Efficacy of a multivalent modified-live virus vaccine containing a Mannheimia haemolytica toxoid in calves challenge exposed with Bibersteinia trehalosi

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Objective—To determine the efficacy of a multivalent modified-live virus (MLV) vaccine containing a Mannheimia haemolytica toxoid to reduce pneumonia and mortality rate when administered to calves challenge exposed with virulent Bibersteinia trehalosi.

Animals—74 Holstein calves.

Procedures—Calves were assigned to 2 treatment groups. Calves in the control group (n = 36) were vaccinated by SC administration of 2 mL of a commercial 5-way MLV vaccine, and calves in the other group (38) were vaccinated by SC administration of a 2-mL dose of a 5-way MLV vaccine containing M haemolytica toxoid (day 0). On day 21, calves were transtracheally administered B trehalosi. Serum was obtained for analysis of antibody titers against M haemolytica leukotoxin. Nasopharyngeal swab specimens were collected from calves 1 day before vaccination (day −1) and challenge exposure (day 20) and cultured to detect bacterial respiratory pathogens. Clinical scores, rectal temperature, and death attributable to the challenge-exposure organism were recorded for 6 days after challenge exposure. Remaining calves were euthanized at the end of the study. Necropsy was performed on all calves, and lung lesion scores were recorded.

Results—Calves vaccinated with the MLV vaccine containing M haemolytica toxoid had significantly lower lung lesion scores, mortality rate, and clinical scores for respiratory disease, compared with results for control calves.


Bibersteinia trehalosi (previously known as Pasteurella trehalosi and Pasteurella haemolytica type T) is a gram-negative bacterial respiratory tract pathogen that causes pneumonia (as well as septicemia) in domestic sheep, goats, and bighorn sheep.1–3 The organism is the most common pathogen found in tonsils of clinically normal American Bison.4,5 It has been identified as a major cause of pneumonia and septicemia in cattle.6–8 Bibersteinia trehalosi can cause peracute bacterial pneumonia in adult dairy cattle, feedlot cattle, and young calves.5 Clinical signs range from pneumonia to sudden death. Lungs often have exudative fibrinous pneumonia, bronchiolitis, and alveolitis.9,10

ABBREVIATIONS

AHDRCC  Animal Health Developmental Research Culture Collection of Zoetis Inc
BRD  Bovine respiratory disease
CI  Confidence interval
lkt  Leukotoxin
MLV  Modified-live virus
OD  Optical density
PBST  PBS solution and 0.05% Tween 20
PFGE  Pulsed-field gel electrophoresis

Bibersteinia trehalosi has been identified in healthy as well as pneumonic or septicemic cattle in the United Kingdom since 2003.10 Increasingly, diagnostic laboratories are isolating B trehalosi from clinically affected cattle.5–8 A report10 from the United Kingdom indicated that B trehalosi was the only pathogen in 18 of 65 (28%) cattle with pneumonia. From 2004 to 2009, B trehalosi was identified in 22 to 45 dairy cattle with pneumonia.5–8 and from 2008 to 2011, 149 cases of respiratory disease attributable to B trehalosi were identified at a diagnostic laboratory in California.4

