Evaluation of the efficacy of multivalent vaccines for the control, treatment and prevention of footrot in merino sheep flocks

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Abstract:

Footrot remains a significant challenge in ruminants, causing substantial economic losses and posing concerns to animal health and welfare. This detrimental disease is caused by the interaction between the bacterium *Dichelobacter nodosus*, susceptible feet and the environment. Control of this disease through traditional management methods, such as antibiotic use, foot paring and foot bathing, are labour intensive, expensive, and dependent on environmental factors and disease identification. Vaccination serves as a treatment and preventative measure for sheep affected with footrot. With ten distinct serogroups of *D. nodosus* (A-I & M), immunity is serogroup-specific. The presence of multiple serogroups in a multivalent vaccine may result in diminished protection due to antigenic competition. The commercial multivalent vaccine, Footvax®, containing serogroups A-I, has been the subject of limited studies, demonstrating varying efficacy. The lack of recent research exploring this vaccine and its effectiveness indicates a critical gap in our current knowledge. This investigation evaluates the efficacy of the commercial multivalent vaccine, Footvax®, on 400 sheep located in the northern midlands of Tasmania. Diagnostic testing through PCR analysis detected multiple serogroups on the farm, including serogroup M, which is absent from this multivalent vaccine. Footvax®'s oil adjuvant induced site lesions in 56% of sheep, posing significant welfare concerns. Both treatment groups demonstrated improvement rates of 55-60% and curative rates of 43%. Vaccinated animals also exhibited elevated antibody titres following initial vaccination, indicating an increased protection against footrot. These findings highlight the efficacy of Footvax® in controlling footrot for short durations and indicate opportunities for improvement in the vaccine formulation.

Key words:

Footrot, *Dichelobacter nodosus*, serogroup, disease control, Footvax®, vaccine response

Introduction:

Footrot is a contagious disease affecting the feet of cloven-hoofed animals. The disease initially presents as inflammation of the interdigital skin and depending on the virulence of the infecting strain may progress to separation of the hoof from the horn (Beveridge, 1941; Stewart and Claxton, 1993). Footrot is caused by the interaction of the bacterium *Dichelobacter nodosus* with the interdigital skin and hoof epithelium of susceptible sheep predisposed by environmental conditions (Beveridge, 1941; Egerton *et al.*, 1969). Severe lameness is observed in affected animals, resulting in reductions in body weight and welfare. In 2022, the cost of footrot in Australia was estimated at $82.2 million (Shephard *et al.*, 2022).

Ten serologically distinct strains (serogroups) of *D. nodosus* (A, B, C, D, E, F, G, H, I and M) are recognised in Australia, and are based on sequence and antigenic variations of the *fimA* gene that encodes for the Type IV fimbriae (Claxton *et al.*, 1983; John *et al.*, 1999; Kennan *et al.*, 2001; Zhou *et al.*, 2001). These Type IV fimbriae are important virulence factors of *D. nodosus* and essential for penetrating the hoof, protease secretions and twitching motility (Egerton *et al.*, 1989; Kennan *et al.*, 2001; Han *et al.*, 2008). Polymerase chain reactions (PCR) assays utilise differences in this *fimA* gene to determine the serogroups present in hoof lesion samples (Dhungyel *et al.*, 2002; McPherson *et al.*, 2018).

Conventional footrot management consists of a combination of foot bathing, foot paring and antibiotic administration (Egerton *et al.*, 1968; Abbott and Lewis, 2005). Although they have proven to be effective at reducing prevalence during non-transmission periods, they are labour intensive and costly (Abbott and Lewis, 2005; Dhungyel *et al.*, 2013). In areas where uniform rainfall patterns are present, footrot often becomes endemic, making it difficult to control and eradicate (Dhungyel *et al.*, 2013). Footrot vaccines are becoming increasingly sought after by producers due to their cost-effective nature, protective and therapeutic effects, and ability to

implement regardless of environmental conditions and current disease status (Dhungyel *et al.*, 2013).

The vaccination strategy implemented is dependent on cost, number of serogroups present, ability to maintain strict biosecurity and whether control or elimination is the desired outcome (McPherson *et al.*, 2021). Outbreak specific mono- and bivalent vaccines have proven successful at controlling, eliminating and eradicating footrot in Bhutan (Gurung *et al.*, 2006), Nepal (Egerton *et al.*, 2002) and Australia (Dhungyel *et al.*, 2008; Dhungyel *et al.*, 2013). When more than two serogroups are present, sequential vaccines are administered with an inter-vaccination interval of three months (Dhungyel *et al.*, 2013). The high costs associated with sampling and specific mono- and bivalent vaccine formulation has resulted in producers opting for multivalent vaccines.

The commercially available multivalent vaccine (Footvax®) contains antigens representing nine of the *D. nodosus* serogroups (A-I). Limited, short-term protection is associated with multivalent vaccines due to antigenic competition (Schwartzkoff *et al.*, 1993; Hunt *et al.*, 1994). Since its commercial release in 1986, studies have tested and evaluated this product in Australia (Schwartzkoff *et al.*, 1993; O'Meara *et al.*, 1993; Raadsma *et al.*, 1994; Hunt *et al.*, 1995) and other countries (Mulvaney *et al.*, 1984; Lewis *et al.*, 1989; Liardet *et al.*, 1989; Duncan *et al.*, 2012; Ennen *et al.*, 2009). These studies have demonstrated varied responses and protection levels following vaccination. As Footvax® is the only multivalent footrot vaccine in Australia, its withdrawal from the market in 2008, due to strict biosecurity rules regarding Bovine Spongiform Encephalitis (BSE), was detrimental to the industry and as such the vaccine was reintroduced in 2020 (Shephard *et al.*, 2022). Despite this recent return to market, Footvax® does not contain M antigens due to the serogroups recent identification and

low prevalence in flocks, making it ineffective in flocks where serogroup M is present (Dhungyel *et al.*, 2015).

