Key Highlights

- This study reaffirms the safety and efficacy of N3PMH and establishes the duration of immunity of the BRSV fraction of N3PMH to be at least 78 days following one dose of vaccine administered intranasally to calves 5 to 7 days of age.
- N3PMH is safe for use in pregnant cows and in calves nursing pregnant cows.
- The primary outcome, lung lesion score (LLS), was significantly (P<0.0001) lower for the calves vaccinated with N3PMH than for calves in the control group.

Duration of Immunity of the Bovine Respiratory Syncytial Virus Fraction of Nasalgen® 3-PMH Administered to Calves 5 to 7 Days of Age

SUMMARY

Nasalgen® 3-PMH (N3PMH) has been shown to be effective for vaccination of healthy cattle, 1 week of age or older against five pathogens implicated in the Bovine Respiratory Disease (BRD) complex: Infectious Bovine Rhinotracheitis (IBR) virus, Parainfluenza 3 virus (PI_3), Bovine Respiratory Syncytial Virus (BRSV), *Mannheimia haemolytica* (MH), and *Pasteurella multocida* (PM). Nasalgen® 3-PMH 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 either one dose of vaccine in which the BRSV fraction was reduced to the minimum protective dose and the other viral and bacterial fractions in N3PMH were at the licensed vaccine dose (22 head) or with one dose of a placebo vaccine from which the BRSV fraction was removed but the other viral and bacterial fractions in N3PMH were at the licensed vaccine dose (22 head). All calves were 5 to 7 days old on the day of vaccination (Day 0). No adverse reactions associated with vaccination were observed. On Day 78 after vaccination, all calves were commingled and challenged with aerosolized virulent BRSV. The challenge was repeated on Day 79. The primary outcome, lung lesion score (LLS), was significantly (P<0.0001) lower for the calves vaccinated with N3PMH than for calves in the control group. The proportion of calves infected with BRSV (BRSV detected in the lung via immunohistochemistry) was lower for the vaccinated group (36%) than for the control group (91%). Maximum titer of BRSV shed in nasal secretions was significantly (P=0.0056) lower for calves vaccinated with N3PMH than for calves in the control group. Duration of nasal shedding of BRSV was significantly (P=0.0003) shorter for calves vaccinated with N3PMH than for calves in the control group. Clinical signs of infection (depression, coughing and nasal discharge) by BRSV were mild and present in 6/22 controls and 3/22 of vaccinates at eight days post-challenge. Results of this study reaffirm the safety and efficacy of N3PMH in calves 1 week of age or older against respiratory disease caused by BRSV and demonstrated the duration of immunity to be at least 78 days following intranasal vaccination of healthy calves with a single dose.

INTRODUCTION

Nasalgen® 3-PMH (N3PMH) vaccine was developed by Merck Animal Health for intranasal administration against viral and bacterial pathogens known to be causal in the Bovine Respiratory Disease complex. N3PMH contains modified live viruses (Infectious Bovine Rhinotracheitis [IBR] virus, Parainfluenza 3 [PI_3], Bovine Respiratory Syncytial Virus [BRSV]), plus avirulent, live *Mannheimia haemolytica* (MH) and *Pasteurella multocida* (PM). This study reaffirms the safety and efficacy of N3PMH and establishes the duration of immunity of the BRSV fraction of N3PMH to be at least 78 days following one dose of vaccine administered intranasally to calves 5 to 7 days of age.

EXPERIMENTAL PROCEDURES

Forty-four Holstein calves (14 males, 30 females) were obtained from a single source, were deprived of colostrum, were identified by unique individual numbers and were transported (two shipments) to the research facility. Prior to arrival, the calves were randomly assigned to be vaccinated intranasally (IN) with N3PMH 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. Each calf was bottle-fed (until able to be fed with a bucket) at least 2 quarts of milk replacer twice daily, had access *ad libitum* to fresh water after 4 days of age and was fed a calf starter diet. Calves were allowed 4 or 5 days to acclimate. Health care was managed by the attending veterinarians. All calves were confirmed (Antigen-capture Enzyme-Linked Immunosorbent Assay) negative for persistent infection with Bovine Viral Diarrhea Virus (BVDV).

