

## Key Highlights

- Nasalgen® 3 (N3) has been shown to be effective for vaccination of healthy cattle, 1 week of age or older against IBR, BRSV and PI<sub>3</sub> that are pathogens implicated in the Bovine Respiratory Disease (BRD) complex.
- The primary outcome, lung lesion score (LLS), was significantly ( $P < 0.0001$ ) less for the calves vaccinated with N3 than for calves in the control group.
- Duration of nasal shedding of BRSV was significantly ( $P = 0.0003$ ) shorter for calves vaccinated with N3 than for calves in the control group.

## Duration of Immunity of the Bovine Respiratory Syncytial Virus Fraction of Nasalgen® 3 in Calves 5 to 7 Days of Age

### 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 virus (PI<sub>3</sub>) that are pathogens implicated in the Bovine Respiratory Disease (BRD) complex. Nasalgen® 3 is safe for use in pregnant cows or in calves nursing pregnant cows. For this study, 44 colostrum-deprived Holstein calves were randomly assigned to be vaccinated intranasally with one dose of N3 (22 head) or with one dose of a placebo vaccine (22 head) that did not contain the BRSV fraction but contained the other viral antigens in N3. All calves were 5 to 7 days old on the day of vaccination (Day 0). No adverse reactions were observed following vaccination. On Day 78 after vaccination, all calves were commingled and challenged with aerosolized virulent BRSV. The primary outcome, lung lesion score (LLS), was significantly ( $P < 0.0001$ ) less for the calves vaccinated with N3 than for calves in the control group. The proportion of calves infected with BRSV (immunohistochemistry score) 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 N3 than for calves in the control group. Duration of nasal shedding of BRSV was significantly ( $P = 0.0003$ ) shorter for calves vaccinated with N3 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. Severity and duration of clinical signs were not different ( $P = 0.807$ ) between the two treatment groups.

### 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. N3 contains modified live viruses (Infectious Bovine Rhinotracheitis [IBR] virus, Bovine Parainfluenza 3 [PI<sub>3</sub>] and Bovine Respiratory Syncytial Virus [BRSV]). This technical bulletin reports the results of research that demonstrate the duration of immunity for the BRSV fraction of N3, and no interference by the other two antigens in N3, after one intranasal administration to calves 5 to 7 days of age.

## EXPERIMENTAL PROCEDURES

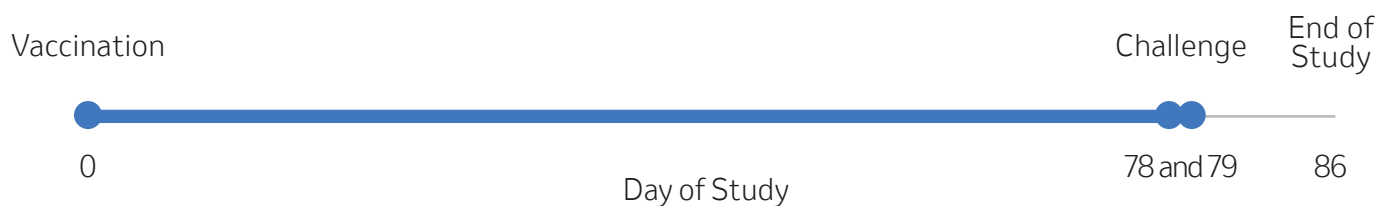
Forty-four Holstein calves (14 males, 30 females) were obtained from a single source, deprived of colostrum, identified by unique individual numbers and transported (two shipments) to the research facility. Prior to arrival, the calves were randomly assigned to be vaccinated intranasally (IN) with N3 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 and had access *ad libitum* to fresh water after 4 days of age and 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). Nasalgen 3 was prepared so that the dose administered contained the minimum protective dose (MPD) of BRSV and contained IBR virus and PI<sub>3</sub> virus at or above titers licensed for release. The placebo vaccine contained the same antigens as N3 but without BRSV. One mL of placebo vaccine was administered into each nostril of 22 calves (15 females, 7 males). Then, one mL of N3 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 was provided prior to the calves vaccinated with N3 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 440 miles to a contract research organization (CRO) 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. (The average of the two scores was recorded and used for analysis.) The total lung score was calculated as follows:

$$\begin{aligned}
 & (\text{Left cranial} \times 0.05) + (\text{Left middle} \times 0.06) + (\text{Left caudal} \times 0.32) + (\text{Right cranial} \times 0.06) \\
 & + (\text{Right posterior cranial} \times 0.05) + (\text{Right middle} \times 0.07) + (\text{Right caudal} \times 0.35) + (\text{Accessory} \times 0.04) \\
 & = \\
 & \text{Total lung lesion score (LLS; \%)}^1
 \end{aligned}$$