The efficacy of the Footvax® vaccine at controlling and eliminating footrot has not been investigated recently, however, previous studies have highlighted the positive effect vaccination can have on animal welfare, production and management costs (Ennen *et al.*, 2009; Dhungyel *et al.*, 2013). This study aims to determine the efficacy of the commercial multivalent vaccine, Footvax®, at controlling, treating, and preventing footrot in a merino flock where multiple serogroups are present. This evaluation will enhance and update existing knowledge surrounding Footvax®, enabling livestock producers to make better informed decisions regarding its suitability for implementation on their farm.

Materials/Methods:

Trial Design:

A mob of one-year hogget merino sheep were selected from a farm located in Cressy, Tasmania, to participate in this randomised control trial. A total of 400 sheep from the mob were randomly assigned to either the control or vaccination group, with every alternate sheep in the race subjected to the vaccination protocol. The control group received no treatment, whilst the vaccination group received two 1mL doses of Footvax® administered via subcutaneous injection into the neck at day 0 and 30. Animals were stood in a 20% zinc sulphate foot bath solution for one hour following each inspection, to ensure appropriate animal welfare standards were met, in line with ethics approval, and to continue with current farm management practices.

A total of three inspections were conducted at 0, 30 and 60 days after the initial vaccination. At each inspection, all feet were inspected and assigned a score (0 to 4) as per the scoring system described by Egerton and Roberts (1971) (Table 1). To ensure an unbiased score, the inspecting veterinarian had no knowledge of which treatment group the animals were in. The highest scores for each foot were added together, taking into account the severity of lesions, to give the total weighted foot score (TWFS) for each sheep (Whittington and Nicholls, 1995). The TWFS for each sheep at each inspection can range from 0 to 64. In addition, the presence and size of any granulomas at the site of injection were also recorded as either small (< 1 cm), large (> 1 cm), or ruptured. Improvement and curative rates were calculated for each treatment group in two stages, day 0 to 30 and day 30 to 60, with results averaged to ensure disease presented at day 30 was taken into consideration.

$$Improvement\ rate\ (\%) = \frac{Number\ of\ sheep\ improved\ at\ completion}{Number\ of\ sheep\ affected\ at\ start} \times 100$$

$$Curative\ rate\ (\%) = \frac{Number\ of\ sheep\ cured\ at\ completion}{Number\ of\ sheep\ affected\ at\ start} \times 100$$

Animal Ethics approval:

This trial and its methods were approved by The University of Sydney's Animal Ethics Committee (AEC Approval Number 2022/2194).

Collection of lesions samples and PCR testing:

Swab samples were collected from the active part of the foot lesions using a sterile cotton tipped swab (CLASSIQSwabs; Coapn Italia, Italy). Foot swabs were collected from up to 20 animals prior to commencement of the trial and at each inspection (0, 30 and 60 days) and placed in 1.5mL screw-cap microcentrifuge tubes (SSIBio, Lodi, USA) containing 300µL of cell lysis buffer (Promega Cooperation, USA). Chromosomal DNA of *D. nodosus* was extracted directly from the swabs using the Gram-negative bacteria protocol from the Wizard Genomic DNA purification kit (Promega Corporation, USA). Briefly, the swabs were discarded from the microcentrifuge tubes and 300µL of nuclei lysis solution added, inverted six times, and incubated at 80°C for 5 minutes. 200µL of protein precipitation solution was added to each tube, vortexed at high speed for 15s to mix the solution, before cooling on ice for 5 minutes. The tubes were then centrifuged at 16,000 x g for 3 minutes, and 800µL of the supernatant aspirated and transferred to a clean, labelled 1.5 mL microcentrifuge tube containing 600µL of isopropanol. The tubes were inverted four times to mix and centrifuged at 16,000 x g for 3 minutes. The supernatant was discarded and 600µL of 70% ethanol was added to each microcentrifuge tube. After inverting six times to wash the DNA pellet, the tubes were centrifuged at 16,000 x g for 3 minutes and ethanol aspirated. Each tube was left to air-dry for 10 minutes before 100µL of DNA rehydration solution was added. The resuspended DNA was incubated at 4°C overnight and tested within 48 hours.

The fimA gene of *D. nodosus* was amplified and detected through conventional multiplex PCR assays as described by Dhungyel *et al.* (2002). A common forward primer and serogroup specific reverse primers were used (Table 2). Serogroups A to I were split into three triplex assays, with each 0.5mL microfuge tube containing a final mixture of 6uL of Multiplex Mastermix (Qiagen, Hilden, Germany), 2µL of Q Solution (Qiagen, Hilden, Germany), 6uL of

nuclease free water, 1uL of forward primer, 1μL of each of the three reverse primers, and 1μL of template DNA. Reaction conditions for serogroups A, B, C, D, E, and F consisted of an initial denaturation phase of 95°C for 15mins, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 60°C for 60s, extension at 72°C for 90s, and a final extension step of 72°C for 10mins. Serogroups G, H, and I utilised the same reaction protocol with a small difference in annealing temperature, 60.2°C. Serogroup M was run in a singleplex assay, with each microfuge tube containing 10μL of Taq Mastermix (Qiagen, Hilden, Germany), 7μL of nuclease free water, 1μL of forward primer, 1μL of serogroup M specific reverse primer and 1μL of template DNA. Thermocycling protocol for this serogroup consisted of initial denaturation of 95°C for 10mins, followed by 35 cycles of denaturation at 95°C for 30s, annealing at 57°C for 30s, extension at 72°C for 60s, and a final extension of 72°C for 5mins. PCR products were run on a 2% agarose gel, stained with 6.5μL of RedSafe dsDNA stain (iNtRON Biotechnology, South Korea), at 100 volts for 90 minutes and photographed under ultraviolet illumination using the Gel Documentation System (Bio-Rad, USA). In each PCR assay a *D. nodosus* prototype strain A1001 (general footrot reference strain) and nuclease-free water were included as positive and negative controls. Successful PCRs were defined as one where an appropriate amplicon was present for the positive control, with no visible amplicons for the negative control.