All calves were 5 to 7 days old and had serum neutralizing (SN) antibody titers to BRSV < 1:2 when vaccinated (Day 0). N3PMH was prepared so that the dose administered contained the minimum protective dose of BRSV and contained IBR virus, PI_3 virus, MH and PM at or above titers licensed for release. The placebo vaccine contained the same antigens as N3PMH but without BRSV. One mL of placebo vaccine was administered into each nostril of 22 calves (15 females, 7 males). Then, 1 mL of N3PMH was administered into each nostril of 22 calves (15 females, 7 males). After vaccination, all calves remained in their respective hutches. For 2 weeks following vaccination, routine care and feeding of the calves in the control group were provided prior to the calves vaccinated with N3PMH to prevent cross-exposure. At about 7 weeks of age, the calves were commingled in one of eight holding pens according to the original randomization assignment; each holding pen contained calves from one shipment and an equal number of calves from each treatment group. Personnel administering the challenge, performing clinical observations, scoring lung lesions or performing laboratory procedures were blinded to the treatment group to which any calf was assigned.

During the post-vaccination period, no adverse events related to the vaccines were observed. Five calves had transient bouts of diarrhea that resolved after prescribed treatment. On Day 78, all calves (44 head) were commingled and shipped approximately 360 miles to a contract research organization where they were challenged by aerosolized/nebulization of a solution (4 mL per calf) that contained virulent BRSV. The challenge was repeated on Day 79. All calves were monitored (clinical signs of infection by BRSV, respiratory rates, and rectal temperatures were recorded) daily from Day 77 (one day before challenge) through Day 86, (post-challenge Day 8). Samples (one swab per nostril) of nasal secretions were obtained on Day 77 and daily from Day 80 to Day 86 for determination of shedding of BRSV. On Day 86, all calves were euthanized. Lungs were removed and examined for pneumonic lesions caused by BRSV, and lesions were scored by two qualified individuals who were blinded to treatment. (The average of the two scores was recorded and used for analysis.) The total lung score was calculated 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 lesion score (LLS; %).[1]

Figure 1. Timeline of events

The total lung lesion scores were the average estimated percentage of lung tissue with pneumonia, caused by BRSV infection, from both observers. For each calf, a sample of lung was taken from an affected area and submitted for immunohistochemistry (IHC) to detect BRSV. An IHC score was assigned as follows:

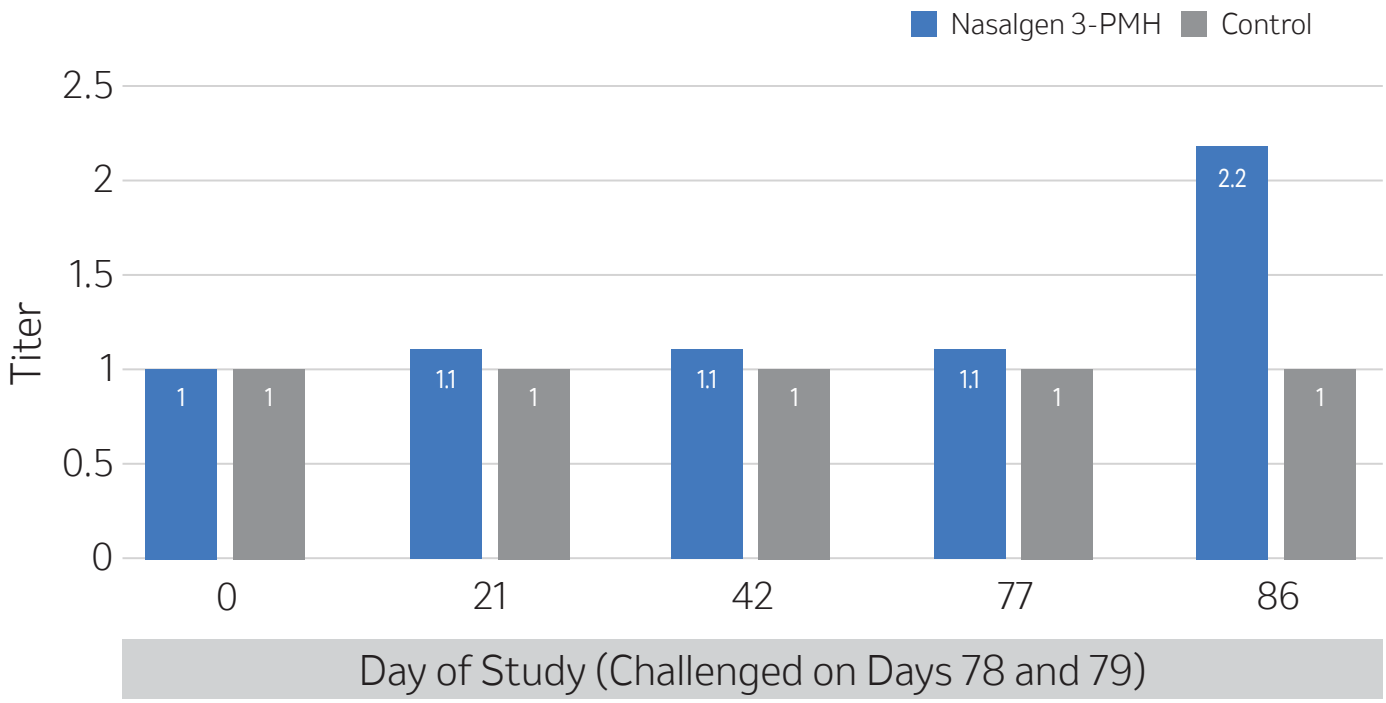
negative = 0; positive: mild = 1; moderate = 2; marked = 3.

