THE ROLE OF SEROGROUP SPECIFIC VACCINES IN THE CONTROL OF FOOTROT IN SHEEP

by

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ABSTRACT

Lameness in sheep is a welfare concern and the most commonly reported cause of lameness in sheep flocks in England is footrot, a polymicrobial disease that requires the presence of the fimbriated bacterium *Dichelobacter nodosus.* There are ten antigenically distinct serogroups of *D. nodosus,* named A-I and M. Serogroups A-I are known to be randomly distributed in English sheep flocks and are all contained in the only commercially available vaccine, Footvax®. The efficacy of multivalent footrot vaccines is limited by antigenic competition. Flock-specific bivalent serogroup vaccines have been used to reduce or eliminate footrot from flocks in Nepal, Bhutan and Australia. This strategy had not been tested under commercial flock conditions in England.

A randomised, double blinded controlled trial was performed in three commercial flocks in South-West England to compare the effect of flock-specific bivalent footrot vaccines against Footvax® and a negative control (saline) on the incidence of lameness and footrot. Within each flock approximately one third of ewes were allocated to each treatment group. The bivalent vaccines contained two of the most prevalent *D. nodosus* serogroups present in the flock being vaccinated, which were identified by direct PCR of interdigital skin swabs from 20-21 ewes with footrot. Ewes from all treatment groups were mixed together and were monitored every week for lameness and foot lesions for 24 weeks. The prevalence of injection site reactions at 4,12,16 and 20 weeks after vaccination was measured. Survival analysis was performed to calculate the relative risk of lameness and footrot between the three treatment groups.

It was possible to identify at least one of the two most prevalent serogroups in two of the three flocks by sampling 21 ewes with footrot. Across the combined flocks, the bivalent vaccine significantly reduced the incidence of lameness and footrot cases compared to saline. The bivalent vaccine was not significantly more protective than Footvax®. The response to vaccination was heterogenous between flocks and within sub-populations of the flocks. Both vaccines were significantly protective in only one flock, in which lameness prevalence was high and the main cause of lameness was footrot. Ewes that had been vaccinated with Footvax® were at three times the odds of developing an injection site reaction compared to ewes that had been vaccinated with the bivalent vaccine.

The aetiology and prevalence of both infectious and non-infectious causes of lameness varies between commercial flocks in England, and the inclusion of footrot vaccination as part of a lameness control strategy needs to be tailored to individual flocks.

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LIST OF ABBREVIATIONS

AIC	Akaike Information Criterion
AHDB	Agriculture and Horticulture Development Board
APHA	Animal and Plant Health Agency
ATC	Animal Treatment Certificate
BCS	Body Condition Score
BSA	Bovine Serum Albumin
BVI	Bivalent Vaccine Included
CH	Cumulative Hazard
CI	Confidence Interval
CODD	Contagious Ovine Digital Dermatitis
D. nodosus	*Dichelobacter nodosus*
EID	Electronic Identification (tag)
FAWC	Farm Animal Welfare Council (Committee)
FR	Footrot
FS	Foot score
HR	Hazard Ratio
ID	Interdigital Dermatitis
LRT	Likelihood Ratio Test
LS	Locomotion Score
MEFS	Modified Egerton Footrot Score
NOAH	National Office of Animal Health
PBS	Phosphate Buffered Saline
PCU	Population Corrected Unit
PCR	Polymerase Chain Reaction
PH	Proportional Hazards
qPCR	Quantitative Polymerase Chain Reaction
RUMA	Responsible Use of Medicines in Agriculture Alliance
SFR	Severe Footrot
SOP	Standard Operating Procedure
VMD	Veterinary Medicines Directorate
WLD	White Line Degeneration

