

## Vaccination of ewes and lambs against parainfluenza<sub>3</sub> to prevent lamb pneumonia

M.L. Thonney<sup>a,\*</sup>, M.C. Smith<sup>b</sup>, R.G. Mateescu<sup>c</sup>, C. Heuer<sup>d</sup>

<sup>a</sup> Department of Animal Science, College of Agriculture and Life Sciences, 114 Morrison Hall, Cornell University, Ithaca, NY 14853-4801, USA

<sup>b</sup> Department of Population Medicine & Diagnostic Science, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

<sup>c</sup> Department of Animal Science, Division of Agricultural Sciences and Natural Resources, Oklahoma State University, Stillwater, OK 74078, USA

<sup>d</sup> EpiCentre, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

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### Abstract

The purpose of this experiment was to test the effectiveness of vaccination against parainfluenza<sub>3</sub> (PI<sub>3</sub>) to prevent pneumonia in lambs in a flock in which over 9% of 2657 lambs born alive in the previous 2 years had died of *Mannheimia (Pasteurella) haemolytica* pneumonia. The experiment was carried out using 176 Dorset ewes and their 1/4 East Friesian lambs born from 15 March to 8 April 2002. One week before the start of the lambing season, 90 ewes were vaccinated with 1 ml of TSV-2<sup>®</sup>, a modified live bovine rhinotracheitis-parainfluenza<sub>3</sub> vaccine, administered intranasally. Alternate litters of lambs within vaccinated (149 lambs) and control (137 lambs) ewe groups received 0.5 ml of TSV-2<sup>®</sup>. Death and treatment data through 66 days of age were analyzed by binary logistic regression with a model that included main effects of ewe or lamb vaccination and the two-way interaction. To account for losses of animals by death or sale, a survival analysis model was used to analyze time from birth to death through 220 days of age. Of 157 lambs delivered by vaccinated ewes, 5.1% were delivered dead, while 8.1% of 149 lambs were delivered dead to control ewes. Total deaths of lambs born alive were fewer and deaths due to pneumonia or unknown causes were fewer in the vaccinated than in the control groups, although the effect of vaccination was not statistically significant. Through 66 days of age when the first lamb was sold, only 10 lambs, or 3.5% of those born alive, died of pneumonia with about equal proportions in the four ewe and lamb vaccination groups. Through 220 days of age, more control lambs died, but the survival analysis found no statistically significant differences among vaccination groups in total death loss nor in the proportion treated, dying, or both due to pneumonia. Among a group of 34 slaughtered lambs distributed across the vaccine treatments, 27 had lung damage evident of pneumonia even though only one lamb had been treated for pneumonia. Thus, although overall deaths were non-significantly higher in control lambs, vaccination against PI<sub>3</sub> did not appear to reduce the incidence of pneumonia in this flock.  
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### 1. Introduction

Pneumonia is a major cause of lamb mortality in many sheep flocks. More than 20% of the non-predator lamb losses reported in the United States National

\* Corresponding author. Tel.: +1 607 592 2541;  
fax: +1 607 255 9829.

E-mail address: [mlt2@cornell.edu](mailto:mlt2@cornell.edu) (M.L. Thonney).

Animal Health Monitoring System sheep survey were due to pneumonia (NAHMS, 2002). While overcrowding, poor ventilation, and inadequate bedding often are contributing factors, lamb pneumonia can be severe even in carefully managed flocks or in flocks kept only on pasture. For example, 80% of the lambs in some New Zealand flocks were infected (Goodwin et al., 2004) even though New Zealand lambs rarely see the inside of a barn. Additionally, while Bighorn Sheep live in well-ventilated environments, they are particularly susceptible to pneumonia (Cassirer et al., 2001).