The experimental unit was the individual calf. Primary outcome variables were lung lesion scores and duration of BRSV morbidity. Morbidity was defined as the presence of a mild, moderate or severe score for depression, dyspnea or coughing, or a moderate or severe score for nasal discharge, a respiratory rate > 60 per minute or a rectal temperature ≥ 104.0° F on any day post-challenge. Supporting outcome variable was nasal shedding of BRSV following 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 challenge. Those SN titers were not subjected to statistical analyses.

RESULTS

No adverse reactions associated with vaccination were observed, and no calf died before the end of the study. On Day 86 (8 days after challenge), six of 22 calves (27.3%) in the control group and three of 22 calves (13.6%) vaccinated with N3PMH had mild clinical signs of infection (ocular and nasal discharge) with BRSV. The duration of morbidity due to BRSV, as defined for this study, was not significantly (Wilcoxon two-sided exact test, $P=0.807$) different for calves in either treatment group; however, the morbidity was ongoing at the end of the study, so the duration of BRSV morbidity analysis may not reflect the true outcome. The other morbidity variables (rectal temperature and respiratory rate) were also not different. All calves were seronegative (SN titer to BRSV < 1:2) prior to vaccination. All calves in the control group remained seronegative throughout the study. Seven of 22 calves vaccinated with N3PMH demonstrated seroconversion by 8 days after challenge. Two of those seven had detectable SN antibodies against BRSV on Day 21.

Figure 2. Geometric mean titer (twofold serial dilution) of serum neutralization (SN) antibodies to BRSV by treatment group.

