Nasalgen® 3-PMH TECH BULLETIN

MERCK Animal Health

Duration of Immunity of the Parainfluenza 3 Fraction of Nasalgen® 3-PMH Administered to Calves 3 to 5 Days of Age

Key Highlights

- This study reaffirms the safety and efficacy of N3PMH and establishes the duration of immunity of the PI_3 fraction of N3PMH to be at least 95 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 duration of nasal shedding of PI_3 virus was significantly ($P<0.0001$) shorter for calves vaccinated with N3PMH than for calves in the control group.

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 one dose of N3PMH (22 head), or with one dose of a placebo vaccine (22 head) that did not contain the PI_3 fraction, but contained the other viral and bacterial antigens in N3PMH. All calves were 3 to 5 days old on the day of vaccination (Day 0). One calf was removed from the control group prior to vaccination. No adverse reactions associated with vaccination were observed. During the post-vaccination period, three calves were euthanized or died for reasons unrelated to vaccination. On Day 95, all calves (40 head) were challenged intranasally with virulent PI_3 virus. All calves (20/20, 100%) in the control group shed PI_3 virus in nasal secretions compared to 18 of 20 (90%) calves vaccinated with N3PMH. Between the two treatment groups, the proportion of calves that shed PI_3 virus was not significantly ($P=0.487$) different. The duration of nasal shedding of PI_3 virus was significantly ($P<0.0001$) shorter for calves vaccinated with N3PMH than for calves in the control group. This study reaffirms the safety and efficacy of N3PMH and establishes the duration of immunity of the PI_3 fraction of N3PMH to be at least 95 days following one dose of vaccine administered intranasally to calves 3 to 5 days of age.

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 PI_3 fraction of N3PMH to be at least 95 days following one dose of vaccine administered intranasally to calves 3 to 5 days of age

EXPERIMENTAL PROCEDURES

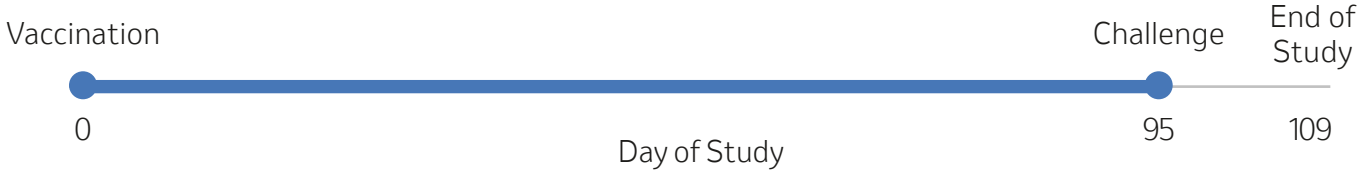
Forty-four Holstein calves (14 males, 30 females) were obtained from a single source, were deprived of colostrum, were identified by unique individual numbers and were transported (two shipments) to the research facility. Prior to arrival, the calves were randomly assigned to be vaccinated intranasally (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. During the study, 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 a calf starter diet. Calves were allowed 2 or 3 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).

On the day before vaccination, one calf was unable to stand and was removed from the study prior to vaccination. All remaining calves (43 head) were 3 to 5 days old and had serum neutralizing (SN) antibody titers to PI_3 < 1:2 (negative) when vaccinated (Day 0). Nasalgen® 3-PMH was prepared so that the dose administered contained the minimum protective dose (MPD) of PI_3 virus and contained IBR, BRSV, MH and PM at or above titers licensed for release. The placebo vaccine contained the same antigens as N3PMH, but without PI_3. One mL of placebo vaccine was administered into each nostril of 21 calves (15 females, 6 males). Then, 1 mL of N3PMH was administered into each nostril of 22 calves (14 females, 8 males). After vaccination, all calves remained in their respective hutches. For 2 weeks following vaccination, care and feeding of the calves in the control group were provided before that for calves vaccinated with N3PMH to prevent cross-exposure. At about 6 weeks of age, the calves were moved to pens according to the original randomization assignment; each pen contained four or five calves from the same shipment. Four days prior to challenge, calves were moved to one of four pens that contained four, five or six calves per pen during the challenge phase of the study. Each challenge pen contained calves from one shipment and a similar number of calves from each treatment group. Three calves were euthanized or died (two from the group vaccinated with N3PMH – one with fibrinopurulent peritonitis and one idiopathic; one from control group – sepsis) for reasons unrelated to vaccination. Two other calves had transient episodes of scours that resolved following prescribed treatment. 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 associated with vaccination were observed.