CHAPTER 1 INTRODUCTION

1.1 Lameness in the sheep industry

1.1.1 The impact of lameness on the sheep industry

Lame sheep are in pain (Ley et al., 1989, McLennan et al., 2016), and so lameness is a serious concern for sheep welfare (Fitzpatrick et al., 2006, Phythian et al., 2011). In 2011, an opinion published by the Farm Animal Welfare Council (FAWC) on sheep lameness concluded that lameness prevalence, estimated to be 10.2% in flocks in England at the time (Kaler and Green, 2009), should be reduced to <5% by 2016 and <2% by 2021 and that this was achievable with the strategies already available to farmers (FAWC, 2011). By 2013, mean lameness prevalence in flocks in England had fallen to 3.5% (Winter*, et al.*, 2015), and global lameness prevalence for all sheep in England was 5%. Farmer surveys from 2018 and 2019 reported mean lameness prevalence in ewes from flocks across Great Britain (Lewis et al., 2021) and the UK (Best et al., 2020) respectively and are not directly comparable with previous surveys of sheep flocks in England. Lewis et al. (2021) reported a mean lameness prevalence of 1.4% and Best et al. (2020) reported a prevalence of 3.2% but in both surveys flock lameness prevalence was >2% in approximately 60% of flocks, which indicates the need for further measures to facilitate control of lameness.

1.1.2 The global and national importance of footrot

Footrot, a bacterial disease*,* is the most common cause of lameness in England, affecting over 90% of 1260 flocks surveyed in 2013 (Winter et al., 2015). The resulting loss of production, along with the costs of treatment and control, has a substantial negative impact

on profitability for the sheep industry (Nieuwhof and Bishop, 2005, Wassink et al., 2010b, Winter and Green, 2017). Footrot is present in flocks in all countries with a significant sheep farming industry, which includes Australia (Best et al., 2018), New Zealand (Zhou and Hickford, 2000) and India (Rather et al., 2011). The economic impact of footrot has prompted some regions and countries, including New South Wales, Australia (Bennett and Hickford, 2011), Switzerland (Greber et al., 2016) and Norway (Asheim et al., 2017), to initiate eradication of the disease.

1.2 The pathogenesis and control of footrot

1.2.1 The clinical appearance of footrot

Footrot develops first as inflammation of the interdigital skin. This is often, but not always, associated with lameness (Kaler et al., 2011). As the lesion progresses hair loss occurs. This stage of footrot is called interdigital dermatitis (ID), scald or strip. If left untreated severe footrot (SFR) develops as the soft horn on the axial surface of the foot is underrun, followed by the solar horn, and eventually the outer hoof wall (Beveridge, 1941). Degradation of the hoof horn causes a characteristic necrotic smell. Disease progression is associated with an increasing degree of pain and reluctance to bear weight on the affected foot (Kaler et al., 2011, McLennan et al., 2016); if not treated promptly it has a detrimental effect on hoof conformation (Kaler et al., 2010b).

1.2.2 Expression and transmission of footrot depends on environmental conditions

Whilst footrot is a polymicrobial disease (Blanchard et al., 2021, Clifton et al., 2022), the bacterium *Dichelobacter nodosus* is necessary for footrot to occur (Beveridge, 1941, Egerton and Parsonson, 1969, Kennan et al., 2010, Witcomb et al., 2014). *D. nodosus* can also be detected on some healthy feet but its presence is short lived in the absence of progression to disease, being detected a maximum of two consecutive weeks in two longitudinal studies (Clifton et al., 2019). The researchers also observed a higher prevalence of footrot during wet weather than prolonged dry weather. Correspondingly, *D. nodosus* was detected in 41% of soil samples in the study conducted during wet conditions compared to only 4.1% when rainfall was low. This adds to existing evidence that the environment has an important role in both sheep to sheep transmission of *D. nodosus* and the progression from exposure to *D. nodosus* to the expression of footrot. Studies by Graham and Egerton (1968) demonstrated that prior damage to the skin surface from wet conditions was a pre-requisite to the development of footrot in healthy sheep, which explained the seasonality associated with footrot transmission in regions of Australia. These regions, and other countries that experience a long season without rainfall, can use this period of *D. nodosus* non-transmission to target measures for the elimination of footrot from flocks (Bennett and Hickford, 2011). For example, sheep that have been non-responsive to treatment or vaccination during the transmission period can be identified and culled (Allworth and Egerton, 2018, Dhungyel et al., 2008, Gurung et al., 2006). Damage to the skin is also the putative reason for an increase in footrot prevalence associated with long or abrasive pasture (Angell et al., 2018, Woolaston, 1993).