Despite good ventilation in the barns at the Cornell sheep farm, 9% of 2657 lambs born alive in the Cornell flock in 2000 and 2001 died of *Mannheimia (Pasteurella) haemolytica* pneumonia. Furthermore, lambs with clinical pneumonia require significant time for treatment and severity of pneumonia is directly related to reduced growth and feed efficiency (Jones et al., 1982; Alley, 1987). While parainfluenza<sub>3</sub> (PI<sub>3</sub>) induces innate immunological responses in lung tissue of infected lambs (Grubor et al., 2004), this virus may contribute to lamb pneumonia by allowing *Mannheimia (Pasteurella) haemolytica* to invade the lung tissue (Davies, 1980; Davies et al., 1980a; Cutlip et al., 1993; Martin, 1996). Although no sheep vaccine is available in the USA, several companies sell a nasally administered cattle vaccine against a combination of Infectious Bovine Rhinotracheitis (IBR) and PI<sub>3</sub>. Vaccination against PI<sub>3</sub> has reduced the incidence of lamb pneumonia in some experiments (Wells et al., 1978; Davies et al., 1980a, 1983; Salisbury, 1984; Lehmkuhl and Cutlip, 1985; Rodger, 1989). A small serological survey showed PI<sub>3</sub> activity in the Cornell flock, as demonstrated by positive serum neutralization titers of 1:4 or higher in 5 of 20 ewes, even though a PI<sub>3</sub> vaccine had never before been used in the flock. The objective of this experiment was to determine if a nasal vaccine against PI<sub>3</sub> administered to ewes prior to lambing or to lambs within 48 h of birth would reduce the incidence of pneumonia in lambs born and housed indoors.

## 2. Materials and methods

### 2.1. Experimental design

The 2 (ewe vaccination or not) × 2 (lamb vaccination or not) factorial experiment was carried out using 176 Dorset ewes and their 1/4 East Friesian lambs born from 15 March to 8 April 2002. A week before the start of the 30-day lambing season when the ewes were given a booster clostridial vaccine, 90 ewes were vaccinated with 1 ml TSV-2® (Pfizer Inc., Animal Health Group, 235 E. 42nd St., New York, NY 10017). TSV-2® is a modified live bovine rhinotracheitis-parainfluenza<sub>3</sub> vaccine

administered intranasally. For 1 to 3 days after lambing, ewes were penned separately with their lambs. Within 2 days of birth at the time of ear tagging, lambs in alternate litters within vaccinated and control ewe groups received 0.5 ml of TSV-2®. Alternate litters – and not alternate lambs – were vaccinated due to the possibility that the live vaccine could be transferred by close contact. Lambs were offered a barley-based creep feed (with soybean meal for supplemental protein, 15% soy hulls for fiber, supplemental vitamins and minerals, decoquinate to prevent coccidiosis, and 2% vegetable oil to eliminate dust) within a week of birth and weaned on day 70 after the start of lambing. After weaning, lambs were sorted by sex and kept in the barn on the same diet and bedded with poor quality hay, some of which was consumed each time the pens were bedded three times weekly.

Lambs were observed daily and any showing signs of pneumonia were treated subcutaneously with oxytetracycline (LA200, Pfizer Inc., Animal Health Group, 235 E. 42nd St., New York, NY 10017) at 2.25 ml/100 kg body weight. Treatments, deaths, and necropsy results were recorded in the *Cewe* database management system (Thonney, 2002). The data base was interrogated to determine numbers of lambs delivered, lambs born alive, lambs that died after being born alive, lambs treated for pneumonia, lamb treatments for pneumonia, and lambs that died of pneumonia based upon necropsy. The first lamb was sold at 66 days of age (included in 60–80 day group in Fig. 1).

A subset of 36 male lambs (18 castrated by rubber banding within 48 h of birth), distributed across the vaccine treatment groups, was slaughtered by Mateescu and Thonney (2005) at ages ranging from 77 to 161 days. The lungs of those lambs were evaluated for lesions evident of pneumonia.

