Eurasia Specialized Veterinary Publication

International Journal of Veterinary Research and Allied Science

ISSN:3062-357X

2021, Volume 1, Issue 1, Page No: 113-121

Available online at: www.esvpub.com/

Field-Based Randomized Controlled Trial Evaluating the Safety and Effectiveness of a Killed Autologous Vaccine Targeting Streptococcus Dysgalactiae Subsp. Dysgalactiae in a Sheep Flock

Bianca Ferreira[1]*, Nelson Duarte[1]

[1]Department of Veterinary Infectious Diseases, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa.

***E-mail** ✉ b.ferreira.vet@icloud.com

ABSTRACT

Neonatal joint-ill in lambs, predominantly caused by Streptococcus dysgalactiae subspecies dysgalactiae (SDSD), leads to elevated rates of morbidity and mortality, highlighting the need for an effective preventive vaccine. A blinded, randomized controlled trial was conducted on a commercial sheep flock in the UK to evaluate an autologous SDSD vaccine. A total of 481 pregnant ewes received two doses of the vaccine, while 509 ewes remained unvaccinated. SDSD-specific antibody titres were measured in both ewes and their lambs, and any adverse reactions or occurrences of joint-ill were recorded. Ten lambs developed joint-ill, evenly split between those born to vaccinated and unvaccinated ewes. Serum samples from 85 vaccinated and 88 control ewes were analyzed using an SDSD antibody ELISA, revealing higher titres in vaccinated ewes. Lambs from vaccinated ewes (n = 87) also showed higher antibody titres than those from unvaccinated ewes (n = 91). Colostrum antibody levels did not differ between groups, and no adverse effects related to vaccination were observed. Although randomization was successful, ELISA data were primarily obtained from crossbred ewes, limiting the power to assess breed-specific differences. Vaccination of ewes did not reduce the incidence of joint-ill in lambs compared to unvaccinated ewes, but it significantly increased SDSD-specific antibody levels in both ewes and their offspring.

Keywords: Drug Design, Computational Drug Discovery, Silico Methods, Molecular Modeling

Received: 16 November 2020
Revised: 05 February 2021
Accepted: 06 February 2021

How to Cite This Article: Ferreira B, Duarte N. Field-Based Randomized Controlled Trial Evaluating the Safety and Effectiveness of a Killed Autologous Vaccine Targeting Streptococcus Dysgalactiae Subsp. Dysgalactiae in a Sheep Flock. Int J Vet Res Allied Sci. 2021;1(1):113-21. https://doi.org/10.51847/tvPjj3GWy5

Introduction

Neonatal infectious arthritis (NIA), commonly known as ‘joint-ill’, is a bacterial infection affecting one or more joints of young lambs, most frequently caused by Streptococcus dysgalactiae subspecies dysgalactiae (SDSD) [1–4]. It generally manifests within the first month of life, causing poor welfare and increased mortality, as well as reduced growth rates in affected lambs [2]. Disease control strategies often rely on prophylactic or metaphylactic use of antibiotics, including products listed under the European Medicines Agency’s categories D and C [2,5–7]. The exact routes of SDSD entry into lambs are not fully understood and may include multiple pathways such as oral exposure, navel infections, ear tag wounds, castration, and tail docking [1,3–5,8]. On endemic farms, SDSD is often widespread in the environment, including lambing pens and grazing fields [9,10], and the pathogen can persist in straw bedding and soil for prolonged periods [8,9], providing ample opportunities for opportunistic infection. Ewes may also serve as direct sources of infection via contaminated teats, milk, vaginal secretions, or wool [3,4,8].

The incubation period of SDSD is not well defined, though some evidence suggests it may range from 24 to 96 hours [11]. Understanding this period can assist clinicians in identifying potential sources and routes of infection, although multiple sources cannot be excluded. Clinical signs are rare in older lambs and adult sheep, despite some carrying the pathogen, particularly in the digestive tract [10]. Early studies showed that blood from recovered lambs could inhibit SDSD growth in vitro, indicating a protective immune response [12].