Collection and testing of blood samples:

Up to 20 randomly selected animals from each treatment were identified and placed into a sub-group for blood collection. Blood samples were collected by jugular venepuncture at each inspection (0, 30 and 60 days). Blood samples were centrifuged at 3,000rpm for 20mins, with the resultant serum separated and stored at 4°C until required for testing. Sera was then diluted

in micro-titre plates using saline in a serial doubling dilution series from 1:10 to 1:20,480. Equal volumes of 100uL of antigen were added to each well, resulting in final serum dilutions within 1:20 and 1:40,960. Micro-titre plates were incubated overnight at 37°C and inspected the following day for floccular agglutination. Agglutination titres were expressed as a reciprocal of the highest serum dilution that resulted in detectable agglutination.

Weather Data:

Weather data, including air temperature and rainfall, were obtained from the Bureau of Meteorology's weather station located at Launceston Airport (Site No. 091311), approximately 18km from the trial site at Connorville farm.

Statistical Analysis:

Data was entered into an Excel spreadsheet and imported to RStudio version 4.3.1 (RStudio, USA) for analysis. TWFS and blood agglutination titres recorded at 0, 30 and 60 days were used for statistical analysis. The factors 'treatment' and 'day' were used as explanatory variables for both data sets, with the addition of 'serogroup' for the blood agglutination data set. Measures of central tendency and dispersion were initially checked, with log transformations conducted on the TWFS data to reduce outlier prevalence. Linear mixed effects models were built to evaluate the explanatory variables association with the outcomes after adjusting for treatment, day, and serogroup. Individual sheep were included as random effects to account for repeated measures. For ease, the blood agglutination data set was split into time periods and analysed separately. Mode assumptions were evaluated using a post hoc analysis, with contrasts between factors obtained and analysed to determine significance.

Results:

Clinical observations:

The majority of sheep inspected during the trial displayed some degree of footrot, with only 8% displaying no clinical disease at any point in time. Approximately 8% of sheep from both treatment groups had foot scores that progressively worsened throughout the trial. The control group exhibited a curative rate of 42.8%, while the vaccinated group demonstrated a curative rate of 43.3%, accompanied by respective improvement rates of 54.9% and 59.6%. When comparing the treatments separately, the vaccinated group was not statistically different at any time point, whereas the control group was statistically different at Day 60 (Figure 1). The interactions between treatment groups were significant at Day 0 ($P = 0.0005$) and 30 ($P < 0.0001$), but not at Day 60 ($P = 0.8797$).

Vaccine Site Reaction:

56% of vaccinated animals exhibited reactions at the site of injection. Throughout the trial duration, 33% and 23% of vaccinated sheep developed small and large reactions, respectively. Only two sheep presented with infected, ruptured lesions at Day 30.

Serogrouping of *D. nodosus*:

The serogroups of *D. nodosus* detected by PCR throughout the trial are reported in Table 3. 14 samples were collected pre-trial, 20 samples were collected at Day 0, and 10 samples were collected at both Day 30 and 60. Serogroups B, C, E, H, I and M were found throughout the trial, with Serogroup M most prevalent overall (Table 3).

Micro-titre serum agglutination test:

Antibody titres in the serum samples increased from the baseline following initial vaccination ($P < 0.05$). The interaction between serogroups and treatments at Day 0 were not statistically significant ($P = 0.4179$). All other interactions between serogroups and treatments at Day 30 and 60 were significant, except Serogroup I at Day 30 ($P = 0.2825$) and Serogroup B at Day 30 ($P = 0.9991$) and 60 ($P = 0.0873$). Serogroups B, C and E did not meet or exceed the minimum antibody level (8) required for protection at any time point (Egerton *et al.*, 1987) (Figure 2).

Environmental data:

During the spring season, temperatures remained within the historical average range, with mean temperatures ranging from 6.5°C to 18.1°C (Figure 3). The season was characterised by average minimum and maximum daily temperatures of 3.8°C and 13.3°C, respectively (Figure 3). Rainfall was consistent during the winter months averaging 68mm per month (Figure 4).

Discussion:

Control of footrot is a complex challenge that frequently involves treatments such as antibiotic injections, foot bathing or vaccination. While the prompt application of antibiotics on individual animals has proved effective (Wassink *et al.*, 2010), employing these treatments at a flock level is impractical due to the expense of products and labour-intensive requirements (Green and Clifton, 2018). Foot bathing, a commonly favoured management practice among farmers, exhibits varied effectiveness and ultimately requires multiple weather dependent applications (Allworth and Egerton, 2018). Consequently, the appeal of vaccination has grown among frustrated farmers due to its ease of administration, therapeutic effect, cost-effective nature and ability to be used regardless of environmental condition or disease status (Dhungyel *et al.*, 2013). Footrot immunity is serogroup specific, requiring the accurate identification of all serogroups present in the flock (Claxton *et al.*, 1983). Multivalent vaccines, containing all serogroups, offer a convenient treatment option for farmers that minimises costs associated with serogroup testing. Previous studies have investigated the effectiveness of the commercial multivalent vaccine, discovering a degree of success at controlling the disease (Mulvaney *et al.*, 1984; Liardet *et al.*, 1989; Ennen *et al.*, 2009; Duncan *et al.*, 2012).

The reported curative rate of the Footvax® vaccine is up to 60% (Coopers®, 2020), exceeding the trials observed rate of 43%. Previous investigations have documented curative rates ranging from 35% (Mulvaney *et al.*, 1984) to 100% (Liardet *et al.*, 1989). It is important to note that Mulvaney *et al.* (1984) and Liardet *et al.* (1989) utilised European sheep breeds, which are known to exhibit more favourable responses to vaccination when compared to the merino breed (Emery et al., 1984). Discrepancies between research findings and the declared Footvax® curative rate may be due to environmental influences and varying trial designs, emphasising the need for comprehensive research to establish a more uniform curative rate that considers causative factors.

