Blinded, controlled field trial of two commercially available *Mycoplasma bovis* bacterin vaccines in veal calves

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**A B S T R A C T**

*Mycoplasma bovis* is an etiologic agent of pneumonia, arthritis, and otitis in young calves, such as those found in the special-fed veal industry. We conducted a blinded, controlled trial of two commercially available *M. bovis* bacterin vaccines for the prevention of respiratory disease in calves associated with *M. bovis* infection. Calves were randomly assigned to a subcutaneous treatment of vaccine A (n = 50), adjuvant A (n = 50), vaccine B (n = 50), or 0.9% sterile saline solution (n = 50) beginning at 27 days of age. Upper-respiratory tract colonization was not impacted by vaccination status. Vaccine A significantly reduced the presence of lung lesions (p = 0.0325), however there was no significant reduction of *M. bovis* in lung lesions. Vaccine B did not significantly reduce total lung lesions or *M. bovis*-specific lung lesions. The relative risk was determined to be 0.56, 1.0, and 1.36 for vaccine A, adjuvant A, and vaccine B, respectively. There was no association between the total specific antibody isotype (IgM, IgG1, IgG2, IgA) concentrations or *M. bovis* antibodies and the *M. bovis*-associated morbidity in the veal calves. Under the field conditions of this study, observed vaccine efficacy for vaccine A and vaccine B was 44% and less than 1%, respectively.

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1. Introduction

*Mycoplasma bovis* infection is an important disease of cattle causing pneumonia, mastitis, otitis, conjunctivitis, and arthritis which is naturally refractory to numerous antimicrobials [19]. Young calves less than 4 months of age are at high risk for respiratory disease due to *M. bovis* [19,28]. *M. bovis* may also act as a predisposing factor that could weaken the host immune system leading to invasion by other pathogenic bacteria or viruses [24]. Between one-quarter and one-third of pneumonia-related illnesses in growing cattle may be attributed at least in part to *M. bovis* infections [18]. Additionally, cattle infected with *M. bovis* may serve as a reservoir of infection by shedding bacteria from the respiratory tract for many months [22]. The largest economic impact of *M. bovis* infections is due to its chronicity and polymicrobial nature of the infections [26].

Special-fed veal represents nearly a 1 billion dollar industry in the United States. In the special-fed veal industry, unaltered male calves are purchased from the dairy industry and raised to ~205 kg. The special-fed veal industry in the US follows “all in/all out” agricultural bio-secure management protocols to reduce exposure to outside diseases. The age of the animal, as well as the chronic nature of the disease, presumed increased spread of disease in intensive housing units, and poor response to most antimicrobial therapies makes veal calves vulnerable to *M. bovis*-associated respiratory disease.

One potential method of bacterial disease control in veal calves is the use of vaccines. Some European and Asian field trials have indicated protection from respiratory disease due to vaccination [8,21,29,31]. Currently there are only a few *M. bovis* vaccines approved in the United States including bacterin and autogenous vaccines. However, there is little evidence of vaccine efficacy as there are few published reports on *M. bovis* vaccines in the United States [17,21]. In an animal host, *M. bovis* has the potential to induce both immune reactive and immunosuppressive functions. This is shown by the activation of TNF-α and nitric oxide in alveolar macrophages as well as inhibition of polymorphonuclear neutrophil (PMN) degranulation, oxidative bursts, and induction
of lymphocyte apoptosis [10,14,23,32,33]. There is a measurable humoral and cellular immune response to *M. bovis*; in cattle, the immune response has been reported to be characterized by Th2-skewed cytokine production [32]. However, as seen in murine models, the immune response of the host may be responsible for some of the lung damage associated with *M. bovis* [7,33]. To appreciate the efficacy of vaccination of individual animals or of entire herds a better understanding of the immune response and distribution of *M. bovis* in affected animals is needed.