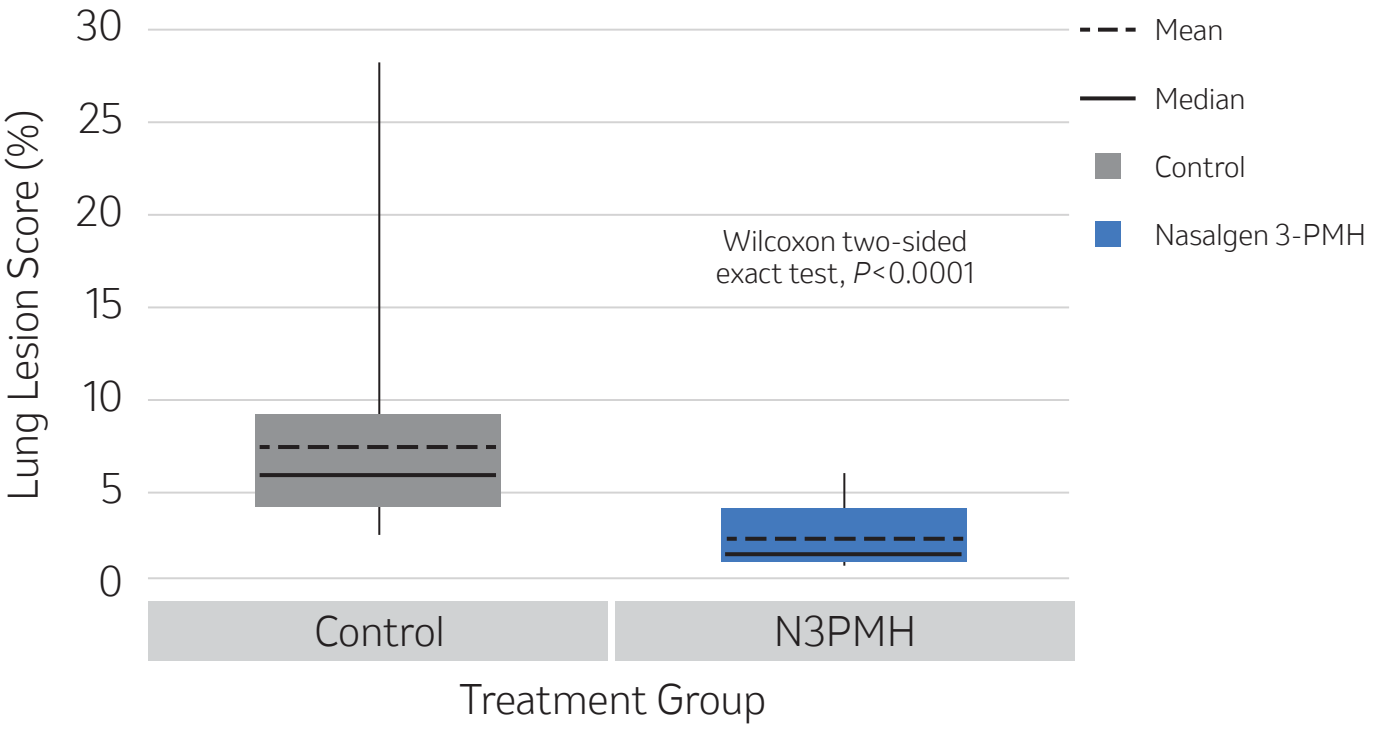


After challenge, all calves had pulmonary lesions due to BRSV. The LLS for the N3PMH group were significantly (Wilcoxon two-sided exact test, $P<0.0001$) lower than those for the control group (Table 1, Figure 3).

Table 1. Quartile summary of analysis of lung lesion scores after challenge with virulent BRSV, by treatment group.