### 2.2. Statistical analysis

Death and treatment data through 66 days of age were analyzed by binary logistic regression with a model that included main effects of ewe or lamb vaccination and the two-way interaction. Deaths from other causes and sales at varying times after 66 days of age (Fig. 1) meant that the time at risk for individual

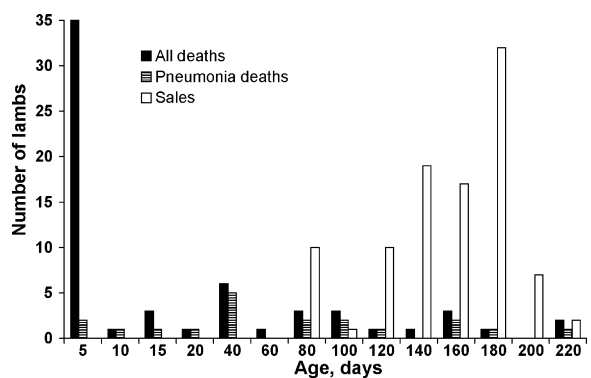


Fig. 1. Distribution of lamb deaths and sales with advancing age. Intervals to 20 days are 5 days, followed by 20-day intervals.

animals varied. Therefore, the time from birth to treatment or death due to pneumonia was analyzed by survival analysis to evaluate fixed effects of sex and vaccination group (four groups of ewe × lamb vaccination), including data from birth through 220 days of age. A power analysis evaluated the sample size used for this experiment relative to the observed effect sizes.

The incidence of pneumonia in the subset of 36 male lambs was evaluated by the same binary logistic regression model as for the main experiment.

### 3. Results

Of the 306 lambs in this experiment, 20 lambs were delivered dead for a stillborn death loss of 5.1% for lambs out of vaccinated ewes (11/157) and 8.1% for lambs out of control ewes (12/149). The live lambing rate was 1.6 lambs/ewe (286/176; Table 1).

The proportion of total deaths was lower in vaccinated lambs and in lambs from vaccinated ewes (Tables 1 and 2), but the differences were not significant. From 6.4 to 14.7% of lambs born alive died by 66 days of age (Table 1) and from 11.0 to 20.0% of lambs born alive died by 220 days of age (Table 2) in each of the four ewe-vaccination, lamb-vaccination groups. For the complete data set to 66 days of age, *P*-values for ewe vaccination, lamb vaccination, and the interaction were 0.19, 0.14, and 0.18, respectively. For the data set through 220 days, the respective *P*-values were 0.18, 0.40, and 0.67.

None of the other response variables were significantly affected by vaccination, even though vaccination efficiency for reduction in total deaths ranged from 27% for lamb vaccination alone to 49% for ewe and lamb vaccination (Fig. 2). From 3.2 to 8.2% of lambs were treated for pneumonia to 66 days of age (Table 1) and from 4.8 to 9.2% of lambs were treated for pneumonia to 220 days of age (Table 2). Deaths from pneumonia ranged from 2.7 to 4.1% for lambs to 66 days of age and from 4.1 to

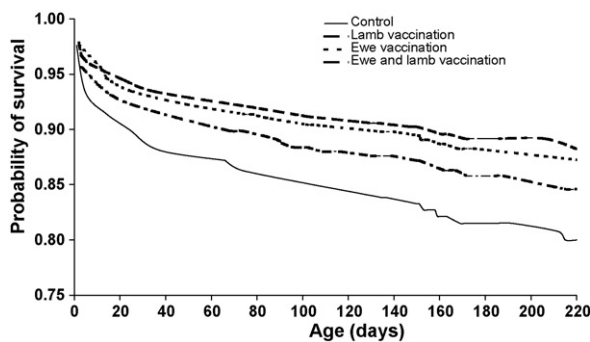


Fig. 2. Proportion of lambs born alive that survived with increasing age.