The few published reports regarding the efficacy of currently available *M. bovis* vaccines impedes the ability to determine if vaccination is a viable strategy for the reduction of *M. bovis*-associated disease in calves. The focus of this study was to determine the efficacy of two commercially produced *M. bovis* bacterins vaccines for the prevention of morbidity associated with *M. bovis* colonization and infection leading to the development of lung lesions. Additional objectives were to compare the vaccinated and control group calves (either adjuvant or sterile saline) with respect to the percentage of nasal colonization and otitis morbidity from *M. bovis*, total immunoglobulin sub-type and cytokine concentrations, *M. bovis*-specific serum immunoglobulin concentrations, and potential of adverse events associated with vaccination.

2. Materials and methods

2.1. Population and sample size

Using a blinded, systematically randomized field trial, we studied 200 unalterd male Holstein calves housed at a single location in northwestern Pennsylvania. The herd was selected because the herd producer reported respiratory problems in previous herds which were suspected, but not confirmed *Mycoplasma* species outbreaks, and a willingness to participate. Calves were followed through the entire growing period from November 2009 through April 2010. Each calf received a diet of non-soy milk replacer containing iron and 40 other essential nutrients and routine veterinary care, including vaccination against BVD (types 1 and 2), IBR, BRSV, and PI3. Calves were monitored for changes in health status daily by the herd owner and −bi-monthly by the research team. When the calves reached sale weight of ~205 kg (approximately 145 days of age), they were shipped to a processing facility. Any individual calves that became ill during the study were treated under the supervision of a veterinarian.

Lung lesion morbidity due to *M. bovis*-associated infection was the major outcome of interest and its anticipated incidence was used to calculate the required sample size. We hypothesized that a 20% reduction in lung lesions would be biologically impactful. The sample size was determined using an alpha value of 95% and a power of 80%. It was determined the ideal sample size per group be 43 calves based upon the prevalence of *M. bovis* (90.5%) in veal calf herds from Pennsylvania [27]. In order to account for attrition a total of 50 calves each were assigned to the following groups: vaccine A (Mycoprene® R, BIOMUNE Co., Lenexa, KS), adjuvant A (all vaccine components except antigen; control group) (BIOMUNE Co., Lenexa, KS), vaccine B (Pulmo-Guard™ Mbp, American Animal Health, Inc., Grand Prairie, TX), and 0.9% sterile saline (control group) (Agri Labs, Ltd., St. Joseph, MO).

2.2. Group assignment, and blinding

Vaccine A and adjuvant A were provided blinded by the manufacturer. Vaccine B was purchased from PBS Animal Health Inc. (Massillon, OH). To ensure differences in air-flow patterns in the barn were not likely to result in an increased risk of infection; groups of 4–5 calves per section were systematically assigned to vaccine A, vaccine B, adjuvant A or sterile saline. The order for the systematic arrangement was randomly assigned using Excel (Microsoft Office Excel 2007). The systematic assignment pattern was followed throughout the barn of 200 calves, resulting in equal groups of 50 calves, spaced throughout the barn.

The calf owners, caretakers, and processing personnel were blinded to individual calf treatments throughout the trial. The researchers involved with data analysis were back-end blinded to the treatment-type administered to each calf.

2.3. Treatment groups

Following Pennsylvania Beef Quality Assurance guidelines [3] and manufacturer’s recommendations, all injections were 2 ml doses given subcutaneously in the neck beginning at approximately 4 weeks of age. According to the manufacturer’s instructions vaccine A and adjuvant A, were administered as three doses to the calves at ~27, 38, and 56 days old. A deviation from Manufacturer’s instructions was made for the doses of vaccine B which were given to the calves as two doses at ~27, and 38 days old instead of calves older than 45 days old. The 0.9% sterile saline solution was administered in calves as two doses at ~27, and 38 days old. Vaccination of calves was administered by a veterinarian.