Based on these observations, vaccinating ewes before lambing may confer protection to lambs through the transfer of SDSD-specific antibodies via colostrum. A successful vaccine is expected to reduce morbidity and mortality, enhance growth performance, and decrease reliance on preventive and therapeutic antibiotics. While autogenous killed vaccines against SDSD have been applied on affected farms with reported benefits, robust clinical trials evaluating their safety and efficacy are limited [10].

This study aimed to evaluate the clinical and immunological effects of a whole-cell killed autogenous vaccine derived from two SDSD isolates from the same farm, using a blinded, randomized controlled trial design.

Methods

Farm background

The study was conducted on a commercial sheep farm in the UK comprising 990 ewes, divided broadly into two groups: Welsh ewes (n = 368) and crossbred ewes (n = 622), primarily Welsh Mules. Lambing commenced in mid-March, with crossbred ewes lambing indoors on straw-bedded yards. These ewes were housed for six weeks prior to lambing, and both ewes and lambs remained indoors for 72 hours post-lambing before being moved to pasture. Welsh ewes lambed outdoors, only being brought inside if intervention was required, such as treatment for infection. In the year prior to the study, around 70 lambs were affected by joint-ill, with similar incidence in previous years.

Vaccine

The autologous vaccine was produced by Ridgeway Biologicals FL09/11/22b (CEVA) using two SDSD isolates obtained from infected lamb joints from the previous lambing season. The bacteria were suspended, inactivated with formaldehyde, emulsified in synthetic oil, and preserved with thiomersal. Each ewe in the vaccination group was scheduled to receive two 2 mL intramuscular doses, 3–4 weeks apart, with the second dose administered 3–4 weeks before lambing.

Safety study

Prior to the main trial, a safety evaluation was conducted in three phases:

1. Two healthy non-pregnant ewes received 2 × 2 mL doses of the vaccine at different intramuscular sites (gluteal and neck muscles) and were monitored closely for one hour, including visual, behavioral, and temperature assessments. Daily monitoring continued for seven days, documenting any temperature changes or injection-site reactions.
2. Subsequently, two healthy pregnant ewes (confirmed via ultrasound and fetal heartbeat visualization) were vaccinated in the same manner and monitored with the same assessments, including a follow-up ultrasound seven days later to confirm fetal viability.
3. Twenty-one days after phase 2, the same two pregnant ewes received a repeat vaccination, with identical monitoring and ultrasound checks as in the previous phase.

Trial design

The study was conducted as a simple, blinded, randomized controlled trial during 2022–2023, including the entire flock. Ewes were randomly assigned to either the vaccination or control group using random numbers **(Figure 1)**. Recruitment occurred in mid-pregnancy (time point 1, **Figure 1**), with each ewe identified by their ear tag and matched to a random number to determine trial allocation. Baseline data, including breed, age, and body condition score, were recorded by JA, LJ, NA, and KK. Ewes in the vaccination group received the autologous vaccine, administered by JA, while control ewes received no placebo or intervention. After 3–4 weeks, all ewes were re-identified, and those in the vaccination group received their second dose. Following this, both researchers and farmers remained blinded to group assignments until the trial concluded.

Post-vaccination, the flock was observed for at least 30 minutes by a veterinarian for immediate adverse reactions. Thereafter, the farmer monitored the flock for signs such as inappetence, isolation, lameness, death, or abortion, with any concerning findings referred promptly to the veterinarian. Close communication between the farmer and veterinarian was maintained throughout the study.