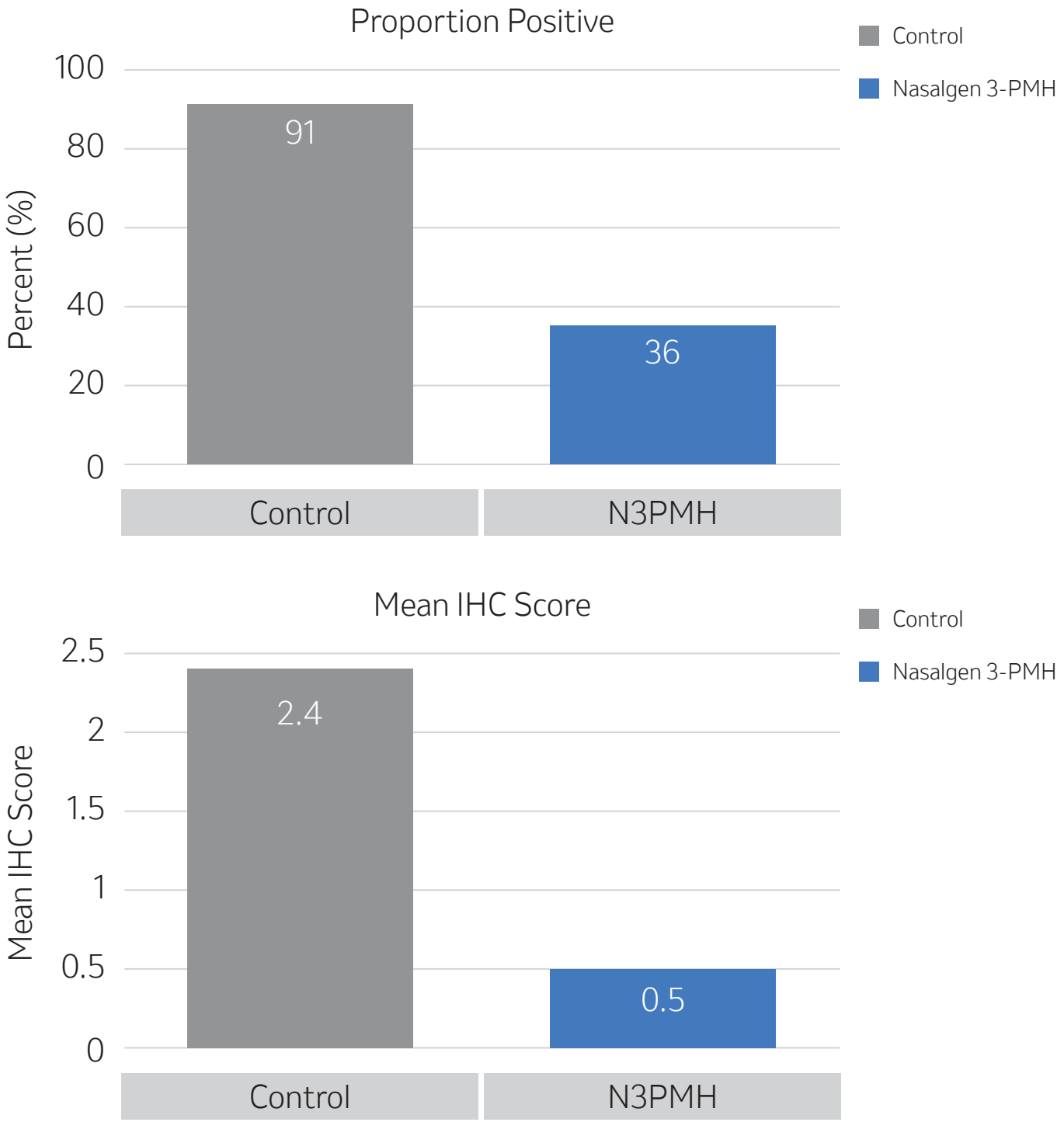
Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	22	7.929	2.89	4.22	5.925	9.31	27.30
N3PMH	22	2.573	0.35	1.11	1.880	4.02	6.85

Figure 3. Lung lesion score (%) post-challenge with virulent BRSV, by treatment group.



