Key Highlights

- This study reaffirms the safety and efficacy of N3PMH and establishes the duration of immunity of the PM fraction of N3PMH to be at least 125 days following one dose of vaccine administered intranasally to calves 2 to 3 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 *Pasteurella multocida* Fraction of Nasalgen® 3-PMH Administered to Calves at 2 to 3 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). N3PMH 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 PM 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 PM fraction was removed but the other viral and bacterial fractions in N3PMH were at the licensed vaccine dose (22 head). All calves were 2 to 3 days old on the day of vaccination (Day 0). The calves were challenged 125 days after vaccination and were observed for seven days post-challenge. On Day 132, surviving calves were euthanized, lungs were removed, lung lesion scores (LLS) were recorded and samples of lung were submitted for bacterial isolation. Lung lesion scores for the calves vaccinated with N3PMH were significantly ($P<0.0001$) lower than those for calves in the control group. After challenge with virulent *P. multocida*, calves vaccinated with N3PMH had significantly ($P=0.0085$) lower maximum rectal temperatures and significantly ($P=0.0251$) shorter duration of fever than calves in the control group. The efficacy of N3PMH was reaffirmed by results of this study, and the duration of immunity to *P. multocida* was determined to be at least 125 days. Results support the claim that N3PMH is safe and effective for intranasal vaccination of calves at 1 week of age or older against respiratory disease caused by *P. multocida*.

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 (BRD) complex. Nasalgen® 3-PMH 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 N3PMH and establishes the duration of immunity of the PM fraction of N3PMH to be at least 125 days following one dose of vaccine administered intranasally to calves 2 to 3 days of age.

EXPERIMENTAL PROCEDURES

Forty-four Holstein calves 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. Each calf was bottle-fed at least 2 quarts of milk replacer twice daily for the first week of the study. From the second week until the calves were approximately 8 weeks old, they were fed at least 2.5 quarts of milk replacer twice daily. Fresh water was provided *ad libitum* to all calves. Post-weaning, the feed met minimum nutritional requirements for animals of that age and complied with standard procedures for the research facility. 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 2 to 3 days old when vaccinated (Day 0), were healthy and had no prior history of vaccination against *Mannheimia haemolytica* or *Pasteurella multocida*. Nasalgen® 3-PMH was prepared so that the dose administered contained the minimum protective dose (MPD) of PM and contained IBR, PI_3 virus, BRSV and MH at or above the titers licensed for release. The placebo vaccine contained the same antigens as N3PMH but without PM. Twenty-two calves (12 males, 10 females) were vaccinated with 1 mL of the placebo vaccine per nostril, and 22 calves (9 males, 13 females) were vaccinated with 1 mL of N3PMH per nostril on Day 0.

On Day 14, one calf (female) in the control group was found dead. Conclusions from results of gross examination and histologic evaluation were that the calf died for reasons unrelated to vaccination.

On Day 125 post-vaccination, the 43 calves were commingled and then randomized and penned by shipment so that each pen contained a similar number of calves from each treatment group. Each calf (22 head vaccinated with N3PMH, 21 head vaccinated with placebo) was challenged intratracheally with virulent *PM*. Calves remained in their assigned pen until the end of the study. On Day 132 (seven days post-challenge), all calves were euthanized, lungs were removed, pulmonary lesions associated with PM were scored and samples of lung tissues were submitted for bacterial isolation. Two observers, acting independently and using the method described by Jericho and Langford (1982),[1] estimated the percent of abnormal lung tissue (Lung Lesion Score [LLS]), and the average of those two estimates was used for analysis. Personnel who administered the challenge, scored the lung lesions or who isolated organisms from samples of lung were blinded to which group the calf was assigned.

Figure 1. Timeline of events

The experimental unit was the individual calf. The primary outcome variable was the lung lesion score (LLS) representing the average percent of lung tissue with pneumonic lesions. Rectal temperature and respiratory rate were supportive variables.

