable BRSV RNA levels (CT level 26=38.6 TCID50/ml and CT level 36=<1 TCID50/ml). Overall there was an effect of treatment (P=0.05) and an effect of time (P<0.0001) noted.
Figure 3. Percentage of Animals with Total Lung Lesion Involvement ≥20%. Group 1 (Vaccinates) and Group 2 (Control). The number of animals in each group with a total lung lesion involvement of 20% or more were compiled. The remaining percent of animals by group had lesion scores that were less than these parameters. There was a statistically significant reduction in lung lesions in Group 1 (Vaccinates) compared to the controls (P<0.05) as well as a reduction in the number of animals with significant lung involvement.
**Figure 4. BRSV RNA in Lung.** Group 1 (Vaccinate) and Group 2 (Control). BRSV RNA lung levels were significantly lower (Group 1 3.97 vs. Group 2 105.85 TCID50/ml p<0.05). Two (2) of the 17 animals in Group 1 were PCR negative (CT value >40) while none (0 of the 16) Group 2 were PCR negative (error bars indicate the standard error).
Figure 5. Nasal IgA OD Values following Vaccination and Challenge. Group 1 (Vaccinate) and Group 2 (Control) IgA OD were compared from post vaccination A) Day 0, B) Day 21, C) Day 71 (day -1 post Challenge) and D) Day 80 (day 8 post challenge). All animals were negative at day 0; 9/17 were positive at day 21 and day 71 (one day prior to challenge) in Group 1 compared to 0/16 in Group 2 at day 21 and 71. At Day 80, 100% of the Group 1 (17/17) were positive compared to 31% (5/16) of Group 2. There was significantly higher average level of IgA antibody for Group at Day 21, 71 and 80 (p<0.05). The average is indicated by the line in each column. The negative OD cutoff is 0.2. *Statistically significant (p<0.05).