2.4. Sample collection

On farm, nasal swabs were collected at 7, 38, 88, and 136 days of age. A sterile rayon-tipped swab with polyurethane plastic shaft (BBL™ Aimes media CultureSwab™, BD, Franklin Lakes, NJ) was inserted in the nostril to a depth of ~4 inches. Swabs were transported on ice and cultured with-in 6 h of collection. Due to the effect that colostral immunoglobulins may have upon infection and response to vaccination, blood was collected upon acclimation to barn (3 days post arrival).

A total of 4 blood samples were collected from each veal calf at 7, 38, 88 and 136 days of age. At each sampling, two 3 ml tubes of blood (BD vacutainer® , BD, Franklin Lakes, NJ) were collected through venipuncture to gather plasma and serum. Samples were centrifuged at 2000 × g at room temperature for 15 min; collected serum was stored at −20 °C until further testing.

At a USDA approved processing facility where lungs were excised post slaughter. A swab was then inserted into an incision made into the trachea to sample both sides of the bronchial bifurcation. Macroscopic lung lesions were identified and excised for examination by culture analysis. All bronchial swabs and lung lesions were transported on ice. Bronchial swabs were cultured within 24 h of collection. In the laboratory, the surface of the lung lesion tissue collected was seared. An incision was then made with a sterile scalpel and a sterile cotton tipped swab (Puritan Medi- cal Products, Guilford, ME) was inserted into the core of the tissue sample and immediately cultured.

2.5. Culture methods

Nasal, bronchial bifurcation, and lung lesion swabs were cultured on blood agar (BA) and MacConkey (MAC) agar at 37 °C in an aerobic environment, and chocolate agar (CA) at 37 °C in a microaerophilic environment. Standard biochemical identification procedures and API (BioMerieux, France) for identification of common bovine respiratory pathogens was used. All swabs were then enriched in Mycoplasma enrichment broth supplemented with penicillin (UC Davis Biological Media Services, Davis, CA) for 48 h before being plated on pleuropneumonia-like organism (PPLO) agar plates. All mycoplasma cultures were grown at 37 °C in a microaerophilic environment. PPLO agar plates were considered negative if no growth was noted by day 10. Confirmation of
mycoplasma growth was performed using PCR of the uvrC M. bovis housekeeping gene as described by Thomas et al. [30] and other common respiratory Mycoplasma species (M. dispar, M. bovirhinis, M. bovigenitalium, M. californicum, M. canadense, and M. alkalesscens) using previously published primer combinations [20].

2.6. Total protein and hematocrit

In order to determine the health of calves prior to vaccination, total protein was determined for all samples using a refractometer (Atago Inc., Tokyo, Japan). Hematocrit readings were also performed on a random selection of calves (n = 31). All calves determined to have abnormal total protein readings were tested again one and two months later.

2.7. Histology

Sections of normal and abnormal lung and trachea tissue were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin–eosin (HE) stain at the Pennsylvania State University Animal Diagnostic Laboratory, University Park, PA.

2.8. Serology

Serum, harvested after clotting, was stored at −20 °C. Serum of all calves at each time point was analyzed for IgM, IgA, IgG1, and IgG2 by ELISA (Bethyl Laboratories, Montgomery, TX) per manufacturer’s instructions and suggested optimization procedures. The optical density of each well was measured at 450 nm using an automated plate reader (ELX800 Microplate Reader, BioTek Instruments, Inc., Winooski, VT). Data was gathered using KC junior (BioTek Instruments, Inc., Winooski, VT).

For each plate duplicate sets of bovine reference standards (Bethyl Laboratories, Montgomery, TX) were analyzed under the same conditions as samples. Reference serum (antibody isotype specific) concentrations of 1000 ng/mL, 500 ng/mL, 250 ng/mL, 125 ng/mL, 62.5 ng/mL, 31.25 ng/mL, 15.625 ng/mL, and 0 ng/mL were prepared and used to calculate the standard curve using linear regression analysis. Duplicate run OD values were averaged. Sample values were converted to a serum concentration.

