Duration of Immunity of the *Mannheimia haemolytica* Fraction of Nasalgen® 3-PMH Administered to Calves 4 to 6 Days of Age

**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 (PI3), Bovine Respiratory Syncytial Virus (BRSV), *Mannheimia haemolytica* (MH) and *Pasteurella multocida* (PM). N3PMH is safe for use in pregnant cows and in calves nursing pregnant cows. For this study, 43 colostrum-deprived Holstein calves were randomly assigned to be vaccinated intranasally with either one dose of vaccine in which the MH fraction was reduced to the minimum protective dose and the other viral and bacterial fractions in N3PMH were at the licensed vaccine dose (21 head) or with one dose of a placebo vaccine from which the MH fraction was removed but the other viral and bacterial fractions in N3PMH were at the licensed vaccine dose (22 head). All calves were 4 to 6 days old on the day of vaccination (Day 0). Calves were challenged with virulent *M. haemolytica* 122 days following vaccination (Day 122). Lung lesion scores (LLS), proportion of calves with fever (rectal temperature > 104.0° F) and duration of fever were all lower for vaccinated calves compared to control calves following challenge. The efficacy of N3PMH was reaffirmed by results of this study, and the duration of immunity to *M. haemolytica* was determined to be at least 122 days. Results 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*.

**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 [PI3], 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 MH fraction of N3PMH to be at least 122 days following one dose of vaccine administered intranasally to calves 4 to 6 days of age.
EXPERIMENTAL PROCEDURES

Forty-three Holstein calves 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. Each calf was bottle-fed at least 2 quarts of milk replacer twice daily for the first week of the study. From the second week until the calves were approximately 8 weeks old, they were fed at least 2.5 quarts of milk replacer twice daily. Fresh water was provided *ad libitum* to all calves. Post-weaning, the feed met minimum nutritional requirements for animals of that age and complied with standard procedures for the research facility. Calves were allowed at least one day to acclimate. Health care was managed by the attending veterinarians. All calves were confirmed (Antigen-capture ELISA) negative for persistent infection with Bovine Viral Diarrhea Virus (BVDV).

All calves were 4 to 6 days old when vaccinated (Day 0), were healthy and had no prior history of vaccination against *Mannheimia haemolytica* or *Pasteurella multocida*. Nasalgen® 3-PMH was prepared so that the dose administered contained the minimum protective dose (MPD) of MH and contained IBR, PI3 virus, BRSV and PM at or above the titers licensed for release. The placebo vaccine contained the same antigens as N3PMH but without MH. Twenty-two calves (8 males, 14 females) were vaccinated with 1 mL of the placebo vaccine per nostril; 21 calves (6 males, 15 females) were vaccinated with 1 mL of N3PMH per nostril on Day 0.

On Day 122 post-vaccination, each calf was challenged intratracheally with virulent MH. Beginning on Day 121 through Day 129, calves were observed daily at about the same time of day (+/- 1 hour), and observations were recorded. During the post-challenge period, calves that died or were euthanized were submitted for necropsy, lungs were removed and pulmonary lesions associated with MH were scored (Jericho and Langford, 1982) independently by two qualified observers. Samples of lung tissues were submitted for bacterial isolation. On Day 129, all surviving calves were euthanized, lungs were removed, pulmonary lesions associated with MH were scored and samples of lung tissues were submitted for bacterial isolation. Two observers, acting independently and using the method described by Jericho and Langford (1982), estimated the percent of abnormal lung tissue (Lung Lesion Score [LLS]) and the average of those two estimates was used for analysis. Personnel who administered the challenge, who scored the lung lesions or who isolated organisms from samples of lungs (or lung tissues) were blinded to which group the calf was assigned.

The experimental unit was the individual calf. The primary outcome variable was the LLS representing the average percent of lung tissue with pneumonic lesions. Mortality, rectal temperature and respiratory rate were supportive variables.
RESULTS
No adverse reactions associated with vaccination were observed. No calves died during the post-vaccination period.

During the post-challenge period, six calves were euthanized due to severe illness before Day 129 of the study – five in the control group and one in the group vaccinated with N3PMH. The proportion of calves that were euthanized because of the effects of the MH challenge (Figure 2) was lower for the calves vaccinated with N3PMH (5%) than for calves in the control group (23%), but that difference was not significant (Fisher’s two-sided exact test, $P=0.1853$).

Figure 2. Proportion of calves that were euthanized after challenge with virulent MH.
Mannheimia haemolytica was isolated from 7 of 21 (33%) samples of lung from calves vaccinated with N3PMH and from 15 of 22 (68.2%) samples of lung from calves in the control group.

Table 1. Quartile summary of analysis for LLS after challenge 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>20.37</td>
<td>0.0</td>
<td>3.26</td>
<td>9.50</td>
<td>43.18</td>
<td>56.19</td>
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<tr>
<td>N3PMH</td>
<td>21</td>
<td>4.91</td>
<td>0.0</td>
<td>0.15</td>
<td>0.70</td>
<td>2.90</td>
<td>44.33</td>
</tr>
</tbody>
</table>

The LLS (Figure 3) for calves vaccinated with N3PMH were significantly (Wilcoxon two-sided exact test, \( P = 0.002 \)) lower than those for calves in the control group.

Figure 3. Lung lesion scores (LLS, %) for calves by treatment group.
Thirteen of 22 (13/22, 59%) calves in the control group and five of 21 (5/21, 23.8%) calves vaccinated with N3PMH had rectal temperatures > 104.0° F (Figure 4) on at least one day post-challenge.

**Figure 4.** Rectal temperatures (%) for calves by treatment group.
Table 2. Quartile summary of analysis for maximum rectal temperature after challenge 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>104.21</td>
<td>102.2</td>
<td>103.2</td>
<td>104.3</td>
<td>105.2</td>
<td>106.0</td>
</tr>
<tr>
<td>N3PMH</td>
<td>21</td>
<td>103.8</td>
<td>101.8</td>
<td>102.8</td>
<td>103.7</td>
<td>104</td>
<td>105.8</td>
</tr>
</tbody>
</table>

The maximum rectal temperatures post-challenge (Figure 5) for calves vaccinated with N3PMH were lower (Wilcoxon two-sided exact test, \( P=0.032 \)) than for calves in the control group.

Figure 5. Maximum rectal temperature post-challenge by treatment group.

Table 3. Quartile summary of analysis for duration of fever after challenge 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>1.5</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
<td>3.0</td>
<td>5.0</td>
</tr>
<tr>
<td>N3PMH</td>
<td>21</td>
<td>0.4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>
The duration of fever (rectal temperature > 104.0° F) post-challenge for calves vaccinated with N3PMH was shorter (Wilcoxon two-sided exact test, \( P=0.01 \)) than for calves in the control group (Figure 6).

**Figure 6.** Duration of fever post-challenge by treatment group.

The maximum respiratory rate post-challenge was not significantly different (Wilcoxon two-sided exact test, \( P=0.178 \)) for calves in either treatment group.

**CONCLUSIONS**

Results of this study reaffirm the efficacy of N3PMH\(^2\) when administered to calves 1 week old or less. In addition, the duration of immunity against *M. haemolytica* was demonstrated to be at least 122 days. Lung lesion scores, proportion of calves with fever (rectal temperature > 104.0° F) and duration of fever were all lower for calves vaccinated at 4 to 6 days of age and subsequently challenged with virulent *M. haemolytica* 122 days later when compared to the control calves. These results 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 MH and establishes the duration of immunity of the MH fraction of N3PMH to be at least 122 days.

**REFERENCES**

