Nasalgen® 3

TECH BULLETIN

MERCK Animal Health

Efficacy of the Bovine Respiratory Syncytial Virus Fraction of Nasalgen® 3 in Calves 4 to 7 Days Old

Key Highlights

- Significant differences in lung lesion scores and viral shedding between Nasalgen 3 vaccinated and control groups confirmed the immunogenic efficacy of the BRSV fraction of N3.
- Also confirmed was the non-interference by the other antigenic fractions in Nasalgen 3. Following administration of a single IN dose of N3 that contained the minimum protective dose of BRSV to healthy calves 4 to 7 days of age, the proportion of calves with pulmonary lesions and lung lesion scores were lower than those for control calves and none of the vaccinated calves had evidence (by IHC) of infection with challenge virus.
- Following challenge with virulent BRSV, a lower proportion of calves vaccinated with N3 shed BRSV in nasal secretions than did calves in the control group. Maximum shedding for calves vaccinated with N3 was lower and for shorter duration than for calves in the control group.

SUMMARY

Nasalgen® 3 (N3) has been shown to be effective for vaccination of healthy cattle 1 week of age or older against Infectious Bovine Rhinotracheitis (IBR) virus, Bovine Respiratory Syncytial Virus (BRSV) and Parainfluenza 3 (PI_3) virus that are pathogens implicated in the Bovine Respiratory Disease (BRD) complex. Nasalgen 3 is safe for use in pregnant cows and in calves nursing pregnant cows. For this study, 44 colostrum-deprived Holstein calves were randomly assigned to be vaccinated intranasally with one dose of N3 (22 head) that contained the minimum protective dose of BRSV or with one dose of a placebo vaccine (22 head) that did not contain the BRSV fraction but contained the other viral antigens in N3. All calves were 4 to 7 days old on the day of vaccination (Day 0). No adverse reactions were observed after vaccination. Three calves in the control group died or were euthanized for reasons unrelated to the vaccine. On Day 30, all calves were commingled, transported and were challenged with aerosolized, virulent BRSV (twice on consecutive days). All calves developed mild clinical signs of respiratory infection. Maximum shedding of BRSV in nasal secretions was significantly ($P=0.0017$) less and duration of shedding was significantly ($P=0.0052$) shorter for calves vaccinated with N3 than for calves in the control group. Calves in both groups were humanely euthanized on Day 38, and their lungs were inspected grossly for lesions associated with BRSV infection. A significantly ($P<0.0001$) greater number of control calves had pulmonary lesions associated with BRSV (19/19, 100%) than calves vaccinated with N3 (6/22, 27.3%). Lung lesion scores were significantly ($P<0.0001$) less for calves vaccinated with N3 than for calves in the control group. Nine of the 19 (47%) samples of lungs from calves in the control group had immunohistochemical (IHC) evidence of infection with BRSV, and that was significantly ($P=0.0003$) greater than none (0%) of the samples from those calves vaccinated with N3. Nasalgen 3 provided protection to calves 4 to 7 days of age, as reflected by the lung lesion scores, IHC findings, magnitude and duration of nasal shedding of BRSV after challenge.

INTRODUCTION

Nasalgen 3 (N3) vaccine has been developed by Merck Animal Health for intranasal administration against viral pathogens known to be causal in the Bovine Respiratory Disease complex. Nasalgen 3 contains modified live viruses (Infectious Bovine Rhinotracheitis [IBR] virus, Bovine Parainfluenza 3 [PI_3] virus and Bovine Respiratory Syncytial Virus [BRSV]). This technical bulletin reports the results of research that demonstrate protective efficacy for the BRSV fraction of N3, and no interference by the other two antigens in N3, after one intranasal administration to calves 4 to 7 days of age.

EXPERIMENTAL PROCEDURES

Forty-four Holstein calves (22 males, 22 females) were obtained from a single source, deprived of colostrum, identified by unique individual numbers and transported (two shipments) to the research facility in De Soto, KS. Prior to arrival, the calves were randomly assigned to be vaccinated intranasally (IN) with N3 or with a placebo vaccine (control group). Calves were housed in individual hutches segregated by treatment group and the groups were physically separated by at least 15 feet. At arrival, calves were fed electrolytes. During the study, each calf was bottle-fed (until able to be fed with a bucket) at least 2 quarts of milk replacer (20% protein) twice daily and had access *ad libitum* to fresh water and to a calf starter diet. Calves were allowed 4 to 5 days to acclimate prior to enrollment. Health care was managed by the attending veterinarians. All calves were confirmed "negative" (antigen-capture Enzyme-Linked Immunosorbant Assay) for persistent infection with Bovine Viral Diarrhea Virus (BVDV).

