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A randomized control trial investigating the effectiveness of a commercial pneumonia vaccine (part I): Pre-weaned lambs

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ABSTRACT

The objective of this controlled vaccine field trial was to determine the effectiveness of a commercial bacterin in increasing colostral immunity to reduce the risk of bacterial pneumonia in pre-weaned lambs in a commercial sheep operation. Pregnant ewes were randomly allocated to vaccination group (Ovipast™ Plus bacterin, n = 1807; unvaccinated, n = 1812). Ewe vaccination did not significantly (P > 0.05) improve lamb pneumonia treatment rates, crude or pneumonia specific mortality rates, or body weight gain. Interestingly, as birth weight increased in lambs from vaccinated ewes, they gained significantly more than lambs from unvaccinated ewes (P = 0.01). There was no difference in culture results from pneumonic lung samples for either *Mannheimia haemolytica* (P = 0.89) or *Bibersteinia trehalosi* (P = 1.00) between lambs from vaccinated and unvaccinated ewes. The results of this study suggest that there was no animal health and welfare benefit from vaccinating ewes with the Ovipast™ Plus bacterin prior to parturition to boost colostral immunity and improve health and growth in their lambs.

1. Introduction

Pre-weaning mortality rates in North America lambs ranges from 10% to 30% with the largest death losses occurring during the first few days of life (Dwyer, 2008). In Canada, lamb mortality rates of 12–16% have been reported during the last 30 years (Ontario Ministry of Agriculture, Food and Rural Affairs, 2012). All major sheep producing countries have identified pneumonia as a common and significant infectious disease (Goodwin et al., 2004). Economic losses from ovine pneumonia are due to increased treatment, feed, and labor costs, reduced growth rates, mortality losses, and carcass discounts (Fernández et al., 2020, West et al., 2009; OMARFRA, 2021).

Pneumonia is a multifactorial disease. Goodwin et al. (2004) described pneumonia as the interaction between one (or more) infectious viral, bacterial and/or parasitic agents, immunological and physiological host factors, and environmental factors. Common causes of ovine pneumonia include parainfluenza virus type 3 (PI-3), and bacteria, such as *Mannheimia haemolytica*, *Pasteurella multocida*, and *Mycoplasma* (Navarro et al., 2019; Australia Livestock Export Corporation Ltd., 2021), which may vary by country. Of the 299 tissue samples collected for an Alberta Livestock and Meat Agency (ALMA) Feedlot Lamb Mortality Report (Van Donkersgoed et al., 2016), *M. haemolytica, P. multocida,* and *Mycoplasma arginini* were most isolated from acute and chronic bronchopneumonic lungs in lambs raised in a large commercial sheep feedlot.

In the presence of some or all these pathogens, pneumonia in sheep is often triggered by stressful events such as weaning or transport, and then amplified by insufficient ventilation systems. Good ventilation serves to reduce airborne pathogen concentration, reduce dust, control ambient temperature, and control humidity and drafts (Callan and Garry, 2002). Other indoor risk factors that can affect the risk of pneumonia include overstocking of pens and poor environmental hygiene. Ideally, ovine pneumonia should be prevented rather than treated, due to medication and labor costs and increasing concerns about the development of antimicrobial resistance (AMR) due to antimicrobial use (Moon et al., 2011).

Vaccination against common ovine pneumonia-causing agents would be an effective way to reduce the occurrence of respiratory disease. Lambs could be protected by passive immunization from ingestion of colostrum containing specific antibodies from their vaccinated dams, and/or protected by active immunization through vaccination prior to disease risks. The effectiveness of vaccinating ewes during pregnancy to

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improve colostral immunity is influenced by colostrum management, vaccine effectiveness, timing of vaccination, stress load, environmental factors, and pathogen load at the time of vaccination (Callan and Garry, 2002). Currently there are no ovine respiratory vaccines licenced for use in Canada. A vaccine trial was conducted in New Zealand to evaluate the effectiveness of the Ovipast™ Plus bacterin (Intervet/Merck Animal Health, Milton Keynes, United Kingdom) in an extensive production system (Goodwin-Ray et al., 2008). In Canada, most lamb production is intensive from birth to weaning (Castonguay, 2013); thus, it is important to evaluate the Ovipast™ Plus bacterin under intensive field conditions, prior to the licensing of the vaccine in Canada. As well, replication of controlled vaccine trials in the field are needed to give producers and veterinarians confidence in the effectiveness of commercial vaccines in sheep flocks.

The Ovipast™ Plus bacterin contains five strains of *M. haemolytica* and four strains of *B. trehalosi,* which are common pathogens in ovine respiratory disease (Van Donkersgoed et al., 2016; Dassanayake et al., 2017). Strains of pathogenic bacteria causing pneumonia in sheep may vary among flocks, regions of a country, and countries, supporting the need to evaluate the effectiveness of the Ovipast™ Plus bacterin's in commercial flocks in Canada, before moving forward with vaccine registration and licensing in Canada.

The objective of the present controlled vaccine field trial was to determine the effectiveness of the Ovipast™ Plus bacterin in reducing morbidity and mortality from pneumonia and improving growth performance in lambs under commercial conditions. If the results of this vaccine trial were positive, this would support moving forward with licensing of the vaccine in Canada. In this scientific article, we report the findings of the pre-weaning phase of the vaccine trial. The results of the growing and finishing phases of this vaccine trial are reported elsewhere (Gardner et al., 2024).

2. Materials and methods

2.1. Trial facility

This project received ethics approval for the animal utilization protocol (AUP#4625) from the Animal Care Committee at the University of Guelph. The vaccine trial was conducted in southern Alberta, Canada, in a commercial sheep operation with approximately 10,000 Rideau Arcott crossbred ewes between Nov. 25th, 2021, and June 1st, 2022. The flock lambed year-round on a weekly rotation, utilizing an OvSynch (Yadav et al., 2020) program, to synchronize groups of approximately 300 ewes each week.

2.2. Vaccine

The Ovipast™ Plus bacterin was imported into Canada by Merck Canada under the authority of the Health of Animals Act and Regulations, after the veterinary researchers received a veterinary biologic's import permit from the Canadian Food Inspection Agency (CFIA, 1493 Application). The vaccine was shipped to the sheep operation overnight on ice by courier and stored in a refrigerator at 3–8°C, which was monitored by a temperature data logger. Ovipast™ Plus bacterin contains the following ingredients per 1 ml: 1) 5×10^8 killed cells each of *M. haemolytica* strains A1 (S1006/77), A2 (S1126/92), A6 (S1084/81), A7 (S1078/81), A9 (S994/77), and *B. trehalosi* strains T3 (S1109/84), T4 (S1085/81), T10 (S1075/81), T15 (S1105/84), 2) aluminum hydroxide gel, and 3) thiomersal (NOAH, 2022). Label instructions indicate that sheep should be vaccinated twice, 4–6 wk apart, with a volume of 2 ml per dose, administered subcutaneously. When the vaccine is administered to pregnant ewes receiving the vaccine for the first time, they should receive the first dose approximately 6 wk prior to lambing, and the second dose 2 wk prior to lambing. Since the flock used in this study had never been vaccinated with the Ovipast™ Plus bacterin, the pregnant ewes received 2 vaccine doses, 4 wk apart, starting 6 wk before the approximate date of parturition, with the booster vaccine 2 wk before lambing.

2.3. Study design

The authors used a sample size equation to detect a difference in proportions between 2 groups (eqn. 2.6, Dohoo et al., 2012), with a 0.5% reduction in mortality (i.e., 1–0.5% crude mortality), using a power of 80% and a confidence interval of 95% (alpha = 0.05) to determine the number of lambs required per vaccine group. Historic pneumonia mortality rates at this sheep operation were 1%. A drop out rate of 20% and a lambing rate of 180% was assumed. The total sample size required was 3333 ewes, or 1666 unvaccinated ewes and 1666 vaccinated ewes.