On Day 95 (Figure 1), all remaining calves (40 head) were challenged intranasally using an atomizer to administer approximately 2 mL of solution containing virulent PI_3 virus per nostril of each calf. All calves were monitored (clinical signs of infection by PI_3, respiratory rates and rectal temperatures were recorded) daily from Day 94 (one day before challenge) through Day 109 (post-challenge Day 14). No calf died or was euthanized after challenge. Samples of nasal secretions (one swab per nostril) were obtained daily from Day 96 to Day 105 for determination of shedding of PI_3. After challenge, a 30-day withdrawal time was imposed and the remaining 40 calves were sold.

The experimental unit was the individual calf. The primary outcome variable was virus isolation from samples of nasal secretions. Supporting outcome variable was clinical disease. Titers of SN antibody to PI_3 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 analysis.

Figure 1. Timeline of events



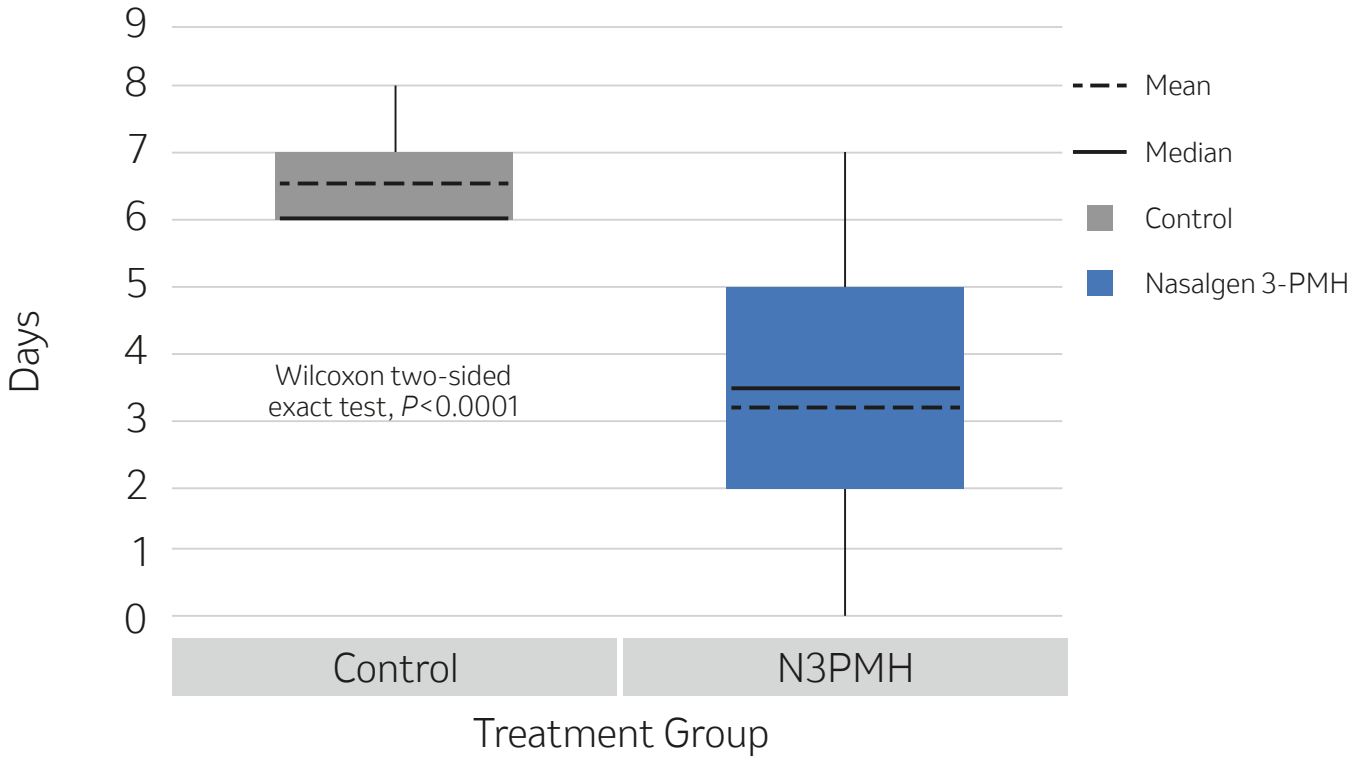
RESULTS

No adverse events associated with vaccination were observed. Nasal shedding of PI_3 virus was defined as presence of the virus in nasal secretions on any day during the post-challenge period. All calves (20/20, 100%) in the control group shed PI_3 virus for an average of 6.6 days with a mean maximum titer of 6.0 $Log_{10}TCID_{50}$/mL. Eighteen of 20 (90%) calves vaccinated with N3PMH shed PI_3 for a mean duration of 3.3 days with a mean maximum titer of 2.6 $Log_{10}TCID_{50}$/mL. Between the two treatment groups, the proportion of calves that shed PI_3 virus was not significantly (Fisher's exact test, $P=0.487$) different. The duration of nasal shedding of PI_3 virus (Table 1, Figure 2) was significantly (Wilcoxon two-sided exact test, $P<0.0001$) shorter for calves vaccinated with N3PMH than that for calves in the control group.

Table 1. Quartile summary of duration (days) of shedding of PI_3 virus in nasal secretions, post-challenge, by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	20	6.6	6.0	6.0	6.0	7.0	8.0
N3PMH	20	3.3	0.0	2.0	3.5	5.0	7.0