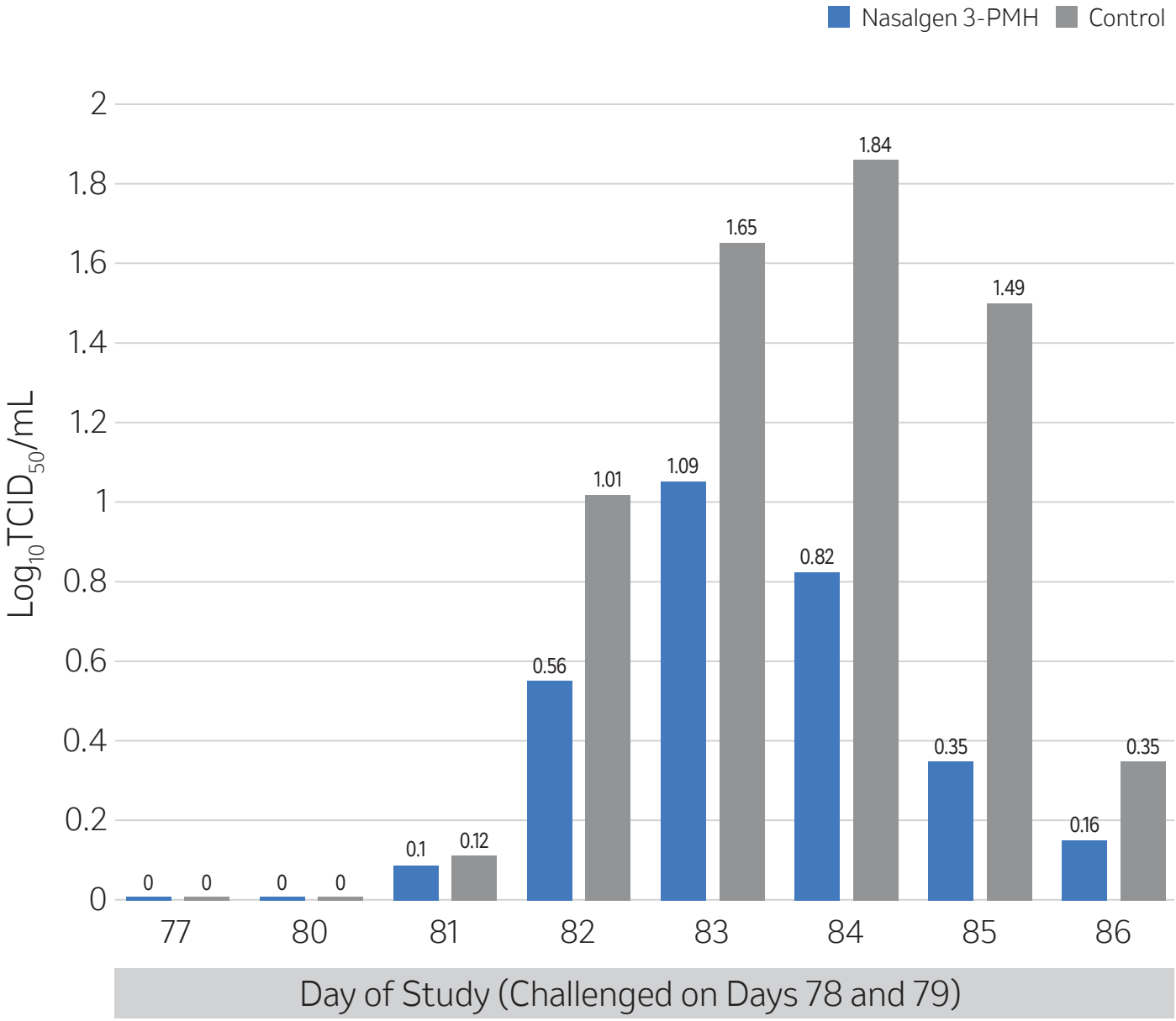
For calves in the control group, 20 of 22 (91%) samples of affected lung were positive for BRSV by IHC staining, and the mean IHC score was 2.4 (Figure 4). For calves vaccinated with N3PMH, eight of 22 (36%) samples of affected lung were positive, and the mean IHC score was 0.5 (Figure 4).

Figure 4. Proportion of calves with IHC positive lungs for BRSV and the mean IHC score (negative = 0; positive mild = 1; moderate = 2; marked = 3) for lungs of calves in each treatment group.



After challenge, BRSV was isolated from nasal secretions of all calves (22/22; 100%) in the control group, and 20 of 22 (91%) of calves vaccinated with N3PMH (Figure 5).

Figure 5. Average titers of BRSV shed in nasal secretions after challenge by day.