The inclusion criteria for ewes included all visually healthy pregnant ewes confirmed pregnant by ultrasound at 9 wk of pregnancy. Ewes were excluded from the vaccine trial if they had aborted prior to the first scheduled vaccination or had pre-term fetuses. Inclusion criteria for pre-weaned lambs participating in the trial was any lamb born to a trial ewe that survived to 2 d of age and received a Canadian Sheep Identification Program (CSIP) ear tag. Lambs were excluded from the trial if they were unhealthy at trial induction or were removed from their mother and put in the nursery. This sheep operation's policy was for each ewe to rear a maximum of 2 lambs to weaning. The selection of which 2 lambs remained with each ewe was based on a policy for the 2 most similar lambs to be kept with each ewe to prevent uneven competition for milk between lambs of the same litter. Pregnant ewes were randomly assigned to either the vaccinated or unvaccinated group by simple randomization. Ewes were processed for vaccination through a chute in groups of 8, which was based on the capacity of the chute system. For each group of 8 animals, a poker chip was blindly selected from a bag which determined the vaccination status of that chute. There was an equal number of poker chips in the bag for both unvaccinated and vaccinated ewes based on the number of animals in that specific group. The vaccine was injected subcutaneously in the left neck as per label directions.

2.4. Animal management and housing

After initial vaccination, due to space limitations, ewes were returned to outdoor pens and vaccinates and non-vaccinates were housed together. When ewes were administered a booster dose of the Ovipast™ Plus bacterin approximately 2 wk prior to lambing (approximately 4 wk later), they were sorted by vaccine group and housed in indoor pens according to vaccine status for the remainder of the trial.

Within 3 h of birth, lambs were weighed, given 2 ml of selenium (Selon-E®, Vetoquinol) intramuscularly, and navels were sprayed with iodine. At 2 d of age, lambs were tail docked using an elastrator ring, given a radio frequency identification CSIP ear tag, a sex tag, a coloured tag for their dam's vaccine status, and this information, along with lamb sex and birth were recorded in the farm's animal health computer software system (FeedIT, ITS Global, Okotoks, Canada). Barn staff were blind to the vaccine status of the tag colour.

The barn had a colostrum replacer protocol in place during the first 24 h following birth. Lambs were checked for colostrum consumption by picking up each lamb and checking its abdominal fill. For any litters of 3 or more lambs, all lambs in the litter were offered LambGro KidGro Colostrum™, containing ≥14% IgG bovine dried colostrum, at a rate of 20 g of powder per 2 kg of body weight, resulting in 93 ml total feeding volume per 2 kg body weight (Grober Nutrition, 2016). Any litter size of less than 3 was checked, and lambs were only offered artificial colostrum if staff were unable to get the lamb drinking from the ewe. Due to staff training, colostrum replacer was offered in a bottle and not tubed. The quantity of colostrum intake was not measured.

The barn staff identified animals for treatment based on daily observations of animals for signs of sickness. Sick lambs were treated

according to a standard veterinary treatment protocol, which was the same for lambs in both vaccine groups.

After birth, lambs were housed in a lambing jug with the ewe for approximately 72 h. Subsequently, ewe-lamb pairs were housed in group pens within the same barn, containing 4–6 ewes with lambs, which had lambed at a similar time. At approximately 1wk of age, ewe-lamb pairs were moved from the lambing barn to a neighbouring barn "A" and placed in 1 of 6 pens according to vaccine status, i.e., 3 unvaccinated pens and 3 vaccinated pens. Each pen housed 44 ewes with their lambs, and they remained in Barn "A" pens for 2 wk. Pens of vaccinated ewes and their lambs were separated from unvaccinated ewes and lambs by a wire gate.

During this 2-wk period, all lambs (approximately 2–3 wk of age) received toltrazuril (Baycox®, Elanco Canada Limited) and tulathromycin (Draxxin®, Zoetis Canada, 2023), to reduce the high risk of coccidiosis and pneumonia in this flock, according to the flock's standard veterinary health protocol. After 2 wk, ewe-lamb pairs were moved into barn "B" with their pen-mates from barn "A", where they were merged into 1 of 2 pens according to vaccine status (1 vaccinated pen and 1 non-vaccinated pen). Each pen consisted of approximately 134 ewe-lamb pairs, and they remained in this barn for 4–5 wk until the lambs were weaned at approximately 8 wk of age. With each movement, vaccine groups were kept separate, and ewe-lamb pairs were kept together. If lambs escaped pens and they still had their coloured trial tag or CSIP ear tag, they were identified in the computer system and returned to their correct pen.

Lambs were weaned abruptly at 8 wk of age. At the time of weaning, lambs were separated from the ewes, weighed to obtain the weaning weight, and sorted by sex and vaccine status, into 4 groups of lambs: (1) unvaccinated ewe lambs, (2) unvaccinated ram lambs, (3) vaccinated ewe lambs, and (4) vaccinated ram lambs. The methodology and results of this vaccine trial in weaned lambs are discussed in another publication (Gardener, *et al.*, 2024).

2.5. Pathology

All ewes that died after vaccination, and lambs that died prior to weaning were necropsied to determine the cause of death, using a standardized procedure (Van Donkersgoed and Ravi, 2018). Staff walked all barns in the morning to collect dead trial animals and necropsies were completed by 1 of the first 2 authors, before noon daily, to reduce autolysis of tissues. A lung score was assigned to any lamb whose primary cause of death was pneumonia. The objective of the lung scoring scale was to quantify the presence and severity of pneumonic lung lesions in the lungs, as well as the presence of pleuritis. The scale used was described by McRae et al. (2016, 2018), where each lobe was allocated a point from 0 to 2: 0 = no lesions, 1 = individual lobe with < 50% affected by pneumonic lesions, and 2 = individual lobe with > 50% pneumonic lesions. Since the ovine lung has 6 lobes: left (L) cranial, L caudal, right (R) cranial, R middle, R caudal, and accessory lobe, each individual animal could receive a maximum score of 12 points. Additionally, a pleuritis score was assigned (scored from 0 to 1): 0 for "not present", and 1 for "present", for a total maximum score of 13 points. A running Excel (Microsoft Office 365; version 16.0.15028.20160) spreadsheet of deaths was kept throughout the duration of the trial where all trial necropsies were recorded.

Lung samples were taken from any lamb diagnosed with pneumonia as the primary cause of death. A total of 50 (24 unvaccinated and 26 vaccinated) samples were cultured for bacterial pathogens, and 27 (15 unvaccinated and 12 vaccinated) samples were cultured for *Mycoplasma* bacteria. The sampling method involved taking a representative sample, approximately 2.5 cm×2.5 cm of tissue, from both the right and left caudal lobes. One half of each lobe sample was frozen for culturing, and the other half was fixed in 10% formalin for histopathology. Frozen and formalin-fixed tissues were shipped to the Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada for bacterial culture and histopathology.

The following procedure was used for bacterial culturing of fresh lung samples, except for culturing of *Mycoplasma spp*. Lung tissue was set up for routine aerobic culture; tissues were seared prior to sampling, an incision was made into the seared tissue, the tissue was scraped, and the scrapings were plated onto Columbia blood agar (BA) and MacConkey agar (MAC). Agar plates were incubated (BA in CO_2 and MAC in atmospheric conditions) for a total of 48 h. Bacterial growth was observed after 24 h and 48 h of incubation. Suspect pathogenic colonies were selected for identification using the MALDI-TOF (spell out and Ref).

For *Mycoplasma*, thawed tissue samples were set up on pig serum (PS) agar plates, yeastolate agar (YA) plates, hyopneumonia agar (HP) plates, and ureaplasma plates (UP), with the corresponding broth media, and subcultured every 2–3 d) (Whitford et al., 1994; Rosendal and Black, 1972). Positive cultures were identified using fluorescent antibody tests performed on agar blocks of *Mycoplasma* colonies. Culture and isolation of live bacteria that may not survive freeze-thaw following transport may not be as sensitive as PCR which can still identify non-viable bacteria in such tissues. However, the fact that multiple species of bacteria and Mycoplasma were isolated in high numbers provides evidence that freezing and transit of lung samples in this study did not significantly affect the outcome.

