Key Highlights

- This study reaffirms the safety and efficacy of N3PMH and establishes the duration of immunity of the IBR fraction of N3PMH to be at least 195 days following one dose of vaccine administered intranasally to calves 3 to 5 days of age.
- N3PMH is safe for use in pregnant cows and in calves nursing pregnant cows.
- The primary outcome variables were acute IBR morbidity and fever after challenge. Each of those variables was significantly (P<0.0001) less for the calves vaccinated with N3PMH than for calves in the control group.

Duration of Immunity of the Infectious Bovine Rhinotracheitis Virus Fraction of Nasalgen® 3-PMH Administered to Calves 3 to 5 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, 45 colostrum-deprived Holstein calves were randomly assigned to be vaccinated intranasally with either one dose of vaccine in which the IBR fraction was reduced to the minimum protective dose and the other viral and bacterial fractions in N3PMH were at the licensed vaccine dose (23 head) or with one dose of a placebo vaccine from which the IBR fraction was removed but the other viral and bacterial fractions in N3PMH were at the licensed vaccine dose (22 head). All calves were 3 to 5 days old on the day of vaccination (Day 0). No adverse reactions associated with vaccination were observed. Four calves (two from each treatment group) were euthanized or died for reasons unrelated to vaccination. On Day 195 after vaccination, 41 calves were challenged with virulent IBR virus by intranasal atomization (2 mL per nostril). The primary outcome variables were acute IBR morbidity and fever after challenge. Each of those variables was significantly (P<0.0001) lower for the calves vaccinated with N3PMH than for calves in the control group. Supportive variables (duration of nasal shedding of IBR virus and maximum titer of IBR virus shed in nasal secretions) were also significantly (P<0.0001 each) lower for the calves vaccinated with N3PMH than for calves in the control group. Results of this study reaffirm the safety and efficacy of N3PMH in calves 1 week of age or older against respiratory disease caused by IBR and demonstrated the duration of immunity to be at least 195 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 IBR fraction of N3PMH to be at least 195 days following one dose of vaccine administered intranasally to calves 3 to 5 days of age.

EXPERIMENTAL PROCEDURES

Forty-six Holstein calves (25 males, 21 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 (blocked by shipment) 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 and had access *ad libitum* to fresh water and a calf starter diet. Calves were allowed 2 or 3 days to acclimate. Health care was managed by the attending veterinarians. All calves were tested (Antigen-capture Enzyme-Linked Immunosorbent Assay) for persistent infection (PI) with Bovine Viral Diarrhea Virus (BVDV). One calf was euthanized prior to vaccination at the discretion of research personnel. Forty-five calves were available for vaccination.

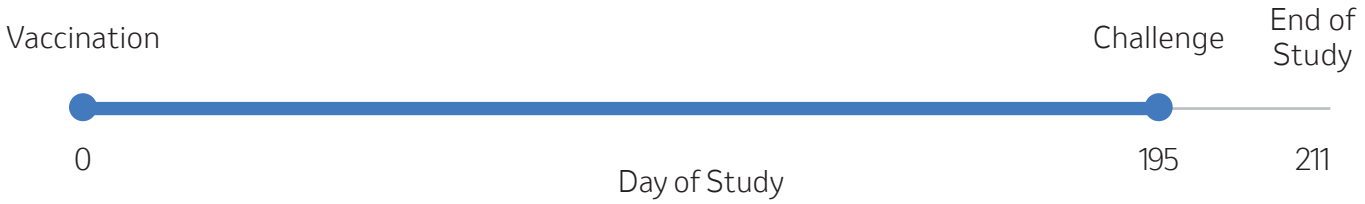
All calves were 3 to 5 days old and had serum neutralizing (SN) antibody titers to IBR < 1:2 when vaccinated (Day 0). N3PMH was prepared so that the dose administered contained the minimum protective dose (MPD) of IBR virus and contained BRSV virus, PI_3 virus, MH and PM at or above titers licensed for release. The placebo vaccine contained the same antigens as N3PMH but without IBR virus. One mL of placebo vaccine was administered into each nostril of 22 calves (12 male, 10 female). Then, 1 mL of N3PMH was administered into each nostril of 23 calves (12 male, 11 female). After vaccination, all calves remained in their respective hutches. For 2 weeks following vaccination, care and feeding of the calves in the control group was provided before that for calves vaccinated with N3PMH to prevent cross-exposure.

During the post-vaccination period, no adverse reactions associated with vaccination were observed. Four calves (two from each treatment group) were euthanized or died for reasons unrelated to vaccination. One was PI positive (testing results were available 1 day post-vaccination/allocation), two because of unresponsive septic arthritis, and one idiopathic death. A few additional calves had transient abnormalities – one calf had diarrhea, cough, weakness, fever, stiff limbs, depression and blood cells in urine, and three calves had lameness – all of which resolved following prescribed treatment. Forty-one, calves (21 N3PMH [11 male, 10 female]; 20 controls [11 male, nine female]) were available for challenge 195 days after vaccination.