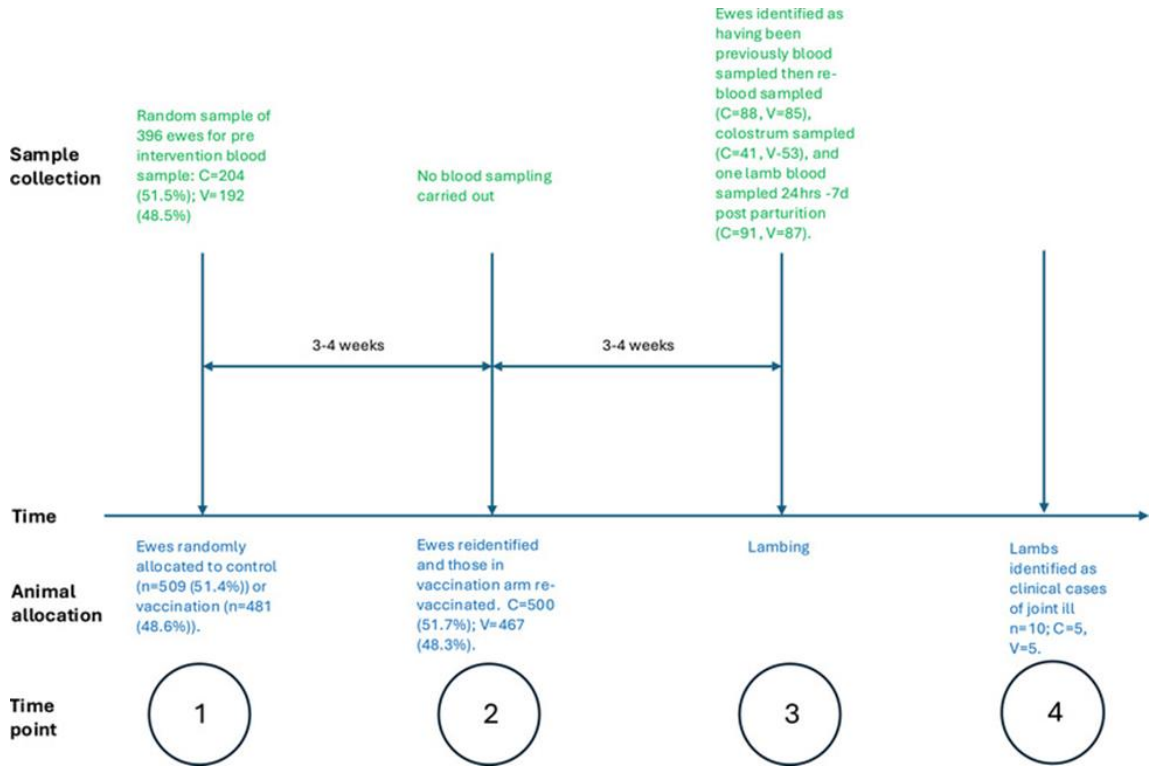

Figure 1. Schematic overview of animal flow throughout the study, showing trial arm allocation, blood and colostrum sampling time points, and identification of clinical cases. C, control arm; V, vaccination arm

A random subset of 200 ewes from each arm was selected for blood sampling before vaccination (time point 1) and at lambing (at least 3 weeks after completion of the full vaccination course; time point 3). Sample size was determined using unpublished pilot ELISA data from 26 Norwegian ewes. Assuming 80% power, 95% confidence level and an effect size of 0.3, a minimum of 175 ewes per group was required; the number was increased to 200 per group to account for potential losses or sampling errors.

In this subset, colostrum was collected from each ewe as soon as possible after parturition (within 24 h) and analysed immediately using a Brix refractometer. One lamb per ewe was blood-sampled between 24 h and 7 days post-lambing; lamb serum total protein was also measured by refractometry. All serum and colostrum samples were tested for antibodies specific to Streptococcus dysgalactiae subsp. dysgalactiae (SDSD) using a validated in-house ELISA. During the lambing period, lambs showing signs of joint ill had joint fluid aspirated from affected joints for bacterial culture and confirmation of SDSD by MALDI-TOF mass spectrometry (Microflex, Bruker Daltonics).