Formalin-fixed tissues were received by the Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada. One representative section from each tissue submitted per case number was trimmed into a single cassette. Tissues were then routinely processed, sectioned at 5 micrometers, and stained with haematoxylin and eosin, prior to histopathological assessment by a board-certified veterinary pathologist. Two sections of lung were examined per slide. Before examining slides, all lung sections were identified as A or B (as viewed with the slide label on the right-hand side).

2.6. Statistical analysis

A timeline spreadsheet containing the events of each trial lamb from 2 d of age through to weaning was completed and uploaded into R/RStudio®. Descriptive statistics were performed by vaccine group using Chi-square Tests for categorical variables and T-Tests for continuous variables, with a threshold of $P < 0.05$ for statistical significance. The variable "treatment for pneumonia" was defined as either yes, for individually sick lambs that were treated for pneumonia, or no, the lamb was never treated for pneumonia. This variable did not include the metaphylaxis treatment with tulathromycin which all lambs received at 3 wk of age. Treatment for pneumonia only included the 1st occurrence of disease.

To determine the effectiveness of the vaccine, univariate regression analyses were performed between vaccination status and pneumonia treatment rates, crude mortality rates, pneumonia mortality rates, and body weight gain. Lambing group was included as a random effect. Potential biologically causal confounders of disease occurrence and vaccination status, such as sex of the lamb, % fed supplemental colostrum, and litter size, were investigated using simple statistics to determine whether there was a significant statistical association ($P \leq 0.05$) between that variable and both vaccination status and the outcome of interest. If there was, then that variable was considered a potential confounder and included as a covariate in the univariate analysis to control its effects on the association of vaccination with the outcome of interest.

To evaluate other risk factors of disease and growth performance in this trial population, a univariable regression analysis was performed between each outcome variable and biologically plausible predictors of disease. Further multivariate models were also developed using a liberal P-value of 0.20 to decide which variables to include in the main effects model. Vaccination status was forced into each main effects model regardless of the P-value because it was a known variable of difference in the trial population. Generalized linear mixed models (GLM) were

created for the categorical outcome variables of crude mortality, pneumonia-specific mortality, and pneumonia-specific treatment rates. All models included a random effect of lambing pen, and backwards analysis was used to create the final models. A P value of < 0.05 was used to determine statistical significance in the final models.

The independence of observations for GLMs was determined using a variance inflation factor (VIF) test, where 1 = no correlation, 1–5 = moderate correlation, and >5 = potentially severe correlation between observations. Continuous variables in GLMs were subjected to the assumption of linearity and investigated in RStudio® using a scatter plot. Studentized residuals, Cook's distance values, and leverage were also determined in RStudio®. The most influential points were determined by combining studentized outliers, leverages, and Cook's distance values, and were plotted on a scatter graph. A Hosmer-Lemeshow test was then used on the model to determine if the model fit the data, the null hypothesis being that the model fits the data.

3. Results

3.1. Descriptive results summary

The final enrollment of breeding ewes onto the trial was 1812 unvaccinated negative controls and 1807 vaccinated. Of those ewes enrolled in the trial, 1794 (99%) unvaccinated were run through the chute to read eartags and 1785 (99%) vaccinated ewes received the second dose of bacterin. Of the unvaccinated and vaccinated ewes enrolled in the trial, 1431 (79.0%) and 1428 lambed (79.0%) contributed lambs to the trial, respectively. A total of 5054 lambs (2543 unvaccinated and 2511 vaccinated) were enrolled in the trial between January 6th, 2022, and April 26th, 2022. Of the 2543 unvaccinated and 2511 vaccinated lambs enrolled, respectively, there were 213 and 236 singles, 992 and 982 twins, 922 and 855 triplets, and 416 and 438 born as quadruplets or more. There was no statistical difference in the distribution of litter sizes between the 2 vaccine groups. Additionally, a total of 342 unvaccinated lambs and 369 vaccinated lambs were offered artificial colostrum, and there was no statistical difference in supplementation between the vaccine groups (P = 0.22, Table 2). There was also no difference in the percent of ram lambs born between the vaccine groups (P = 0.58, Table 2) (Table 1).

Table 1
Causes of mortality in nursing lambs in a Canadian sheep flock participating in a controlled vaccine field trial to determine the effectiveness of the Ovipast™ Plus bacterin - 2021–2022.

Cause of Death	Unvaccinated	Ovipast™ Plus
Diarrhea	106	131
Starvation	78	92
Pneumonia	36	33
Other	20	15
Crush/injury/trauma	17	14
Undetermined due to autolysis	20	11
Unknown	12	11
Omphalitis	13	10
Liver abscesses	10	11
Septic	9	7
Enterotoxemia	8	6
No necropsy	6	4
Intestinal/intussusception	3	2
Nervous signs	2	3
Watery mouth	2	3
Pleuritis	1	2
Kidney obstruction/abscess	2	1
Laryngitis	1	1
Peritonitis	1	1
Impaction	1	1
Bloat	1	0
Melena	1	0
Total	350	361

Throughout the pre-weaning period, 1229 lambs were identified as sick by the health crew and treated for pneumonia, 637 unvaccinated and 592 vaccinated, and this difference was not significant (P = 0.24). The other 160 treatments (72 unvaccinated, 88 vaccinated) were for different diseases, and there was no difference in treatment rates between the vaccine groups (P = 0.20). Of the lambs that were treated for pneumonia, 29 died from pneumonia, with a crude case fatality rate of 2.4% (29/1229). Of the lambs that died of pneumonia (n = 69), 42% were identified as sick by the health crew and treated individually for pneumonia (29/69), whereas 55% received only the prophylactic treatment of tulathromycin (Draxxin®, Zoetis Canada, 2023) at 3 wk of age (36/69). The remaining 6% that died from pneumonia were treated for a different disease; thus, they were misdiagnosed (4/69). The leading causes of death were diarrhea, starvation, and pneumonia, respectively (Table 2).

There were no significant differences ($P \geq 0.05$) in pneumonia treatment rates, crude mortality rates, pneumonia specific mortality rates, birth weight, weaning weight, average daily gain, or trial removals between lambs borne from vaccinated or unvaccinated ewes (Tables 2 and 3).

3.2. Culture and histopathology results

There was no difference between vaccine groups in pathogenic respiratory bacteria cultured from pneumonic lungs (Table 3). *Salmonella dublin* was cultured, along with *P. multocida*, from a lamb that died from bronchopneumonia at approximately 2 wk of age.

3.3. Univariable regression summary

Five outcomes were evaluated using vaccine status as a univariable predictor: (1) crude mortality (GLM), (2) pneumonia-specific mortality (compared to those that did not die or died of a different cause) (GLM), (3) pneumonia-specific mortality (compared to those that died of another cause) (GLM), (4) treatment for pneumonia (GLM) and, (5) weight gain from birth to weaning (linear model) (Table 4). In addition to vaccine status, the weight gain model also included a continuous variable for days to weaning to prevent bias based on how long each animal was in the pre-weaning phase. All univariate models showed that vaccination did not significantly affect any of the outcomes of interest (P-value range: 0.26–0.63, Table 4).

3.4. Multivariable regression model results

In the first mixed multivariable logistic regression disease risk model of "**crude mortality**", there were 4 significant predictor variables (Table 5). There was a statistically significant sex effect, with the odds of ram lambs dying from any cause 1.34 times greater than the odds of ewe lambs dying from any cause (95% CI: 1.13–1.58, $P < 0.001$). Birth weight had a significant quadratic relationship with crude mortality (Fig. 1). There was a significant interaction between litter size and artificial colostrum supplementation. The odds of dying from any cause was 2 times higher for lambs born as twins that received colostrum replacement product (CRP) compared to lambs born as quadruplets that received CRP (95% CI: 1.09–3.70, P = 0.03) (Fig. 2). Colostrum replacement product administration by itself was not statistically significant, except when included as an interaction term with litter size.