In the past, field isolates of B trehalosi were likely to be classified within the Mannheimia haemolytica com-
plex, which may have obscured the role of *B trehalosi* in disease in cattle. Some of the increase in apparent prevalence is the result of previous misdiagnosis of *B trehalosi*, which is phenotypically similar to *M haemolytica*. The use of PFGE has been beneficial in the assessment of the source of respiratory Pasteurellaceae in epidemiological investigations of wild and domestic ruminants and has confirmed the role of *B trehalosi* as a primary pathogen. The name *B trehalosi* was proposed by scientists following molecular evaluation of this group of organisms and the determination that the organism differs sufficiently from other Pasteurella spp and thus deserves a group to itself on the basis of DNA-DNA relatedness and on the basis of amplified fragment length polymorphism and 16s rRNA sequencing. Although genetically different to the extent that it is now a separate genus and species, *B trehalosi* shares some pathogenic characteristics with *M haemolytica*. The gene *lktA* is key to expression of lkt, which is a major virulence factor of *M haemolytica*: *lktA* has been found in *B trehalosi*. There is evidence that rabbit antisera to all serotypes of *M haemolytica* 1 through 12 (including what is currently referred to as *B trehalosi*) cytotoxin (*lkt*) cross neutralizes toxin from *M haemolytica* A1. Fibrinogen-binding proteins have been found in supernatants of both *M haemolytica* and *B trehalosi*, and evaluation of western blots has revealed that the fibrinogen molecules are of a similar size. A protease has been identified in *B trehalosi* that is similar to a protease in *M haemolytica*. For both organisms, prechallenge sera neutralizes protease activity, which results in lower lung lesion scores and suggests an immune response to protease is important in protection against both *B trehalosi* and *M haemolytica*. In the study that was intrinsic to the name change of this organism to *B trehalosi*, 9 of 43 isolates evaluated were isolated from cattle with pneumonia.

Antimicrobial treatment cannot reliably control the peracute nature of infections attributable to *B trehalosi*; therefore, a vaccine is needed to aid in control of this bacterial pathogen in domestic as well as wild ruminants. The purpose of the study reported here was to evaluate the efficacy of a commercial vaccine containing multivalent MLV plus an *M haemolytica* toxoid for protection of calves against pneumonia after challenge exposure with *B trehalosi*.

**Materials and Methods**

**Animals**—Young (1 to 2 days old) top grade Holstein bull calves (*n* = 74) were purchased from sale barns and transported to a commercial farm. Requirements were that calves weigh ≥ 45.5 kg and be vigorous and healthy. At the time of purchase, all calves had negative results for persistent infection with bovine viral disease virus, as determined by use of an antigen-capture ELISA performed by personnel at a commercial laboratory.

Calves were raised on the commercial farm until they were 9 to 10 weeks old, when they were moved to a Zoetis Research farm in Richland, Mich. Before transport to the research farm, calves were judged to be healthy and had negative results for *M haemolytica*, *Pasteurella multocida*, and *B trehalosi* on nasopharyngeal swab specimens. In addition, serum antibody titers against *M haemolytica* lkt and *P multocida* outer membrane proteins were tested with ELISAs by use of cut-off values that had been used to indicate cattle would be susceptible to challenge exposure with Pasteurellaceae in preliminary studies conducted by our research group.

At the research farm, calves were housed in groups in rooms (14.94 × 8.23 × 3.05 m). Calves were housed such that they could not directly or indirectly contact calves in other rooms for the duration of the study. Animal identification and assignment per room were provided to the investigators. Calves then were randomly allocated to treatments within each room in accordance with a generalized randomized block design with the blocking factor based on room. The random treatment allocation plan was generated with a computer program that used a random number generator function. There were 26 calves in one room, 24 calves in a second room and 24 calves in a third room; the 2 latter rooms had 12 calves of each treatment group, whereas the other room had 12 calves of one treatment group and 14 calves of the other treatment group. Animals remained in their assigned room for the duration of the study. The study was approved by the Animal Use and Care Committee of Zoetis Inc.

**Study design**—Before assignment to study groups, all calves were clinically assessed (Appendix). Each calf had a clinical score of 0 and rectal temperature < 39.4°C and was free of *B trehalosi* and *M haemolytica*. Calves (*n* = 36) in one group were injected SC with a single 2-mL dose of a commercially available 5-way MLV vaccine containing bovine herpesvirus 1, bovine viral diarrhea virus types 1 and 2, parainfluenza virus type 3, and bovine respiratory syncytial virus, and calves in the other group (*n* = 38) were injected SC with a single 2-mL dose of a 5-way MLV vaccine containing the same 5 viral agents and *M haemolytica* toxoid. Day of vaccination was designated as day 0.