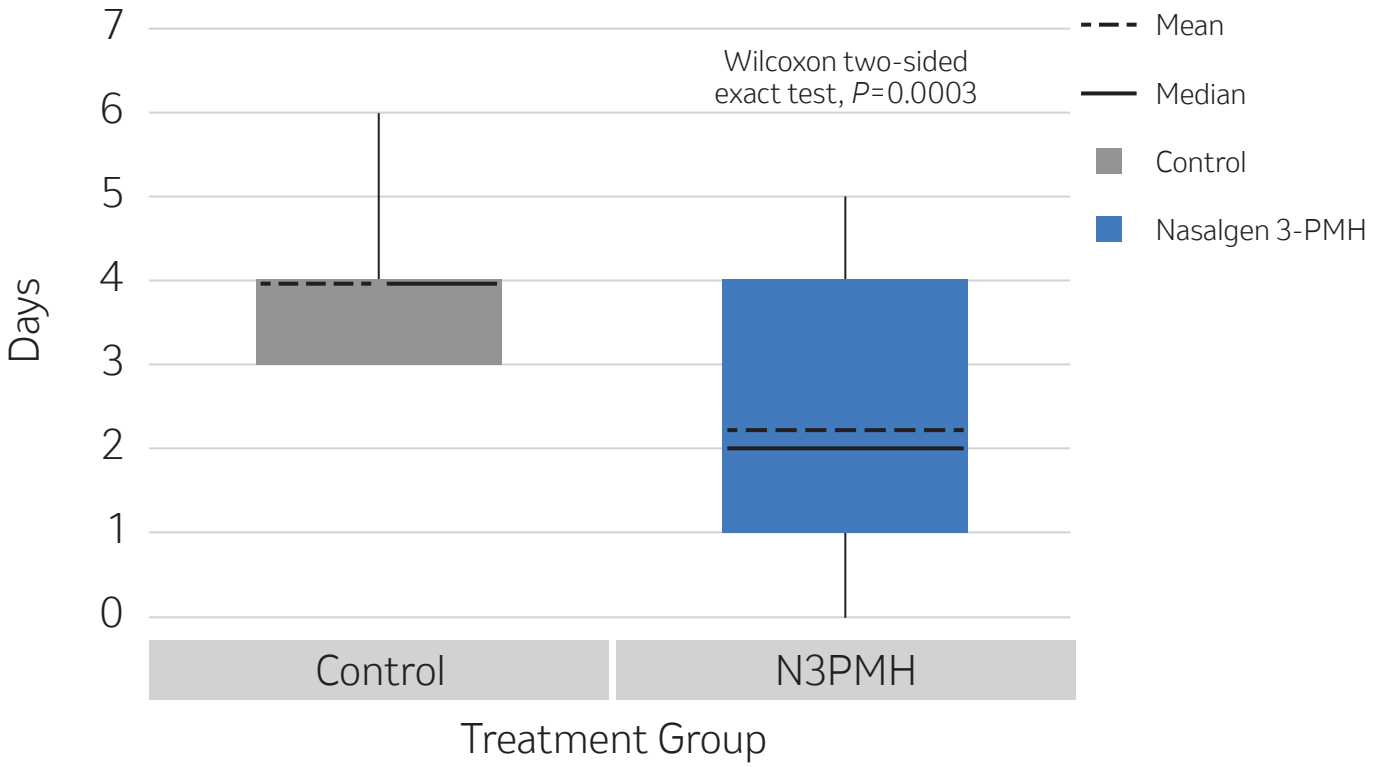


The duration of nasal shedding of BRSV post-challenge (Table 2, Figure 6) was significantly (Wilcoxon two-sided exact test, $P=0.0003$) less for the N3PMH group than for the control group.

Table 2. Quartile summary of analysis of duration of nasal shedding of BRSV after challenge with virulent BRSV, by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	22	4.0	3.0	3.0	4.0	4.0	6.0
N3PMH	22	2.3	0.0	1.0	2.0	4.0	5.0

Figure 6. Duration of shedding of BRSV in nasal secretions after challenge.