When calves were approximately 7 weeks of age, they were moved to a building where they were randomized to one of nine holding pens so that each holding pen contained calves (four or five) from one shipment and a similar number of calves (at least one) from each treatment group. Five days prior to challenge, the calves were moved to another building, were commingled and randomly assigned to one of 10 pens so that each pen contained calves (four or five) from one shipment and a similar number of calves (at least one) from each treatment group.

On Day 195 after vaccination, 41 calves were challenged with virulent IBR virus by intranasal atomization (2 mL per nostril). All calves were monitored through Day 211 (16 days post-challenge) for clinical signs (including rectal temperature) of disease caused by IBR virus. Nasal secretions from each calf (one swab per nostril) were sampled the day before challenge (Day 194) and daily from Day 196 through Day 209 (Day 1 through Day 14 post-challenge) to detect IBR virus. After compliance with all relevant withdrawal times, the remaining 41 calves were sold.

Figure 1. Timeline of events

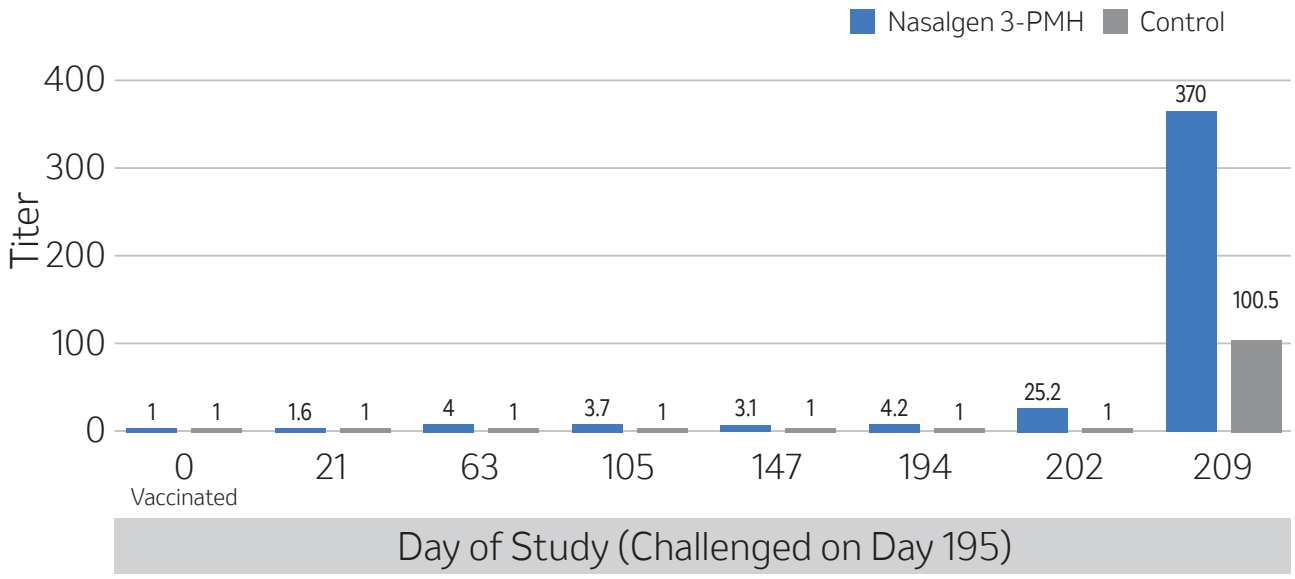


The experimental unit was the individual calf. The primary outcome variables were acute IBR morbidity and fever after challenge. For this study, IBR morbidity was defined as the presence of moderate to severe clinical signs of nasal or ocular discharge, nasal lesions, dyspnea, depression, anorexia and/or cough on any day post-challenge. Fever was defined as a rectal temperature ≥ 104° F for two or more consecutive days after challenge. The supporting variable was nasal shedding of IBR virus after challenge. Titers of SN antibody to IBR virus 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 quantitated for statistical analyses. Personnel administering the challenge, performing clinical observations or performing laboratory procedures were blinded to the treatment group to which any calf was assigned.