In the second multivariable logistic regression disease risk model of "**pneumonia-specific mortality**", lambs that died of pneumonia were compared to the entire trial population (Table 6). There was a significant sex effect, with ram lambs being 2.13 times more likely to die of pneumonia than an ewe lamb (95% CI: 1.27–3.57, P = 0.004).

In the third mixed multivariable logistic regression disease risk model of "**pneumonia-specific mortality**" compared to death from other causes (n = 711) (Table 7) there was a significant sex effect on lambs that died of pneumonia. The odds of a ram lamb dying from

Table 2
Simple descriptive statistics of lamb parameters of pre-weaned lambs in a Canadian sheep flock participating in a controlled vaccine field trial to determine the effectiveness of the Ovipast™ Plus bacterin: 2021–2022.

	Unvaccinated			Ovipast™ Plus			P	95% CI	
	Rams	Ewes	Total	Rams	Ewes	Total		Unvaccinated	Ovipast™ Plus
N inducted			2543			2511	NA	NA	NA
N rams			1312			1316	0.58	-0.02–0.04	
N fed supplemental colostrum			342			369	0.22	-0.01–0.03	
N Singles			213			236	0.22	-0.01–0.03	
N Twins			992			982	0.97	-0.03–0.03	
N Triplets			922			855	0.11	-0.05–0.00	
N Quadruplets (or more)			416			438	0.30	-0.01–0.03	
Lamb movements from induction to weaning:									
N inducted	1312	1231	**2543**	1316	1195	**2511**	NA	NA	NA
N died	190	160	**350**	205	156	**361**	0.56	-0.01–003	
N removed	35	41	**76**	26	28	**54**	0.07	-0.02–0.00	
N weaned	1088	1030	**2118**	1085	1011	**2096**	NA	NA	NA
Production Outcomes:									
Average birth weight (kg)	4.24	3.98	**4.12**	4.18	3.95	**4.07**	0.41	4.08–4.15	4.03–4.11
Average weaning weight (kg)	15.67	14.86	**15.28**	16.08	14.86	**15.49**	0.17	15.11–15.44	15.33–15.66
Average days to weaning	50.21	50.73	**50.47**	51.26	50.64	**50.96**	0.004	50.25–50.68	50.74–51.53
ADG (birth to weaning)	0.23	0.21	**0.22**	0.23	0.21	**0.22**	0.17	0.22–0.22	0.22–0.23
Treatment and Pneumonia related outcomes:									
Treatment with an antimicrobial+	396	313	**709**	380	300	**680**	0.54	-0.03–0.02	
Treatment for pneumonia+	363	274	**637**	334	258	**592**	0.24	-0.04–0.01	
Pneumonia mortality	24	12	**36**	24	9	**33**	0.85	-0.01–0.01	

+ These antimicrobial treatments represent additional treatments of individual animals clinically sick with disease, and do not include metaphylactic treatment of all lambs at 3 weeks of age to reduce pneumonia risks.
NA = not applicable

Table 3
Comparison of culture results from lung samples of pre-weaned lambs diagnosed with fatal pneumonia in a Canadian controlled vaccine field trial to determine the effectiveness of the Ovipast™ Plus bacterin: 2021–2022.

	Pre-wean Confirmed Pneumonia		Pre-wean %		
Isolation rate	**Unvaccinated**	**Ovipast™ Plus**	**Unvaccinated**	**Ovipast™ Plus**	**P**
Number of samples cultured	24	26			
Mannheimia haemolytica (type I or II)	15	18	62.50%	69.23%	0.89
Bibersteinia trehalosi	1	1	4.17%	3.85%	1.00
Pasteurella multocida	8	5	33.33%	19.23%	0.34
Trueperella pyogenes	5	7	20.83%	26.92%	0.74
Moraxella bovoculi	1	3	4.17%	11.54%	0.61
Isolation rate	**Unvaccinated**	**Ovipast™ Plus**	**Unvaccinated**	**Ovipast™ Plus**	**P**
Number of samples cultured	15	12			
Mycoplasma ovipneumoniae	11	8	73.33%	66.67%	1.00
Mycoplasma arginini	10	7	66.67%	58.33%	0.71

pneumonia compared to dying from other causes was 1.88 times greater than the similar odds of death in an ewe lamb (95% CI: 1.08–3.28, P = 0.03).

The variable "wk of age" represented the range of ages when the lambs died. This value was not normally distributed and was therefore categorized into 0–2 wk, 2–4 wk, 4–6 wk, and >6 wk of age. Most lamb deaths fell into the 0–2-wk category, making it the most biologically relevant as the referent category. All 3 age categories at time of death: 2–4, 4–6, and >6 wk, were statistically significant, with the odds of death from pneumonia versus other causes of death being 4.10 times at 2–4 wk (95% CI: 1.74–9.65, P = 0.001), 8.41 times at 4–6 wk (95% CI: 3.52–20.10, P < 0.001), and 14.34 times at > 6 wk (95% CI: 6.18–33.28, P < 0.001) more likely compared to a lamb that died at 0–2 wk of age (Fig. 3).

In the fourth mixed multivariable logistic regression disease risk model of "**pneumonia treatment**", there was a significant sex effect (Table 8). The odds of a ram lamb being treated for pneumonia was 1.35 times greater than the odds of an ewe lamb being treated for pneumonia (95% CI: 1.18–1.54, P < 0.001). For birth weight, there was a significant quadratic relationship, where lambs born at approximately 4 kg of body weight had the highest probability of being treated for pneumonia. The probability of treatment was lower at lighter and higher birth weights (Fig. 4).

All models fulfilled the assumptions of normality of residuals, homoscedasticity of the variance, and independence. Only one variable was identified as an outlier during leverage diagnostics. Observation 4054 demonstrated irregularities in data recording that could not be confirmed; therefore, this animal was removed from the final models, and the models were updated. The removal of this datapoint did not change the results of any of the models. Season was not a significant variable in any of the multivariate models.

4. Discussion

The commercial vaccine used in this trial is labeled for use in reducing "pasteurellosis", which is a common infectious respiratory disease in small ruminants, caused by various species *Pasteurella* bacteria (Daphal et al., 2018). Pasteurellosis can be a challenge to define epidemiologically as an outcome in a vaccine trial; therefore, the following objective and subjective measures were used because they are quantifiable: 1) treatment rates for pneumonia, 2) crude lamb mortality rates, 3) mortality rates from pneumonia, 4) mortality rates from pneumonia compared to death from other causes), and 5) body weight gain. Based on the results of this controlled field trial, vaccination of

Table 4
Effectiveness of the Ovipast™ Plus bacterin in ewes during gestation and effects on pre-weaning lamb health and performance: controlled vaccine field trial in a Canadian sheep operation.

Crude mortality	Category	OR	P
Vaccine Status	Unvaccinated	referent	-
	Ovipast™ Plus	1.05	0.54
Pneumonia specific mortality	Category	OR	P
Vaccine status	Unvaccinated	referent	-
	Ovipast™ Plus	0.93	0.76
Pneumonia mortality versus other causes of death	Category	OR	P
Vaccine Status	Unvaccinated	referent	-
	Ovipast™ Plus	0.88	0.61
Treatment for pneumonia	Category	OR	P
Vaccine Status	Unvaccinated	referent	-
	Ovipast™ Plus	0.91	0.26
Weight gain from birth to weaning	Category	Coefficient (g)	P
Vaccine Status	Unvaccinated	referent	-
	Ovipast™ Plus	0.18	0.33
Days to wean	-	0.29	<0.001

Lambing group was included as a random effect in the univariate analysis.