All calves were 4 to 7 days old when vaccinated (Day 0). Nasalgen 3 was prepared so that the dose administered contained the minimum protective dose (MPD) of BRSV fraction and contained IBR and PI_3 virus at or above titers licensed for release. The placebo vaccine contained the same antigens as N3 but without BRSV. One mL of placebo vaccine was administered into each nostril of 22 calves (13 females, 9 males) and one mL of N3 was administered into each nostril of 22 calves (9 females, 13 males). After vaccination, daily observation, care and feeding of the calves in the control group preceded that for the calves in the group vaccinated with N3. Prior to challenge, three calves in the control group died or were euthanized for reasons unrelated to vaccination (bloat [one calf], bacterial septicemia [two calves]). Therefore, 19 calves remained in the control group and 22 calves remained in the N3 group. Some calves had diarrhea (seven), lethargy and/or anorexia (four) and scours and lethargy (two) which responded after treatment and did not influence the outcome of the study. On Day 30, the remaining 41 calves were clinically healthy, were commingled and were transported approximately 350 miles to a contract research organization (CRO). After arriving at the CRO, all calves were commingled in a single pen for challenge with aerosolized/nebulized virulent BRSV (4 mL dose/calf). On Day 31, all calves were challenged a second time with aerosolized/nebulized virulent BRSV. Calves were observed daily for clinical signs associated with infection by BRSV and rectal temperatures were recorded daily from Day 29 through Day 38 (Day before [-1] to Day 8 post-challenge).

Nasal secretions from each calf (one swab per nostril) were sampled immediately prior to challenge and daily from Day 32 through Day 38 (Day 2 through Day 8 post-challenge) to detect BRSV. Titers of serum neutralization (SN) antibodies against BRSV were determined with samples obtained from each calf on study Day 0, Day 21 and Day 30. On Day 38 (8 days post-challenge), blood was collected for serum neutralization (SN) antibodies and all calves were humanely euthanized. The lungs were examined and scores assigned for visible lesions caused by infection with BRSV. A score for both lungs (Total Lung Score) was calculated based on the estimation of the percent of lesions (Jericho and Langford, 1982) in each lobe as follows: (Left Cranial x 0.05) + (Left Middle x 0.06) + (Left Caudal x 0.32) + (Right Cranial x 0.06) + (Right Posterior Cranial x 0.05) + (Right Middle x 0.07) + (Right Caudal x 0.35) + (Accessory x 0.04) = Total Lung Score (TLS). For each calf, a sample of lung was taken from an affected area, or from the right cranial lobe if no lesion was present, placed in buffered formalin and submitted for detection of BRSV by immunohistochemistry (IHC). Disposal of all calves was by appropriate methods.

The experimental unit was the individual calf. The primary outcome variable was the lung lesion score (percent lung lesions) after challenge. The supporting variable was nasal shedding of BRSV after challenge. Exploratory variables were (by treatment group) the proportion of calves with pulmonary lesions, the proportion of samples of lungs that were "positive" for BRSV by IHC and the proportion of calves that shed BRSV in nasal secretions after challenge. Titers of SN antibody to BRSV were used as an enrollment criterion ("negative") as an indicator of biosecurity ("negative"), and as a general indicator of antigenic/immunologic response ("positive") to vaccination and/or to the challenge. Those SN titers were not quantitated for statistical analyses. Personnel who administered the challenge and performed clinical observations, scored lung lesions and/or performed laboratory procedures were blinded to which treatment group any calves were assigned.

RESULTS

No adverse reactions were observed during the study. All calves were seronegative (SN antibody titer < 1:2) to BRSV prior to vaccination and calves in the control group remained seronegative throughout the 38-day study. Calves vaccinated with N3 responded serologically and demonstrated an anamnestic response post-challenge.