Serum of all calves at each collection point was analyzed for M. bovis-specific antibodies via semi-quantitative ELISA (Biovet Inc., St. Hyacinthe, Canada) per manufacturer’s instructions. The optical density of each well was measured at 450 nm using an automated plate reader according to the same conditions as reported above for isotype antibody ELISA. Positive and negative controls, provided by the manufacturer were run with each plate.

A subset of calves was selected for further testing. A total of 5 calves (10%) from each treatment subgroup were randomly selected for cytokine screening. Sera from each collection date were sent to Aushon Biosystems, Inc. (Billerica, MA) for the bovine chemiluminescent multiplex enzyme-linked immunosorbent assay (ELISA) panels for IL-1β, IL-2, IL-4, IL-6, IFN-γ, and TNF-α.

2.9. Statistical methods

The statistical significance of M. bovis morbidity was determined using Fisher’s exact test and 2 × 2 contingency tables. Categorical outcome variables were compared using chi-square tests. Quantitative outcome variables were compared using independent sample t-tests. Vaccine efficacy was determined using the relative risk, a ratio of the risk among the vaccinated and the risk among the unvaccinated [2]. Due to differences in the proprietary adjuvants for each vaccine, Vaccine A and adjuvant A were compared and vaccine B and sterile saline were compared. ELISA data were compared using two-factor ANOVA with repeated measures. A p-value of <0.05 with two-sided tests was considered significant for all analyses. Statistical analyses were performed using GraphPad Prism v. 5 (GraphPad Software, Inc., La Jolla, CA).

3. Results

3.1. Baseline data and vaccine-associated adverse events

After attrition (unidentifiable ear tags at slaughterhouse (n = 13) the vaccine A, adjuvant A, vaccine B, and sterile saline groups included 45, 45, 48, and 49 calves, respectively. The average readings for total protein (TP) and hematocrit upon entry to the barn were 5.9 g/dL and 24%, respectively. Nineteen calves were shown to have TP levels less than 5.0 g/dL upon entry into the barn, likely due to poor transfer of colostrum from the dam. All calves were found to be of acceptable health for vaccine regimen. A total of 7 subcutaneous granuloma vaccine associated adverse events were noted at injection sites after vaccination with vaccine A (n = 2) and adjuvant A (n = 5). Upon examination of the carcasses, no granulomas were found. No adverse reactions at injection sites were noted for vaccine B or 0.9% sterile saline solution. The average weight gain over the course of the study for calves receiving vaccine A, adjuvant A, vaccine B, and saline was 167 ± 30.7, 168 ± 31.8, 171 ± 31.2, and 170 ± 37.4 pounds, respectively.

3.2. Lung infection

A total of 81 lungs containing macroscopic lesions were identified at the abattoir. At slaughter, lung lesions were identified in a total of 14, 25, 24, and 18 calves treated with vaccine A (n = 43), adjuvant A (n = 43), vaccine B (n = 48), and sterile saline (n = 49), respectively. Of the lung lesions from calves receiving vaccine A, adjuvant A, vaccine B, and sterile saline, 3, 10, 7, and 8 of the collected lesions were culture positive for M. bovis, respectively (Table 1). M. bovis was the only Mycoplasma species identified. Histopathological examination found interstitial pneumonia ranging from minimal to mild interstitial pneumonia to severe broncho-interstitial pneumonia for the collected lesions identified to have M. bovis (Fig. 1). The trachea histopathology samples demonstrated minimal to mild chronic tracheitis (31.6%) or were reported to be normal. Other bacterial respiratory pathogens cultured from the lung lesions of calves treated with vaccine A were Pasteurella multocida (n = 1); adjuvant A, P. multocida (n = 1) and Mannheimia haemolytica (n = 1); vaccine B, P. multocida (n = 1), Klebsiella pneumoniae (n = 1), and Arcanobacterium pyogenes (n = 2); and for sterile saline, P. multocida (n = 1), K. pneumoniae (n = 1), and A. pyogenes (n = 1).

