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Pasteurella Vaccination of Ewes

Abstract
Pasteurella haemolytica is a major contributor to neonatal pneumonia in lambs; which continues to be a major problem. Experimentation was conducted to determine the efficacy of vaccinating pregnant ewes to reduce the incidence of pneumonia in newborn lambs. Vaccines utilized in this experimentation included three different commercial Pasteurella haemolytica vaccines intended for use in cattle and an experimental vaccine prepared in our laboratory. Only one of the commercial vaccines increased levels of anti-Pasteurella antibodies in serum of the ewes at time of lambing, but lambs from all three groups of vaccinated ewes had higher levels of antibodies than control lambs. Some lambs in all groups developed pneumonia during the neonatal period.

Ewes administered the experimental vaccine had significantly higher levels of serum antibodies at lambing time. This increase was reflected in increased levels in serum of lambs from the vaccinated ewes. However, the antibodies appeared not to be protective, since as many lambs in the treatment group developed pneumonia as did in the control group.

Keywords
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Disciplines
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Pasteurella Vaccination of Ewes

A.S. Leaflet R1470

Merlin Kaeberle, professor, veterinary microbiology, Arnold McClain, sheep manager, McNay Farm, and Daniel Morrical, professor, animal science

Summary

*Pasteurella haemolytica* is a major contributor to neonatal pneumonia in lambs; which continues to be a major problem. Experimentation was conducted to determine the efficacy of vaccinating pregnant ewes to reduce the incidence of pneumonia in newborn lambs. Vaccines utilized in this experimentation included three different commercial *Pasteurella haemolytica* vaccines intended for use in cattle and an experimental vaccine prepared in our laboratory. Only one of the commercial vaccines increased levels of anti-*Pasteurella* antibodies in serum of the ewes at time of lambing, but lambs from all three groups of vaccinated ewes had higher levels of antibodies than control lambs. Some lambs in all groups developed pneumonia during the neonatal period.

Introduction

Neonatal pneumonia continues to be a major problem in Iowa sheep flocks. *Pasteurella haemolytica* bacteria of several serotypes of the A biotype are the primary cause of this disease. The pneumonia occurs most commonly during the first three weeks of life and may be responsible for appreciable mortality in some flocks. Management practices and early treatment may control the disease to some extent, but better methods are needed.

Vaccination incorporated into management practices is one potential method for controlling this disease. If resistance of the lambs could be enhanced through antibodies transferred from the ewe, beneficial effects might be expected. This approach requires a vaccine that enhances the level of protective antibodies in the ewe and results in increased transfer of those antibodies to the newborn lamb via the colostrum. No commercial vaccine currently is available for use in sheep. The problem is complicated by the multiple serotypes of *P. haemolytica* contributing to pneumonia in lambs. Furthermore, the specificity of antibodies primarily responsible for protecting against *P. haemolytica* infection remains somewhat controversial. Researchers have reported immunity to be type specific and mediated by capsular, outer membrane protein or leukotoxin antibodies. A new generation of *Pasteurella* vaccines for use in cattle are based on the potential immunogenicity of bacterial extracts and leukotoxin included in the preparations. A problem with their use in sheep is that they are composed of antigenic material from a single serotype, *Pasteurella haemolytica* A-1.

A question commonly posed by sheep producers has been as follows: Can the cattle vaccine be used to protect sheep? This study was conducted to determine the efficacy of commercial *Pasteurella* vaccines and an experimental vaccine prepared in our laboratory.

Materials and Methods

Ewes in the McNay Farm flock scheduled to lamb in February were randomly assigned to treatment groups. These ewes were either vaccinated or left as unvaccinated controls. Vaccines were administered following collection of blood for serum in mid-January, and the ewes and their lambs were bled for serum 24 to 48 hours after lambing. Serum was harvested and stored at -20°C until tested for antibodies.

Experiment I was conducted in 1994 with treatment groups of 10 animals each as follows:
- A - commercial vaccine A
- B - commercial vaccine B
- C - commercial vaccine C
- D - controls, no vaccination.

One-half the recommended cattle dose (2 ml.) was administered with 0.5 ml. injected subcutaneously on each side of the neck.