**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:

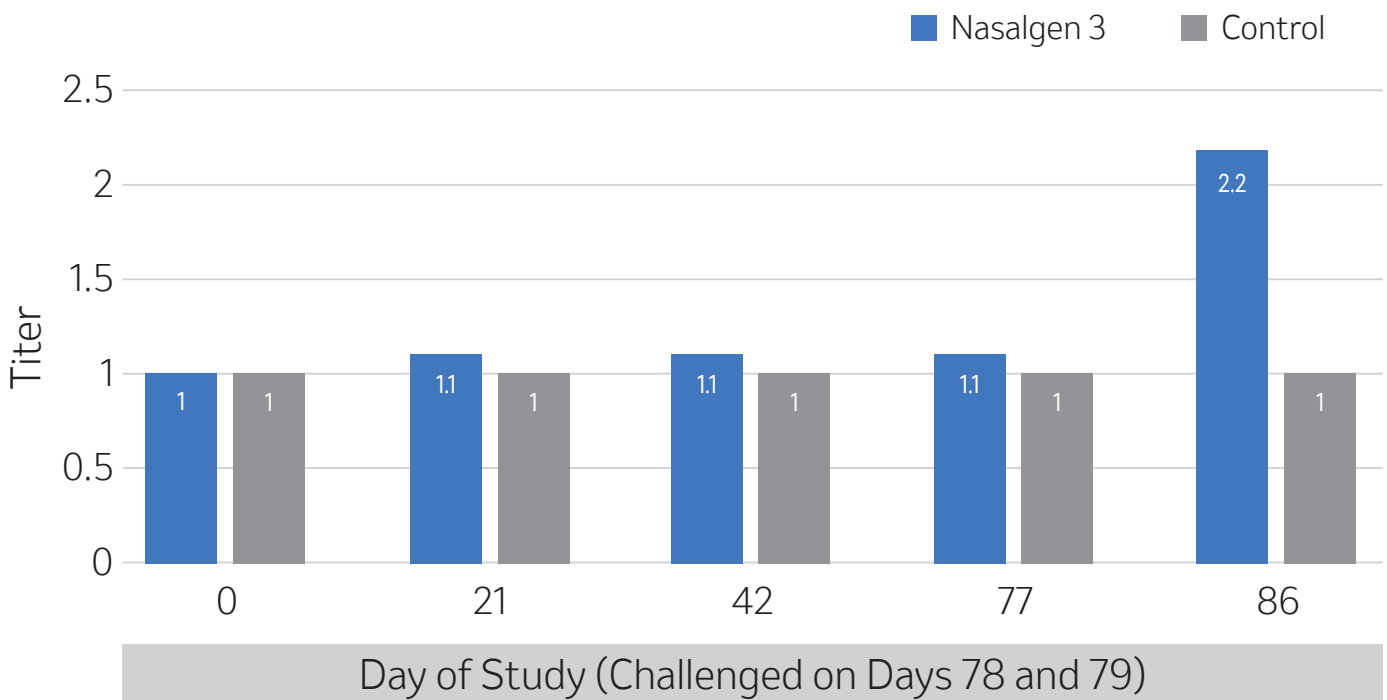
negative = 0; positive: mild = 1; moderate = 2; marked = 3.

The experimental unit was the individual calf. Primary outcome variables were lung lesion scores (LLS) 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  $\geq 104.0^{\circ}\text{F}$  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 quantitated for 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 N3 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 N3 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.