The SDSD-specific ELISA, developed by Moredun Research Institute [13], used plates coated with 100 µL/well of a paraformaldehyde-inactivated clinical SDSD isolate (1:20 dilution in carbonate coating buffer, pH 9.6) overnight at 4 °C. After six washes with PBS-Tween-20 (PBS-T), wells were blocked with 5% donkey serum in PBS for 1 h at room temperature. Test sera were added and serially diluted twofold in sample diluent (2% donkey serum in PBS-T). Each plate included duplicate samples, a standard curve of known high-titre positive serum (1:100 to 1:12,800) and four blank wells containing only diluent. After 1 h incubation, plates were washed, incubated with HRP-conjugated rabbit anti-sheep IgG (1:20,000), washed again, and developed with TMB substrate for 15 min in the dark. The reaction was stopped with H_2SO_4 and optical density read at 450 nm (Thermo Scientific MultiSkan FC).

Statistical analyses

Baseline characteristics of ewes in each arm were compared using descriptive statistics and chi-squared tests. A two-sample test of proportions assessed deviation from the expected 1:1 allocation ratio (hypothesised proportion 0.5).

ELISA data distribution was examined with histograms and formally tested for normality using Shapiro–Wilk tests. Because data were non-normally distributed, differences in ELISA titres between control and vaccinated groups were evaluated using the Kruskal–Wallis rank test. Within the control group, pre-vaccination (time point 1) and lambing (time point 3) serum ELISA values for the same ewes were compared using the Wilcoxon signed-rank test.

Null hypotheses tested

Primary clinical outcome

No difference in the incidence of joint ill in lambs born to vaccinated versus unvaccinated ewes.

Secondary outcomes

2. No difference in baseline (time point 1) serum SDSD-specific ELISA values between control and vaccinated ewes.
3. No difference in serum SDSD-specific ELISA values at lambing (time point 3) between vaccinated and control ewes.
4. No difference in colostrum SDSD-specific ELISA values at lambing between vaccinated and control ewes.
5. No difference in colostrum Brix percentage between vaccinated and control ewes.
6. No difference in lamb serum SDSD-specific ELISA values between offspring of vaccinated and control ewes.
7. No difference in lamb serum total protein concentration between offspring of vaccinated and control ewes.
8. No difference in serum SDSD-specific ELISA values at lambing compared with baseline values within the control ewes.

P-values ≥ 0.05 were interpreted as weak evidence against the null hypothesis; lower p-values were considered strong evidence of a true difference.

Results

Participant flow

A total of 990 ewes were enrolled in the study; their baseline characteristics are presented in **Table 1**. Of these, 509 (51.4%) were allocated to the control arm and 481 (48.6%) to the vaccination arm. A two-sample test of proportions showed no significant deviation from the expected 1:1 allocation (hypothesised proportion 0.5; p = 0.4).

Comparison of baseline characteristics between the two arms using chi-squared tests revealed a statistically significant difference only for age (p < 0.001); no significant differences were observed for other characteristics **(Table 1)**.

Table 1. Initial characteristics of the ewes included in the study

Variable	Vaccinated *n* (%)	Controls *n* (%)	Statistical comparisons between groups
Group (*n* = 990)	481 (48.6)	509 (51.4)	$p = 0.9^{*}$
Age (years) (*n* = 981)			
1	115 (46.8)	131 (53.3)	
2	84 (65.1)	45 (34.9)	
3	110 (50.2)	109 (49.8)	
4	46 (43.0)	61 (57.0)	
5	110 (50.0)	110 (50.0)	
6	9 (15.0)	51 (85.0)	$p < 0.001^{\dagger}$
Breed (*n* = 990)			
Crossbred	299 (48.1)	323 (51.9)	
Welsh	182 (49.5)	186 (50.5)	$p = 0.7^{\dagger}$
Body condition score (*n* = 365)			
1	1 (100)	0 (0.0)	