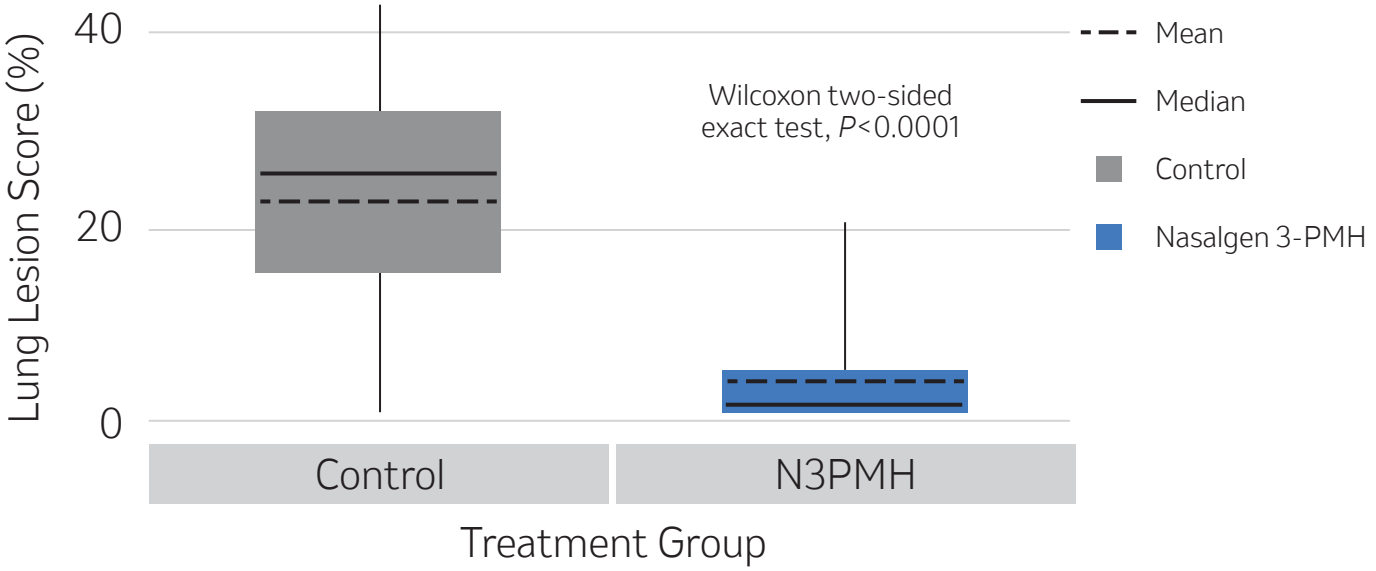
RESULTS

No adverse reactions associated with vaccination were observed. One calf in the control group died on Day 14, and no calf in either group died after challenge. The LLS (Table 1, Figure 2) for the group vaccinated with N3PMH were significantly (Wilcoxon two-sided exact test, $P<0.0001$) lower than that for the control group.

Table 1. Quartile summary of analysis of lung lesion score (%) after challenge with virulent PM by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	21	22.241	0.596	15.438	23.436	30.404	45.440
N3PMH	22	4.061	0.047	1.121	2.340	4.885	20.175

Figure 1. Lung lesion scores (%) by treatment group.