Experiment II was conducted in 1995 with treatment groups as follows:
- A - 23 ewes, administered 0.5 ml. experimental vaccine subcutaneously at each of two sites
- B - 23 ewes, no vaccination

The vaccine consisted of equal amounts of sodium salycylate-extracted outer membrane constituents of four *Pasteurella haemolytica* organisms representing serotypes 1, 2, 6, and 8. Dosage per ewe was 1 mg. of protein in 0.5 ml. saline solution emulsified in 0.5 ml. of Freund’s incomplete adjuvant.

Antibody titers in sera of ewes and lambs were determined with an ELISA. The antigen utilized for testing sera from Experiment I was from a serotype A-1 culture. The antigen was prepared by suspending organisms grown on agar medium in RPMI 1640 medium,
treatment with sodium salicylate, removal of the bacteria by centrifugation, dialysis against tris-buffered saline solution, and precipitation with cold ethanol. Antigen utilized for Experiment II was prepared by the same procedure but represented a pool made of equal quantities of extract from the four different serotypes. The number of animals utilized for analysis of data was reduced from the number in the original groups. Only combinations of ewes and surviving lambs where testing indicated nursing and transfer of maternal antibodies are included in the reported findings.

Results and Discussion

Most of the ewes had relatively high titers of antibodies against outer membrane components of *P. haemolytica* prior to vaccination, although there was marked variability among animals. These antibody levels are indicated in figures 1, 2, and 3 where the titer is reported as the positive/negative ratio (a P/N ratio of 25 reflects a titer of approximately 1:14,000).

In experiment I, only one of the commercial vaccines significantly enhanced the titer of the ewes at time of lambing and could be due in part to the relatively low mean titer in that group of ewes prior to vaccination. Antibody levels in serum of ewes at the time of lambing may be somewhat misleading, since large quantities of immunoglobulins are transferred to the colostrum during the last three weeks of gestation. This is reflected in the levels of antibodies in lambs from vaccinated ewes, which were significantly higher than in lambs from unvaccinated ewes. These antibodies, however, did not appear to be protective since pneumonia developed in lambs from all groups [Table 1]. Lambs developing pneumonia had moderate levels of antibodies, levels that were higher than some lambs that were not diagnosed with clinical pneumonia.

The experimental vaccine utilized in experiment II markedly enhanced antibody levels in vaccinated ewes and their lambs (figure 3). The response of the ewes and transfer to the lambs was better than with the commercial vaccines utilized in experiment I. However, once again these antibodies did not seem to protect the lambs from clinical pneumonia, since 19 lambs developed clinical pneumonia—9 from control ewes and 10 from vaccinated ewes. The susceptibility of lambs to *P. haemolytica* pneumonia when they have appreciable levels of antibodies to the microorganism presents a dilemma. One would expect those antibodies to be protective but this seems not to be the case. Possible reasons for this circumstance are as follows: (1) the specificity of the antibodies and (2) the isotype of the antibodies. By far the highest level of antibodies transferred from the ewe to her lambs belong to the IgG1 isotype. Protective antibodies (anti-leukotoxin?) may be present in the ewe but may be primarily of the IgG2 isotype and consequently are not efficiently transferred to the lambs. This needs to be confirmed by additional experimentation. The answer to the problem may be a vaccine that will induce a high level of protective antibodies of the IgG1 isotype in the ewe.
Figure 1. Mean antibody titers of ewes before vaccination with commercial Pasteurella haemolytica vaccines and after lambing.

* Significant treatment effect  $P > 0.05$

Figure 2. Mean antibody titers in ewes before vaccination with commercial vaccines and their lambs after nursing.

* Significant differences  $P > 0.05$
Figure 3. Serum antibody titers of ewes pre- and post vaccination with an experimental vaccine and their lambs after nursing.

![Graph showing antibody titers](image)

* Significant treatment effect  P > 0.05

Table 1. Mean antibody levels in all lambs compared with levels in lambs that subsequently developed clinical pneumonia.

<table>
<thead>
<tr>
<th>Group</th>
<th>All Total</th>
<th>Lambs Mean Ab</th>
<th>Pneumonic Number</th>
<th>Lambs Mean Ab</th>
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<td>Controls</td>
<td>19</td>
<td>8.9</td>
<td>2</td>
<td>3.9</td>
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<td>19.5</td>
<td>2</td>
<td>15.8</td>
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<tr>
<td>Vaccine B</td>
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<td>19.5</td>
<td>1</td>
<td>8</td>
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<td>Vaccine C</td>
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