3.3. Upper-respiratory tract colonization

Upper-respiratory tract (URT) colonization was neither significantly different for vaccine A compared to adjuvant A nor vaccine B compared to sterile saline (Fig. 2). The cumulative incidence at day 136 of M. bovis-associated URT colonization for vaccine A, adjuvant A, vaccine B, and saline were 94.0% (95% CI, 83.1–97.9%), 92.0% (95% CI, 80.7–94.4%), 96.0% (95% CI, 85.8–99.7%), and 90.0% (95% CI, 78.2–96.1%), respectively. M. bovis was recovered from a total of 62.0% (95% CI, 47.6–74.9%), 49.0% (95% CI, 37.1–60.9%), 56.0% (95% CI, 42.3–69.3%), and 59.0% (95% CI, 45.2–71.8%) of swabs collected at the bronchial bifurcation of calves treated with vaccine A, adjuvant A, vaccine B, and sterile saline, respectively.

3.4. Efficacy of vaccination on M. bovis-associated lung lesions

It was determined that vaccine A significantly reduced the number of lung lesions identified in veal calves compared to adjuvant A
Table 1
Summary of lung lesions.

<table>
<thead>
<tr>
<th></th>
<th>Vaccine A</th>
<th>Adjuvant A</th>
<th>Vaccine B</th>
<th>0.9% Saline</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total calves</td>
<td>43</td>
<td>45</td>
<td>48</td>
<td>49</td>
<td>185</td>
</tr>
<tr>
<td>Lung lesions</td>
<td>14</td>
<td>25</td>
<td>24</td>
<td>18</td>
<td>81</td>
</tr>
<tr>
<td>with <em>Mycoplasma bovis</em></td>
<td>3</td>
<td>10</td>
<td>7</td>
<td>8</td>
<td>28</td>
</tr>
</tbody>
</table>

Fig. 1. Histopathology of lung lesions-associated with *Mycoplasma bovis*. (A and B) Severe broncho-interstitial pneumonia with intrabronchiolar accumulations of neutrophils and cellular debris. Marked peribronchial lymphoid hyperplasia is also evident, characteristic of *Mycoplasma* infection. (C) Mild to moderate interstitial pneumonia characterized by interstitial thickening. (D) Normal calf trachea.

Fig. 2. Cumulative incidence of *Mycoplasma bovis* infection. Cumulative incidence of *M. bovis* upper-respiratory tract (URT) colonization of vaccine A (solid line), adjuvant A (solid with a dot line), vaccine B (dashed line), and 0.9% sterile saline solution (dotted line).

(p = 0.0325), but did not significantly reduce *M. bovis*-specific lung lesions (p = 0.0690). It was determined that vaccine B neither significantly reduced the total number of lung lesions identified in veal calves as compared to 0.9% sterile saline (p = 0.2218), nor significantly reduced the number of veal calves with *M. bovis* lung lesions (p = 1.000). It was determined that calves in the center of the systematically randomized groups of five for the vaccinated were less likely to develop lung lesions than the assigned control group. The relative risk of developing a lung lesion for vaccine A compared to adjuvant A and vaccine B compared to sterile saline was 0.56 and 1.36, respectively. The vaccine efficacy related to overall reduction of lung lesions in veal calves of vaccine A compared to adjuvant A was 44%. Vaccine B compared to sterile saline showed no vaccine efficacy (defined as less than 1%).