RESULTS

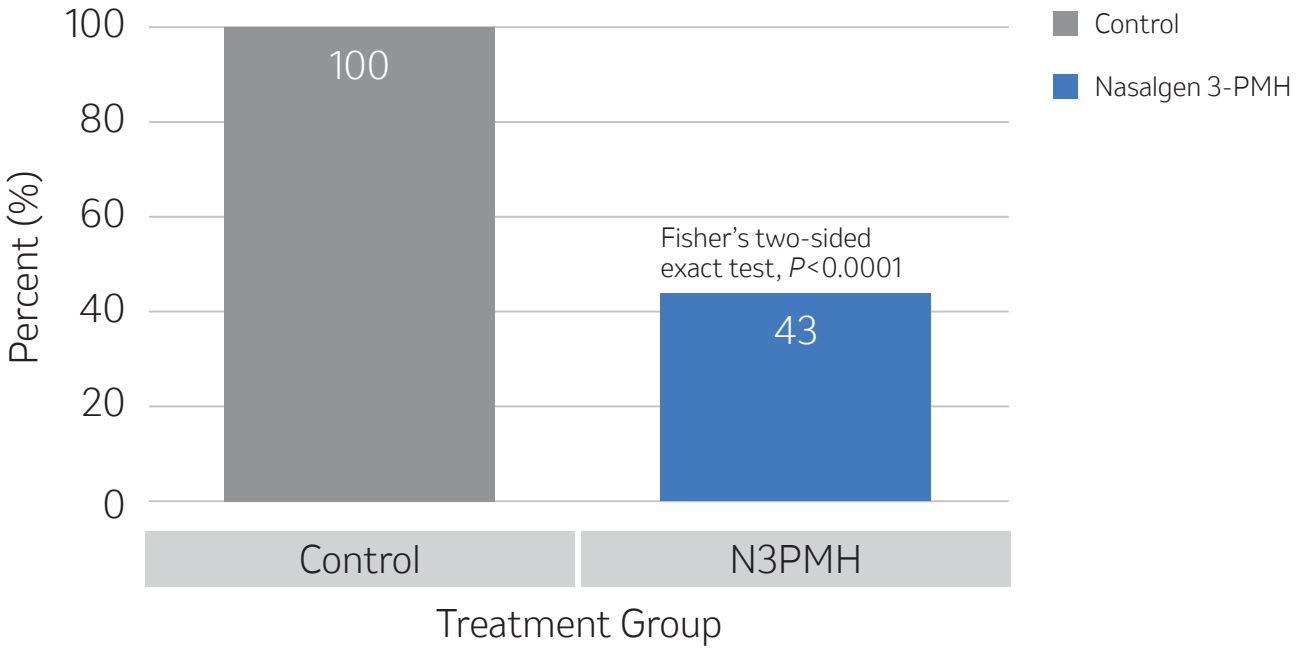
No adverse reactions associated with vaccination were observed during the study. All calves were seronegative (SN antibodies < 1:2) to IBR virus prior to vaccination on Day 0. Eighty-one percent of vaccinated calves (17/21) developed SN titers > 1:2 prior to challenge, and 90% (19/21) had titers >1:2 seven days after challenge. Calves in the control group remained seronegative until 14 days post-challenge (Day 209, Figure 2). Calves vaccinated with N3PMH demonstrated an anamnestic response after challenge, which suggests stimulation of memory cells resulting from the vaccine.

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



During the post-challenge period, the proportion of calves vaccinated with N3PMH (9/21, 43%) that had clinical signs (Figure 3) of IBR morbidity on any study day was significantly (Fisher's two-sided exact test, $P<0.0001$) lower than that of the calves in the control group (20/20, 100%).

Figure 3. Proportion of calves with clinical signs of infection by IBR virus, by treatment group.

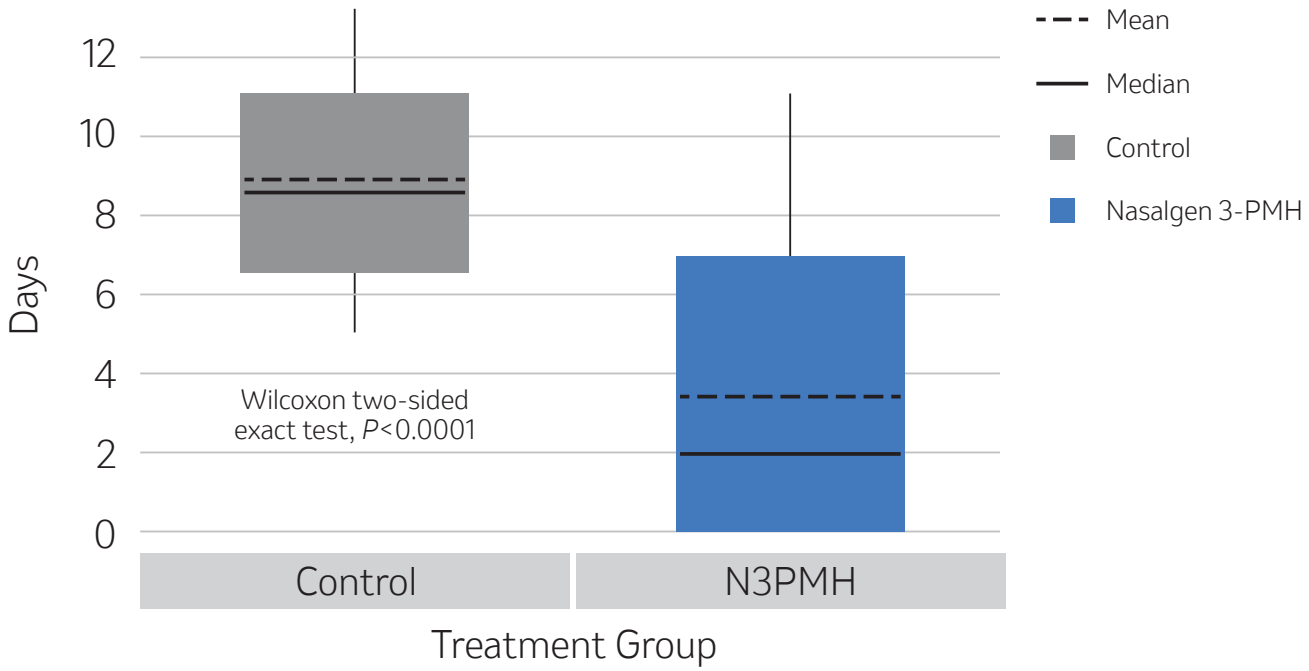


The duration of IBR morbidity (moderate to severe clinical signs of disease and/or fever ≥ 104.0° F), post-challenge, was significantly (Wilcoxon two-sided exact test, $P<0.0001$) shorter for calves vaccinated with N3PMH than for calves in the control group (Table 1, Figure 4).