The unexpected similarity in curative and improvement rates between the control and vaccinated animals in this study challenges previous findings in this field (Mulvaney *et al.*, 1984; Liardet *et al.*, 1989). The significant decline in disease prevalence within the control group at day 60 was unexpected and the likely cause of the similar curative and improvement rates. These decreases appeared to occur independent of any management interventions, as similar reductions were not observed in the vaccinated group. The consistent, favourable weather conditions experienced throughout the trial indicate that environmental factors were likely not driving these findings (Roberts and Egerton, 1969; Whittington, 1995; Dhungyel *et al.*, 2013). A reduced overall prevalence of contagious disease within the flock, attributed to the therapeutic and prophylactic effects of vaccination (Dhungyel *et al.*, 2013), may have contributed to a reduced likelihood of new disease development, accounting for these results. As these findings are unique within this field of study, further investigation is required to validate these findings and determine the potential advantages of vaccinating solely disease-affected animals for the entire mob.

Footvax® has been documented to provide protection for up to 10-12 weeks following vaccination (Hunt *et al.*, 1994; Coopers®, 2020). An extended observation period of at least 120 days post-initial vaccination would have been optimal for a comprehensive examination of the vaccines impact on the flock and its protective efficacy after 12 weeks. However, due to time constraints associated with deadlines, the trial was concluded at the 60 day mark. Subsequent comprehensive investigations are required to gain a greater understanding of the influence time has on the interaction between vaccination and disease prevalence.

Footrot is commonly associated with areas characterised by uniform rainfall patterns (Dhungyel *et al.*, 2013), similar to the climatic conditions observed in Cressy during the trial duration. Despite the farms implementation of rigorous foot bathing methods upon departure

293 from the shed, control and eradication of this disease remains a challenge. This difficulty can
294 be attributed to the continuous moisture present in the pasture, previously identified as a crucial
295 factor for the persistence and spread of footrot (Graham and Egerton, 1968). The combination
296 of consistent rainfall, which sustain soil moisture, and mild winter temperatures, stimulated
297 luscious pastures and created an environment that favoured footrot expression (Beveridge,
298 1941; Egerton *et al.*, 1969). These findings align with the knowledge that footrot transmission
299 occurs when mean ambient temperatures exceed 10°C (Graham and Egerton, 1968). Although
300 the trial took place prior to spring, the weather throughout the current year created optimal
301 conditions for footrot expression and spread. These environmental conditions played an
302 important role in providing the appropriate circumstances to evaluate the efficacy of the
303 multivalent vaccine.

304 Antigenic competition is described as the tendency of individual vaccine components to
305 generate a reduction of antibodies (Hunt *et al.*, 1994; Raadsma *et al.*, 1994). Although the
306 mechanisms behind this phenomenon remain extensively debated throughout literature, it is
307 widely acknowledged that antigenic competition is responsible for the limited efficacy
308 commonly observed in multivalent vaccines. Our findings support this knowledge, with
309 inadequate levels of vaccinal antibodies observed across the majority of targeted serogroups
310 (Thorley and Egerton, 1981). Vaccination had the least effect on serogroup B antibody titres
311 and is likely attributed to antigenic competition between the two serogroup B sub-types
312 included in the Footvax® vaccine (Infopest, 2023). Although groups were randomly allocated,
313 initial variations in the disease status of the groups were evident. The vaccinated group
314 exhibited slightly elevated antibody titres at day 0, potentially contributing to greater protection
315 and subsequent decreased expression of the disease. To enhance future trial designs, the
316 incorporation of pre-vaccination weights and body condition assessments could provide
317 balanced groups and valuable insights into the welfare implications of the vaccine.

The limited development of natural immunity following recovery from footrot is associated with the localised infection confined to the avascular epidermis (Beveridge, 1941). Prior research has suggested that infection may lead to slight increases in antibody titres, whereas vaccination develops a greater protection against footrot (Egerton and Roberts, 1971). Our investigation supports these findings, revealing slight elevations in antibody titres within the control group. Individual comparisons indicated that sheep that had been cured of footrot exhibited a greater antibody response for specific serogroups, likely corresponding to the serogroup causing disease. Although a decline in elevated antibody titres within the control group was not observed, due to the short duration of the trial, studies in goats discovered antibody titres resulting from *D. nodosus* infection declined rapidly following recovery, returning to pre-infection levels within 3 months (Ghimire *et al.*, 2002). Although goats and sheep share similar characteristics, footrot infections in goats typically manifest milder symptoms and exhibit varying behaviours when compared to those observed in sheep (Ghimire *et al.*, 1999). Consequently, future research should prioritise the use of sheep to determine the extent and duration of protection provided from disease recovery.

Analysis of serogroups within the flock provides crucial insights into the complexities associated with ovine footrot epidemiology. The Australian system of classification of *D. nodosus* utilises slide agglutination with unabsorbed antisera (Claxton *et al.*, 1983) and PCR (Dhungyel *et al.*, 2002), while the alternative method of absorbed antisera in tube agglutination tests is used in Britain (Thorley and Day, 1986) and the United States of America (Gradin *et al.*, 1993). Advancements in serogroup-specific PCR have demonstrated its greater sensitivity, accuracy and speed compared to conventional agglutination methods (Dhungyel *et al.*, 2002). Modern PCR techniques were employed in this trial, with direct isolation of bacteria from foot swabs utilised (McPherson *et al.*, 2018), as opposed to the previously time-consuming process of culturing bacteria pre-PCR (Dhungyel *et al.*, 2002). The inclusion of the recently developed

343 forward and reverse primers for serogroup M has allowed for consistent PCR detection
344 methods across all serogroups (Dhungyel *et al.*, 2017). Analysis of the flock revealed the
345 presence of multiple serogroups, including B, C, E, H, I and M. Serogroup M was first
346 documented in Australia on King Island, Tasmania, in 2015 (Dhungyel *et al.*, 2015), and has
347 likely spread to other parts of Tasmania including this flock. The absence of this serogroup
348 from Footvax® presents significant challenges in effectively managing and eradicating flocks
349 with serogroup M.