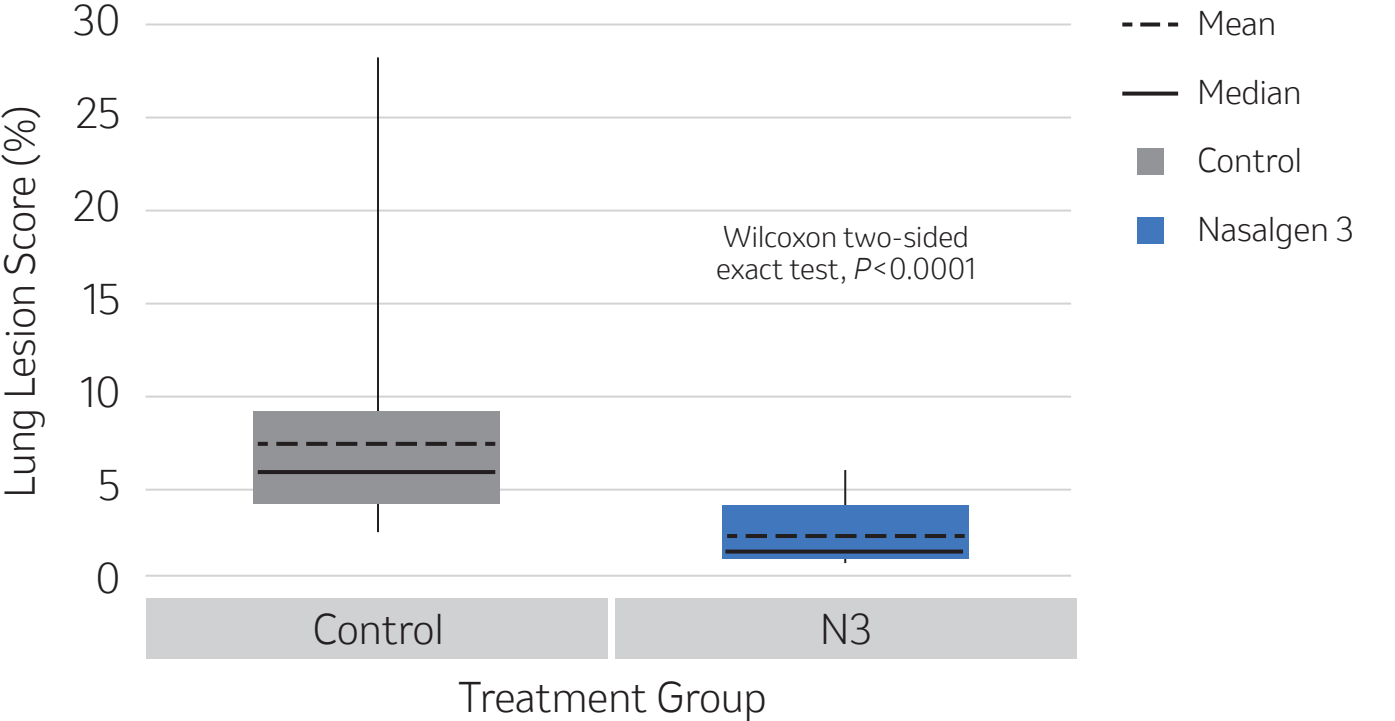


After challenge, all calves had pulmonary lesions due to BRSV. The LLS for the N3 group were significantly (Wilcoxon two-sided exact test;  $P < 0.0001$ ) less than those for the control group (Table 1, Figure 3).