Table 1. Quartile summary of analysis for duration of IBR morbidity (days) post-challenge, by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	20	8.7	5.0	6.5	8.5	11.0	13.0
N3PMH	21	3.5	0.0	0.0	2.0	7.0	11.0

Figure 4. Duration of IBR morbidity (days) post-challenge, by treatment group.



After challenge, fever was observed in a lower proportion of calves, was of lower maximum temperature and was of shorter duration for calves vaccinated with N3PMH than for those in the control group.

The proportion of calves with fever (rectal temperature ≥ 104° F for two or more consecutive days after challenge) was significantly (Wilcoxon two-sided exact test, $P<0.0001$, Figure 5) less for calves vaccinated with N3PMH (9/21, 43%) than for the calves in the control group (20/20, 100%).

Figure 5. Proportion of calves with fever (rectal temperature ≥ 104° F for two or more consecutive days after challenge) after challenge by treatment group.

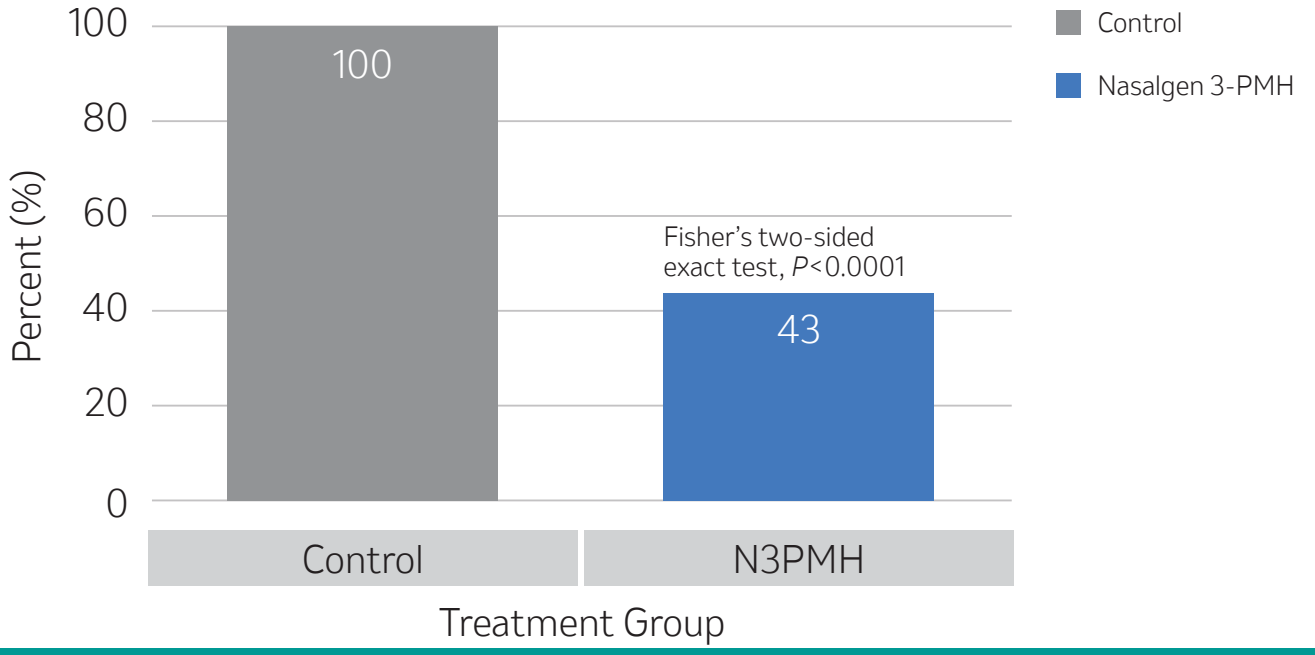
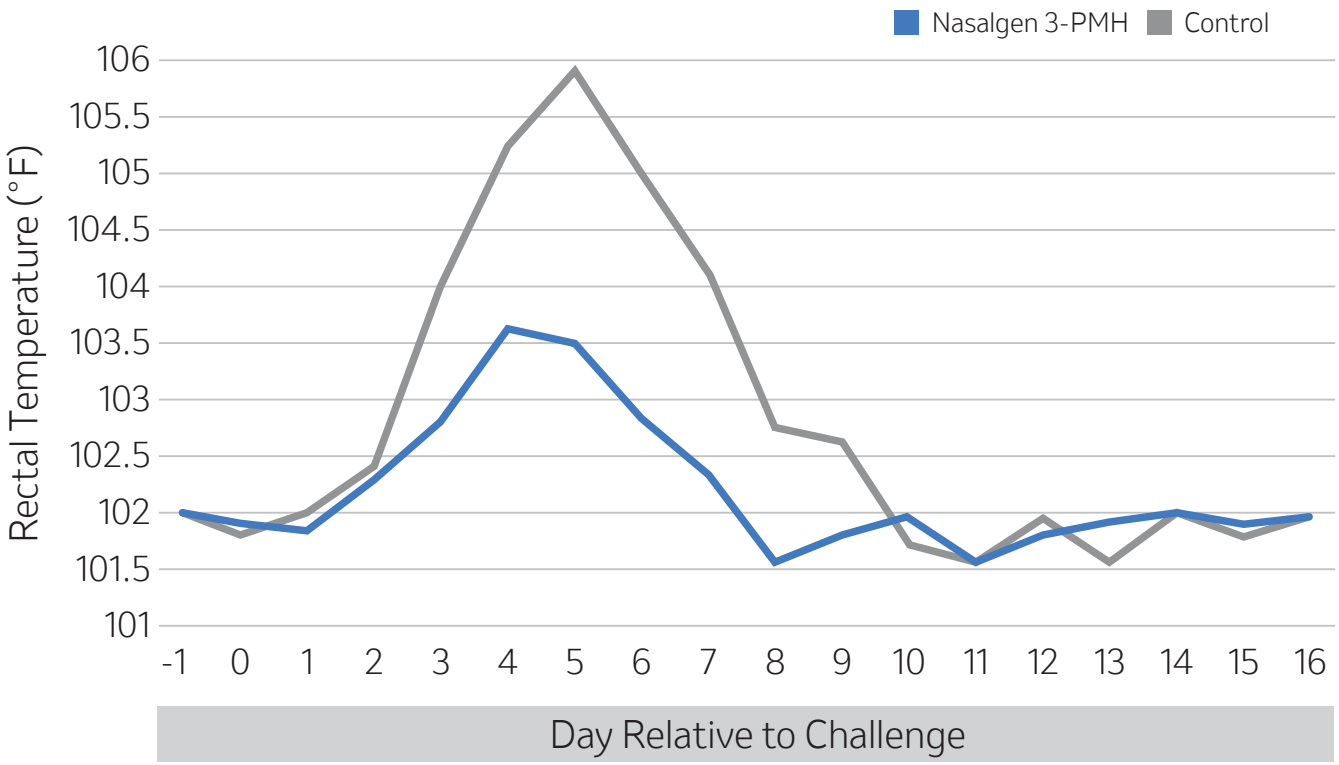