Table 5
A generalized linear mixed effects model evaluating risk factors associated with crude mortality in pre-weaned lambs in a Canadian sheep operation participating in a controlled vaccine field trial to determine the effectiveness of the Ovipast™ Plus bacterin: 2021–2022.

Predictors	Category	Odds Ratios	P	CI
(Intercept)	-	8.32	<0.001	2.95 – 23.49
Vaccination Status	Unvaccinated	referent	-	-
	Ovipast™ Plus	1.01	0.94	0.84 – 1.21
Sex	Ewe	referent	-	-
	Ram	1.34	0.001	1.13 – 1.58
Birth Weight (kg)	-	0.21	<0.001	0.13 – 0.34
Birth Weight^2 (kg)	-	1.13	<0.001	1.07 – 1.20
Supplemental Colostrum	Did not receive	referent	-	-
	Received	1.38	0.15	0.89 – 2.13
Litter Size	Twin	referent	-	-
	Single	1.05	0.81	0.73 – 1.50
	Triplet	0.75	0.01	0.60 – 0.94
	Quadruplet (or more)	1.12	0.38	0.87 – 1.45
Supplemental Colostrum*Litter Size	Received*Twin	referent	-	-
	Received*Single	2.06	0.20	0.67 – 6.31
	Received*Triplet	1.06	0.83	0.61 – 1.84
	Received*Quadruplet (or more)	0.50	0.03	0.27 – 0.92
Random Effects				
σ^2	3.29			
$\tau_{00\ \text{Vaccinination status*Lambing group}}$	0.01			
$\tau_{00\ \text{Lambing group}}$	0.06			
ICC	0.02			
$N_{\text{Vaccination status}}$	2			
$N_{\text{Lambing group}}$	13			
Observations	5054			
Marginal R^2 / Conditional R^2	0.08 / 0.10			

ewes twice during gestation with the Ovipast™ Plus bacterin, to improve colostral immunity in lambs, did not reduce pneumonia treatment rates or crude or pneumonia-specific mortality rates, or improve growth performance.

Potential reasons for vaccine failure in this trial include: 1) the presence of other respiratory pathogens not included in the bacterin, 2) variation in the bacterial strains present on the farm versus those in the bacterin, 3) a low pneumonia mortality rate (1.4%), 4) vaccine type and components, and 5) background colostral immunity in both vaccinated and unvaccinated ewes.

For budgetary reasons, all lungs in the study were not cultured for pathogens, and those that were, were limited to what pathogens could be identified. For example, no testing for viruses was undertaken; therefore, we cannot determine the role viruses, such as PI-3, adenovirus, and respiratory syncytial virus, may have played in causing respiratory disease during this trial. Previous research at this sheep operation did not find these viruses present in fatal cases of pneumonia in post-weaned lambs (Van Donkersgoed, 2016). It appears that *M. haemolytica* was still playing a significant role on this farm, because it was cultured from many cultured lungs (> 60%) in both vaccinated and unvaccinated groups. Only 4% of the lungs were culture positive for *B. trehalosi*, suggesting it was not a common lung pathogen. Other pathogens of significance in descending order of prevalence, that were cultured include: *M. ovipneumoniae*, *M. arginini, T. pyogenes, P. multocida,* and *Moraxella bovoculi* (Table 3). Their presence and importance should be considered in future development of respiratory vaccines for sheep.

No typing of the *M. haemolytica* strains was performed to determine if there was any difference between isolates cultured here and those in the vaccine strains. Villard et al. (2008) conducted a study on 8 strains of *M. haemolytica* of serotypes A1 and A2, as well as 3 strains of *B. trehalosi* of serotypes T3 and T4, all of which were strains in the Ovipast™ Plus bacterin used in the present study. The authors found that although all strains of *B. trehalosi* were stable throughout multiple rounds of sub-culturing, both strains of *M. haemolytica* showed patterns of variation by PFGE and phenotypic analysis, and A2 showed variation by both phenotypic and capsular typing. Additionally, Vougidou et al. (2013) investigated the variation in the (leukotoxin) LktA gene of *M. haemolytica.* The authors stated that recombination was common among the LktA sequence, more specifically in the N-terminus region, which is important for the cytotoxic traits of the toxin during disease progression. Since there is no cross-reactivity of antibodies targeting these different characteristics, a mismatch between the vaccine and host flock strains could be a potential reason for vaccine failure (González et al., 2019).

Another potential reason for the inability to detect vaccine differences could be the low pre-weaning pneumonia mortality rate on this farm. Although a large portion of lambs in both the unvaccinated (25%) and vaccinated (23.6%) groups were treated for pneumonia, only a small percent of these in each vaccine group died from pneumonia, 1.4% and 1.3% respectively. It should be noted that this barn had a previously established veterinary health protocol to metaphylactically treat all lambs with tulathromycin at 3 wk of age to reduce losses from pneumonia. Tulathromycin is labelled to treat and control respiratory disease from *M. haemolytica*, *P. multocida*, *H. somni*, and *M. bovis* (Draxxin®, Zoetis Canada, 2023). It is possible that administration of tulathromycin to all lambs at 3 wk of age reduced pneumonia-associated morbidity and mortality and the ability to detect a vaccine effect. Tulathromycin was

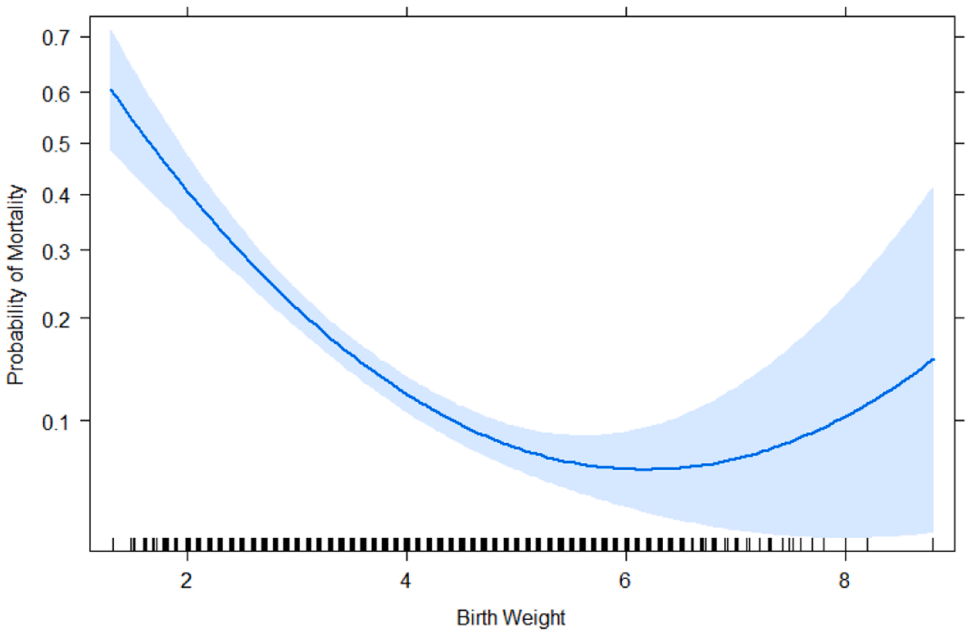

Fig. 1. Generalized linear mixed model: effects of birth weight on crude mortality, using data from a randomized controlled vaccine trial to determine the effectiveness of the Ovipast™ Plus bacterin in pre-weaned lambs raised on a commercial Canadian sheep operation.*Colostrum = 0 indicates lambs did not receive artificial colostrum and colostrum = 1 indicates they did receive artificial colostrum.