1.2.3 Host factors have a role in disease expression

The risk of expression of footrot following exposure to *D. nodosus* also depends on host factors, namely natural resistance (Anaya et al., 2021, Emery et al., 1984, Escayg et al., 1997, Skerman and Moorhouse, 1987), induced immunity (section 1.3.2) and foot conformation (Kaler et al., 2010b). Nieuwhof et al. (2008) estimated a heritability for footrot resistance of 0.12 and 0.19 in two different breeds. However, the large influence of environmental and pathogen factors on expression of footrot complicates estimates of heritability (Conington et al., 2008, Russell et al., 2013, Walkom et al., 2022). This hinders strategies for breeding footrot resistant sheep, together with the polygenic nature of footrot resistance (Mucha et al., 2015, Raadsma et al., 2018). Therefore, there is a greater emphasis on control measures that have a more rapid impact on footrot prevalence.

1.2.4 Characteristics of D. nodosus and its virulence factors

D. nodosus is a Gram-negative anaerobe, rod-shaped in appearance with prominent poles from which protein filaments, or 'pili', extend. Also known as Type IV fimbriae, these structures are found in other Gram-negative bacteria such as *Pseudomonas aeruginosa* (Mattick, 2002). They are essential for virulence as they enable the twitching motility that allows movement of the bacteria into the deeper, anaerobic parts of the skin (Depiazzi and Richards, 1985, Han et al., 2008). Mutagenesis of the *fimA* (*pilA*) gene, which codes for the major fimbrial subunit protein, and other genes involved in fimbrial assembly, has also demonstrated that these fimbrial structures secrete extracellular proteases (Han et al., 2007, Kennan et al., 2001). These proteases are required to digest the extracellular matrix at the skin-horn junction, allowing *D. nodosus* to penetrate the epidermis beneath the hoof horn and cause separation of these layers (Depiazzi et al., 1991).

D. nodosus produces three proteases; AprV2 and AprV5 are acidic in nature and have been shown to been essential for virulence, whilst the role of a third, similar but basic protease, BprV, has yet to be defined (Han et al., 2012, Kennan et al., 2010, Riffkin et al., 1995). The genes encoding the proteases exist as 'benign' variants, but there is poor correlation between the *AprV2*/*AprB2* genotype and the ability to digest elastase *in vitro*, a standardised test for virulence that has a high correlation with footrot phenotype in Australian flocks (McPherson et al., 2017). Additionally, the more severe clinical manifestations of footrot in Sweden have been associated with *AprB2 D. nodosus* strains (Frosth et al., 2015). Differentiation of benign from virulent forms of footrot is important in regions that have control or eradication programmes that target only SFR (Collins and Bowring, 2020, Locher et al., 2015, Smith et al., 2021a). In England, SFR is endemic and the *AprV2* genotype is likely to be universally present in flocks with the SFR phenotype (Monaghan et al., 2021) and so the *AprV2/ AprB2* genotype is unlikely to warrant consideration in control strategies for footrot at this stage.