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

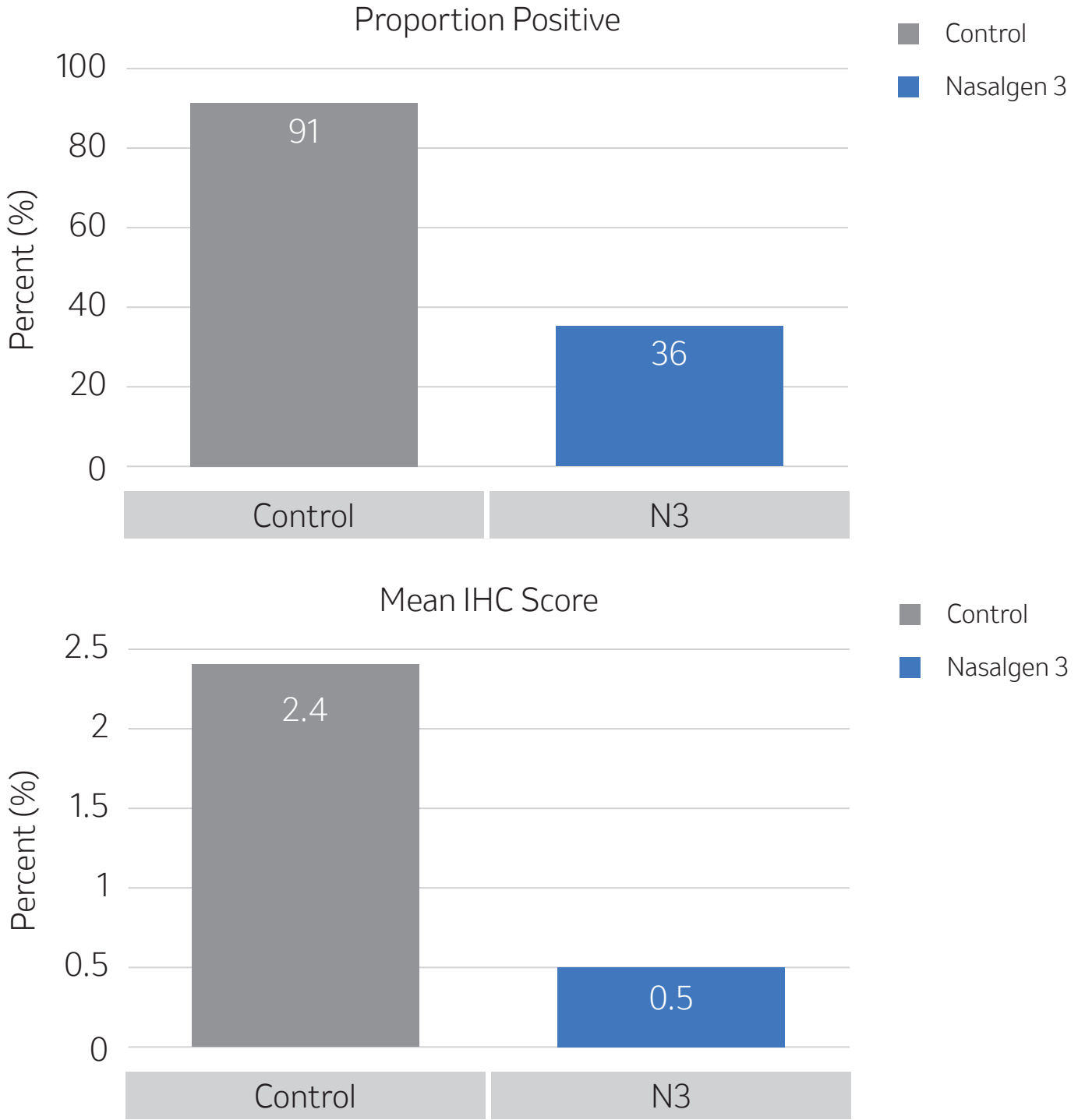
Treatment Group	N	Mean	Minimum	Lower Quartile	Median	Upper Quartile	Maximum
Control	22	7.929	2.89	4.22	5.925	9.31	27.30
N3	22	2.573	0.35	1.11	1.880	4.02	6.85

