Nasalgen® 3

TECH BULLETIN

MERCK Animal Health

Efficacy of the Bovine Parainfluenza 3 Virus Fraction of Nasalgen® 3 in Calves 6 or 7 Days Old

Key Highlights

- Results of this study demonstrate protective efficacy of the PI_3 fraction of Nasalgen 3 and confirmed the non-interference by the other antigenic fractions in N3 when administered intranasally to healthy calves 6 or 7 days old.
- Vaccination with a single dose of Nasalgen 3 that contained the minimum protective dose of PI_3 virus resulted in a lower proportion of calves that shed PI_3 virus in nasal secretions, in shorter duration of viral shedding and in lower titers of PI_3 virus shed in nasal secretions, compared to vaccination with a placebo.
- 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 PI_3.

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, 40 colostrum-deprived Holstein calves were randomly assigned to be vaccinated intranasally with N3 (26 head) that contained the minimum protective dose of PI_3 virus or a placebo vaccine (14 head) that did not contain the PI_3 fraction but contained the other viral antigens in N3. All calves were 6 to 7 days old on the day of vaccination (Day 0). No adverse reactions were observed after vaccination. One calf from the control group died, and four calves vaccinated with N3 died or were euthanized for reasons unrelated to vaccination. On Day 39 (first shipment) or Day 32 (second shipment), all calves were challenged by intranasal administration of virulent PI_3 virus. After challenge, PI_3 virus was isolated from nasal secretions of significantly (P=0.0131) fewer calves vaccinated with N3 (13/22, 59%) than from calves (13/13, 100%) in the control group. The maximum titer (Log_{10} $TCID_{50}$/mL) of PI_3 virus shed in nasal secretions was significantly lower, and the duration of nasal shedding was significantly (P<0.0001) shorter for calves vaccinated with N3 than from calves in the control group. Nasalgen 3 provided protection to calves 6 to 7 days of age as reflected by the proportion of calves that shed PI_3 virus after challenge and by duration of shedding of PI_3 virus.

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 PI_3 viral fraction of N3 and no interference by the other two antigens in N3 after one intranasal administration to calves 6 or 7 days old.

EXPERIMENTAL PROCEDURES

Holstein calves were obtained from a single source, identified by unique individual numbers, deprived of colostrum and transported (two shipments) to the study site in De Soto, KS. Calves were randomly assigned to be vaccinated intranasally (IN) with N3 or with a placebo vaccine (control group). Calves were housed in individual hutches that were segregated by treatment group and the two groups were physically separated by at least 15 feet. Calves were bottle-fed at least 2 quarts of milk replacer twice daily until they could be fed from a bucket. They had access *ad libitum* to fresh water. As part of the daily routine, calves in the control group were fed and cared for before those vaccinated with N3. Calves were allowed 5 days to acclimate prior to enrollment. Health care was managed by the attending veterinarian. All calves were confirmed (antigen-capture Enzyme-Linked Immunosorbent Assay) to not be persistently infected with Bovine Viral Diarrhea Virus (BVDV).

Because the calves arrived on different days, the day of vaccination (Day 0) was also different by 7 days. All calves were 6 or 7 days old on the respective day of vaccination. Forty calves (24 males, 16 females) were clinically healthy, had SN antibody titers to PI_3 ≤ 1:2 and were enrolled in the study. Nasalgen 3 was prepared so that the dose administered contained the minimum protective dose (MPD) of PI_3 virus and contained IBR and BRSV at or above titers licensed for release. The placebo vaccine contained the same antigens as N3 but without the PI_3 virus. One dose of placebo vaccine was administered (2mL into the left nostril) of 14 calves (9 males, 5 females) and 2 mL of N3 were administered into the left nostril of 26 calves (13 males, 13 females).

No adverse events attributable to the vaccine were observed following vaccination.

Calves were observed daily and general health was recorded. Immediately prior to challenge, calves were penned five or six to a pen according to the established randomization protocol. All calves were challenged with virulent PI_3 virus administered IN (2 mL of challenge material per nostril) on the same day (39 days following vaccination for calves in the first shipment and 32 days following vaccination of calves in the second shipment). All calves were monitored daily for 14 days after challenge.

The experimental unit was the individual calf. The primary outcome variable was nasal shedding of PI_3 virus. Supporting variables were clinical disease and duration of clinical disease. For purposes of this study, clinical disease was defined as the presence of depression, nasal discharge, ocular discharge, dyspnea or coughing, or fever (rectal temperature ≥ 104.0°F) on any day after challenge. Personnel who administered the challenge and performed clinical observations and/or who performed laboratory procedures were blinded to the treatment group to which a calf was allocated.

Figure 1. Proportion of calves, in each treatment group, that shed PI_3 virus in nasal secretions on any day post-challenge.