3.5. Antibody response

The serum antibody isoclass response (IgM, IgA, IgG1 and IgG2) was assessed via ELISA at 7, 38, 88, and 136 days of age. The trends across the sampling times for the averaged IgM, IgA, IgG1, and IgG2 values for vaccine A, adjuvant A, vaccine B, and sterile saline are...
shown in Fig. 3. The differences across sampling time points for each immunoglobulin tested for all treatment groups was significant \((p < 0.0001)\). No significant differences between vaccinated or control calves was detected for IgM, IgA, IgG1, or IgG2. The largest differences between vaccine A and adjuvant A and between vaccine B and saline occur at 88 days for each immunoglobulin tested (56 days post final dose of vaccine A and adjuvant A; 38 days post final dose of vaccine B and saline). There was a significant difference in the IgG1 antibody subclass response of vaccine A calves which had URT colonization of \(M.\ bovis\) and those without URT colonization at day 7 \((p = 0.01)\) and day 88 \((p = 0.05)\), while a significant difference between vaccine B calves was found for IgG2 subclass response at day 38 \((p = 0.03)\) and for IgA at day 88 \((p = 0.006)\). There were no significant differences for the IgM antibody isoclass regardless of URT status.

At three days post-arrival to the barn, \(M.\ bovis\) seropositivity was noted for of 38.0% (95% CI 25.8–51.9%), 20.0% (95% CI 11.1–33.2%), 28.0% (95% CI 17.4–41.8%), and 36.0% (95% CI 24.1–49.9%) of calves in vaccine A, adjuvant A, vaccine B, and saline, respectively. At 138 days of age 100% of calves in each intervention group had antibodies detected by ELISA to \(M.\ bovis\). There was no correlation between antibody level prior to vaccination and the vaccination success.

### 3.6. Cytokine response

A subset of 5 calves was randomly selected from each treatment group for cytokine assays. The average cytokine concentrations (pg/mL) for days 7, 38, 88, and 138 for IL-1β, IL-2, IL-4, IL-6, IFN-γ, and TNF-α are shown in Fig. 4. Most notably, calves receiving either vaccine A or vaccine B showed a peak in IL-1β and TNF-α concentration at day 88. Calves receiving either adjuvant A or saline remained stable across sampling for IL-1β, and TNF-α (Fig. 4). Additionally, an increase in IL-2 concentrations was seen for vaccine A and saline at 88 days. Differences between vaccinates and the respective controls were no significant for IL-1β, IL-2, IL-4, IL-6, IFN-γ, nor TNF-α.

### 3.7. Additional results

Otitis, identified by characteristic unilateral or bilateral ear droop, was noted in 16 calves throughout the study period. Otitis was present in calves vaccinated with vaccine A \((n = 4)\), adjuvant A \((n = 6)\), vaccine B \((n = 3)\), and saline \((n = 3)\) groups. Fifteen of the 16 calves with otitis also had at least one \(M.\ bovis\) positive nasal swab during the study period. Vaccination with either vaccine A or vaccine B did not result in a significant reduction of otitis morbidity. No cases of arthritis were noted in this study.

### 4. Discussion

Protection from \(M.\ bovis\)-associated respiratory disease has been reported for killed whole cell bacterin vaccines in older calves \([8,13,21,29,31]\), but there have been reports of adverse events associated with vaccination against \(M.\ bovis\) \([4,5]\). Efficacy has also been reported for a saponin adjuvanted bacterin vaccine in 3 week old dairy calves in Europe \([21]\). The efficacy of the commercially available bacterin vaccine in the United States, marketed for stocker and feedlot cattle, has been reported by Maunsell et al. \([17]\) in young dairy calves from Florida. The study reported that the bacterin vaccine was ineffective at preventing nasal colonization and \(M.\ bovis\)-associated disease in pre-weaned endemically infected dairy herds from Florida \([17]\).