1.5	0 (0.0)	5 (100)	
2	89 (45.6)	106 (54.4)	
2.5	47 (50.0)	47 (50.0)	
3	35 (50.7)	34 (49.3)	
3.5	1 (100)	0 (0.0)	$p = 0.2^{\dagger}$
Litter size (n = 461)			
1	45 (52.9)	40 (47.1)	
2	164 (48.7)	173 (51.3)	
3	21 (53.9)	18 (46.2)	$p = 0.7^{\dagger}$

Note: * denotes p-values obtained from a one-sample proportion test, whereas † denotes p-values derived from a χ^2 test.

Of the 990 ewes initially enrolled, 967 completed the study in their assigned group: 500 (51.7%) in the control group and 467 (48.3%) in the vaccinated group. Fourteen ewes received only one vaccine dose (either the first or second), and nine were lost to follow-up. A two-sample proportion test showed no significant deviation from an expected 50:50 allocation between the control and vaccinated arms (p = 0.3).

Blood samples were collected at enrollment from a random subset of 396 ewes (prior to vaccination in the vaccinated group). Of these, 204 (51.5%) were from the control group and 192 (48.5%) from the vaccinated group (p = 0.9). At lambing (≥3 weeks after the second vaccine dose), 178 of these ewes were recaptured and resampled. Most ewes that could not be resampled belonged to the Welsh breed, as catching them at pasture post-lambing risked mismothering. This yielded paired (pre- and post-vaccination) blood samples for 88 (50.9%) control ewes and 85 (49.1%) vaccinated ewes.

Colostrum was collected from 41 (43.6%) control ewes and 53 (56.4%) vaccinated ewes. Blood samples were also obtained from lambs, with 91 (51.1%) from control-group dams and 87 (48.9%) from vaccinated-group dams.

Vaccine safety

No vaccine-related adverse reactions were observed, apart from a transient increase in body temperature 24 hours after vaccination.

Outcomes

Primary outcome

Null hypothesis 1: Vaccination would not reduce the incidence of joint-ill in lambs born to vaccinated versus unvaccinated ewes.

Only ten cases of joint-ill were diagnosed (compared with approximately 70 cases in the previous year). Five affected lambs were born to unvaccinated ewes and five to vaccinated ewes **(Table 2)**. Because of the low number of cases, no further statistical analysis of risk factors was conducted. The null hypothesis could not be rejected.

Table 2. Characteristics of lambs diagnosed with joint-ill

Ewe ID	Treatment Group	Ewe Age (years)	Ewe Breed	Litter Size	Lamb Sex	Ewe Serum ELISA (pre-vaccination)	Ewe Serum ELISA (at lambing)	Colostrum ELISA	Lamb Serum ELISA (early post-partum)	Colostrum Brix (%)	Lamb Total Serum Protein (g/L) *
7	Control	6	Crossbred	2	Male	519.3	1612.9	589.2	161.8	30	38
16	Control	5	Crossbred	2	Male	32.3	145.1	–	160.2	30	–
329	Control	5	Crossbred	2	Male	292.7	5.2	663.2	667.6	30	49
363	Control	2	Crossbred	2	Male	1276.0	937.0	370.8	835.5	29	47
385	Control	1	Crossbred	2	–	–	–	–	–	–	–
96	Vaccinated	3	Crossbred	3	–	–	–	–	–	30	–

128	Vaccinated	3	Crossbred	2	Male	163.1	191.0	–	321.7	–	43
246	Vaccinated	5	Crossbred	2	–	–	–	–	–	18	–
12	Vaccinated	2	Crossbred	2	Female	466.2	524.7	–	1340.8	30	42
342	Vaccinated	3	Crossbred	2	Female	474.2	467.4	428.6	55.5	19	28

Secondary outcomes (Table 3)

Analysis of the ELISA results revealed predominantly right-skewed distributions across the study groups, and Shapiro–Wilk testing confirmed non-normality; consequently, non-parametric methods were applied for statistical comparisons.