Twenty-one days after vaccination, calves were challenge-exposed with *B trehalosi* strain AHDRCC 40499. The challenge organism was acquired at a diagnostic laboratory from a calf with pneumonia. The challenge organism was identified by use of biochemical methods including a commercial microbial identification system and 16sRNA; subsequently, identity of a subset was confirmed by use of matrix-assisted laser desorption ionization–time-of-flight (ie, MALDI-TOF) and to possess *lkt* genes by use of PFGE.

**Challenge exposure**—Calves were challenge exposed by transtracheal injection with 120 mL of a log-arithmetic-phase broth culture of *B trehalosi* AHDRCC 40499. Culture of *B trehalosi* was initiated by inoculating 50 mL of warmed HP medium into a 250-mL baffled flask that contained a loop (approx 100 µL) of frozen stock. The flask was incubated at 37°C with shaking (130 revolutions/min) for 15 to 18 hours. An aliquot (2 mL) of the culture was added to each 100 mL of HP medium in an appropriately sized baffled flask, which was incubated at 37°C with shaking (150 revolutions/min) until the OD (measured at 650 nm) approxi-
mated that for a solution containing 1.0 × 10^9 CFUs/mL. The flask was immediately placed on ice, and the contents were used as a stock solution that was diluted in PBS solution to achieve a final challenge-exposure dose of 6.4 × 10^8 CFUs in 120 mL. Diluted challenge-exposure material was maintained on ice until administered. Aliquots of the challenge-exposure material as well as the stock solution were retained in the laboratory and used to perform plate counts to determine the actual dose used for challenge exposure.

Challenge exposure was conducted in the rooms used to house the calves. Briefly, calves were restrained in a standing position. Hair over the tracheal region of each calf was clipped, and the area was disinfected with alcohol wipes. Then, 4 mL of lidocaine solution was infused SC into the tissues over the trachea (diameter of infiltrated area, 1 to 2 cm). A 16-gauge trochar was directed caudally through the skin and into the trachea at the location of the local anesthesia. A cannula was inserted through the trochar and advanced to the bifurcation of the trachea; it was then retracted 2 to 4 cm to ensure the tip was located in the distal aspect of the trachea and not in a major bronchus. A total of 120 mL of diluted challenge-exposure material was then infused through the cannula, which was followed by flushing with 60 mL of cold HP medium. The cannula was then removed.

Clinical monitoring—Calves were observed, clinical scores of disease were assigned, and rectal temperatures were recorded daily after challenge exposure by individuals who were unaware of the treatment group of each calf. Calves that died or were euthanized because of clinical disease after challenge exposure were submitted for necropsy. All remaining calves were euthanized by captive bolt 6 days after challenge exposure (ie, day 27) and submitted for necropsy.

Sample collection—Blood samples were collected from each calf on days –1, 20, and day of death or euthanasia (day 27 for most calves in the study). Samples were allowed to clot at room temperature (22.2°C). Serum was harvested, divided into 2 aliquots, and submitted for testing or stored frozen.

Serum titers against M haemolytica lkt—Serum samples were assayed for antibodies against M haemolytica lkt by use of a sandwich ELISA developed at Zoetis Inc. Each well of a 96-well plate was coated with 60 mL of goat anti-bovine IgG diluted 1:5,000 in 1% casein and incubated for 1 to 4 hours at 37°C. The buffer was L of 1% casein in µnatantk diluted 1:500 in 1% casein in PBST was added to each well. The plate was sealed and incubated for another 60 ± 10 minutes at 37°C. The plate was then washed 3 times with PBST (300 µL/well). The PBST was decanted, and 100 µL of substrate was added to each well. Substrate was prepared with equal parts of 2 components of a peroxidase substrate. The plate was protected from light and incubated at 27 ± 2°C for approximately 10 minutes.