The purpose of this study was to determine the efficacy of two commercially available \(M.\ bovis\) bacterin vaccines for use in veal calves. The vaccine efficacy related to reduction of lung lesions in veal calves was 44% and <1% for vaccine A and vaccine B, respectively. Vaccine efficacy in this study may be different from previous studies due to the strain of bacteria used in the vaccines, the adju-
vant used (the adjuvant of both vaccines used in this study are proprietary; only the adjuvant of vaccine A was acquired for this study), the method of inactivation, the high URT colonization (44%) of the total study calves prior to vaccination for this endemically infected herd, the impact of herd immunity, and the housing differences of veal calves compared to other calves. The vaccines used in this study were ineffective at preventing URT colonization with M. bovis in calves. Vaccine A and vaccine B were also ineffective at preventing M. bovis infection of the bronchus. This indicates that M. bovis infection at the intersection of the upper-respiratory and lower-respiratory tract is not affected by vaccination. Although serum IgG1 response was shown to increase throughout the duration of the study there was not a significant difference between calves vaccinated with vaccine A and adjuvant A or vaccine B and sterile saline.

Previous research has shown no relationship between indirect hemagglutination titers and IgM or IgG concentrations in calves [6]. Local immune responses are likely better indicators of M. bovis protection than serum antibody responses [12]. The role of specific antibody levels in susceptible and immunized animals is poorly understood, but it has been suggested that IgG responses of vaccinated calves and naturally infected calves are similar [5]. The lack of a significant difference between serum IgA concentrations, in vaccinated and control calves is likely due to the similarity of infection status across the herd and that vaccines may not be designed to stimulate the mucosal system. It has been suggested that IgA antibodies are unlikely to be produced following subcutaneous vaccination with killed Mycoplasma species [11]. The results of this study are in agreement with previously reported immune responses to a M. bovis vaccine subcutaneously administered to heifer calves [17]. The lack of notable differences in the tested antibody isotype responses for vaccinated and control calves may be due to the constant stimulation of the immune response in all calves due to the endemic nature of M. bovis infections in the study herd.

It is unclear how vaccination would impact naïve calves instead of calves which had already begun to show M. bovis seropositivity prior to vaccination. A total of 30.5% of all calves in the barn, regardless of intervention group were seropositive at three days post arrival to the barn indicating that it is likely at least a portion of these calves had become M. bovis seropositive at their origination farms. Presumably, M. bovis antibodies which are transferred to new-born calves via maternal colostrum may indicate the prevalence in the maternal population of a farm if one single batch of colostrum is not used to feed all new-born calves. Calves were...
purchased from a multi-state area and therefore it is unknown if an individual calf received colostrum from the dam or from bulk colostrum. Previous studies throughout Europe have found the seropositivity to range from 2.2% to 14% [1,15]. The high seroprevalence of *M. bovis* antibody results in this study indicate a need for further studies.

It has been reported that in cattle an immune response to *M. bovis* is characterized by a Th2-skewed cytokine response [33]. The increased levels of IL-1β and TNF-α in vaccine A and B as compared to adjuvant A and saline suggest that a pro-inflammatory effect in vaccinated calves, which is likely in epithelial cells. In murine models it has been shown that IL-1β augments the TNF-α responses in lung epithelial cells [25]. However, in this study there is no evidence to suggest that the origin of the observed cytokine response was epithelial. The increase in IL-2 found in the vaccine A and saline groups may be the result of adaptive immune responses in those calves. It is likely that the early (~7 days age) sampling impacted the cytokine and IgG specific antibody titers reported, which may represent the maternal transfer of antibody to the calf. The changes noted in IL-4, IL-6, and IFN-γ level for the subset of calves selected may be impacted by the colostal status of calves upon entry into the barn which are shown to stabilize by the second and third samplings. The detectable differences between intervention groups for IL-1β, IL-2, and TNF-α are not significant and therefore it is recommended that larger sampling groups are tested in future studies to determine the nature of the differences.