Null hypothesis 2: There would be no difference in baseline serum ELISA levels between control and vaccinated ewes at study entry (time point 1).

At time point 1, the control ewes (n = 88) had a median ELISA value of 422.5 (IQR: 174.0–816.1), whereas the vaccinated ewes (n = 85) had a median of 441.8 (IQR: 144.1–703.3). The Kruskal–Wallis test indicated no significant difference (p = 0.5), so this hypothesis remained supported.

Null hypothesis 3: Serum ELISA levels at lambing (time point 3) would not differ between vaccinated and control ewes.

At time point 3, median values were 571.0 (IQR: 191.7–1159.6) in controls and 873.2 (IQR: 442.1–1532.0) in vaccinated ewes. Kruskal–Wallis testing revealed significantly higher levels in vaccinated ewes (p = 0.02). Adjusting for baseline values, the change from pre-vaccination to lambing was 156.0 (IQR: −215.4 to 657.1) for controls and 393.7 (IQR: 58.4–946.1) for vaccinated ewes, again showing a significant increase (p = 0.02), leading to rejection of this null hypothesis.

Null hypothesis 4: Colostrum ELISA levels at lambing would be similar between groups.

Median colostrum ELISA was 1010.4 (IQR: 589.2–2243.4) in controls (n = 41) and 1134.1 (IQR: 428.6–1883.3) in vaccinated ewes (n = 53), with no significant difference (p = 0.8), so the hypothesis was retained.

Null hypothesis 5: There would be no difference in colostrum Brix percentage between the groups at lambing.

Median Brix readings were 29% (IQR: 24–30%) for control ewes (n = 211) and 30% (IQR: 25–30%) for vaccinated ewes (n = 217), showing no statistical difference (p = 0.1). This null hypothesis was therefore not rejected.

Null hypothesis 6: Serum ELISA levels in lambs would not differ based on maternal vaccination status at lambing.

Median serum ELISA was 662.3 (IQR: 211.3–1469.2) in lambs from control ewes (n = 91) and 1171.9 (IQR: 372.3–1903.5) in lambs from vaccinated ewes (n = 87). The Kruskal–Wallis test indicated significantly higher levels in the vaccinated group (p = 0.02), leading to rejection of this hypothesis.

Null hypothesis 7: Total serum protein in lambs would be unaffected by maternal vaccination at lambing.

Both groups showed a median serum total protein of 6.9 g/dL (IQR: 6.1–7.7 in controls, 6.0–7.7 in vaccinated; n = 90 and 87), with no significant difference (p = 0.7). The null hypothesis was not rejected.

Null hypothesis 8: Serum ELISA in control ewes would remain unchanged between baseline and lambing.

Among control ewes only (n = 88), comparison of values at time points 1 and 3 revealed an overall increase (median 422.5 [IQR: 174.0–816.1] at baseline vs. 571.0 [IQR: 191.7–1159.6] at lambing; p = 0.02), resulting in rejection of this null hypothesis.

Table 3. ELISA values and statistical comparisons between control and vaccinated groups

Measurement	Vaccinated group median (IQR)	Control group median (IQR)	Statistical test (Kruskal–Wallis)
Baseline serum ELISA (n = 88 controls; n = 85 vaccinated) – Time point 1	441.8 (144.1–703.3)	422.5 (174.0–816.1)*	p = 0.5
Serum ELISA at lambing (n = 88 controls; n = 85 vaccinated) – Time point 3	873.2 (442.1–1532.0)	571.0 (191.7–1159.6)*	p = 0.02
Change in serum ELISA from baseline to lambing (n = 88 controls; n = 85 vaccinated) – Time point 3	393.7 (58.4–946.1)	156.0 (−215.4 to 657.1)	p = 0.02
Colostrum ELISA at lambing (n = 41 controls; n = 53 vaccinated) – Time point 3	1134.1 (428.6–1883.3)	1010.4 (589.2–2243.4)	p = 0.8

Lamb serum ELISA at lambing (n = 91 controls; n = 87 vaccinated) – Time point 3	1171.9 (372.3–1903.5)	662.3 (211.3–1469.2)	p = 0.02

*Values compared within the control group using a Wilcoxon signed-rank test, p = 0.02.