Figure 6. Average daily rectal temperatures from the day before challenge (-1, Day 194) through 16 days post-challenge (Day 211), by treatment group.

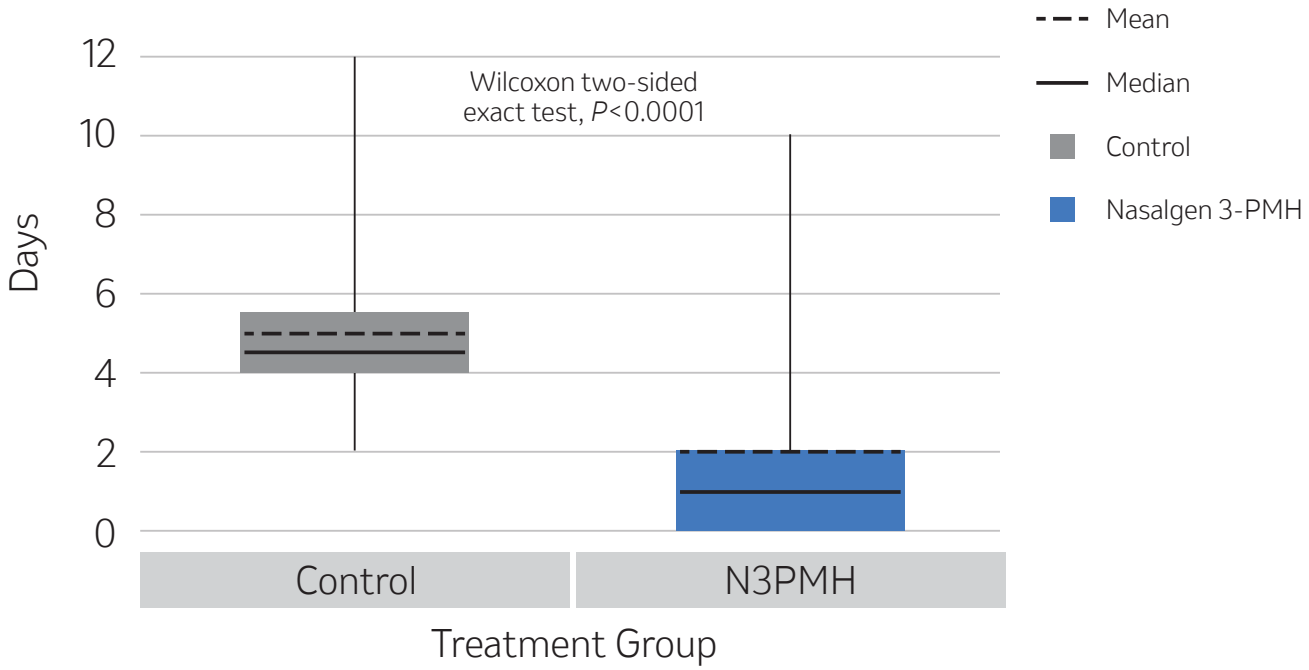


The duration of fever (Table 2, Figure 7), post-challenge, included the first and last day of fever and was significantly (Wilcoxon two-sided exact test, $P<0.0001$) shorter for calves vaccinated with N3PMH than for calves in the control group.