Importantly, it was shown that *M. bovis* colonization was established prior to vaccination. If the adaptive immune responses were unable to develop there may have been chronic inflammation, which may limit vaccine efficacy. It should be noted that vaccine A is labeled for use in calves 3 weeks of age or older while vaccine B is labeled for use in calves 45 days of age or older. Calves in this study were vaccinated at 27, 38, and 56 days of age or 27 and 38 days of age for vaccine A and vaccine B, respectively. It is possible that vaccination at 27 and 38 days of age does not elicit the same immune response as in calves 45 days of age or older, as recommended for vaccine B. Calves should not be vaccinated within 21 days or 60 days prior to slaughter for vaccine A or vaccine B, respectively. Vaccination at a later age, as recommended by vaccine B, plus the need for 60 days between vaccination and slaughter will likely prove to be challenging for veal producers due to the total length of time that calves are fed prior to processing (~145 days). The age of calves at time of slaughter may be one reason why no differences were noted in weight gain of vaccinated versus unvaccinated calves. The vaccination protocol was selected based upon the early age of URT colonization and lung infection observed in endemic mastitis exposed veal calves from Pennsylvania in previous work [27].

The systematic assignment of calves in groups of five was selected in order to control for the possibility of air flow differences in the barn. Calves were randomly assigned a specific stall upon entry to the barn. The random assignment should minimize the influence of herd immunity. It was determined that the calves in the center of the group of five were not significantly less likely to have lung lesions. Therefore, it is unlikely that herd immunity is acquired by vaccination with vaccine A or vaccine B for *M. bovis*-associated lung lesions. It is possible that clusters greater than five are necessary to identify herd immunity effects for *M. bovis*-associated lung lesions. Based on this data, it is unlikely that a selection bias from the randomization method resulted in a significant impact upon the measured outcomes in this study.

It has been suggested that calves with TP values less than 5.0 g/dL have failed of passive transfer of antibody and are 3–6 times more likely to die before 6 months of age, although mortality in special-fed veal herds is generally less than in other calf raising operations [9,34]. In order to assess the overall health of calves upon entry into the barn, total protein and hematocrit were performed. A total of 19 calves were found to have TP values below 5.0 g/dL upon entry into the veal raiser barn. Six of the 19 calves (0.3158; 95% CI 0.1516–0.5420) were found to have lung lesions at slaughter. Forty-three percent of calves with a TP value greater than or equal to 5.0 g/dL were found to have lung lesions at slaughter (0.4345; 95% CI 0.3618–0.5101). These results are similar to previously reported results of another *M. bovis* bacterin vaccine, indicating that TP concentrations do not play a role in the incidence of respiratory disease [17]. The average weight gain of calves was not significantly impacted by vaccination against *M. bovis*. These results agree with previous reports that antimicrobial treatment rates for respiratory disease and weight gain do not differ between vaccinated and unvaccinated groups [16]. In this study, treatment group did not impact the presence of otitis cases in the veal calves.

To the best of the authors’ knowledge this is the first report of a blinded, controlled field trial of these two *M. bovis* vaccines available in the United States in young calves. A distinction was made between lung lesions and lung lesions-associated with *M. bovis* in order to estimate the specific impact of *M. bovis* upon development of lung lesions in vaccinated calves. However it should be noted that it is not always possible to isolate or detect *M. bovis* in tissues and vaccination may have prevented immunosuppression which would have resulted in polymicrobial infections leading to better vaccine efficacy. Vaccination with either vaccine A or vaccine B was not efficacious in preventing upper-respiratory tract colonization of *M. bovis*, nor effective in preventing *M. bovis*-specific lung lesions in special-fed veal calves. Vaccine A was shown to have a vaccine efficacy of 44% in preventing the presence of lung lesions. Vaccination resulted in a detectable difference in IL-1β and TNF-α following the final dose of vaccine. The results of this study suggest that vaccine A is more likely to be efficacious for use in young veal calves (starting at 3 weeks of age). The results of this study should not be used to infer the ability of either vaccine to effectively prevent *M. bovis* colonization or disease in animals of other ages. This research demonstrates the difficulty of implementing current vaccination strategies for use with special-fed veal calves which are much younger than most dairy or beef calves when vaccinated. As well as the inherent higher risk for sharing respiratory pathogens due to the close proximity of uninfected to infected calves.

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