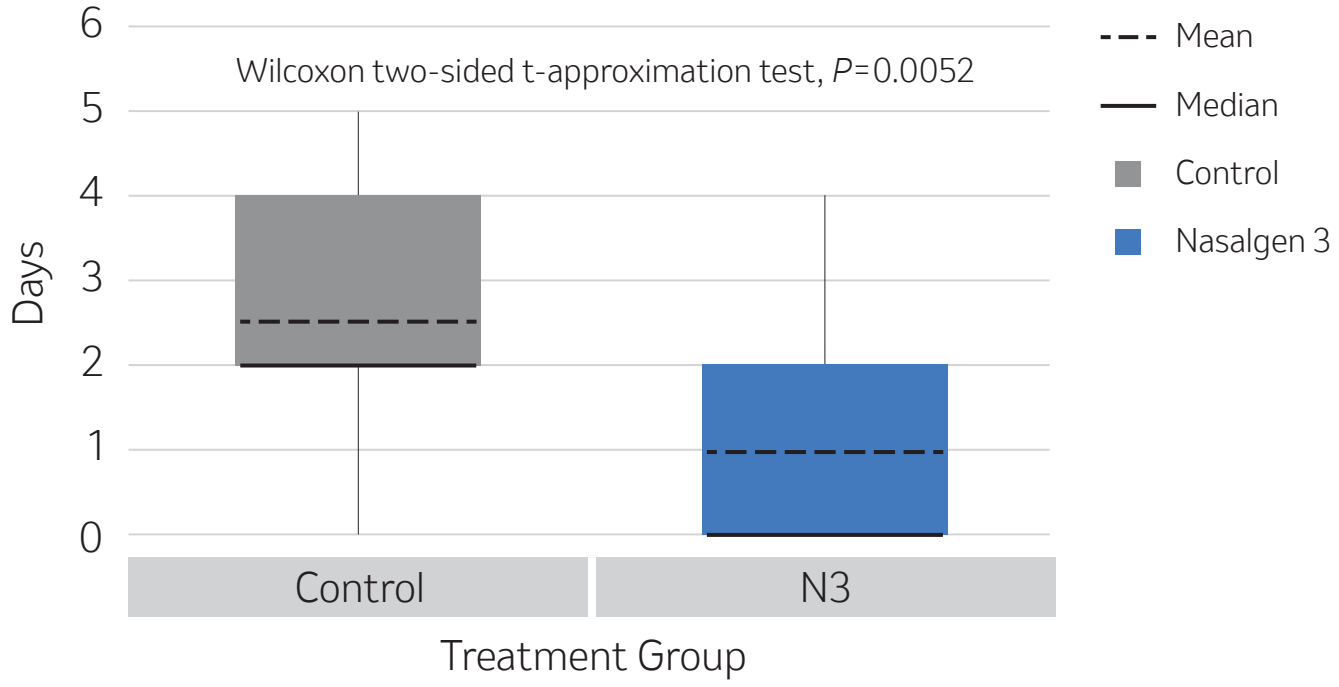
All calves exhibited mild clinical signs of disease after challenge. Those signs consisted primarily of mild depression, dyspnea, nasal discharge, fever, tachypnea and/or cough.

After challenge, a significantly (Fisher's exact two-sided test, $P=0.0013$) lower proportion of calves vaccinated with N3 (7/22, 32%) shed BRSV in nasal secretions than did those in the control group (16/19, 84%). The duration of nasal shedding of BRSV post-challenge was significantly (Wilcoxon two-sided t-approximation test, $P=0.0052$) shorter for calves vaccinated with N3 than for calves in the control group (Table 1, Figure 1).

Table 1. Quartile summary of analysis of duration of nasal shedding of BRSV post-challenge by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	19	2.5	0	2	2	4	5
N3	22	1	0	0	0	2	4

Figure 1. Duration of nasal shedding of BRSV post-challenge by treatment group.