Table 1  
Number (and proportions) of lambs born, treated lambs, lamb treatments, and lambs dying to 66 days of age

Vaccination	Ewe Lamb	Ewes lambing	Lambs delivered <sup>a</sup> (per ewe)	Lambs born alive (per ewe)	Deaths <sup>b</sup> (%)	Due to pneumonia		Deaths from pneumonia or unknown causes <sup>b</sup> (%)	
						Lambs treated <sup>b</sup> (%)	Lamb treatments <sup>b</sup> (per lamb)	Deaths <sup>b</sup> (%)	Deaths <sup>b</sup> (%)
Yes	Yes	45	157 (1.7)	73 (1.6)	7 (9.0)	6 (8.2)	8 (0.11)	3 (4.1)	5 (6.8)
	No	45	76 (1.7)	76 (1.7)	6 (7.6)	5 (6.6)	10 (0.13)	3 (3.9)	4 (5.3)
No	Yes	43	149 (1.7)	62 (1.4)	4 (6.4)	2 (3.2)	4 (0.06)	2 (3.2)	2 (3.2)
	No	43	75 (1.7)	75 (1.7)	11 (14.7)	4 (5.3)	9 (0.12)	2 (2.7)	6 (8.0)
Total		176	306 (1.7)	286 (1.6)	28 (9.7)	17 (5.9)	31 (0.11)	10 (3.5)	17 (5.9)

<sup>a</sup> Ewe vaccination groups only.

<sup>b</sup> Of those born alive.

Table 2  
Number (and proportions) of treated lambs, lamb treatments and number of lambs dying to 220 days of age of lambs born alive

Vaccination		Deaths (%)	Due to pneumonia				Deaths from pneumonia or unknown causes (%)
Ewe	Lamb		Lambs treated (%)	Lamb treatments (per lamb)	Deaths (%)	Lambs treated or dead (%)	
Yes	Yes	8 (11.0)	6 (8.2)	8 (0.1)	3 (4.1)	8 (11.0)	5 (6.8)
	No	9 (11.8)	7 (9.2)	12 (0.2)	5 (6.6)	10 (13.2)	6 (7.9)
No	Yes	9 (14.5)	3 (4.8)	5 (0.1)	6 (9.7)	9 (14.5)	6 (9.7)
	No	15 (20.0)	6 (8.0)	12 (0.2)	5 (6.7)	9 (11.0)	10 (13.3)
Total		39 (14.3)	22 (7.7)	37 (0.1)	19 (6.6)	36 (12.6)	27 (9.4)

9.7% for lambs to 220 days, but were unrelated to PI<sub>3</sub> vaccination.

Other death losses were due to arthritis, birth defect, grain overload, hypothermia, listeriosis, omphalitis, starvation, urolithiasis, or unknown causes (Table 3). A determination of cause of death was attempted by necropsy of all dead lambs, but in some cases a necropsy could not be done or cause of death could not be determined. Given the possibility that some of the lambs that died of unknown causes could have died of pneumonia, the effect of vaccination on deaths from a combination of pneumonia and unknown causes was analyzed (Tables 1 and 2), with no additional encouraging results for vaccination to 66 days but a non-significant advantage for vaccination to 220 days.

Data from the subset of 34 male lambs that were slaughtered by Mateescu and Thonney (2005) at ages from 77 to 161 days are shown in Table 4. Only one

lamb – in the vaccinated ewe, control lamb group – had been treated for pneumonia, and it was 15 to 20 days of age at the time of treatment. None of the other lambs had exhibited clinical signs of pneumonia. All of the lambs that were vaccinated – regardless of whether their dams were vaccinated – had lung lesions associated with having had pneumonia. Eight of 14 control lambs from vaccinated ewes and five of six control lambs from control ewes had lung lesions associated with having had pneumonia.

#### 4. Discussion

Lambs within litters were expected to respond more similarly than lambs from different litters and, because litters varied in size (Table 1), this could have affected the results. This cluster effect was not accounted for in the statistical analysis for two reasons. First, intraclass correlations among lambs within litters were very low for all of the response variables (~0.016) resulting in variance inflation factors of only about 1.01. Second, our results were non-significant and including a cluster effect in the logistic regression model could only increase the within-treatment variances, making it even less likely for vaccination effects to be statistically significant (McDermott and Schukken, 1994).