The OD of plates was determined at 405 and 490 nm by use of a spectrophotometer; the OD for 490 nm was subtracted from the OD for 405 nm. The assay was considered valid and the plate value determined when the positive control sample was between 0.8 and 1.2 net absorbance units, the negative control sample was < 0.3 net absorbance units, and the blank was < 0.12 net absorbance units. The cutoff for the positive control sample was determined as the mean absorbance for the positive control sample divided by 5. A sample titer was calculated as the last titer point at which the mean absorption value for a sample minus the positive cutoff value was greater than zero.

Nasopharyngeal swab specimens were collected on days –1 (before vaccination) and 20 (before challenge exposure). Samples were submitted for standard bacterial culture to detect B trehalosi, P multocida, M haemolytica, Histophilus somni, and Trueperella pyogenes. Swab specimens and tissues were collected from the lungs of calves on the day of necropsy. Lung swab specimens were cultured to detect B trehalosi, P multocida, M haemolytica, H somni, and T pyogenes.

Determination of lung lesion scores—Lung lesion scores (total percentage of consolidated lung in each lobe) were determined by an individual who was unaware of the treatment group of each calf. Percentage consolidation in each lung lobe was determined manually and was weighted by use of the following ratios of individual lung lobe to total lung mass: cranial part of the left cranial lobe, 5%; caudal part of the left cranial lobe, 6%; left caudal lobe, 32%; cranial part of the right cranial lobe, 6%; caudal part of the right cranial lobe, 3%; right medial lobe, 7%; right caudal lobe, 35%; and accessory lobe, 4%. Weighted lung lobe values were summed for all lobes to yield the percentage consolidation of the lungs for each calf.

Data analysis—Arc-sin square root transformation was applied to the percentage consolidation of lung values, which was followed by analysis with a general linear mixed model that had treatment as a fixed ef-
fect and block (room) as a random effect. When there was a significant ($P \leq 0.05$) overall treatment effect, linear combinations of parameter estimates were used in a priori contrasts to make comparisons between treatments. Back-transformed least squares means, SEMs, and 95% CIs were calculated from least squares parameter estimates obtained from the analyses.

For mortality data, a calf was described as having survived (euthanized at the end of the study on day 27) or as having died or been euthanized (died or euthanized before day 27) on the basis of data collected on the final disposition form and summarized by treatment. When death or euthanasia of a calf was related to the challenge exposure (ie, before day 27), then death was coded as a binary variable ($0 =$ survived; $1 =$ died or was euthanized) and analyzed with a generalized mixed model procedure by use of a binomial error and logit link. The model included treatment as a fixed effect and block (room) as a random effect. Least squares means and back-transformed least squares means and 95% CIs were reported. When there was a significant ($P \leq 0.05$) treatment effect, comparison of mortality rates was performed. Death or euthanasia related to challenge exposure was summarized by use of the prevented fraction with 95% confidence limits. For clinical observation data, the number of calves with a clinical score $\geq 2$ (acute or severe BRD) after challenge exposure was summarized.

## Results

### Clinical signs of respiratory tract disease, mortality rate, and lung lesion scores

Calves developed typical clinical signs of respiratory tract disease after challenge exposure. Acute BRD (clinical score $\geq 2$ on at least 1 day after challenge exposure) was observed during the 6 days after challenge exposure in significantly ($P = 0.002$) more control calves (26/36 [72.2%]) than in calves vaccinated with the 5-way MLV vaccine containing $M$ haemolytica toxoid ($13/38$ [34.2%]). Table 1. Control calves had a significantly ($P = 0.049$) higher mortality rate (12/36 [33.3%]), compared with the mortality rate for the calves vaccinated with the 5-way MLV vaccine containing $M$ haemolytica toxoid ($5/38$ [13.2%]). Mean percentage consolidated lung for the control calves (43%) was significantly ($P = 0.012$) higher than the percentage for the calves vaccinated with the 5-way MLV vaccine containing $M$ haemolytica toxoid (30%). There were no significant differences in the mean rectal temperature between groups during the study.