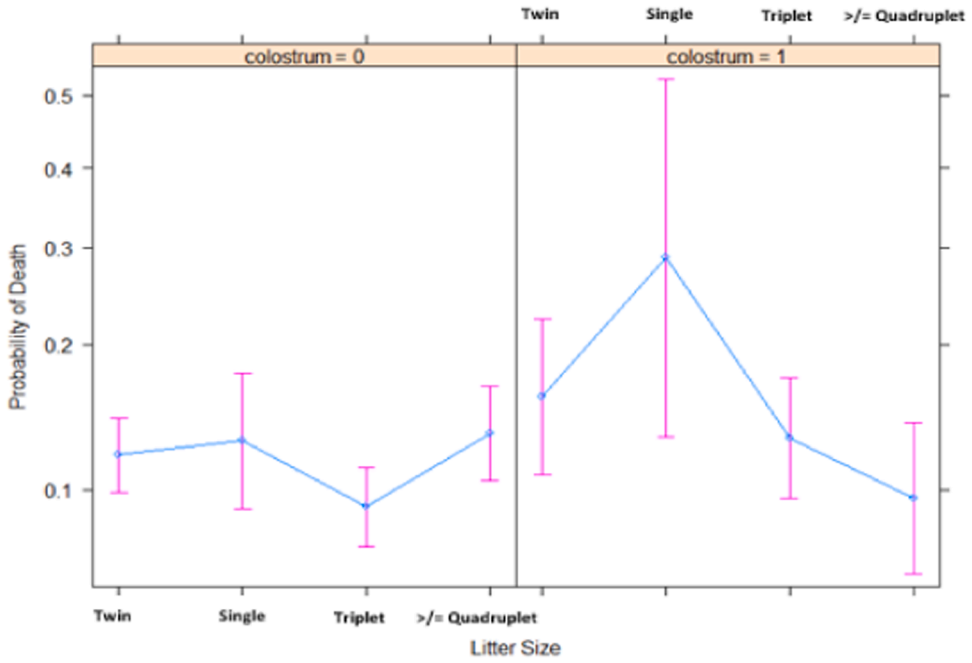

Fig. 2. Generalized linear mixed model: Interaction between supplemental colostrum and litter size, on the outcome of crude mortality, using data from a randomized controlled field trial to determine the effectiveness of the Ovipast™ Plus bacterin in a commercial Canada sheep operation.

given to both vaccine groups; thus, it would not have created any directional misclassification bias in the vaccine trial results. Based on the prevalence of pneumonia mortality in this population, with a sample size of 2543 non-vaccinates and 2511 vaccinates, this study had the power to detect a mortality difference of 1.4–0.6% between vaccine groups (using 95% confidence and 80% power), but this vaccine effect size was not observed in this trial.

Another possible reason for vaccine failure could be the vaccine type and the antigen components. Killed whole cell bacterins generate a type 2 immune response, which is antibody-mediated, and generally considered less protective than the immune response to a modified-live vaccine (Meeusen et al., 2007; Tizard, 2020). There have been bacterins developed against *M. haemolytica* for cattle, and they may have increased effectiveness because they contain leukotoxoids. Leukotoxin (Lkt) is a known cause of pneumonia caused by *M. haemolytica* (Oppermann et al., 2017), and antigen production against Lkt is the focus of *M. haemolytica* vaccine production in cattle. Although studied mainly in cattle *in vitro,* all ruminant leukocytes are vulnerable to cytolysis induced by the Lkt produced from *M. haemolytica* (Jeyaseelan et al., 2002). In a recent experimental challenge study, vaccination of

Table 6
A generalized linear mixed effects model evaluating risk factors associated with pneumonia mortality in pre-weaned lambs in a Canadian sheep operation participating in a controlled vaccine field trial to determine the effectiveness of the Ovipast™ Plus bacterin: 2021–2022.

Predictors	Category	Odds Ratios	P	CI
(Intercept)	-	0.01	<0.001	0.01 – 0.01
Vaccination Status	Unvaccinated	referent	-	-
	Ovipast™ Plus	0.92	0.74	0.57–1.48
Sex	Ewe	referent	-	-
	Ram	2.13	0.004	1.27–3.57
Random Effects				
σ^2	3.29			
$\tau_{00\ \text{Vaccination status*Lambing group}}$	0			
$\tau_{00\ \text{Lambing group}}$	0			
$N_{\text{Vaccination status}}$	2			
$N_{\text{Lambing group}}$	13			
Observations	5054			
Marginal R^2 / Conditional R^2	0.04 / NA			

Table 7
A generalized linear mixed effects model evaluating risk factors associated with pneumonia mortality compared to other causes of death in pre-weaned lambs in a Canadian sheep operation participating in a controlled vaccine field trial to determine the effectiveness of the Ovipast™ Plus bacterin: 2021–2022.

Predictors	Category	Odds Ratios	P	CI
(Intercept)	-	0.01	<0.001	0.00–0.03
Vaccination Status	Unvaccinated	referent	-	-
	Ovipast™ Plus	0.94	0.81	0.56–1.58
Sex	Ewe	referent	-	-
	Ram	1.88	0.03	1.08–3.28
Weeks of Age	0–2	referent	-	-
	2–4	4.10	0.001	1.74–9.65
	4–6	8.41	<0.001	3.52–20.10
	>6	14.34	<0.001	6.18–33.28
Random Effects				
σ^2	3.29			
$\tau_{00\ \text{Vaccinination status*Lambing group}}$	0			
$\tau_{00\ \text{Lambing group}}$	0			
$N_{\text{Vaccination status}}$	2			
$N_{\text{Lambing group}}$	13			
Observations	711			
Marginal R^2 / Conditional R^2	0.260 / NA			

lambs twice with the Ovipast™ Plus bacterin did not increase Lkt ELISA antibodies (Van Donkersgoed, et al., 2024), which may explain, in part, failure to see a vaccine benefit.

In other studies, investigating the transfer of colostral immunity to *M. haemolytica* in vaccinated versus unvaccinated dams, the research was conducted on farms with no history of respiratory disease, which would have prevented interference from antibodies generated from natural infections (Prado et al., 2006). In that study, both groups conferred IgG to calves, and given that both *M. haemolytica*, *P. multocida* are normal commensal residents of the respiratory tract of sheep (Ural, 2021), a similar transfer of colostral antibodies is to be expected from ewes to lambs regardless of vaccination. Since *M. haemolytica* and *B. trehalosi* were cultured from lung samples of animals that died from pneumonia in this flock prior to the study taking place (Van Donkersgoed 2016), baseline antibodies were more than likely in this vaccine study. It is possible that naturally acquired antibodies present in the trial ewes were already sufficient to produce colostral antibodies to protect some lambs from unvaccinated ewes against respiratory disease, reducing our ability to see a vaccine effect, especially in lambs less than 4 wk of age. It is also possible that antibodies generated prepartum in ewes from vaccination were insufficient in the colostrum to be protective; or, there were adequate amounts of leukotoxin IgG in the colostrum, but the type of antibody present i.e., IgG_1 versus IgG_2, did not provide a protective effect in the lambs (Lacasta et al., 2015).

Until an effective vaccine is developed to reduce pre-weaning morbidity and mortality from pneumonia, producer and veterinary focuses should be on alternate management strategies to reduce disease risks. Strategies related to animal husbandry, such as good colostral management, good environmental management, reduction of stressors, and good lambing management (i.e., genetic management to reduce the number of lambs/litter) should be employed. Further analyses of the flock's trial data yielded valuable information regarding risk factors associated with preweaning morbidity and mortality that could be used to prevent and reduce future disease risks on this farm.