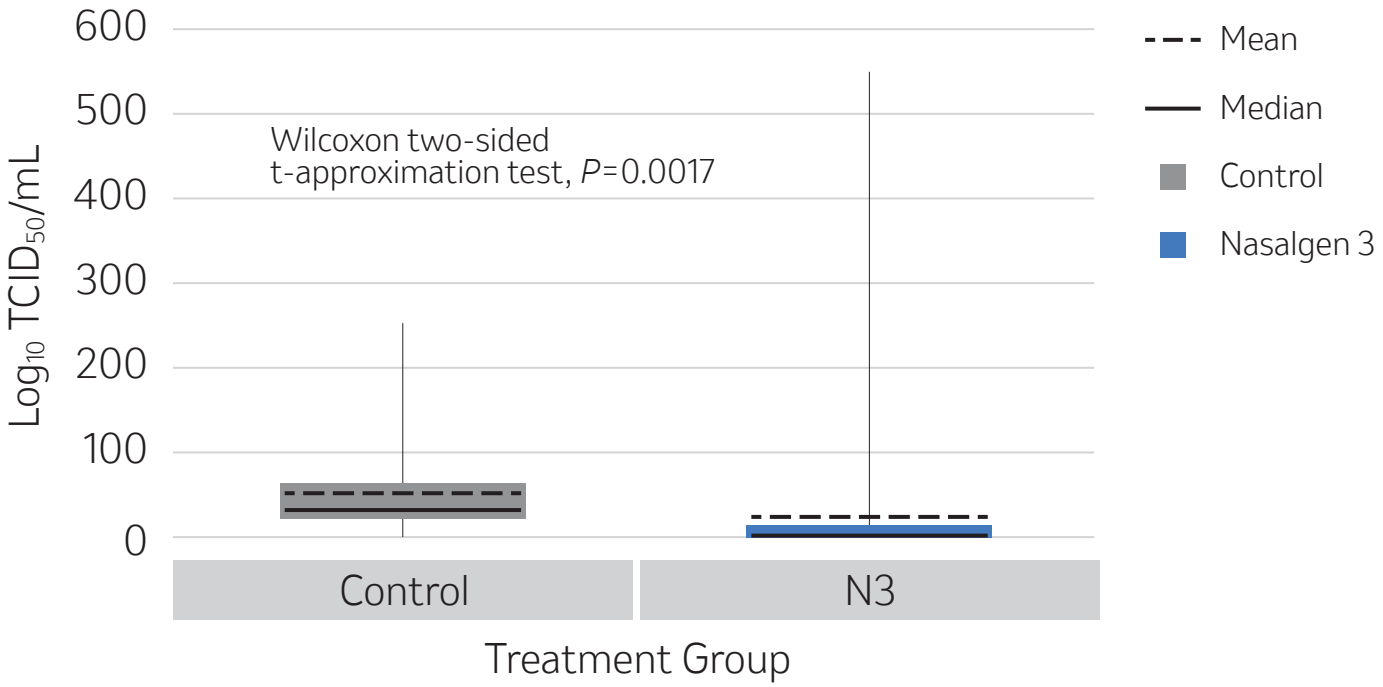


Maximum titers of BRSV shed in nasal secretions of calves vaccinated with N3 were significantly (Wilcoxon two-sided t-approximation test, P=0.0017) less than that for calves in the control group (Table 2, Figure 2).

Table 2. Quartile summary of analysis of maximum titers ($TCID_{50}$/mL) of BRSV shed in nasal secretions post-challenge by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	19	52.07	0	18.6	27	57.2	256
N3	22	37.03	0	0	0	18.6	543

Figure 2. Maximum titers ($TCID_{50}$/mL) of BRSV shed in nasal secretions after challenge with virulent BRSV.