Serum testing and bacterial culture of nasopharyngeal swab specimens and lung tissues—All of the calves had serum anti-lkt antibody titers $\leq 1:3,200$ at the start of the study (Table 2). This titer had been found to be the break point at which animals were susceptible to experimental challenge exposure in preliminary studies conducted by our research group. Mean anti-lkt antibody titer remained low for the control calves and was 248 (95% CI, 181 to 339) at the time of challenge exposure (day 21). Calves vaccinated with the 5-way MLV vaccine containing $M$ haemolytica toxoid had a significantly ($P < 0.001$) higher mean antibody titer (5,254; 95% CI, 3,867 to 7,138) before challenge exposure. After challenge exposure with $B$ trehalosi, mean antibody titers increased in both groups of calves, although the mean antibody titer for the calves vaccinated with the 5-way MLV vaccine containing

### Table 1—Lung lesion scores, mortality rate, and clinical BRD rate for 36 calves vaccinated by SC administration of a commercial 5-way MLV vaccine (control group) and 38 calves vaccinated by SC administration of a 5-way MLV vaccine containing Mannheimia haemolytica toxoid (5-way toxoid) and challenge exposed with Bibersteinia trehalosi 21 days later.

<table>
<thead>
<tr>
<th>Group</th>
<th>Lung lesion score (%)</th>
<th>Mortality rate (%)</th>
<th>Calves with clinical score $\geq 2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>43 (35–50)</td>
<td>33.3 (19.8–50.3)</td>
<td>72.2 (55.3–84.5)</td>
</tr>
<tr>
<td>5-way toxoid</td>
<td>304 (23–57)</td>
<td>13.2 (5.5–28.3)</td>
<td>34.2 ll(20.8–50.7)</td>
</tr>
</tbody>
</table>

Values reported are least squares mean (95% CI). $^*$Represents the percentage of consolidated lung for all lung lobes. TClinical score was assessed by use of a scale of 0 to 3 (0, healthy calf; 1, nonspecific illness; 2, acute BRD; and 3, severe BRD). $^{†,‡,§,ll}$Within a column, value differs significantly ($^{‡}$p $= 0.012$; $§$p $= 0.049$; $ll$P $= 0.002$) from the value for the control group.

### Table 2—Serum anti-lkt antibody titers for 36 calves vaccinated (day 0) by SC administration of a commercial 5-way MLV vaccine containing Mannheimia haemolytica toxoid (5-way toxoid) and challenge exposed with Bibersteinia trehalosi on day 21.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day –1</th>
<th>Day 20</th>
<th>Day 22 to 27*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>367 (269–501)</td>
<td>248 (181–339)</td>
<td>2,714 (1,911–3,852)</td>
</tr>
<tr>
<td>5-way toxoid</td>
<td>417 (307–566)</td>
<td>5,254 (3,867–7,138)</td>
<td>7,566 (5,506–10,393)</td>
</tr>
</tbody>
</table>

Values reported are least squares mean (95% CI). $^{*}$Calfes were observed for 6 days after challenge exposure; titers were determined in samples obtained from calves on the day they died or were euthanized because of illness attributable to $B$ trehalosi. $^{†}$Control calves and 5 calves vaccinated with the 5-way MLV vaccine containing $M$ haemolytica toxoid (24 control calves and 33 calves vaccinated with the 5-way MLV vaccine containing $M$ haemolytica toxoid), which were euthanized on day 27. $^{‡}$Within a column, value differs significantly ($P < 0.001$) from the value for the control group.
M *haemolytica* toxoid remained significantly (\(P < 0.001\)) higher (7,566; 95% CI, 5,506 to 10,393), compared with the mean antibody titer for the control calves (2,714; 95% CI, 1,911 to 3,852).