Age was identified as a risk factor for dying of pneumonia. The first 2 wk of life were the lowest risk of dying from pneumonia, compared to other illnesses. After 6 wk of age, the risk of death from pneumonia compared to other causes of death increased by 14.34 times, compared to the first 2 wk of life (Fig. 3). This finding is consistent with other published literature that the peak incidence of mortality due to pneumonia in lambs was after 23 d of age (Lacasta et al., 2008), and greatest between 30 and 50 d of age (Navarro et al., 2019). There was a 27.5% pneumonia treatment rate in pre-weaned lambs in this flock despite metaphylactic treatment with tulathromycin at 3 wk of age. Of the 1389 lambs that were treated, 1229 (88.5%) were treated for pneumonia, highlighting the importance of respiratory disease in pre-weaned lambs raised intensively. Based on the proportion of animals that only received one treatment for pneumonia and did not die, the recovery rate for lambs being treated for pneumonia appeared high (98%), assuming all those treated for disease were sick from pneumonia. The staff may have overtreated animals that were not sick, or the animals may have been sick from another disease that was misdiagnosed as pneumonia, which would create misclassification bias; however, this misclassification bias would not have been directional in this trial as the staff that pulled and treated sick lambs were blind to vaccine status. Misclassification bias could reduce the ability to see a true vaccine effect because of the lack of objective diagnostic tests to confirm respiratory disease in live lambs.

Lamb sex was significantly associated with the odds of dying, which agrees with the published literature (Gama et al. 1991; Binns et al. 2002). These authors proposed (Gama et al., 1991; Nash et al., 1996) that the reason for higher mortality in rams is due to increased birth weights, leading to dystocia, as well as the stress of castration. In the present study, birth weight could potentially be impacting the sex effect because the average birth weight of ram lambs (4.21 kg) was significantly higher than the average birth weight of ewe lambs (3.97 kg). All ram lambs on this operation were left intact; therefore, castration did not play a role in mortality rates in the present study. Birth weight is likely not the main reason male lambs having a greater odds of dying, because the regression model also identified a significant association between mortality and birth weight, without a significant interaction between sex and birth weight. The role of sex in pneumonia in sheep is likely complex, but worthy of further investigation as this effect was observed in the post-weaning phase of this trial (Gardner et al., 2024) and in another respiratory vaccine-controlled field trial (Van Donkersgoed, et al., 2024).

The impact of birth weight on crude mortality is best modeled as a positive quadratic (U-shaped) with lambs of low and high birth weights having the highest probability of dying compared to lambs of moderate birth weights (Fig. 1). This has been reported previously in other studies (Gama et al., 1991). The quadratic troughs at approximately 6.2 kg suggest, that on this farm specifically, lambs with this birth weight had the lowest probability of dying. Lambs with a higher birth weight likely had increased odds of dying due to dystocia complications, and lambs

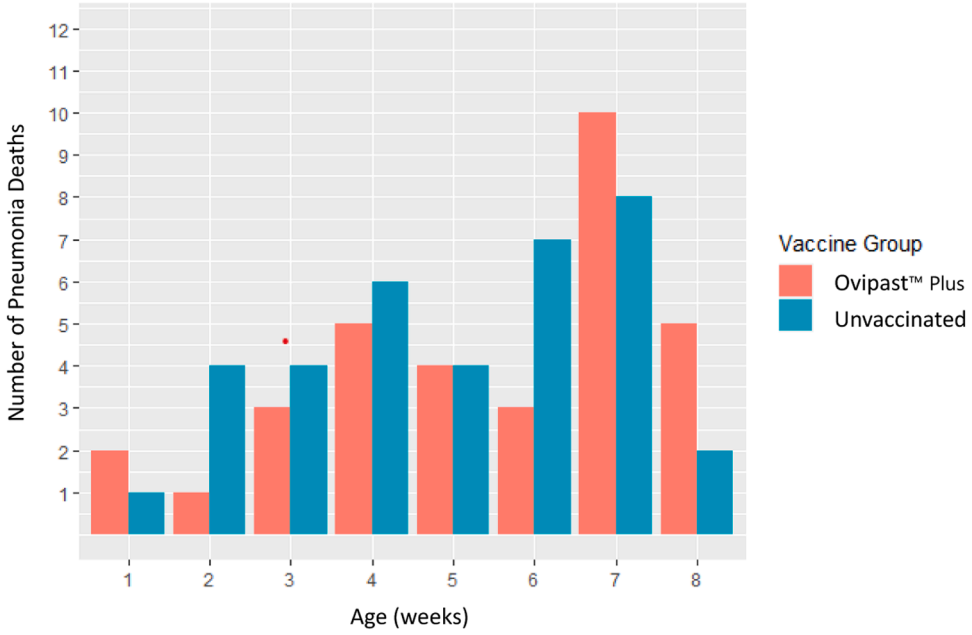

Fig. 3. Occurrence of pneumonia deaths in nursing lambs from vaccinated and unvaccinated ewes in a controlled vaccine field trial conducted on a commercial Canadian sheep operation to determine the effectiveness of the Ovipast™ Plus bacterin in ewes to improve lamb pre-weaning health.

Table 8
A generalized linear mixed effects model evaluating risk factors associated with treatment rates for pneumonia in pre-weaned lambs in a Canadian sheep operation participating in a controlled vaccine field trial to determine the effectiveness of the Ovipast™ Plus bacterin: 2021–2022.

Predictors	Category	Odds Ratios	P	CI
(Intercept)	-	0.08	<0.001	0.03 – 0.21
Vaccination Status	Unvaccinated			
	Ovipast™ Plus	0.91	0.24	0.78 – 1.06
Sex	Ewe			
	Ram	1.35	<0.001	1.18 – 1.54
Birth Weight (kg)	-	2.02	0.003	1.28 – 3.18
Birth Weight^2 (kg)	-	0.91	0.001	0.87 – 0.96
Random Effects				
σ^2	3.29			
$\tau_{00\ \text{Vaccination status*Lambing group}}$	0.01			
$\tau_{00\ \text{Lambing group}}$	0.13			
ICC	0.04			
$N_{\text{Vaccination status}}$	2			
$N_{\text{Lambing group}}$	13			
Observations	5054			
Marginal R^2 / Conditional R^2	0.012 / 0.054			

with a lower birth weight had increased odds of dying as they may have been born with less or no brown fat, and thus, were more prone to hypothermia (Binns et al., 2002). Birth weight also had a significantly protective quadratic relationship with treatment rates for pneumonia. The birth weight with the greatest probability of being treated for pneumonia was approximately 4.1 kg, with approximately a 22% probability of being treated for pneumonia.

The last risk factors of significance for disease and growth were litter size and colostrum. These two variables did not act independently. The effect of colostrum replacement on mortality was modified by the size of the litter a lamb was born into. Receiving artificial colostrum was defined as a lamb that consumed any amount of artificial colostrum within 24 h of birth when offered (i.e., if offered and did not drink, the lamb would not have been recorded as receiving any colostrum). A quadruplet lamb receiving colostrum replacement product had 2 times greater odds of surviving compared to a twin that received artificial colostrum. This is further explained in Fig. 2, where singles receiving artificial colostrum had the greatest odds of mortality of any litter size. On this farm, the protocol for feeding artificial colostrum specified that any lamb from a litter size of 3 or more was to automatically receive supplementary artificial colostrum at birth through to 24 h of age. Any lamb born to an ewe that had a single, twins, or triplets only received artificial colostrum if the lamb(s) were clearly not drinking within the first few h of life due to reasons related to either lamb (e.g., anoxia, hypothermia) or maternal issues (e.g., poor mothering, udder edema). When lambs were fed supplemental colostrum, it was administered via a bottle; therefore, intake of a sufficient volume of colostrum to prevent failure of passive transfer of immunity could not be guaranteed, like with a stomach tube. Based on this health protocol, survivability may have been lower in singles given colostrum, because only those lambs already struggling to drink were given supplemental colostrum. There was no guarantee that they would ingest the colostrum if offered; therefore, they were already predisposed to mortality and the statistical association is not causal. The singleton lamb receiving colostrum was most likely either sick postnatally, or the ewe was not feeding it adequately due to a variety of reasons (e.g., poor mothering instinct, udder conformation, or mastitis), which would predispose the lamb to not being fed well in the future or having a compromised immunity, leading to lower weight gain. As well, the singleton lamb may have experienced more dystocia due to higher birth weights. In Canada, there are no colostrum replacement products made from sheep colostrum. All commercial products are manufactured with bovine-sourced colostrum, and they may be lacking the protective antibodies specific for sheep pathogens.