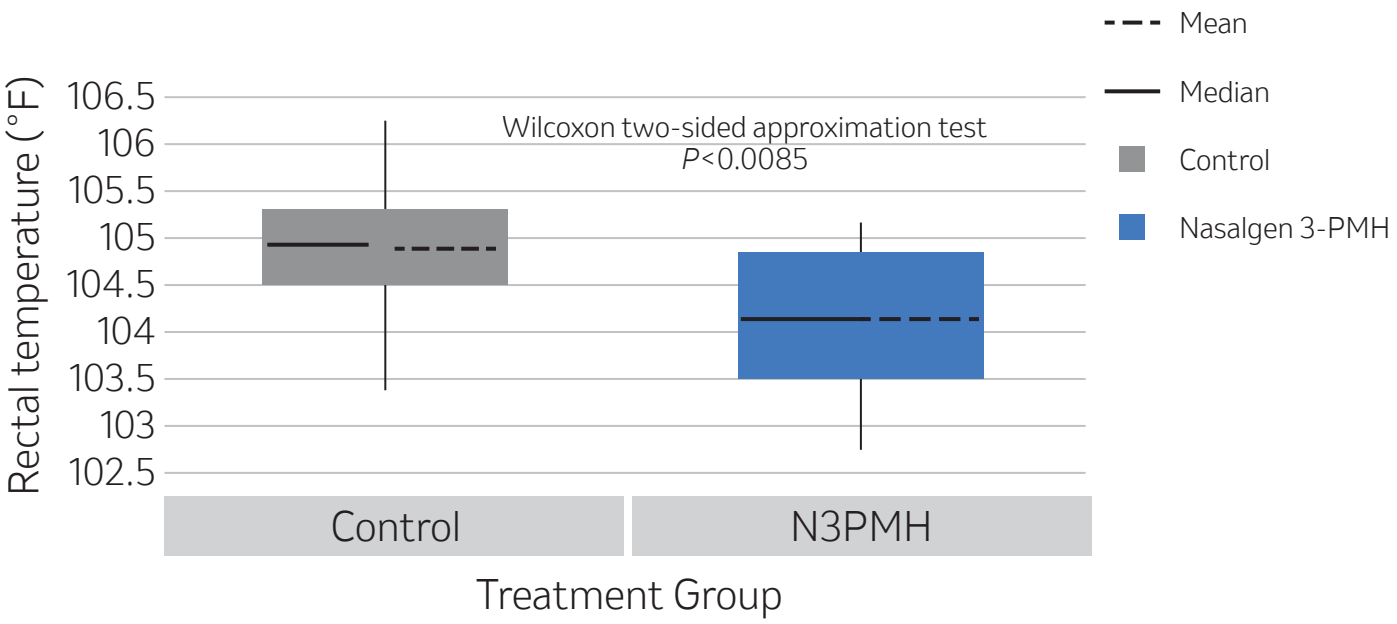
Pasteurella multocida was isolated from (7 of 22, 31.7%) samples of lung from calves in the N3PMH-vaccinated group and from all (21 of 21, 100%) samples of lung from calves in the control group.

Eighteen of 21 calves (85.7%) in the control group and 12 of 22 calves (54.5%) vaccinated with N3PMH had fever (rectal temperature > 104.0° F) on at least one day post-challenge. The maximum rectal temperatures (Table 2, Figure 2) for calves vaccinated with N3PMH were significantly (Wilcoxon two-sided t-approximation test, $P=0.0085$) lower than those for calves in the control group.

Table 2. Quartile summary of analysis of maximum rectal temperatures (F°) after challenge with virulent PM by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	21	104.89	103.4	104.5	104.9	105.2	106.2
N3PMH	22	104.14	102.8	103.5	104.15	104.8	105.2

Figure 2. Maximum rectal temperature post-challenge by treatment group.