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



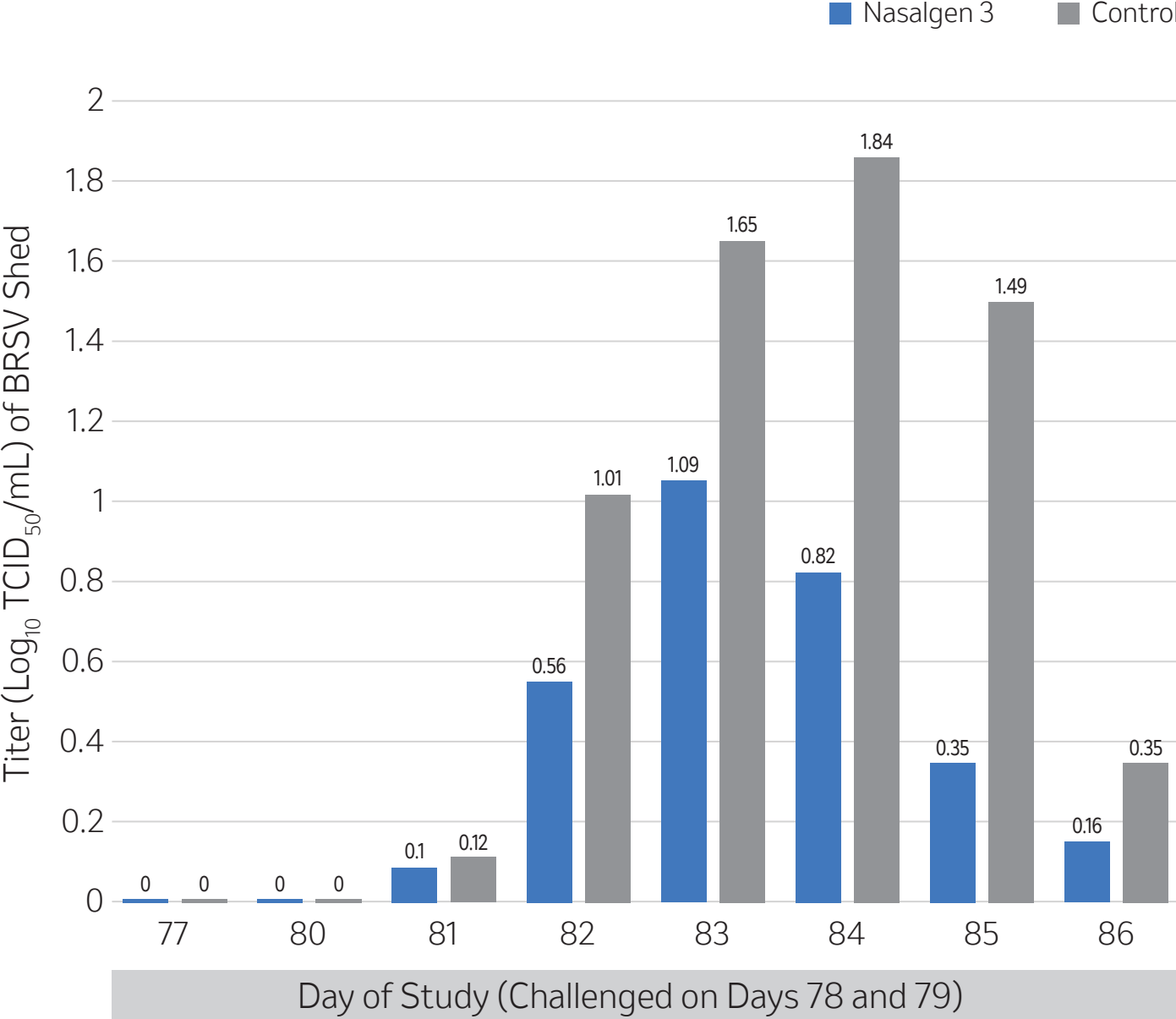
For calves in the control group, 20 of 22 (91%) samples of affected lung were “positive” for BRSV by IHC staining, and the mean IHC score was 2.4 (Figure 4). For calves vaccinated with N3, eight of 22 (36%) samples of affected lung were positive and the mean IHC score was 0.5 (Figure 4).

**Figure 4.** Proportion of calves with IHC-positive lungs, and the mean immunohistochemical staining (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 N3 (Figure 5).