Discussion

Study design and limitations

Overall, randomisation was largely effective, with similar proportions of sheep allocated to each trial arm across the analysed subcategories. Minor discrepancies were noted in the age distribution, particularly for ewes aged 2, 4, and 6 years, between the control and vaccinated groups; however, these were considered unlikely to have influenced the outcomes.

The ELISA analysis was primarily restricted to crossbred ewes, as following up Welsh ewes that lambed outdoors posed practical and welfare challenges. This limitation reduced the total number of samples analysed and consequently decreased the precision of the resulting estimates.

While the ELISA assay employed was specific for SDSD IgG, it has not yet been calibrated against an exact IgG concentration. Therefore, although relative comparisons can be made between groups, individual ELISA values cannot be directly translated into precise IgG concentrations.

Generalisability

Given the study was conducted under real-world farm conditions, similar outcomes could be expected in comparable settings. A whole-flock or cluster-level vaccination approach, rather than individual randomisation, might enhance the vaccine's effect by promoting herd immunity and potentially reducing environmental contamination with SDSD.

Safety

In the safety study, the vaccine's only observed physiological effect was a transient increase in body temperature, aside from its intended serological response. No adverse events, such as abortions, inappetence, or mortality, were noted during the main field trial, indicating that the vaccine was well-tolerated.

Interpretation of results

Vaccination did not reduce the incidence of joint-ill in lambs, with five cases observed in both the control and vaccinated groups. The overall number of cases (n = 10) was substantially lower than expected, as the farmer reported around 70 cases in previous seasons. This apparent reduction could be due to chance or possibly a decreased environmental load of SDSD resulting from vaccination. Studies using cluster randomisation alongside environmental sampling could help clarify this effect.

Pre-vaccination serum ELISA values were highly variable, which is consistent with expectations in an endemic flock, as IgG levels are influenced by individual exposure, timing of exposure, and the ewe's immune response. At lambing, ELISA values increased in both groups, with vaccinated ewes showing a significantly greater rise, suggesting a vaccine-induced response. The specific IgG concentration required for protection remains unknown, highlighting the need for in vivo challenge studies to determine protective thresholds.

The increase in ELISA values in control ewes was unexpected. A signed-rank test indicated that this rise was unlikely to have occurred by chance, suggesting that other factors were involved. Close housing of ewes during lambing, both indoors and outdoors, may have increased the likelihood of environmental SDSD exposure. In other infectious diseases, ewes experience periparturient immune suppression, such as the "periparturient rise" seen with gastrointestinal nematodes [14], increasing susceptibility to infection. Similarly, reduced immunity to SDSD at lambing could have contributed to increased environmental shedding of the pathogen. Further investigation is warranted to understand these mechanisms.

No differences were observed in colostrum ELISA values between groups. In this field study, uncontrolled timing of lambing and variable intervals between birth and colostrum collection could have allowed some lambs to suckle before sampling, potentially lowering colostrum antibody density. This hypothesis is supported by the finding that lambs born to vaccinated ewes had higher serum ELISA values than those from control ewes, reflecting maternal vaccination responses. To assess whether this difference was due to systematic variations in colostrum intake, serum total protein levels were compared, with no differences detected, suggesting that effective maternal transfer, rather than variations in colostrum volume or timing, accounted for the elevated lamb ELISA values. Therefore,

successful vaccination against joint-ill depends not only on vaccine administration but also on lambs receiving sufficient high-quality colostrum shortly after birth.