1.2.5 Variations in the FimA protein are the basis of serogroup classification

Fimbriae are immunogenic (Egerton et al., 1987, Every and Skerman, 1982, Walker et al., 1973). Ten serogroups, designated A-I and M, have been identified on the basis of the slide-agglutination test (Claxton et al., 1983, Ghimire et al., 1998, Walker et al., 1973). This test is an observation of aggregation between *D. nodosus* fimbriae and antisera raised to specific *D. nodosus* strains. Serogroups are antigenically distinct because of a variation between them of 35 to 50 amino acids in the fimbrial subunit protein FimA (Mattick et al., 1991). Serogroups have no or weak antibody cross reactivity, and consequently do not generate

cross-protective immunity, an essential consideration for pilus based footrot vaccines (Stewart et al., 1985a). There is no relationship between *D. nodosus* serogroups and the severity of footrot lesions (Monaghan et al., 2021).

Specific PCR primers for serogroups A-I have been published, allowing serogroups to be identified directly from swabs taken from the feet of sheep without the need for less sensitive and more costly culture-based methods (Best et al., 2018, Dhungyel et al., 2002, McPherson et al., 2018). A validated primer for serogroup M has not yet been published.

1.2.6 Managements associated with the control of footrot

In recent years, evidence-based effective control measures for footrot have been identified that have reduced the prevalence of lameness in UK sheep flocks (Winter et al., 2015). Correspondingly, mean flock lameness prevalence increased from 3.3% to 4.1% between 2013 and 2015 in association with a decrease in the use of these control measures in a longitudinal study of 154 flocks (Prosser et al., 2019). Treatment of sheep that are lame with footrot within 3 days of onset of lameness with both systemic and topical antibiotics typically cures footrot in 2-10 days (Kaler et al., 2011, Kaler et al., 2010a, Wassink et al., 2010b). Antibiotic treatment reduces the bacterial load on the affected foot (Staton et al., 2021) and therefore infectivity to other sheep. The *D. nodosus* load is higher on feet with ID than those that have progressed to underrunning footrot (Maboni et al., 2016, Witcomb et al., 2015). Consequently, treatment early in the onset of lameness is necessary to prevent onward spread, and separation of lame sheep is associated with reduced lameness prevalence (Witt and Green, 2018).

Not trimming feet therapeutically increases the rate of recovery from footrot (Kaler et al., 2010a) and is positively associated with increased revenue per ewe (Lima et al., 2020) and lower flock lameness prevalence (Best et al., 2020). Routine foot trimming appears to have no benefit or is detrimental to foot health (Lewis et al., 2021, Reeves et al., 2019).

The use of footbathing as a treatment for SFR is a risk factor for lameness (Lewis et al., 2021, Winter et al., 2015), whereas footbathing to control ID is associated with a lower prevalence of lameness (Winter et al., 2015, Witt and Green, 2018). Routine footbathing is associated with an increased prevalence of granulomas and 'shelly hoof', two non-infectious foot conditions (Reeves et al., 2019).

Flock lameness prevalence can be reduced by culling sheep that are lame more than once in a year (Witt and Green, 2018) or culling sheep with poor foot conformation at the start of an intervention programme; these sheep can act as carriers of *D. nodosus,* re-infecting the flock (Best et al., 2021b, Kaler et al., 2010b).

There is one vaccine against footrot that is licensed in the UK, Footvax® (MSD Animal Health). Vaccination against footrot is a tool that has not been used widely, despite having been available for several decades, but the percentage of vaccinated flocks in England appears to be increasing. In 2013, 16.4% of farmers in England vaccinated some or all of their flock with Footvax® (Winter et al., 2015) compared to 26.6% of flocks in 2018 (Lewis et al., 2021). Recent non-randomised surveys reported a higher uptake of Footvax® in UK flocks at 36.1% (Best et al., 2020) and 30.6% (Hall et al., 2022). The introduction of Footvax® to flocks with a high prevalence of lameness (>10%) did not reduce lameness prevalence

compared to flocks that did not vaccinate during a 12-18 month intervention trial (Witt and Green, 2018). More recently, an association between vaccination with Footvax® for >5 years and lower lameness prevalence has been reported by multiple studies (Best et al., 2020, Lewis et al., 2021, Prosser et al., 2019). Despite dissatisfaction being expressed by some who have used Footvax®, farmers still favour vaccination as an approach to control footrot (Wassink et al., 2010a).