350 The exclusive identification of serogroups H and I on day 60 was unexpected and sheds light
351 on potential limitations within the study. A varying number of lesion swabs ranging from 10
352 to 20 were obtained from animals with severe footrot lesions. Vaccinated animals exhibited
353 predominantly milder symptoms, resulting in an imbalanced representation of overall
354 serogroups within the flock. It is also possible that certain management practices may have
355 contributed to the presence of these serogroups. *D. nodosus* has been found to persist in the
356 environment for up to two weeks (Cederlöf *et al.*, 2013), and therefore the introduction of
357 serogroups H and I to this mob may have occurred through pasture contamination from other
358 infected mobs. To address these challenges, future studies should employ a more
359 comprehensive sampling approach to ensure an accurate depiction of serogroups existing
360 within the mob. Additionally, the implementation of two-week pasture gaps between groups
361 would minimise infection associated with environment contamination.

362 The development of vaccine site granulomas following Footvax® administration is a
363 significant concern in the context of sheep welfare. Footvax®, containing 60% light mineral
364 oil NF and 4.5% manide oleate, has been documented in the data sheet to induce large local
365 swelling at the vaccination site for a considerable proportion of sheep (Infopest, 2023).
366 Swelling is typically of a short duration, but in some cases formation of discharging abscesses

can occur (Infopest, 2023), found in 1% of vaccinated sheep in this investigation. Although this vaccine does not have a withholding period, recommendations have been made to avoid in animals 2-3 months prior to sale or slaughter due to local pigment changes in the wool and economic losses associated with carcass trimming (Infopest, 2023). Limited research has documented the incidence of site lesions following vaccination with Footvax®, but one study reported site reactions in up to 44% of vaccinated animals (Ennen *et al.*, 2009). Although it was anticipated that prior vaccination of this mob a year earlier at marking and weaning would decrease the overall occurrence of lesions due to prior exposure, the observed prevalence exceeded this previous study, with 56% developing granulomas at the site of injection (Ennen *et al.*, 2009). Oil-based vaccines are associated with significant site reactions, but their longer protective effect over alum-precipitated aqueous vaccines have resulted in their continuous use (Ross and Titterington, 1984). Recent research has indicated that water-in-oil based adjuvants are effective in mono and bivalent footrot vaccines (Egerton *et al.*, 2002, Dhungyel *et al.*, 2008, Dhungyel *et al.*, 2013), demonstrating an alternative to the current multivalent adjuvant. Further investigations comparing reactions and effectiveness of various adjuvants in multivalent vaccines is critical to reduce the current animal welfare implications associated with Footvax®.

In conclusion, the results of this trial enhance existing knowledge by continuing to highlight the variable efficacy of Footvax® in controlling footrot. Future improvements to the vaccine, including the inclusion of serogroup M, are imperative to provide producers with a more comprehensive solution for their flock. The significant prevalence of site lesions indicates the need for an extensive evaluation of alternative adjuvants to improve associated animal welfare standards. Although the current vaccine represents a cost-effective option, the study findings shed light on its limitations, emphasising the necessity for reassessment and refinement.

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Conflict of Interest Statement:

The authors declare no conflict of interest.

References:

Abbott, K. A. & Lewis, C. J. 2005. Current approaches to the management of ovine footrot. *The Veterinary Journal*, 169, 28-41.

Allworth, M. B. & Egerton, J. R. 2018. Comparison of footbathing and vaccination to control ovine footrot in an experimentally infected flock. *Australian veterinary journal*, 96, 395-399.

Beveridge, W. I. B. 1941. *Foot-Rot in Sheep: A Transmissible Disease due to Infection with Fusiformis nodosus(n. sp.). studies on Its Cause, epidemiology, and Control*, Melbourne, H.E. Daw for Council for Scientific and Industrial Research.

Cederlöf, S. E., Hansen, T., Klaas, I. C. & Angen, Ø. 2013. An evaluation of the ability of Dichelobacter nodosus to survive in soil. *Acta veterinaria scandinavica*, 55, 4-4.

Claxton, P. D., Ribeiro, L. A. & Egerton, J. R. 1983. Classification of Bacteroides nodosus by agglutination tests. *Australian veterinary journal,* 60**,** 331-334.

Coopers®. 2020. Coopers ® Animal Health Footrot Control & Eradication. [PDF] Available at: https://www.coopersanimalhealth.com.au/wp-content/uploads/sites/335/2022/06/GetPdf-40.pdf.

Department of Regional NSW DPI 2023. Footrot in sheep and goats - Primefact 1533. 6 ed.: Department of Primary Industries.

Dhungyel, O. P., Lehmann, D. R. & Whittington, R. J. 2008. Pilot trials in Australia on eradication of footrot by flock specific vaccination. *Veterinary microbiology,* 132**,** 364-371.

Dhungyel, O. P., McPherson, A. S. & Whittington, R. J. 2017. Virulent footrot diagnosis. North Sydney, NSW: The University of Sydney.

Dhungyel, O. P., Schiller, N. & Whittington, R. 2015. Identification and characterization of serogroup M Dichelobacter nodosus from sheep with virulent footrot. *Veterinary Microbiology,* 176**,** 378-381.

Dhungyel, O. P., Schiller, N., Eppleston, J., Lehmann, D., Nilon, P., Ewers, A. & Whittington, R. 2013. Outbreak-specific monovalent/bivalent vaccination to control and eradicate virulent ovine footrot. *Vaccine,* 31**,** 1701-1706.

Dhungyel, O. P., Whittington, R. J. & Egerton, J. R. 2002. Serogroup specific single and multiplex PCR with pre-enrichment culture and immuno-magnetic bead capture for identifying strains of D. nodosus in sheep with footrot prior to vaccination. *Molecular and cellular probes,* 16**,** 285-296.

Dhungyel, O., Schiller, N., Eppleston, J., Lehmann, D., Nilon, P., Ewers, A. & Whittington, R. 2013. Outbreak-specific monovalent/bivalent vaccination to control and eradicate virulent ovine footrot. *Vaccine*, 31, 1701-1706.