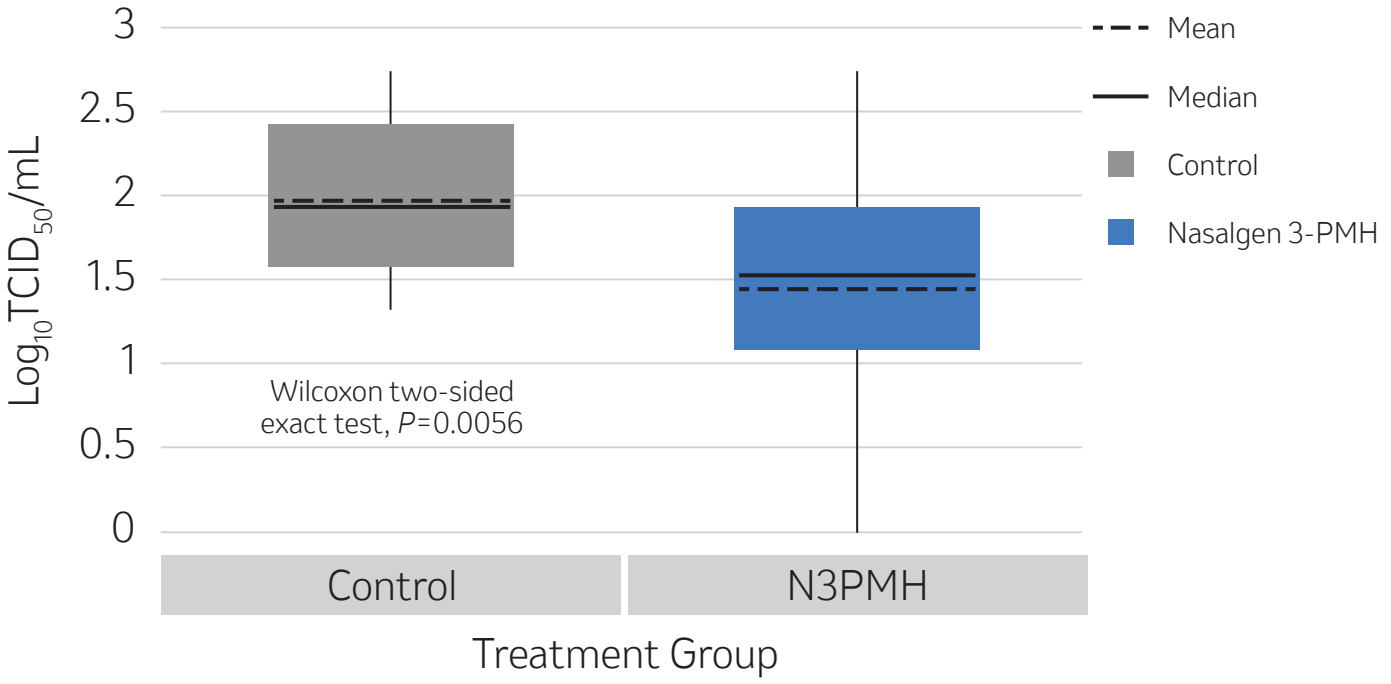


Maximum titers ($Log_{10}TCID_{50}$/mL) of BRSV shed in nasal secretions (Table 3, Figure 7) were significantly (Wilcoxon two-sided exact test, P=0.0056) less for calves in the N3PMH group than for calves in the control group.

Table 3. Quartile summary of analysis of maximum titers ($Log_{10}TCID_{50}$/mL) of BRSV shed in nasal secretions after challenge with virulent BRSV, by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	22	1.99	1.43	1.59	1.92	2.41	2.73
N3PMH	22	1.49	0.0	1.11	1.51	1.92	2.73

Figure 7. Maximum titers ($TCID_{50}$/mL) of BRSV shed in nasal secretions post-challenge, by treatment group.



CONCLUSIONS

Calves that were vaccinated at 5 to 7 days of age with a single intranasal dose of N3PMH that contained the minimum protective dose of BRSV antigen had significantly lower lung lesion scores compared to calves vaccinated with a placebo vaccine following challenge with virulent BRSV virus at both 78 and 79 days after vaccination. For calves in the control group, 20 of 22 (91%) samples of affected lungs were BRSV IHC positive compared to just eight of 22 (36%) calves vaccinated with N3PMH. The IHC results also showed less BRSV in lungs of calves vaccinated with N3PMH. The duration of nasal shedding of BRSV after challenge was significantly shorter and maximum titer shed was lower for vaccinated calves than for those in the control group. Clinical signs (depression, cough and nasal discharge) were mild and present in 6/22 controls vs. 3/22 of vaccinates at 8 days post-challenge. Results of this study reaffirm the safety and efficacy of Nasalgen® 3-PMH[2], demonstrate the duration of immunity of at least 78 days following intranasal vaccination of healthy calves, and support the claim that Nasalgen® 3-PMH is safe and effective for intranasal vaccination of calves at 1 week of age or older against respiratory disease caused by BRSV.

REFERENCES

[1]Jericho KWF, Langford EV. 1982. Aerosol Vaccination of Calves with *Pasteurella haemolytica* Against Experimental Respiratory Disease. *Can J Comp Med.* 46:287-292.

[2]Efficacy of the Bovine Respiratory Syncytial Virus Fraction of Nasalgen® 3-PMH in Calves 4 to 7 Days of Age Technical Bulletin; Merck Animal Health.

Report No. BLI-090R, dated October 5, 2018, entitled “Duration of Immunity (DOI) for the Bovine Respiratory Syncytial Virus (BRSV) Fraction in Product Code 11C8.20, Administered Intranasally to One Week Old Calves.”

Nasalgen® 3-PMH

MERCK Animal Health