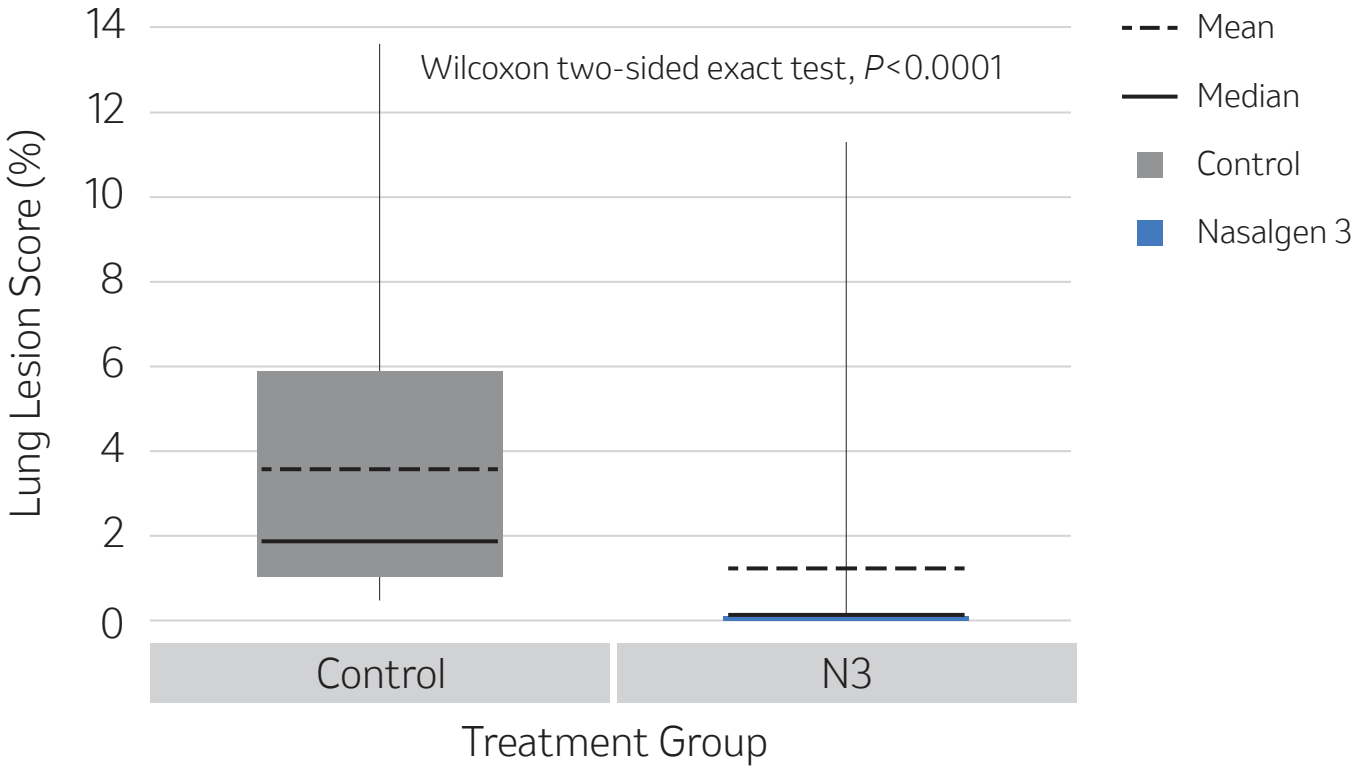


All calves were euthanized before clinical signs resolved in some of the calves, so the duration of clinical signs could not be determined. Pulmonary lesions associated with BRSV were present in all calves in the control group (19/19, 100%) and in significantly (Fisher's exact two-sided test, $P<0.0001$) fewer calves vaccinated with N3 (6/22, 27%). The lung lesion scores (%) for calves vaccinated with N3 were significantly (Wilcoxon two-sided exact test, $P<0.0001$) less than those for calves in the control group (Table 3, Figure 3).

Table 3. Quartile summary of analysis of lung lesion score after challenge with virulent BRSV for calves in each treatment group.