Duncan, J. S., Grove-White, D., Moks, E., Carroll, D., Oultram, J. W., Phythian, C. J. & Williams, H. W. 2012. Impact of footrot vaccination and antibiotic therapy on footrot and contagious ovine digital dermatitis. *Veterinary record*, 170, 462-462.

Egerton, J. R. & Roberts, D. S. 1971. Vaccination against ovine foot-rot. *Journal of Comparative Pathology*, 81, 179-185.

Egerton, J. R., Cox, P. T., Anderson, B. J., Kristo, C., Norman, M. & Mattick, J. S. 1987. Protection of sheep against footrot with a recombinant DNA-based fimbrial vaccine. *Veterinary microbiology*, 14, 393-409.

Egerton, J. R., Dhungyel, O. P., Abbott, K. A., Kristo, C., Ghimire, S. C., Shrestha, H. K., Joshi, H. D. & Joshi, B. R. 2002. Eradication of virulent footrot from sheep and goats in an endemic area of Nepal and an evaluation of specific vaccination. *Veterinary record,* 151**,** 290-295.

Egerton, J. R., Parsonson, I. M. & Graham, N. P. H. 1968. Parenteral Chemotherapy of Ovine Foot-rot. *Australian veterinary journal,* 44**,** 275-283.

Egerton, J. R., Roberts, D. S. & Parsonson, I. M. 1969. The aetiology and pathogenesis of ovine foot-rot. I. A histological study of the bacterial invasion. *Journal of Comparative Pathology,* 79**,** 207-215.

Egerton, J. R., Yong, W. K. & Riffikin, J. R. 1989. Antigens of Bacteroides nodosus. *Footrot and Foot Abscess of Ruminants*. 1 ed. Boca Raton, FL: CRC Press.

Emery, D. L., Stewart, D. J. & Clark, B. L. 1984. The comparative susceptibility of five breeds of sheep to foot-rot. *Australian veterinary journal*, 61, 85-88.

Ennen, S., Hamann, H., Distl, O., Hickford, J., Zhou, H. & Ganter, M. 2009. A field trial to control ovine footrot via vaccination and genetic markers. *Small ruminant research,* 86**,** 22-25.

Ghimire, S. C., Egerton, J. R. & Dhungyel, O. P. 1999. Transmission of virulent footrot between sheep and goats. *Australian veterinary journal*, 77, 450-453.

Ghimire, S. C., Whittington, R. J., Dhungyel, O. P., Joshi, H. D. & Egerton, J. R. 2002. Diagnosis of footrot in goats: application of ELISA tests for response to antigens of Dichelobacter nodosus. *Veterinary microbiology*, 87, 237-251.

Gradin, J. L., Sonn, A. E. & Petrovska, L. 1993. Serogrouping of Bacteroides nodosus isolates from 62 sources in the United States. American Journal of Veterinary Research, 54, 1069-1073.

Graham, N. P. H. & Egerton, J. R. 1968. Pathogenesis of ovine foot-rot: the role of some environmental factors. *Australian veterinary journal,* 44**,** 235-240.

Green, L. & Clifton, R. 2018. Diagnosing and managing footrot in sheep: an update. *In practice (London 1979),* 40**,** 17-26.

Gurung, R. B., Dhungyel, O. P., Tshering, P. & Egerton, J. R. 2006. The use of an autogenous Dichelobacter nodosus vaccine to eliminate clinical signs of virulent footrot in a sheep flock in Bhutan. *The veterinary journal (1997),* 172**,** 356-363.

Han, X., Kennan, R. M., Davies, J. K., Reddacliff, L. A., Dhungyel, O. P., WHITTINGTON, R. J., Turnbull, L., Whitchurch, C. B. & Rood, J. I. 2008. Twitching Motility Is Essential for Virulence in Dichelobacter nodosus. *Journal of Bacteriology*, 190, 3323-3335.

Hunt, J. D., Jackson, D. C., Brown, L. E., Wwood, P. R. & Stewart, D. J. 1994. Antigenic competition in a multivalent foot rot vaccine. *Vaccine,* 12**,** 457-464.

Hunt, J. D., Jackson, D. C., Wood, P. R., Stewart, D. J. & Brown, L. E. 1995. Immunological parameters associated with antigenic competition in a multivalent footrot vaccine. *Vaccine,* 13**,** 1649-1657.

490 Infopest. 2023. Footvax® Label information. [online] Available at:
491 https://websvr.infopest.com.au/LabelRouter?LabelType=L&Mode=1&ProductCode=
492 51170.

493 John, G. H., Smith, R., Abraham, K. J. & Ellis, R. P. 1999. Identification and grouping of
494 Dichelobacter nodosus , using PCR and sequence analysis. *Molecular and cellular*
495 *probes*, 13, 61-65.

496 Kennan, R. M., Dhungyel, O. P., Whittington, R. J., Egerton, J. R. & Rood, J. I. 2001. The
497 type IV fimbrial subunit gene (fimA) of Dichelobacter nodosus is essential for
498 virulence, protease secretion, and natural competence. *Journal of bacteriology*, 183,
499 4451-4458.

500 Lewis, R. D., Meyer, H. H., Gradin, J. L. & Smith, A. W. 1989. Effectiveness of vaccination
501 in controlling ovine footrot. *Journal of animal science*, 67, 1160-1166.

502 Liardet, D. M., Chetwin, D. H., McNerney, D. M. & Hindmarsh, F. H. 1989. Reduction of
503 the prevalence of footrot on New Zealand farms by vaccination. *New Zealand*
504 *veterinary journal*, 37, 129-130.

505 McPherson, A. S. 2018. Development of a conventional polymerase chain reaction test for
506 the detection of Dichelobacter nodosus serogroup M. In: McPherson, A. S., Aspects
507 of the aetiopathogenesis and diagnosis of ovine footrot. [PhD thesis]. Sydney:
508 University of Sydney, pp. 109-124.

509 McPherson, A. S., Dhungyel, O. P. & Whittington, R. J. 2018. Detection and Serogrouping of
510 Dichelobacter nodosus Infection by Use of Direct PCR from Lesion Swabs To
511 Support Outbreak-Specific Vaccination for Virulent Footrot in Sheep. *Journal of*
512 *clinical microbiology*, 56.