Conclusions

Vaccination did not reduce the number of lambs affected by joint-ill compared to unvaccinated ewes. However, vaccination significantly increased SDSD-specific ELISA values in both ewes and their lambs. Further research is needed to determine the herd immunity potential of the vaccine and the SDSD-specific IgG concentrations required to confer protection against infection.

Acknowledgments: This study was supported by Arwain DGC and AHDB, and Naseem Ahmed was supported by the University of Liverpool MRes programme.

Conflict of Interest: None

Financial Support: None

Ethics Statement: The study was approved under the Animals (Scientific Procedures) Act 1986, Home Office Project License Number PP1744936. Ethical approval for the study was obtained from the University of Liverpool, number VREC 1242. The vaccine trial was licensed by the Veterinary Medicines Directorate with Animal Test Certificate ATC/B 56268/0002. Written informed consent was obtained from the farmer.

References

1. Watkins GH, Sharp MW. Bacteria isolated from arthritic and omphalatic lesions in lambs in England and Wales. Vet J. 1998;156(2):235-8.
2. Ridler A, Hickson R, Griffiths K, Pettigrew E, Kenyon P. Effects of Streptococcus dysgalactiae polyarthritis on lamb growth and mortality and risk factors for disease. Small Rumin Res. 2019;177:25-8.
3. Lacasta D, Ferrer LM, Ramos JJ, Loste A, Bueso JP. Digestive pathway of infection in Streptococcus dysgalactiae polyarthritis in lambs. Small Rumin Res. 2008;78(2-3):202-5.
4. Cornell RL, Glover RE. Joint-ill in lambs. Vet Rec. 1925;5(21):833-9.
5. Rutherford SJ, Jeckel S, Ridler A. Characteristics of sheep flocks affected by Streptococcus dysgalactiae arthritis. Vet Rec. 2015;176(17):435.
6. European Medicines Agency. EMA categorisation of antibiotics in the European Union. 2019. www.ema.europa.eu/en/documents/report/categorisation-antibiotics-european-union-answer-request-european-commission-updating-scientific_en.pdf
7. Jackson LP, Higgins HM, Duncan JS. A cross-sectional survey of farmer-reported prevalence and farm management practices associated with neonatal infectious arthritis ("joint-ill") in lambs on UK sheep farms. Front Vet Sci. 2024;11:1489751.
8. Rutherford SJ, Rycroft AN, Ridler AL. Sources of Streptococcus dysgalactiae in English and Welsh sheep flocks affected by infectious arthritis (joint-ill). Vet Rec. 2014;174(23):579.
9. Jackson LP. Preventing neonatal infectious arthritis in lambs: sources, transmission and characterisation of Streptococcus dysgalactiae. PhD Thesis. Department of Livestock & One Health, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool; 2024.
10. Smistad M, Tollersrud TS, Austbø L, Porcellato D, Wolff C, Asal B, et al. Molecular detection and genotype characterization of Streptococcus dysgalactiae from sheep flocks with outbreaks of infectious arthritis. Vet Microbiol. 2021;262:109221.
11. Blakemore F. Joint-ill polyarthritis of lambs in East Anglia. Vet Rec. 1939;51(26):1207-19.
12. Blakemore F, Elliott SD, Hart-Mercer J. Studies on suppurative polyarthritis (joint-ill) in lambs. J Pathol Bacteriol. 1941;52(1):57-83.
13. Jackson LP, Timofte D, Ballingall KT, Duncan JS. The development, validation and application of an indirect enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to Streptococcus dysgalactiae subspecies dysgalactiae in lamb and ewe sera. Res Vet Sci. 2025;187:105604.

14. Hamer K, McIntyre J, Morrison AA, Jennings A, Kelly RF, Leeson S, et al. The dynamics of ovine gastrointestinal nematode infections within ewe and lamb cohorts on three Scottish sheep farms. Prev Vet Med. 2019;171:104752.