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

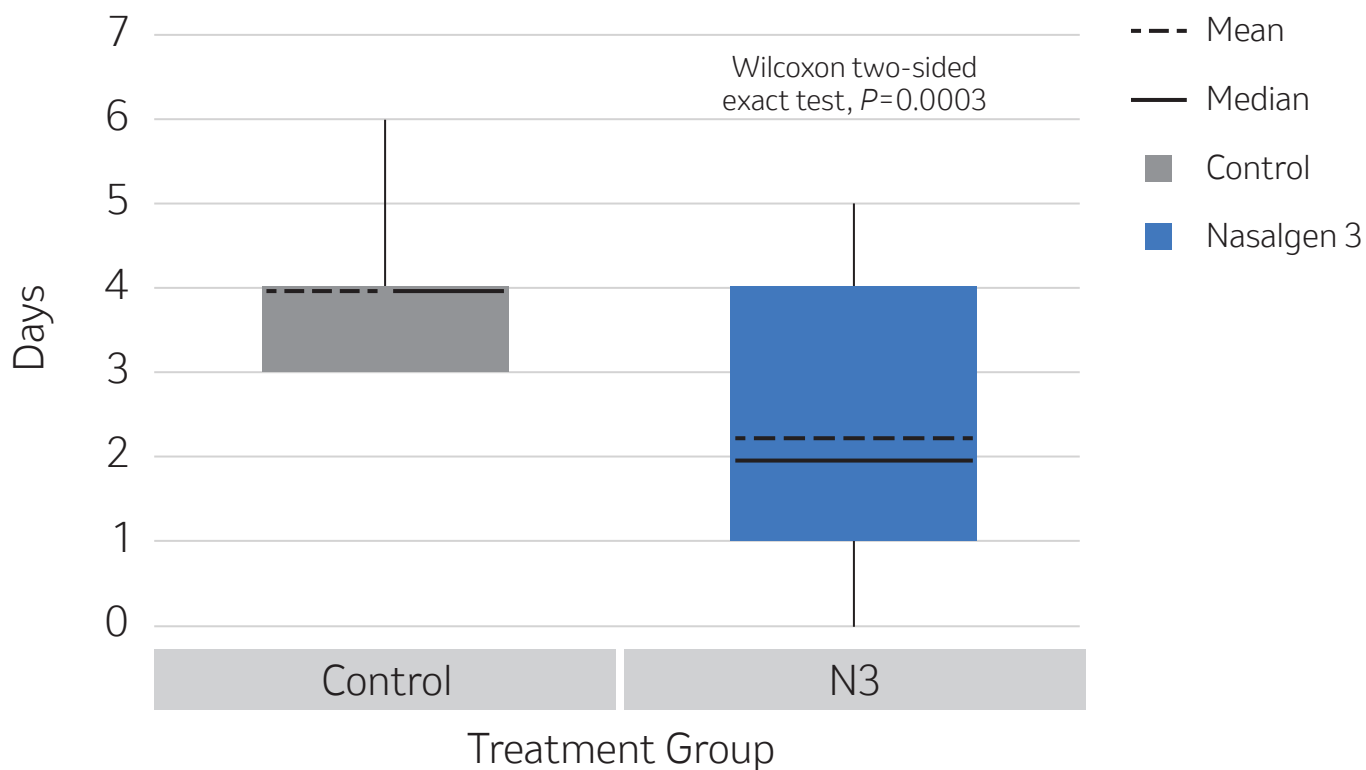


The duration of nasal shedding of BRSV post-challenge (Table 2, Figure 6) was significantly (Wilcoxon two-sided exact test;  $P=0.0003$ ) less for the N3 group than for the control group.