513 McPherson, A. S., Whittington, R. J., Hall, E., Cook, E. J., Jones, J. V., Qi Ang, Y., McTavish,
514 E. L. & Dhungyel, O. P. 2021. A comparison of multivalent and bivalent vaccination
515 strategies for the control of virulent ovine footrot. *Vaccine,* 39**,** 1736-1745.

516 Mulvaney, C. J., Jackson, R. & Jopp, A. J. 1984. Field trials with a killed, nine-strain, oil
517 adjuvanted Bacteroides nodosus footrot vaccine in sheep. *New Zealand veterinary*
518 *journal*, 32, 137-139.

519 O'Meara, T. J., Egerton, J. R. & Raadsma, H. W. 1993. Recombinant vaccines against ovine
520 footrot. *Immunology and cell biology*, 71, 473-488.

521 Raadsma, H. W., O'Meara, T. J., Egerton, J. R., Lehbach, P. R. & Schwartzkoff, C. L. 1994b.
522 Protective antibody titres and antigenic competition in multivalent Dichelobacter
523 nodosus fimbrial vaccines using characterised rDNA antigens. *Veterinary immunology*
524 *and immunopathology,* 40**,** 253-274.

525 Roberts, D. S. & Egerton, J. R. 1969. The aetiology and pathogenesis of ovine foot-rot: II. The
526 pathogenic association of Fusiformis nodosus and F. necrophorus. *Journal of*
527 *comparative pathology,* 79(2), 217-227.

528 Ross, A. D. & Titterington, D. M. 1984. Injection site lesions of footrot vaccines in sheep.
529 *New Zealand veterinary journal*, 32, 6-8.

530 Schwartzkoff, C. L., Egerton, J. R., Stewart, D. J., Lehrbach, P. R., Elleman, T. C. & Hoyne,
531 P. A. 1993. The effects of antigenic competition on the efficacy of multivalent footrot
532 vaccines [sheep]. *Australian veterinary journal,* 70**,** 123-126.

533 Shephard, R., Webb Ware, J., Blomfield, B. & Niethe, G. 2022. Priority list of endemic
534 diseases for the red meat industry - 2022 update. *Meat and Livestock Australia Limited*,
535 B.AHE.0327.

536 Stewart, D. J. & Claxton, P. D. 1993. Ovine footrot: clinical diagnosis and bacteriology. In:
537 CORNER, L. A. & BAGUST, T. J., editors. 1993. Australian Standard Diagnostic
538 Techniques for Animal Diseases. CSIRO for the standing committee on Agriculture
539 and Resource Management, East Melbourne, Australia, 1-27.

540 Thorley, C. M. & Day, S. E. J. 1986. Serotyping survey of 1296 strains of Bacteroides nodosus
541 isolated from sheep and cattle in Great Britain and Western Europe. Footrot ib
542 ruminants. Proceedings of a Workshop, Melbourne 1985, 131-134.

543 Thorley, C. M. & Egerton, J. R. 1981. Comparison of alum-absorbed or non-alum-absorbed
544 oil emulsion vaccines containing either pilate or non-pilate Bacteroides nodosus cells
545 in inducing and maintaining resistance of sheep to experimental foot rot. *Research in*
546 *veterinary science,* 30**,** 32-37.

547 Wassink, G. J., King, E. M., Grogono-Thomas, R., Brown, J. C., Moore, L. J. & Green, L. E.
548 2010. A within farm clinical trial to compare two treatments (parenteral antibacterials
549 and hoof trimming) for sheep lame with footrot. *Preventive veterinary medicine,* 96**,**
550 93-103.

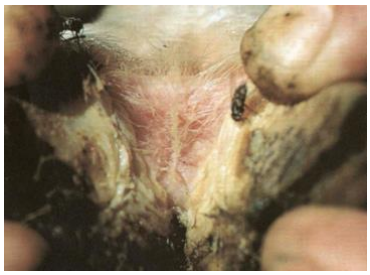
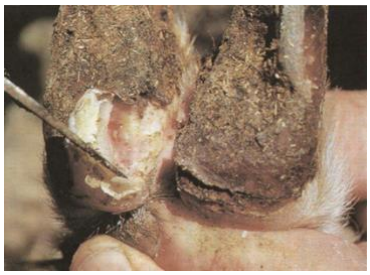
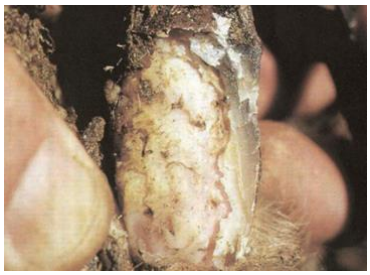
551 Whittington, R. J. & Nicholls, P. J. 1995. Grading the lesions of ovine footrot. *Research in*
552 *veterinary science*, 58, 26-34.

553 Whittington, R. J. 1995. Observations on the indirect transmission of virulent ovine footrot in
554 sheep yards and its spread in sheep on unimproved pasture. *Australian veterinary*
555 *journal*, 72, 132-134.

556 Zhou, H., Hickford, J. G. H. & Armstrong, K. F. 2001. Rapid and accurate typing of
557 Dichelobacter nodosus using PCR amplification and reverse dot-blot hybridisation.
558 *Veterinary microbiology*, 80, 149-162.

Tables:

Table 1: Footrot scoring system adapted from Egerton and Roberts (1971) with images obtained from (Department of Regional NSW DPI, 2023).

Score	Clinical Findings	Image
0	Healthy foot.	
1	Slight to moderate interdigital dermatitis.	
2	Severe interdigital dermatitis; hair loss.	
3	Extensive erosion of interdigital skin; separation of the soft horn and sole (underunning).	
4	Severe underunning of the horn and sole, extending to the abaxial wall; white necrotic exudate present	

562 **Table 2:** Summary of Primers utilised in direct PCR.