The present experiment was designed to determine if reduced viral shedding by vaccinated ewes or if anti-

Table 3  
Numbers of lambs dying from causes other than pneumonia

Vaccination		Other causes of death	Age from birth	
Ewe	Lamb		66 days	220 days
lambs (% dead of lambs born alive)				
Yes	Yes	Omphalitis	1 (1.4)	1 (1.4)
		Starvation <sup>a</sup>	1 (1.4)	1 (1.4)
		Unknown	2 (2.7)	2 (2.7)
		Urolithiasis	0 (0.0)	1 (1.4)
	No	Grain overload	1 (1.3)	1 (1.3)
		Hypothermia	1 (1.3)	1 (1.3)
		Listeriosis	0 (0.0)	1 (1.3)
		Unknown	1 (1.3)	1 (1.3)
No	Yes	Arthritis	0 (0.0)	1 (1.6)
		Starvation <sup>a</sup>	2 (3.2)	2 (3.2)
	No	Birth defect	1 (1.3)	1 (1.3)
		Pyelonephritis	1 (1.3)	1 (1.3)
		Starvation <sup>a</sup>	3 (4.0)	3 (4.0)
	Unknown	4 (5.3)	5 (6.7)	

<sup>a</sup> Starvation was diagnosed if the lamb had no obvious fat tissue and no milk in the stomach.

Table 4  
Incidence of pneumonia in 34 slaughtered male lambs

Vaccination		Slaughtered lambs	Evidence of pneumonia
Ewe	Lamb		
Yes	Yes	8	8
	No	14	8
No	Yes	6	6
	No	6	5

bodies to PI<sub>3</sub> obtained by lambs from colostrum could protect them at young ages and, if not, if vaccination shortly after birth could protect them. The design also provided the opportunity to test if ewe and lamb vaccination offered better protection than either vaccination alone. Death losses from pneumonia were lower than expected, but neither main effect of ewe or lamb vaccination nor the interaction were significant. Overall death losses in vaccinated groups were lower, but not significantly so, through 66 days of age prior to the sale of any lambs. The survival analysis, which accounted for the time that each lamb was at risk of treatment or death, also showed that vaccination had no effect to 220 days of age, but vaccination could have contributed to the overall lower death loss (Fig. 2) with a non-significant vaccination efficiency of 49% for the ewe vaccination–lamb vaccination group.

The 14.3% death loss for lambs born alive in this experiment is within the range of that reported by others (Hight and Jury, 1970; Dalton et al., 1980; Oltenacu and Boylan, 1981; Hulet et al., 1984; Johnston et al., 1999; Matos et al., 2000; Wassmuth et al., 2002; Carson et al., 2002). In addition to pneumonia, lambs died from a variety of causes (Table 3). Subclinical pneumonia might have increased the susceptibility of lambs to some of these other causes of death, and it is likely that it could have been the cause of death for those cases in which a cause could not be determined. Given that the proportion dying from pneumonia or unknown causes to 220 days of age was 1.4 to 2 times higher in the control ewe–control lamb group than in the three vaccinated groups (Table 2), it is possible that vaccination could have had an effect, although in this experiment it was non-significant.

In an experiment with 18 neonatal lambs, half were challenged with virulent PI<sub>3</sub> virus to determine the expression of the innate immune system (Grubor et al., 2004). Viral antigens were present in lung tissue at days 3 and 6 postinoculation but none were detected by day 17 postinoculation, indicating that the innate immune system can control PI<sub>3</sub> viral infections in lambs. However, an injury to the lungs by a viral organism like PI<sub>3</sub> may be an important catalyst for subsequent infection by organisms like *Mannheimia (Pasteurella) haemolytica*, *P. multocida*, and *Mycoplasma*, the bacterial species most often found to have caused lung damage in pneumonia (Davies et al., 1983; Alley, 2002). This prompted several previous tests of vaccination against PI<sub>3</sub> to control lamb pneumonia, as well as the current experiment.

