Efficacy of the *Pasteurella multocida* Fraction of Nasalgen® 3-PMH in Calves 1 to 4 Days Old

**SUMMARY**

Nasalgen® 3-PMH (N3PMH) 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), Parainfluenza 3 virus (PI3), *Pasteurella multocida* (PM) and *Mannheimia haemolytica* (MH) that are pathogens implicated in the Bovine Respiratory Disease (BRD) complex. 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) that contained the minimum protective dose of PM or a placebo vaccine (22 head) that did not contain the *Pasteurella multocida* fraction but contained the other viral and bacterial antigens in N3PMH. All calves were 1 to 4 days old on the day of vaccination (Day 0). No adverse reactions were observed after vaccination. Before the challenge, two calves in the control group died of causes unrelated to vaccination. On Day 26 (post-vaccination), all calves were challenged with virulent *Pasteurella multocida* administered intratracheally. Maximum rectal temperatures and maximum respiratory rates for calves in the N3PMH group were not significantly \( (P > 0.05) \) different from those for calves in the control group. On Day 33 (7 days post-challenge), all calves were humanely euthanized. Their lungs were harvested and lung lesions were scored. The N3PMH-vaccinated group had significantly \( (P < 0.0001) \) lower lung lesion scores compared to those for the control group. Nasalgen 3-PMH provided protection to calves 1 to 4 days old, as reflected by the reduced lung lesion scores.

**INTRODUCTION**

Nasalgen 3-PMH vaccine has been 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, Bovine Parainfluenza 3 [PI3] virus, Bovine Respiratory Syncytial Virus [BRSV]), plus avirulent, live *Pasteurella multocida* (PM) and *Mannheimia haemolytica* (MH). This technical bulletin reports the results of research that demonstrates protective efficacy for the PM fraction of N3PMH, and no interference by the other four antigens in N3PMH, after one intranasal administration to calves 1 to 4 days old.
EXPERIMENTAL PROCEDURES

Forty-four Holstein calves (26 males, 18 females) were obtained from a single source, identified by unique individual numbers, deprived of colostrum and transported (two shipments) to the study site at De Soto, KS. Calves were randomly assigned to be vaccinated intranasally (IN) with N3PMH or with a placebo vaccine (control group). Prior to and after vaccination, calves were housed in individual hutches. Throughout the study, each calf was bottle-fed at least 2 quarts of milk replacer twice daily and fresh water was provided ad libitum. Calves were allowed to acclimate for at least a day prior to enrollment. Health care was managed by the attending veterinarians. All calves were confirmed “negative” for persistent infection with Bovine Viral Diarrhea Virus (BVDV). Only calves that were clinically healthy at the time of vaccination and that had no prior vaccination against PM or MH were enrolled.

All calves were 1 to 4 days old on the day of vaccination (Day 0). N3PMH was prepared so that the dose administered contained the minimum protective dose (MPD) of PM and contained IBR virus, PI3 virus, BRSV and MH at or above titers licensed for release. The placebo vaccine contained the same antigens as N3PMH but without PM. One mL of placebo vaccine was administered into each nostril of 22 calves (14 males, eight females), and 1 mL of N3PMH was administered into each nostril of 22 calves (12 males, 10 females).

Prior to the challenge, calves from the two treatment groups were commingled by shipment and allocated to one of 10 pens. On Day 26 (post-vaccination), virulent PM was inoculated intratracheally into each calf. After the challenge, the calves remained (commingled) in their respective pens for the duration of the study. Calves were observed daily, at approximately the same time each day, and observations were recorded. On Day 33 (7 days after challenge), blood was collected from each calf, calves were euthanized, lungs were harvested and lung lesions were scored and recorded. Lung lesions were scored by two individuals according to the procedure described by Jericho and Langford (1982).

The individual calf was the experimental unit. The primary outcome variable was the percent of lung lesions (lung lesion score – LLS). Rectal temperature and respiratory rate were supporting variables. Personnel who administered the challenge and scored the lung lesions and/or performed laboratory procedures were blinded to the treatment group to which a calf was allocated.

RESULTS

No adverse events attributable to the vaccine were observed. During the post-vaccination period, two calves in the control group died (one due to pneumonia of unknown etiology and one due to intestinal perforation) of conditions unrelated to vaccination. A few additional calves were transiently affected by conditions (three bloat, two loose feces, one not finishing milk, one slow to stand and eat) that resolved after prescribed treatment, that were not related to vaccination and that did not influence the outcome of the study.

None of the calves in either treatment group died after the challenge and before euthanasia. After the challenge, maximum rectal temperatures and maximum respiratory rates were not significantly (P=0.0786; P=0.8955, respectively) different for calves in either treatment group. The proportion of calves affected with lung lesions was lower for calves vaccinated with N3PMH (14/22=64%) than for calves vaccinated with the placebo (20/20=100%).

The LLS (%) for calves in the N3PMH-vaccinated group were significantly (shipment-stratified, Wilcoxon two-sided test, P<0.0001) less than those for the calves in the control group (Table 1, Figure 1).
Table 1. Quartile summary of analysis of lung lesion score (%) after challenge with virulent PM by treatment group.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N</th>
<th>Mean</th>
<th>Minimum</th>
<th>Lower Quartile</th>
<th>Median</th>
<th>Upper Quartile</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>9.5</td>
<td>0.91</td>
<td>4.21</td>
<td>7.82</td>
<td>13.45</td>
<td>25.47</td>
</tr>
<tr>
<td>N3PMH</td>
<td>22</td>
<td>2.0</td>
<td>0</td>
<td>0</td>
<td>0.53</td>
<td>0.93</td>
<td>16.05</td>
</tr>
</tbody>
</table>

Figure 1. Lung lesion score (%) after challenge with virulent PM by treatment group.

Pasteurella multocida was isolated from the lungs of 18.2% of calves (4/22) vaccinated with N3PMH and from 35% of calves (7/20) vaccinated with the placebo.

CONCLUSIONS
Results of this study demonstrate protective efficacy of the Pasteurella multocida fraction of Nasalgen 3-PMH and confirmed the non-interference by the other antigenic fractions in N3PMH when a single dose with minimum protective titers of PM was administered intranasally to healthy calves 1 to 4 days old. Nasalgen 3-PMH significantly lowered the mean lung lesion scores associated with virulent PM. Results of this study 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 Pasteurella multocida.

REFERENCES