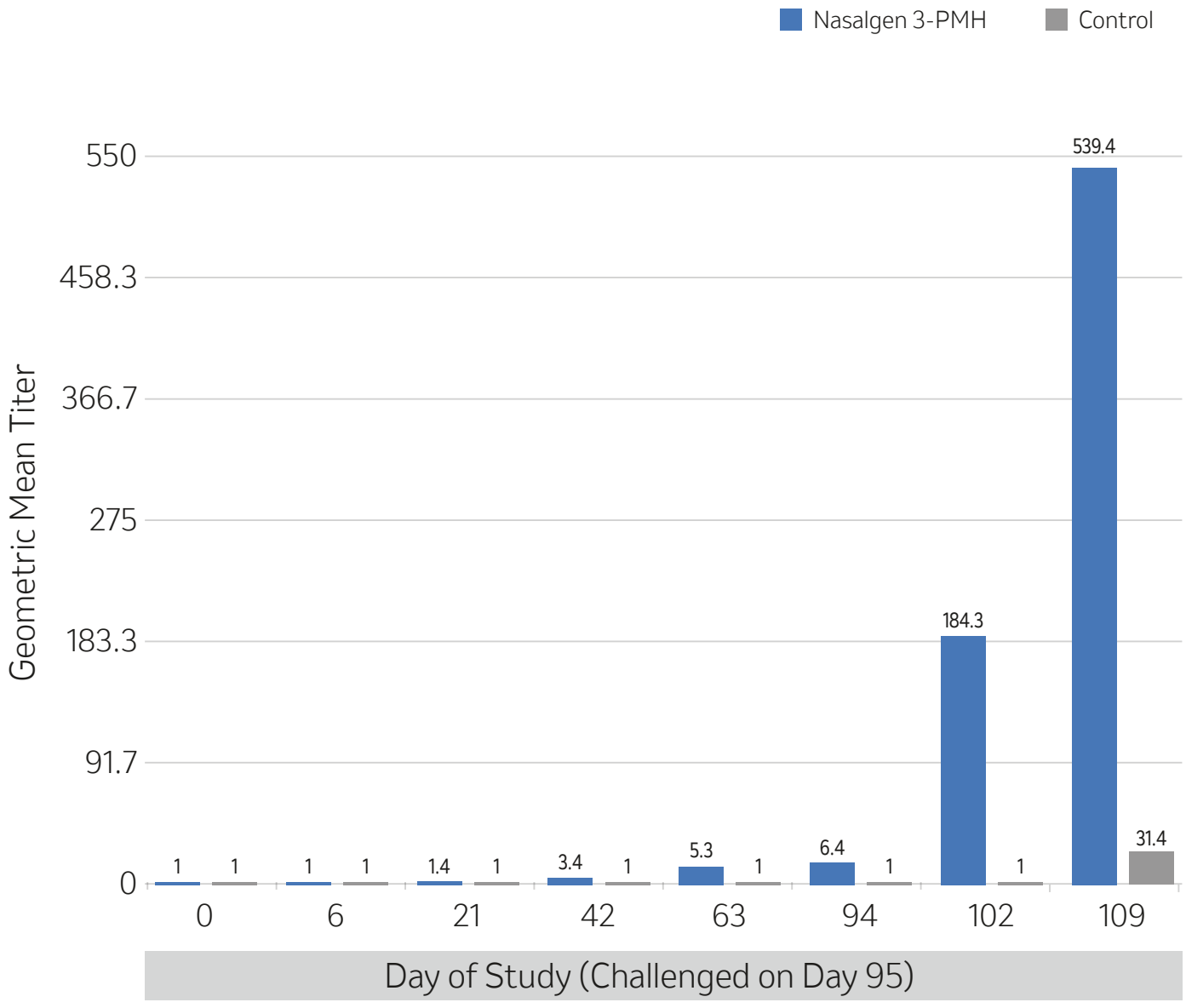
Figure 2: Duration (days) of nasal shedding of PI_3 virus, post challenge, by treatment group.



No calf in either treatment group exhibited clinical signs that were more severe than "mild." The proportion of calves with clinical signs on any day after challenge was not significantly (Fisher's two-sided exact test, $P=0.231$) different for calves in either treatment group.

All calves were seronegative (SN antibody titer < 1:2) to PI_3 virus prior to vaccination (Figure 3). Calves in the control group remained seronegative until Day 109 (14 days post-challenge). Calves vaccinated with N3PMH responded serologically and demonstrated an anamnestic response post-challenge.

Figure 3. Geometric mean titer (twofold dilution) of serum neutralization (SN) antibodies to PI_3 by treatment group.



CONCLUSIONS

The study reported here demonstrated that nasal shedding of PI_3 virus was of shorter duration for calves that were vaccinated with N3PMH at minimum protective dose than those that received a placebo vaccine and challenged 95 days after vaccination. As expected, clinical signs of infection with PI_3 were mild and not significantly different between the treatment groups. Results of this study reaffirm the safety and efficacy of the PI_3 fraction of N3PMH.[1] The duration of immunity to the PI_3 fraction of N3PMH was demonstrated to be at least 95 days following one dose of vaccine, at minimum protective dose, administered intranasally to calves 3 to 5 days of age. This supports 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 PI_3.

REFERENCES

[1]Efficacy of the Bovine Parainfluenza 3 Virus Fraction of Nasalgen® 3-PMH in Calves 3 to 5 Days of Age. Technical Bulletin; Merck Animal Health USDA-reviewed duration of immunity report.

Report No. BLI-089R, dated September 27, 2018, entitled "Duration of Immunity of the Bovine Parainfluenza 3 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."

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