Table 2. Quartile summary of analysis for duration of fever post-challenge, by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	20	5.0	2.0	4.0	4.5	5.5	12.0
N3PMH	21	2.0	0.0	0.0	1.0	2.0	10.0

Figure 7. Duration of fever post-challenge, by treatment group.

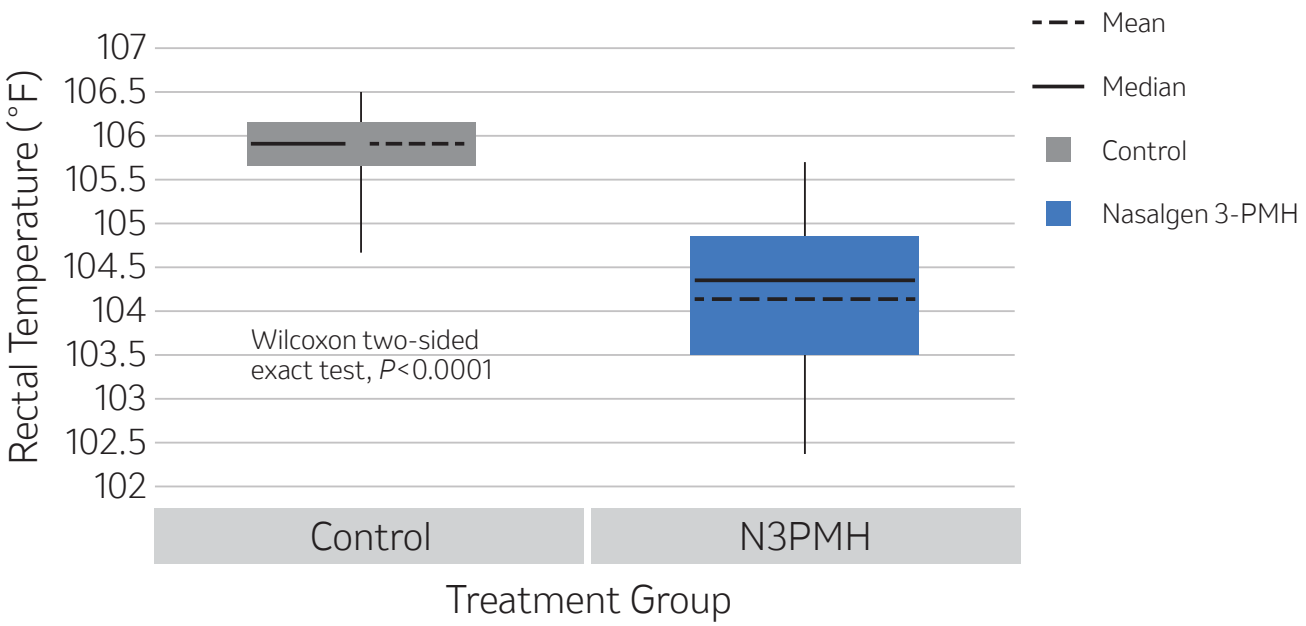


The maximum rectal temperature, post-challenge, was significantly (Wilcoxon two-sided exact test, $P<0.0001$) lower for calves vaccinated with N3PMH than for calves in the control group (Table 3, Figure 8).

Table 3. Quartile summary of analysis for maximum rectal temperature, post-challenge, by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	20	105.9	104.7	105.6	105.9	106.2	106.5
N3PMH	21	104.2	102.4	103.5	104.3	104.9	105.7

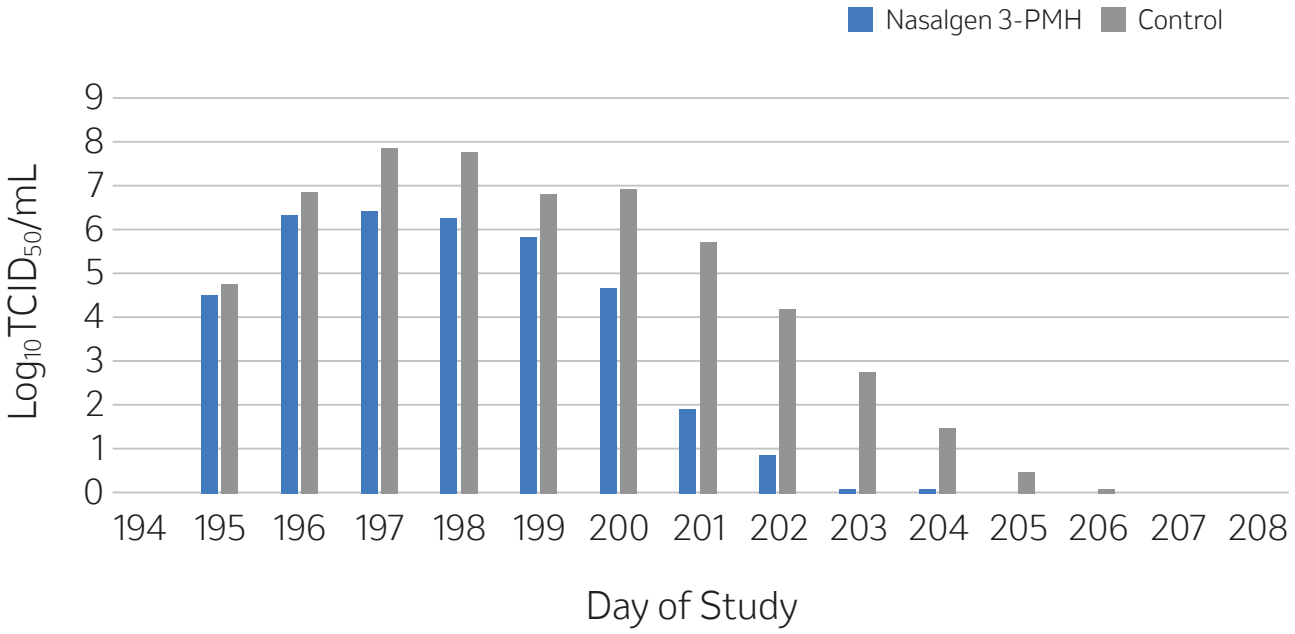
Figure 8. Maximum fever post-challenge, by treatment group.