This project met the target enrollment numbers of ewes, but it did not meet the targeted enrollment of lambs in the first phase of the vaccine trial evaluating the effects of ewe vaccination on lamb pre-weaning health and performance. Since the ewes were housed and bred in groups of 300, the overall number enrolled was rounded up rather than

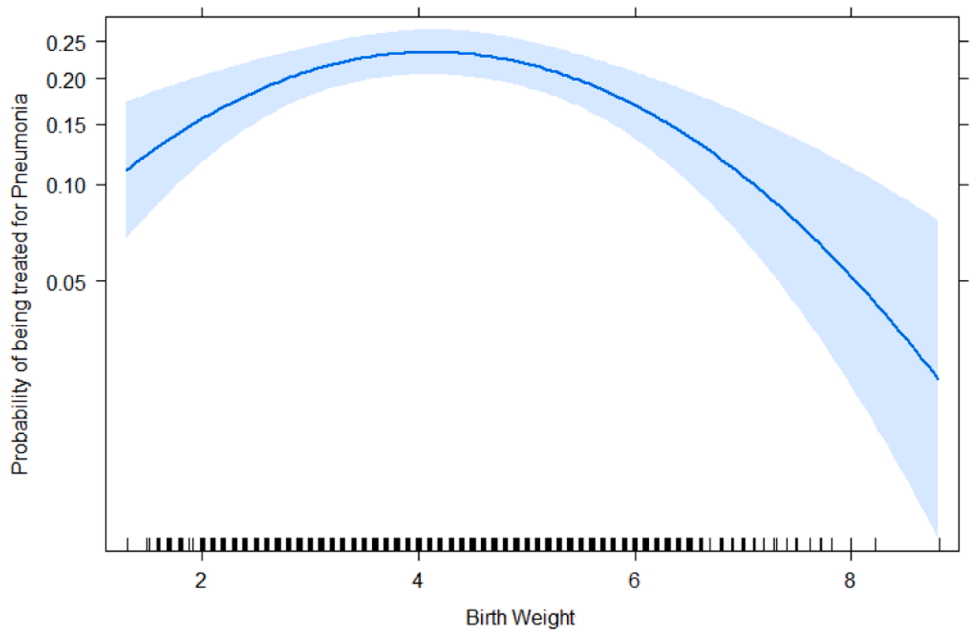

Fig. 4. Generalized linear mixed model: the effect of lamb birth weight on treatment for pneumonia, using data from a controlled vaccine field trial in a Canadian sheep operation, to determine the effectiveness of the Ovipast™ Plus bacterin in ewes to improve preweaning lamb health.

down to avoid breaking up the last lambing group. Falling short on lamb enrollment occurred due to the higher than predicted drop-out rate of ewes at 21.1%, as well as a 5.8% mortality rate for ewes prior to lambing. Reasons for ewe withdrawal explained most of the reduced lamb numbers, because this resulted in a complete loss of her lambs (i.e., no live lambs, no lambs left with the ewe, or the ewe was open). To reach the targeted 6000 lambs enrolled, the ewe enrollment numbers would have had to be 4230 total (2215 per vaccine group) to account for the 26.9% drop out rate, assuming there was an ideal lambing rate of 1.8 lambs per ewe. The average litter size for ewes inducted in the trial was 3 lambs per ewe, resulting in a lambing percentage of 300% (this value includes those stillborn and those that died prior to tagging at 2 d of age), which was higher than the percentage estimated a priori and used for the sample size calculation. The average number of lambs kept with each ewe for the trial (total lambs minus stillborn and those that died prior to tagging and moved to the nursery) was 2, making the enrollment rate greater than the anticipated 1.8 lambs per ewe. Overall, these numbers support the fact that ewe withdrawal was the reason for under-enrollment. The under-enrollment likely would not have affected the vaccine effectiveness results of the trial, as the P-values for all models were not close to the significance threshold (range: 0.24–0.94).

In this Canadian trial, there was not a significant difference in ewe withdrawal rates between vaccine groups for any of the following periods: 1) between their first and second dose of Ovipast™ Plus bacterin ($P = 0.12$), 2) between the second vaccination and lambing ($P = 0.98$), and 3) between lambing and weaning of lambs ($P = 0.11$). There was also no significant difference in ewe crude mortality rates between vaccine groups ($P = 0.83$). Throughout the trial, ewe mortality between the first and second dose of Ovipast™ Plus ($P = 1.00$), between the second dose and lambing ($P = 0.51$), and between lambing and weaning did not differ between vaccine groups ($P = 0.65$). With respect to vaccinating ewes, these results suggest there was no positive or negative effect of the vaccine on ewes.

The withdrawals in lambs during the pre-weaning period was close to being significant ($P = 0.07$), with most of the difference attributed to a loss in the trial tag. This reiterates the concern mentioned in the NZ trial (Goodwin-Ray et al., 2008), that loss of ear tags can be a major source of lamb withdrawals in sheep vaccine trials. Therefore, use of 2 unique individual animal ear tags in a trial, if one tag was lost, would reduce trial losses.

There were limitations in the present controlled vaccine field trial. Firstly, as previously discussed, no blood samples were taken from ewes or lambs to evaluate baseline titres of antibody levels or post vaccination antibody levels to determine the humoral immune response from vaccination in ewes or whether ewe vaccination increased passive immunity in their lambs. Evaluating these antibody titres may have helped explain why the vaccine failed to reduce lamb morbidity or mortality rates. Secondly, there is a lack of recent benchmarking data for lamb and sheep mortality in Canada, making some of the study outcomes standalone, without an accurate value for comparison.

Bias may have occurred in this trial in a couple of areas. Firstly, the first author was aware of the vaccine status of the animals on trial, and she performed most of the necropsies. Mortality is an objective outcome and can not be biased by the person who does the necropsy, only the cause of death could potentially be biased, but we are not aware of any directional vaccine bias on her part because she had no preconceptions on the effectiveness of the vaccine in this flock. Additionally, there was a systematic protocol for necropsies, along with an objective lung scoring and lung sampling procedure for bacterial culture, that was followed on each lamb, and everyone who performed necropsies was trained on these protocols to reduce diagnostic misclassification bias. The primary author was not involved in treatment decisions. Treatment decisions were made by the barn staff who were blind to vaccine status and followed a standardized veterinary flock treatment protocol, and these veterinarians were not involved in the research project. One of the strengths of this randomized controlled vaccine trial design compared to previous studies is that the present study was conducted in a single flock of animals, all born and raised on the same farm. Using a single origin flock reduces variability that can occur when studying flocks from different farms. In the present study, the authors can confidently state that all animals were treated the same and raised in the same environment, which would have reduced variability in other factors that may confound vaccine results, if not controlled through randomization, stratification, elimination, or statistical analyses.

In conclusion, the Ovipast™-Plus bacterin administered twice to pregnant dams did not reduce pneumonia treatment rates, crude mortality rates, pneumonia-specific mortality rates, or improve weight gain in pre-weaned lambs. There were some interesting interactions found

between weight gain and other risk factors, which require further investigation to understand their clinical significance. Further research is required to identify effective ovine respiratory vaccines for use in this flock and in other sheep operations in Canada.

CRediT authorship contribution statement

M.D. Gardner: Writing – original draft, Validation, roject administration, Methodology, Investigation, Formal analysis, Data curation, **J. Van Donkersgoed**: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization, **M.T. Spinato**: Writing – review & editing, Writing – original draft, Resources, Methodology, **C.A. Bauman**: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis.

Declaration of Competing Interest

No conflict.

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