Two uncontrolled clinical trials were reported without statistical analysis of the data. Salisbury (1984) vaccinated 1700 ewes and their lambs against PI<sub>3</sub> and lamb deaths declined from 80 to 90/year to 15 in the year

following vaccination. Rodger (1989) vaccinated ewes and lambs against PI<sub>3</sub> in seven flocks comprising 3330 ewes. Pneumonia losses declined to zero from the 3 to 11% range of pneumonia losses observed the previous year.

In trials where lambs were challenged with PI<sub>3</sub> and *P. haemolytica* organisms after PI<sub>3</sub> vaccination, vaccination reduced lamb deaths (Wells et al., 1978) or pulmonary lesions (Davies et al., 1980b). Administration of IBR-PI<sub>3</sub> vaccine to ewes prior to lambing and to their lambs at birth resulted in reduced shedding of the PI<sub>3</sub> virus in vaccinated lambs compared to control lambs after challenge with PI<sub>3</sub> virus (Lehmkuhl and Cutlip, 1985).

In three controlled field trials, Davies et al. (1983) found that PI<sub>3</sub> vaccination decreased the incidence of significant pneumonic lung lesions at the time of rising natural PI<sub>3</sub> titers, but this effect was statistically significant only in the first trial with 40% of controls showing lesions, and not in the other two trials where 18% of controls were infected.

Results from the present experiment are in agreement with those of Davies et al. (1983) that statistically significant reductions in clinical losses due to vaccination against PI<sub>3</sub> are difficult to detect in field experiments. The power analysis indicated that either a higher infection rate or an experiment with considerably more lambs would be required to detect significant differences. Assuming that 12% of control lambs were treated or died due to pneumonia, 313 lambs would have been required in each experimental group to show a 50% decrease due to vaccination with 80% power ( $\beta = 0.2$ ) and 90% confidence ( $\alpha = 0.10$ ). Given the current sample size of 72 lambs/group, 12% observed treatment or death due to pneumonia in controls, and  $\alpha = 0.10$ , the study had 25% power to detect a vaccine efficacy of 50%. Thus, without much higher natural infection rates or a much higher vaccination efficiency, field experiments to detect significant differences due to vaccination will need to be from larger flocks than are available in the Northeastern USA or across multiple flocks with major detectable levels of pneumonia. A higher vaccine efficiency had been expected in the present experiment.

While only about 7% of the lambs born alive died from pneumonia (Table 2), based upon the data in Table 4 it is likely that more than 75% of all lambs had pneumonia at some point in their lives and that most of those went undetected. Thus, vaccination with PI<sub>3</sub> appeared to be ineffective in preventing subclinical pneumonia in this experiment, suggesting that monitoring lung damage from abattoir samples may be necessary to evaluate overall effectiveness of methods of preventing pneumo-

nia (Goodwin et al., 2004). This approach could also increase the power of experiments to detect significant vaccination efficiency.

Although cross-vaccination of control lambs was minimized through vaccinating each litter of lambs, the vaccinated ewes were mixed with controls before and after lambing and vaccinated and control lambs were mixed together after one to three days in a lambing pen with their littermates and mothers. Thus, because overall death losses from pneumonia were lower in this experiment than in the same flock in previous years, it is possible that the live vaccine could have been transferred to control animals to reduce the overall incidence of clinical pneumonia.

## 5. Conclusion

Overall deaths were non-significantly higher in control lambs, but vaccination against PI<sub>3</sub> did not affect the proportion of lambs treated for pneumonia nor deaths due to pneumonia. The proportion of control lambs dying from pneumonia was reduced compared to previous years and the possible transfer of the live vaccine to control animals cannot be ruled out. It remains possible that this vaccine may be useful in reducing lamb pneumonia in susceptible sheep flocks.

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