Primers	Gene	Sequence (5'-3')	Product size (bp)	Reference
Forward Primer	*fimA*(all)	CCTTAATCGAACTCATGATTG	-	Dhungyel et al. (2002)
Reverse A	fimA(A)	AGTTTCGCCTTCATTATATTT	415	
Reverse B	fimA(B)	CGGATCGCCAGCTTCTGTCTT	283	
Reverse C	fimA(C)	AGAAGTGCCTTTGCCGTATTC	325	
Reverse D	fimA(D)	TGCAACAATATTTCCCTCATC	319	
Reverse E	fimA(E)	CACTTTGGTATCGATCAACTTGG	363	
Reverse F	fimA(F)	ACTGATTTCGGCTAGACC	241	
Reverse G	fimA(G)	CTTAGGGGTAAGTCCTGCAAG	279	
Reverse H	fimA(H)	TGAGCAAGACCAAGTAGC	409	
Reverse I	fimA(I)	CGATGGGTCAGCATCTGGACC	189	
Forward M	fimA(M)	AGCWGTAATCAGTGGTACTTAT	-	McPherson (2018)
Reverse M	*fimA*(M)	TGATCCATAAGTAATAGTTACGAC	94	

563

564 **Table 1:** Summary of direct PCR results throughout the trial.

Time Period	Number of Swabs	Serogroups										
		A	B	C	D	E	F	G	H	I	M	Negative
Pre-Trial	14	-	5	-	-	-	-	-	-	-	2	8
Day 0	20	-	8	3	-	5	-	-	-	-	8	4
Day 30	10	-	-	1	-	2	-	-	-	-	6	2
Day 60	10	-	4	2	-	-	-	-	8	3	5	-

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Figures:

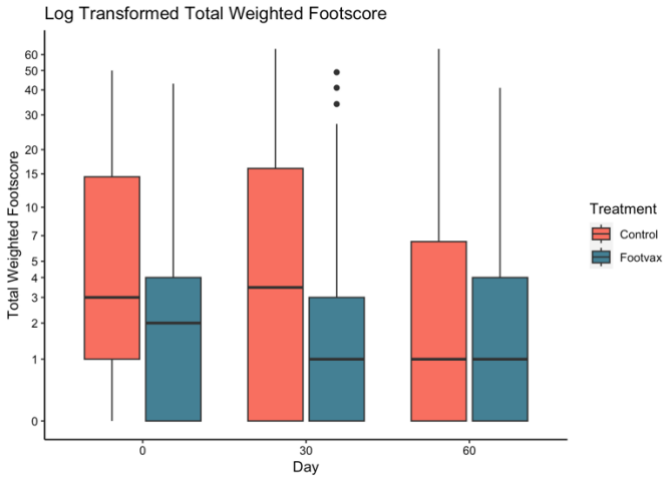

Figure 1. Comparison of log transformed Treatment Total Weighted Footscore (TWFS) at each time period.

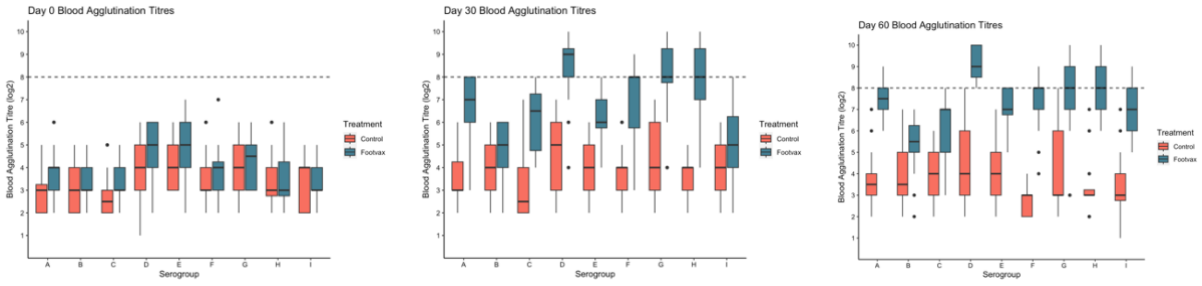

Figure 2. Comparison of Blood Agglutination Titres between the treatment groups at each time point. The dashed line represents the likely protective antibody levels (Egerton *et al.*, 1987).

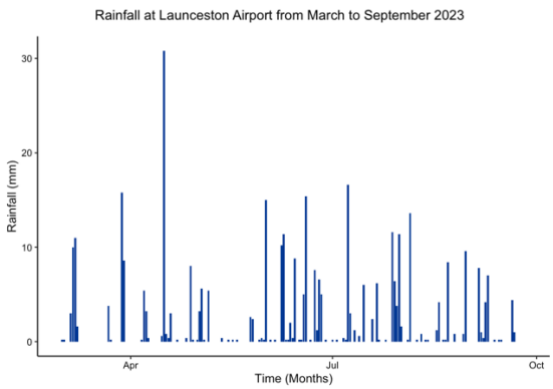

Figure 3. Rainfall data at Launceston Airport from March to September 2023.

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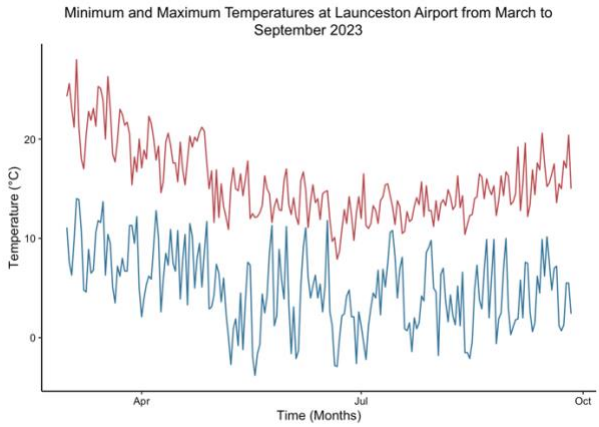

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579 **Figure 4.** The Maximum (red line) and minimum (blue line) temperatures at Launceston Airport from March to

580 September 2023.