After challenge, viral shedding in nasal secretions was lower for calves vaccinated with N3PMH compared to that for calves in the control group as indicated by peak viral titers and duration of shedding.

Average titers (Log_{10} Tissue Culture Infectious Dose Fifty Percent [$Log_{10}TCID_{50}$]/mL) of IBR virus shed daily in nasal secretions by treatment group are presented in Figure 9.

Figure 9. Average titers ($Log_{10}TCID_{50}$/mL) of IBR virus shed daily in nasal secretions by treatment group.



Before challenge, none of the calves were shedding IBR virus in nasal secretions. After challenge, the duration of shedding of IBR virus (Table 4, Figure 10) and the maximum titer ($Log_{10}TCID_{50}$/mL) of IBR virus shed (Table 5, Figure 11), in nasal secretions were each significantly (Wilcoxon two-sided exact test, $P < 0.0001$) lower for calves vaccinated with N3PMH than for calves in the control group.

Table 4. Quartile summary of analysis for duration of shedding of IBR virus in nasal secretions post-challenge, by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	20	9.9	8.0	9.0	10.0	10.0	12.0
N3PMH	21	7.0	6.0	6.0	7.0	8.0	10.0

Figure 10. Duration of shedding of IBR virus in nasal secretions post-challenge, by treatment group.