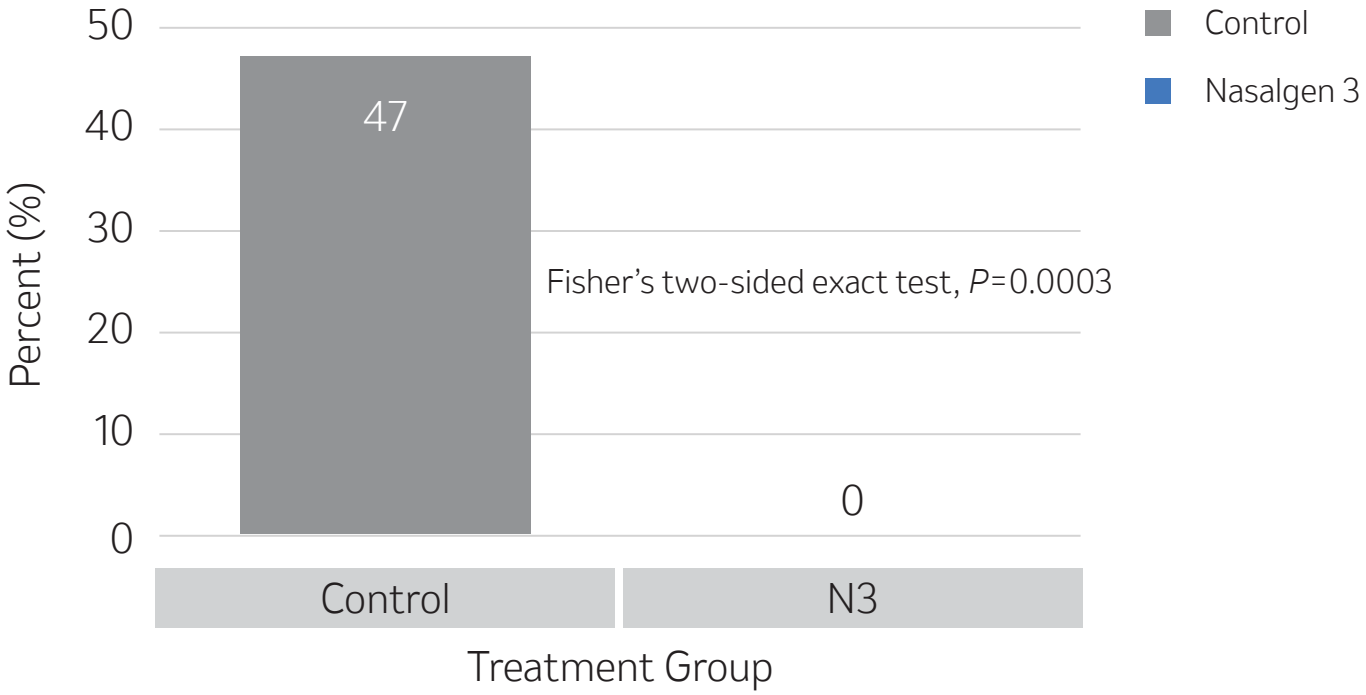
Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	19	3.88	0.2	0.9	2.1	5.9	13.7
N3	22	1.15	0	0	0	0.1	11.3

Figure 3. Lung lesion scores (%) post-challenge by treatment group.



None of the 22 (0%) calves vaccinated with N3 were "positive" for BRSV by IHC which was significantly (Fisher's two-sided exact test, *P*=0.0003) less than for samples from nine of 19 (47%) calves in the control group (Figure 4) that were "positive."

Figure 4. More samples (47%) of lung from calves in the control group were infected by BRSV than for calves vaccinated with N3 (0%) (Fisher's two-sided exact test, *P*=0.0003) as detected by immunohistochemistry (IHC).



CONCLUSIONS

Significant differences in lung lesion scores and viral shedding between N3-vaccinated and control groups confirmed the immunogenic efficacy of the BRSV fraction of Nasalgen 3. Also confirmed was the non-interference by the other antigenic fractions in Nasalgen 3. Following administration of a single IN dose of N3 that contained the minimum protective dose of BRSV to healthy calves 4 to 7 days of age, the proportion of calves with pulmonary lesions and lung lesion scores were lower than those for control calves and none of the vaccinated calves had evidence (by IHC) of infection with challenge virus. Nine of nineteen (9/19, 47%) calves in the control group had evidence (by IHC) of infection with challenge virus. Three important points are emphasized by that finding: 1) The BRSV used for challenge was virulent and infected the calves in the control group; 2) Nasalgen 3 prevented infection by the virulent BRSV used for challenge; 3) infection or protection was not distinguished clinically because clinical signs were mild and not different between treatment groups and duration of clinical disease could not be determined as the calves were not observed until resolution. Following challenge with virulent BRSV, a lower proportion of calves vaccinated with N3 shed BRSV in nasal secretions than did calves in the control group. Maximum shedding for calves vaccinated with N3 was lower and virus was shed for shorter duration than for calves in the control group. Results of this study support the claim that Nasalgen 3 is safe and effective for intranasal vaccination of calves at 1 week of age or older against respiratory disease caused by BRSV.

REFERENCES

Data on File: USDA-approved efficacy report for N3.
Jericho KWF, Langford EV. Aerosol vaccination of calves with *Pasteurella haemolytica* against experimental respiratory disease. *Can J Comp Med.* 1982;46:287-292.

MAHCattle.com • 800-521-5767

US-NAL-200200005

MERCK Animal Health