Nasopharyngeal swab specimens obtained on days –1 and 20 had negative results for bacterial culture of *B trehalosi* and *M haemolytica*. Lang swab specimens obtained at necropsy yielded *B trehalosi* for 25 of 36 (69.4%) control calves and 24 of 38 (63.2%) calves vaccinated with the 5-way MLV vaccine containing *M haemolytica* toxoid. None of the calves in either group had specimens that yielded *H somni*. One control calf had specimens that yielded *T pyogenes* and *M haemolytica*, and 1 calf vaccinated with the 5-way MLV vaccine containing *M haemolytica* toxoid had specimens that yielded *P multocida*. This indicated that the lung lesion scores were most likely attributable to infection with *B trehalosi*.

**Discussion**

*Bibersteinia trehalosi* is a pathogen of cattle that causes acute pneumonia and septicemia in a wide range of age groups. The acute nature of *B trehalosi* infections and the sometimes poor response to antimicrobial treatment suggest the need for a vaccine to prevent infections and the sometimes poor response to antitoxin.

The acute nature of *B trehalosi* infection and the sometimes poor response to antibiotic treatment suggest the need for a vaccine to control infection. Currently, there are no vaccines with label claims for prevention of diseases attributable to *B trehalosi* in cattle. Several vaccines have had variable results for controlling respiratory tract disease attributable to *B trehalosi*, but most of those studies were conducted in domestic and bighorn sheep. A decrease in survival rate and an increase in incidence of respiratory tract disease were reported in lambs of bighorn ewes vaccinated IM with 2 mL of a commercial vaccine labeled for cattle that included *P multocida* bacterin and *M haemolytica* leukotoxoid. In another study in a large commercial sheep flock, 2 doses of an experimental vaccine containing *M haemolytica* strains A1, A2, A6, A7, and A9 and *B trehalosi* strains T3, T4, T10, and T15 administered SC in the neck did not improve average daily gain, reduce severity of respiratory tract disease, or reduce the recovery of either pathogen during postmortem examination. In a third study, a 2-mL dose of an *M haemolytica*–*P multocida* bacterin leukotoxoid did not reduce pharyngeal colonization by *M haemolytica* or *B trehalosi* in pack goats, despite good induction of lkt-neutralizing antibodies.

There have been other reports that use of experimental vaccines reduced disease attributable to *B trehalosi*. An unidentified vaccine or vaccines against *B trehalosi* improved disease control in sheep. In another study, a protease produced by a *B trehalosi* isolate was found to be immunogenic, and serum antibodies were associated with a reduction in lung lesions in bighorn sheep challenge exposed with *B trehalosi*. In a separate study, bighorn sheep vaccinated several times with culture supernatants from *M haemolytica* A2 and *B trehalosi* as well as with an *M haemolytica* A1 vaccine had a reduction in the severity and incidence of pneumonia and an increase in survival rate. The sheep in that study also had high titers of lkt-neutralizing and bacterial surface-specific antibodies. In a study in which investigators evaluated use of an iron-regulated protein–based *B trehalosi* vaccine in sheep, there was good efficacy in reducing the mortality rate after challenge exposure. However, to our knowledge, no vaccines have been tested for efficacy in cattle.

The vaccination trial reported here was conducted to evaluate the efficacy of a 5-way MLV vaccine containing *M haemolytica* toxoid with inactivated lkt. Investigators have found that this vaccine induces good protection in cattle in the face of challenge exposure with *M haemolytica*. Although it has been suggested that cattle isolates of *B trehalosi* typically are nonhemolytic and less likely to produce lkt, evaluation of a large number of isolates from across the United States suggests that the prevalence of lkt genes in *B trehalosi* is much higher. The fact that *B trehalosi* possesses several other antigens related to those expressed by *M haemolytica* suggests that cross protection by a vaccine is plausible. Apparent failures in sheep and wild ungulates may have been attributable to a variety of reasons, such as differences in the sample of animals used as indicators of protection, prevalence of disease in the herd or flock at the time of the study, and other stressors (especially handling of wild ungulates) that could reduce efficacy of vaccines. Although similarities exist genetically with the lktA gene, there are significant differences among Pasteurellaceae, including *B trehalosi*, in the lktA gene as well as in lktB, lktC, and lktD genes and periplasmic iron-regulated proteins. This may also explain some of the differences in protection among vaccines.