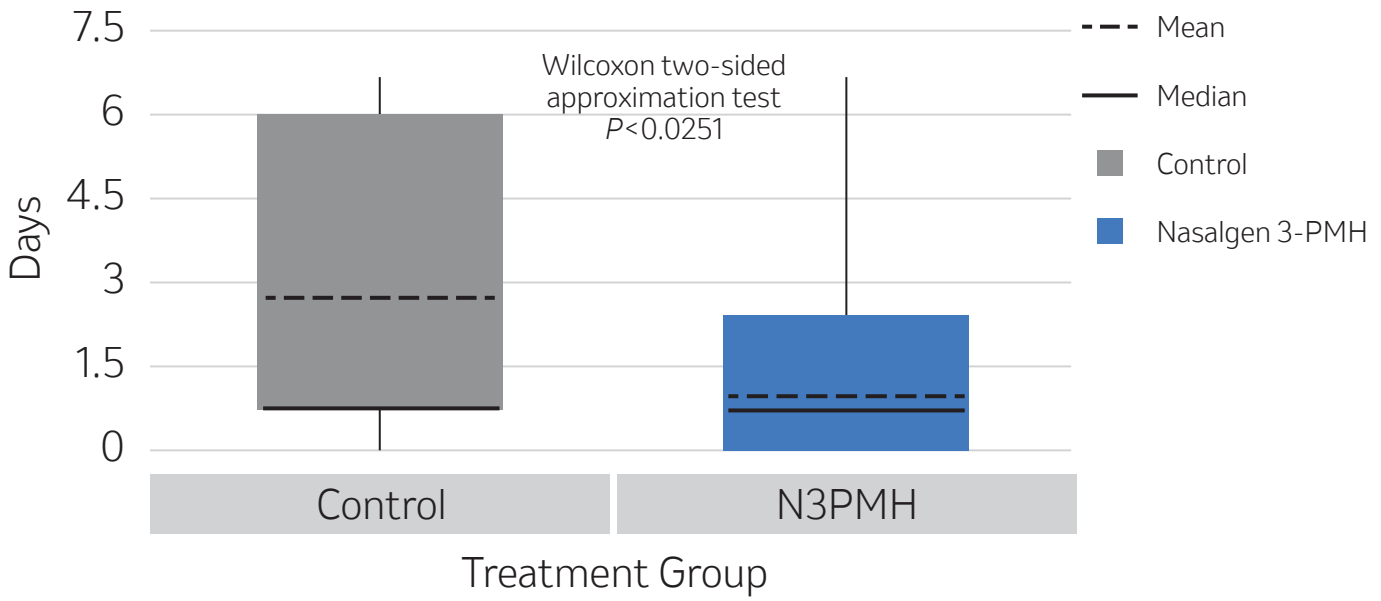


The duration of fever (Table 3, Figure 3) for calves vaccinated with N3PMH was significantly (Wilcoxon two-sided t-approximation test, $P=0.0251$) shorter than that for calves in the control group.

Table 3. Quartile summary of analysis of duration of fever (rectal temperatures > 104° F) for at least one day after challenge with virulent PM by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	21	2.9	0.0	1.0	1.0	6.0	7.0
N3PMH	22	1.2	0.0	0.0	1.0	2.0	7.0

Figure 3. Duration (days) of fever post-challenge by treatment group.



All calves in both groups had respiratory rates > 40 breaths per minute on at least one day post-challenge. The maximum respiratory rate for calves in either treatment group was not significantly different (Wilcoxon two-sided t-approximation test, $P=0.1829$).

CONCLUSIONS

Results of this study reaffirm the efficacy of N3PMH[2] when administered to calves 1 week old or less. In addition, the duration of immunity was demonstrated to be at least 125 days. Lung lesion scores, proportion of calves with fever (rectal temperature > 104.0° F) and duration of fever were all lower for calves vaccinated at 2 to 3 days of age and challenged with virulent *PM* 125 days later when compared to control calves. These results 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 PM and establishes the duration of immunity of the PM fraction of N3PMH to be at least 125 days.

REFERENCES

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

[2]Efficacy of the *Pasteurella multocida* Fraction of Nasalgen® 3-PMH in Calves 1 to 4 Days of Age. Technical Bulletin; Merck Animal Health.

Report No. BLI-088R, dated October 5, 2018, entitled "Duration of Immunity (DOI) for the *Pasteurella multocida* Fraction in Product Code 11C8.20, Administered Intranasally to One Week Old Calves."

Nasalgen® 3-PMH

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