**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	3	3	4	4	6
N3	22	2.3	0	1	2	4	5

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



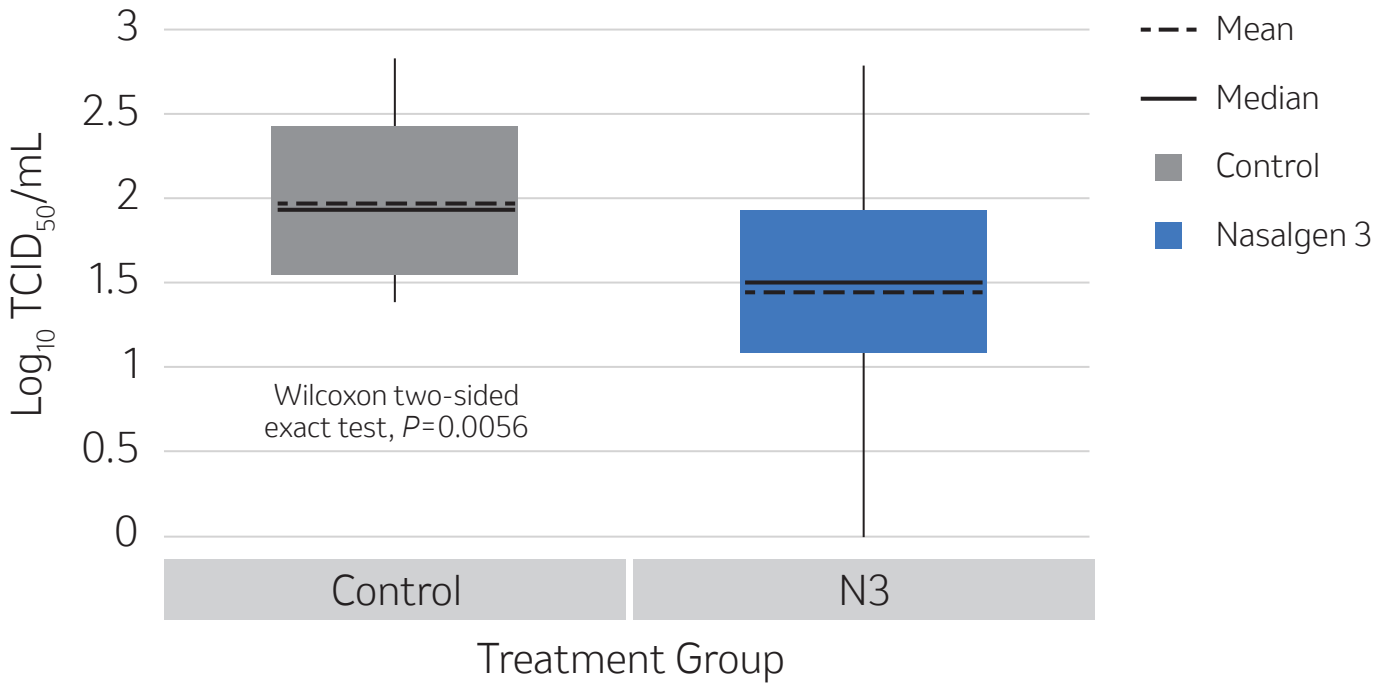


Maximum titers ( $\text{Log}_{10} \text{TCID}_{50}/\text{mL}$ ) of BRSV shed in nasal secretions (Table 3, Figure 7) were significantly (Wilcoxon two-sided exact test;  $P=0.0056$ ) less for calves in the N3 group than for calves in the control group.

**Table 3.** Quartile summary of analysis of maximum titers ( $\text{Log}_{10} \text{TCID}_{50}/\text{mL}$ ) 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
N3	22	1.49	0	1.11	1.51	1.92	2.73

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



## CONCLUSIONS

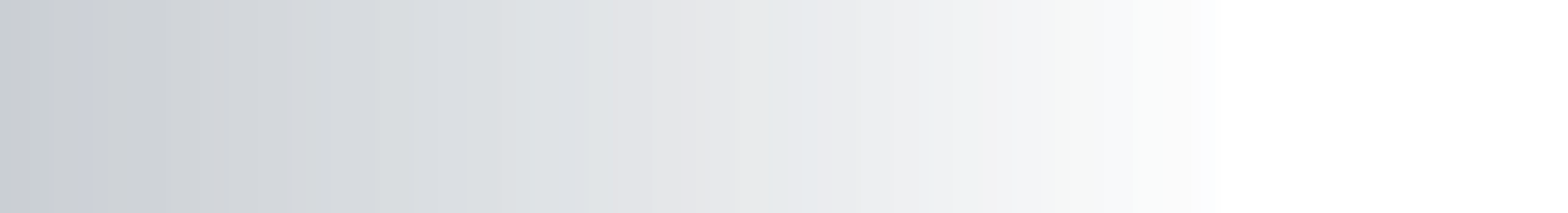
When challenged 78 days after vaccination, lung lesion scores were significantly less in calves that were vaccinated with a single dose of N3 than in calves in the control group. For calves in the control group, 20 of 22 (91%) samples of affected lungs were BRSV IHC positive compared to just eight of 22 (36%) calves vaccinated with N3. The IHC results showed less BRSV in lungs of calves vaccinated with N3. 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<sup>2</sup>, demonstrate the duration of immunity of at least 78 days following intranasal vaccination of healthy calves, and 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 BRSV.

## REFERENCES

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

<sup>2</sup>Efficacy of the Bovine Respiratory Syncytial Virus Fraction of Nasalgen 3 in Calves 4 to 7 Days of Age Technical Bulletin; Merck Animal Health.

Data on file at Merck Animal Health and USDA.



The logo for Nasalgen 3 features a cluster of blue dots of varying sizes, arranged in a pattern that suggests a spray or a cloud of particles, positioned above the product name. The text "Nasalgen" is in a bold, black, sans-serif font, and the "3" is in a blue, sans-serif font. A registered trademark symbol (®) is located between the "n" and the "3".

# Nasalgen<sup>®</sup>3