1.3 The efficacy of footrot vaccines

1.3.1 The efficacy of multivalent footrot vaccines

Serogroup antigens are the basis of current vaccines against footrot and the protective effect is greater when they contain higher quantities of fimbriae, or fimbriae only rather than whole cells (Stewart et al., 1986, Stewart et al., 1985a, Stewart et al., 1982). Multivalent footrot vaccines were introduced because of the lack of cross protectivity between heterologous serogroups (Stewart et al., 1986, Stewart et al., 1985a, Stewart et al., 1991) and the recognition that multiple serogroups occur within flocks (Claxton et al., 1983). Field and experimental trials of multivalent vaccines in Scotland (Hindmarsh, 1989) and Australia (Allworth and Egerton, 2018, Lambell, 1986) demonstrated a protective efficacy of 46-84% over a period of 4-5 months. In the USA, the protection from a multivalent vaccine was equivalent to footbathing (Bulgin et al., 1985).

Footvax® contains *D. nodosus* fimbriae for serogroups A-H, including two B serotypes, and whole cell serogroup I. The datasheet recommends administering a second dose after 4-6 weeks if footrot prevalence remains significant, or prevailing conditions are conducive to the

disease; revaccination 'may be required every 4-5 months' (NOAH, 2021). A field trial in England compared the curative and protective effects of Footvax® in previously unvaccinated lambs (Duncan et al., 2012). Lambs were coinfected with contagious ovine digital dermatitis (CODD), another infectious cause of lameness, and were grazing in wet conditions. The prevalence of footrot in both vaccinated and unvaccinated lambs decreased by 30% after 9 weeks. New footrot infections occurred in 13% of vaccinated lambs compared to 28% of unvaccinated lambs, resulting in a vaccine efficacy of 62% for footrot protection.

1.3.2 Antigenic competition and protective immunity in sheep

Vaccination with *D. nodosus* fimbrial subunit protein induces a marked rise in serum agglutinating antibody titre, which is protective against SFR caused by the homologous serogroup (Egerton et al., 1987, Every and Skerman, 1982, Raadsma et al., 1994, Stewart et al., 1985a, Stewart et al., 1991). There is a positive, although not always linear, relationship between the agglutinating antibody titre and its protective capability (Raadsma et al., 1994). Hunt et al. (1994) demonstrated that agglutinating antibody titre more than doubled when the amount of pilus antigen included in a monovalent vaccine was increased tenfold. In contrast, the inclusion of pilus antigen from other *D. nodosus* serogroups in the vaccine resulted in a decrease in agglutinating antibody specific to the serogroup contained in the monovalent vaccine, and this was proportional to the number of additional antigens. Hunt et al. (1994) also demonstrated a significantly higher prevalence of severe footrot lesions and a shorter duration of protection in sheep receiving a decavalent vaccine than those receiving the monovalent vaccine. Raadsma et al. (1994) demonstrated both a decrease in agglutinating antibody titre against each antigen and a decrease in protection against footrot

as the number of *D. nodosus* antigens in a footrot vaccine increased. A decavalent vaccine stimulated less than 30% of the agglutinin specific to one serogroup compared to a monovalent vaccine containing the same dose of the homologous fimbrial antigen. Schwartzkoff et al. (1993a) investigated the effect of simultaneously injecting multiple antigens at separate sites on the sheep but still observed the same proportional reduction in agglutinating antibody titre compared to monovalent vaccines. McPherson et al. (2021) compared four different formulations of multivalent footrot vaccines, two containing six serogroups and two containing nine serogroups, against six combinations of commercial bivalent vaccine. Predicted mean antibody titres for sheep that received a bivalent vaccine were significantly higher than for sheep that received a multivalent vaccine. The inhibitory effect of increasing the number of antigens in a vaccine is antigenic competition. The mechanism behind it has not been fully established (Dhungyel et al., 2014). However, McPherson et al. (2021) reported no significant difference in mean antibody titres between sheep that received either a six or nine serogroup vaccine, indicating that the effect of antigenic competition can be overcome to an extent by the formulation of the vaccine adjuvant.

