Efficacy of the Mannheimia haemolytica Fraction of Nasalgen® 3-PMH in Calves 2 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 MH or a placebo vaccine (22 head) that did not contain the Mannheimia haemolytica fraction but contained the other viral and bacterial antigens in N3PMH. All calves were 2 to 4 days old on the day of vaccination (Day 0). No adverse reactions were observed after vaccination. On Day 25 (post-vaccination), all calves were challenged with virulent Mannheimia haemolytica administered intratracheally. Mortality caused by the challenge was significantly (P=0.0022) lower for calves vaccinated with N3PMH than for those in the control group. 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 32 (7 days post-challenge), all calves were humanely euthanized, their lungs were harvested and lung lesions were scored. Lung lesion scores for calves vaccinated with N3PMH were significantly (P<0.0001) lower than those for calves in the control group. Nasalgen 3-PMH provided protection to calves 2 to 4 days old as reflected by mortality and lung lesion scores after MH challenge.

INTRODUCTION
Nasalgen 3-PMH (N3PMH) 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 demonstrate protective efficacy for the MH fraction of N3PMH and no interference by the other four antigens in N3PMH after one intranasal administration to calves 2 to 4 days old.

Key Highlights
• Results of this study demonstrate protective efficacy of the Mannheimia haemolytica fraction of Nasalgen 3-PMH and confirmed the non-interference by the other antigenic fractions in N3PMH when a single dose with minimum protective dose of MH was administered intranasally to healthy calves 2 to 4 days old.
• N3PMH resulted in significantly lower lung lesion scores and mortality due to challenge with M. haemolytica, even though clinical signs of respiratory rate and/or rectal temperatures were not different between the treatment groups.
• N3PMH is safe and effective for intranasal vaccination of calves at 1 week of age or older against respiratory disease caused by M. haemolytica.
EXPERIMENTAL PROCEDURES

Forty-four Holstein calves (20 males, 24 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 2 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 MH and contained IBR virus, P13 virus, BRSV and PM at or above titers licensed for release. The placebo vaccine contained the same antigens as N3PMH but without MH. One mL of placebo vaccine was administered into each nostril of 22 calves (11 males, 11 females), and one mL of N3PMH was administered into each nostril of 22 calves (9 males, 13 females).

Prior to the challenge, calves from the two treatment groups were commingled and allocated to 11 pens (four calves per pen, both shipments represented). On Day 25 (post-vaccination), virulent MH was inoculated intratracheally into each calf. After 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. Any calf that died or was euthanized during the post-challenge period was submitted for necropsy where the lungs were harvested, lung lesions were scored and recorded, and samples were obtained for bacterial isolation. On Day 32 (7 days after challenge) blood was collected from each remaining calf, calves were humanely 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, respiratory rate and post-challenge mortality were supporting variables and were subjected to exploratory analyses. Personnel who administered the challenge and scored the lung lesions and/or performed bacterial isolation procedures were blinded to the treatment group to which a calf was allocated.
RESULTS

No adverse events attributable to the vaccine were observed. No calves were removed from the study after vaccination and prior to challenge. After challenge, maximum rectal temperatures (shipment stratified, Wilcoxon two-sided test, \( P=0.2919 \)) and maximum respiratory rates (shipment stratified, Wilcoxon two-sided test, \( P=0.0654 \)) were not significantly different for calves in either treatment group.

On Day 26 (one day after challenge), three calves in the N3PMH group were euthanized. In the control group, six calves were euthanized and one calf was found dead. On Day 27 (two days after challenge), one calf in the N3PMH group was found dead. Six calves in the control group were euthanized, and one calf was found dead. Postmortem findings led to the conclusion that each of the calves that died or was euthanized had pneumonia due to the challenge. Mortality due to challenge was significantly less (shipment stratified, Cochran-Mantel-Haenszel test, \( P=0.0022 \), Figure 1) for calves vaccinated with N3PMH (18.2%) than for calves in the control group (63.6%).

Figure 1. Mortality (%) of calves euthanized or that died post-challenge with MH, by treatment group.

Lung lesion scores for calves vaccinated with N3PMH were significantly (shipment stratified, Wilcoxon two-sided test, \( P<0.0001 \)) less than for calves in the control group (Table 1, Figure 2).

Table 1. Quartile summary of analysis of lung lesion scores (%) 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>22</td>
<td>27.84</td>
<td>0.12</td>
<td>15.30</td>
<td>29.49</td>
<td>42.76</td>
<td>54.92</td>
</tr>
<tr>
<td>N3PMH</td>
<td>22</td>
<td>5.45</td>
<td>0</td>
<td>0.66</td>
<td>2.06</td>
<td>5.51</td>
<td>32.8</td>
</tr>
</tbody>
</table>
Mannheimia haemolytica was isolated from the lungs of 63.6% of calves (14/22) vaccinated with N3PMH and from 95.5% of calves (21/22) vaccinated with the placebo.

CONCLUSIONS
Results of this study demonstrate protective efficacy of the Mannheimia haemolytica fraction of Nasalgen 3-PMH and confirmed the non-interference by the other antigenic fractions in N3PMH when a single dose with minimum protective dose of MH was administered intranasally to healthy calves 2 to 4 days old. N3PMH resulted in significantly lower lung lesion scores, and of mortality, due to challenge with M. haemolytica, even though clinical signs of respiratory rate and/or rectal temperatures were not different between the treatment groups. Those findings suggest that additional research is important in order to consistently correlate clinical signs and actual lung lesions that contribute to mortality associated with BRD. Results of this study support the claim that N3PMH is safe and effective for intranasal vaccination of calves at 1 week of age or older against respiratory disease caused by M. haemolytica.

REFERENCES