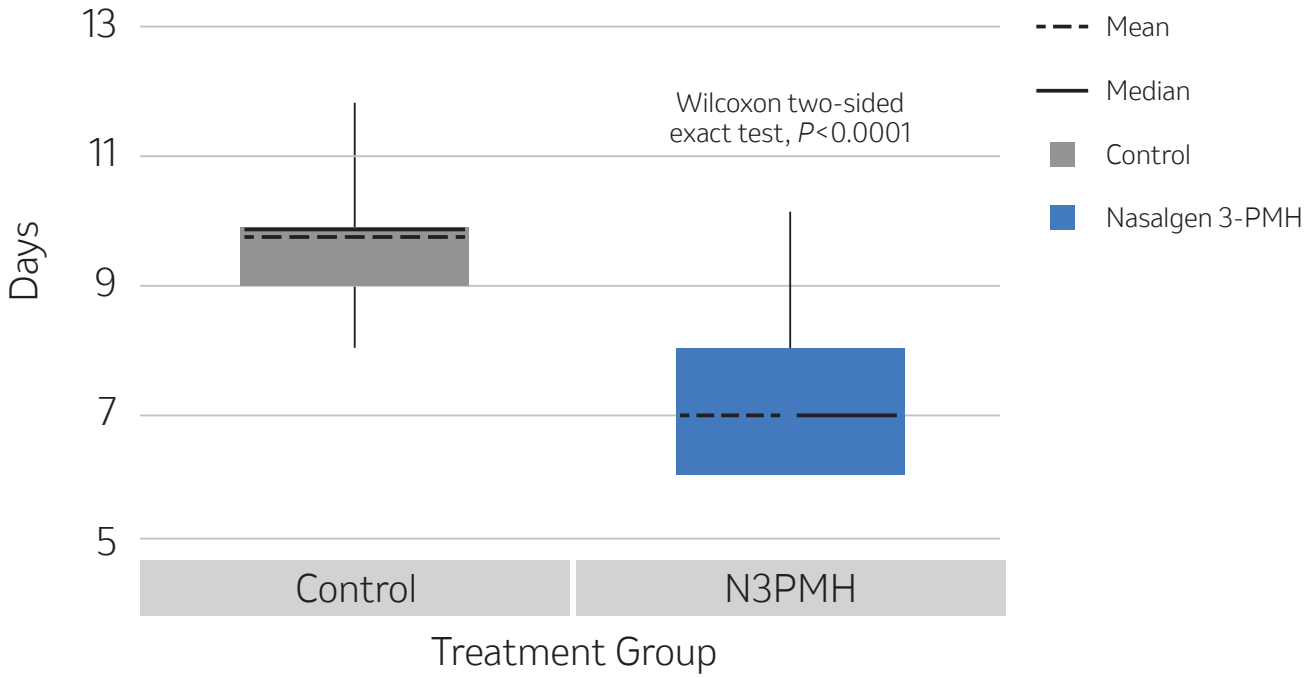
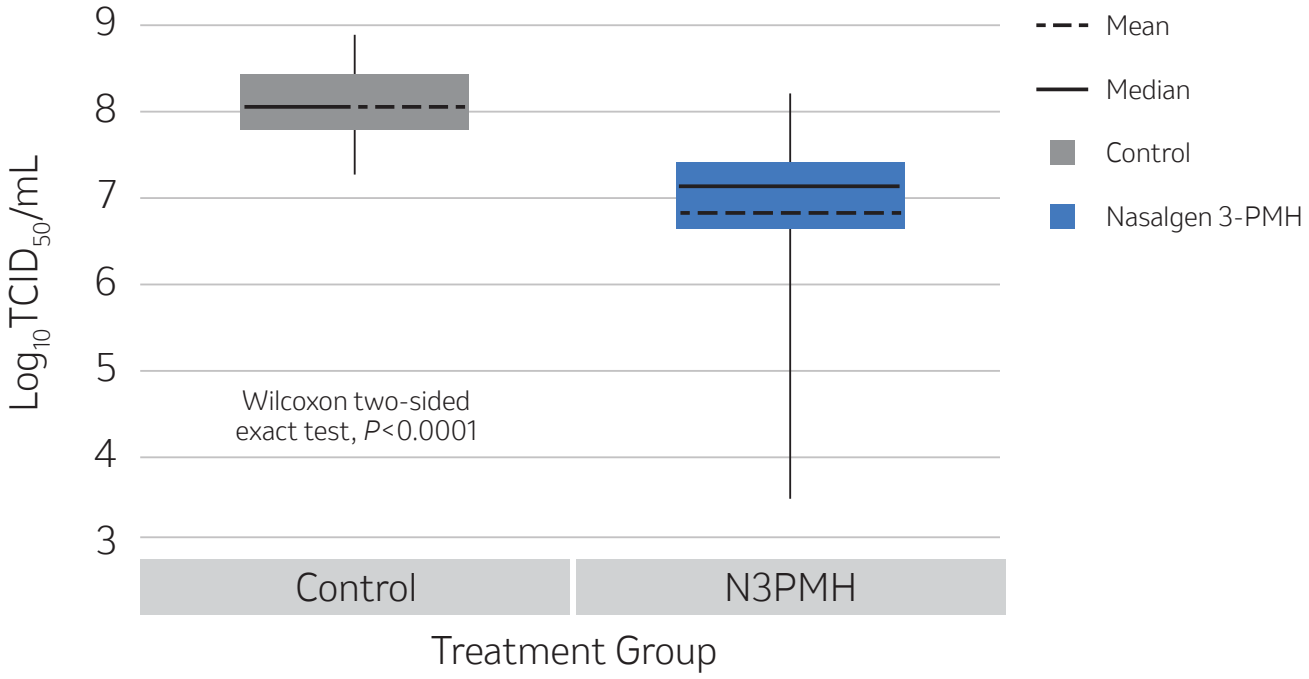


Table 5. Quartile summary of analysis for maximum titer of IBR virus shed in nasal secretions post-challenge, by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	20	8.1	7.3	7.7	8.1	8.4	8.9
N3PMH	21	6.9	3.5	6.7	7.3	7.5	8.3

Figure 11. Maximum titer of IBR virus shed in nasal secretions post-challenge, by treatment group.



CONCLUSIONS

A single intranasal dose of Nasalgen® 3-PMH that contained the minimum protective dose of IBR antigen provided significant protection when compared to placebo vaccine in neonatal calves that were challenged with virulent IBR virus 195 days after vaccination. The proportion of calves with acute IBR morbidity, duration of IBR morbidity, proportion of calves with fever, duration of fever and maximum rectal temperature was significantly ($P<0.0001$) lower for the calves vaccinated with N3PMH than for calves in the control group. Duration of nasal shedding of IBR virus and maximum titer of IBR virus shed in nasal secretions were also significantly ($P<0.0001$ each) lower for the calves vaccinated with N3PMH compared to those for calves in the control group. Results of this study reaffirm the safety and efficacy of Nasalgen® 3-PMH[1], demonstrate the duration of immunity of at least 195 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 IBR virus.

REFERENCES

[1]The Efficacy of the Infectious Bovine Rhinotracheitis Fraction of Nasalgen® 3-PMH in Calves 4 to 7 Days Old.

Report No. BLI-095R, dated June 18, 2019, entitled "Duration of Immunity of the Bovine Rhinotracheitis Virus Fraction Contained in Bovine Rhinotracheitis-Parainfluenza 3-Respiratory Syncytial Virus-*Mannheimia haemolytica*-*Pasteurella multocida* Vaccine, Modified Live Virus, Avirulent Live Culture, Administered Intranasally to One Week Old Calves."

Nasalgen® 3-PMH

MERCK Animal Health