In the present study, the 5-way MLV vaccine containing *M haemolytica* toxoid reduced lung lesion scores, mortality rate, and clinical signs of disease in calves challenge exposed with a virulent isolate of *B trehalosi*. Serologic testing confirmed that calves vaccinated with the 5-way MLV vaccine containing *M haemolytica* toxoid had high antibody titers against *M haemolytica* lkt. The PFGE analysis of the strain of *B trehalosi* used for the challenge exposure confirmed the presence of lktB and lktD genes. In an unpublished study, 18 of 40 *B trehalosi* isolates from 14 states obtained from 1999 to 2010 contained both lktB and lktD genes. This suggests that at least in situations whereby lkt is produced by *B trehalosi*, an *M haemolytica* vaccine with inactivated lkt may protect calves against infection. Field reports support the use of *M haemolytica* toxoid in protecting cattle and controlling outbreaks of pneumonia attributable to *B trehalosi* in cattle. Results of the present study supported that a 5-way MLV vaccine containing *M haemolytica* toxoid can help prevent pneumonia caused by *B trehalosi* in cattle.

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**References**


b. Central States Testing, Elizabethtown, Ky.


e. Bovi-Shield GOLD One Shot, Zoetis Inc, Kalamazoo, Mich.

f. California Animal Health and Food Safety Lab, University of California-Davis, Tulare, Calif.

g. Biolog Microbial ID Systems, Biolog Inc, Hayward, Calif.

h. HP proprietary medium used to culture fastidious respiratory pathogens, Zoetis Inc, Kalamazoo, Mich.

i. Lidocaine hydrochloride injection 2%, Sparhane Laboratories, Lenexa, Kan.
j. Large animal tracheal wash kit, Semi-Flex, product No. 64900, Har-Vei, Spring Valley, Wis.
l. Horseradish peroxidase–conjugated goat anti-bovine IgG (H+L), Jackson Laboratory, Bar Harbor, Me.
m. ABTS peroxidase substrate, Kirkegaard and Perry Laboratories Inc, Gaithersburg, MD.

o. PresponeSHM, Boehringer Ingelheim, St Joseph, Mo.
q. Pulmoguard PH-M, Agri Laboratories Ltd, St Joseph, Mo.

References


Appendix appears on the next page
### Appendix

Clinical evaluation scoring used for all calves from 2 days before challenge exposure until the end of the study.*

<table>
<thead>
<tr>
<th>Score</th>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Healthy calf</td>
<td>Calf is alert and active; stands, moves, and responds to stimuli quickly and steadily; and has continuous interest in its surroundings.</td>
</tr>
<tr>
<td>1</td>
<td>Nonspecific illness</td>
<td>Clinical signs not necessarily specific for acute BRD infection; clinical signs may include nasal discharge, and the calf may hold its head low and be mildly lethargic.</td>
</tr>
<tr>
<td>2</td>
<td>Acute BRD</td>
<td>Clinical signs are moderate and specific for acute BRD and may include a marked increase in respiratory effort. Calf is lethargic and has signs of depression and a reduced interest in its surroundings, walks slowly, and may stagger. Calf is slow to respond to stimuli and is frequently recumbent. Repeated coughing and nasal discharge are not common but may be evident.</td>
</tr>
<tr>
<td>3</td>
<td>Severe BRD</td>
<td>Clinical signs may include acute respiratory distress (with open-mouth breathing or grunting) and marked lethargy and signs of depression. Calf is gaunt and recumbent, has little or no response to stimuli, and has difficulty when attempting to stand or move.</td>
</tr>
</tbody>
</table>

*Calves with a score of 3 were euthanized for humane reasons; surviving calves were euthanized 6 days after challenge exposure.