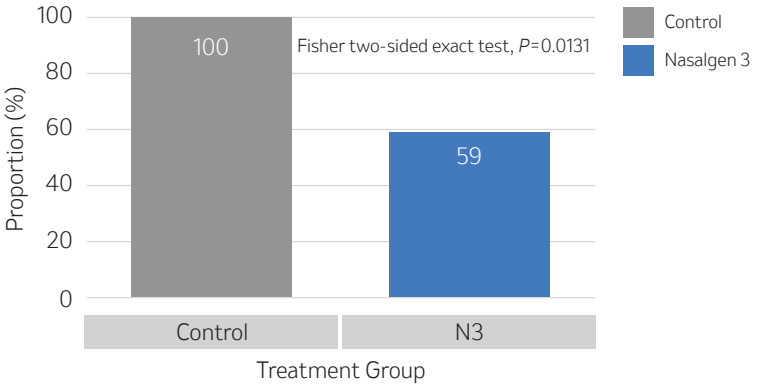


Figure 2. Maximum titer (Log_{10} $TCID_{50}$/mL) of PI_3 virus shed in nasal secretions post-challenge by treatment group.

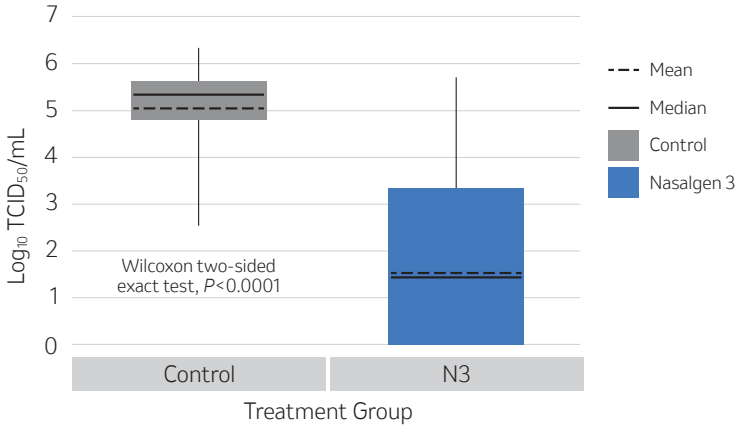
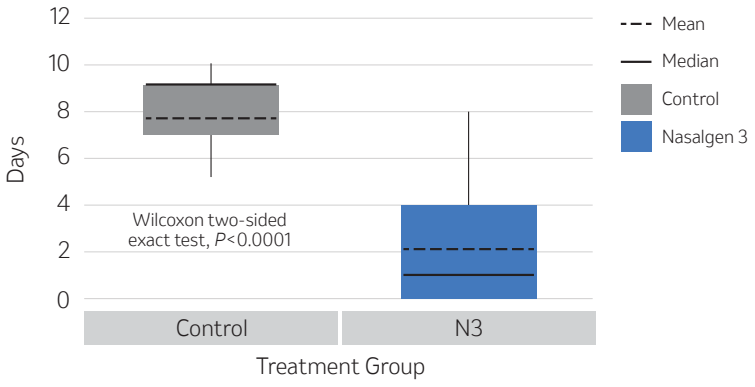


Figure 3. Duration of shedding of PI_3 virus in nasal secretions, post-challenge by treatment group.



RESULTS

No adverse reactions were observed. After challenge, seven of 13 (54%) calves in the control group and nine of 22 (41%) calves vaccinated with N3 developed clinical signs of infection by PI_3. Those signs were mild to moderate nasal discharge, ocular discharge, cough and/or fever. On Day 4 and Day 5 post-challenge, one calf vaccinated with N3 developed fever ≥ 104°F. No other clinical signs developed and the fevers resolved.

Prior to challenge, samples of nasal secretions were "negative" for PI_3 virus. After challenge, all (100%) of the calves in the control group and only 13 of 22 (59%) of the calves vaccinated with N3 shed PI_3 virus in nasal secretions (Fisher two-sided exact test, $P=0.0131$, Figure 1).

Maximum titer (Log_{10} $TCID_{50}$/mL) of PI_3 virus shed in nasal secretions post-challenge was significantly (Wilcoxon two-sided exact test, $P<0.0001$) lower for calves vaccinated with N3 than for calves in the control group (Table 1, Figure 2).

Table 1. Quartile summary of maximum titer (Log_{10} $TCID_{50}$/mL) of PI_3 virus shed in nasal secretions post-challenge by treatment group.

Treatment Group	*N*	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	13	5.008	2.5	4.9	5.3	5.7	6.3
N3	22	1.786	0	0	1.7	3.3	5.7

The duration of nasal shedding of PI_3 virus post-challenge was significantly (Wilcoxon two-sided exact test, $P<0.0001$) shorter for calves vaccinated with N3 than for calves in the control group (Table 2, Figure 3).

Table 2. Quartile summary of duration 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	13	7.92	5	7	9	9	10
N3	22	2.05	0	0	1	4	8

CONCLUSIONS

Results of this study demonstrate protective efficacy of the PI_3 fraction of Nasalgen 3 and confirmed the non-interference by the other antigenic fractions in Nasalgen 3 when administered intranasally to healthy calves 6 or 7 days old. Vaccination with a single dose of Nasalgen 3 that contained the minimum protective dose of PI_3 virus resulted in lower proportion of calves that shed PI_3 virus in nasal secretions, resulted in shorter duration of viral shedding and resulted in lower titers of PI_3 virus shed in nasal secretions, compared to vaccination with a placebo. 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 PI_3.

REFERENCES

Data on File: USDA-approved efficacy report for N3.

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