1.3.3 Variation in host response to footrot vaccination

The immune response to a vaccine is variable between individuals for reasons including age, health status, presence of maternal antibodies and differences in genetic ability to produce an immune response (Heininger et al., 2012). Low and high vaccine responders are recognised in human and livestock vaccine trials and are defined by the difference in measured antibody response, which is partly under genetic control (Jouneau et al., 2020,

Lipsit et al., 2022, Mentzer et al., 2015, Raadsma et al., 1999). The ability of footrot vaccines to prevent or treat disease varies between individual sheep (Dhungyel et al., 2008, Smith et al., 2021b) and was observed to vary between breeds of sheep in experimental trials (Skerman et al., 1982, Stewart et al., 1985a). The mechanism underlying the difference in responses has not been elucidated (Bhardwaj et al., 2014, Skerman et al., 1982). It is recommended that non-responders to vaccination are culled during footrot elimination programmes (Dhungyel et al., 2008, Dhungyel et al., 2013). Amend et al. (2021) investigated the heritability of antibody titres to antigen derived from Footvax® in Merinoland sheep as a potential target for breeding sheep that are more footrot resistant. Two doses of Footvax® were given to half sibling groups, four weeks apart. There were differences in the antibody titres between the groups from 2 weeks after the second dose of vaccine, and the heritability at 28 weeks was moderate at 0.25. Breeding programmes therefore remain a potential strategy for improving the response to footrot vaccination but have not been tested in any long term studies under UK sheep farming conditions.

1.3.4 Serogroup specific vaccination

Targeted, monovalent or bivalent serogroup vaccines were used in the elimination of severe footrot in Nepal (Egerton et al., 2002), Bhutan (Gurung et al., 2006) and Australia (Dhungyel et al., 2008, Dhungyel et al., 2013). These countries had distinct disease transmission periods. It is important to note that, other than in Bhutan, sheep were vaccinated in conjunction with other control measures, namely antibiotic treatments and culling of sheep that had persistent footrot following vaccination. Egerton et al. (2002) reported the elimination of virulent footrot from all 40 flocks in a region in Nepal, where the disease had

been endemic for 30 years. Only serogroups B and E were detected in the flocks. Footrot disappeared within a year of control measures being introduced, at which stage only half of the flocks had received monovalent or bivalent vaccinations.

A monovalent, whole cell vaccine was used with no other treatment or control measures in a single flock in Bhutan with a 10 year history of footrot (Gurung et al., 2006). The vaccine targeted serogroup B, the only serogroup detected in the flock. The initial prevalence of footrot was 14.6%: it had been eliminated by day 30 after vaccination. The vaccine programme was repeated the following year only, and three years later footrot was still absent when all feet were examined. In this case the role that vaccination played is unclear because there was no control group, although other flocks in the region remained diseased.

Dhungyel et al. (2008) used an autogenous, whole cell monovalent vaccine in a commercial flock of 660 Merinos infected with only serogroup F. Using the same footrot lesion scoring criteria as Egerton et al. (2002) the prevalence of lesions decreased from an estimated 44% before vaccination to 0.5% at 4 months post vaccination. Similar results were achieved in a flock of 3006 sheep in which only serogroup C had been detected. Dhungyel et al. (2013) trialled this strategy on a further 12 large commercial flocks infected with between 1 and 7 serogroups. The relative prevalence and virulence of the *D. nodosus* isolates from each flock was used to select serogroups for inclusion in flock-specific monovalent or bivalent recombinant fimbrial vaccines. When more than two serogroups were detected in a flock, re-sampling and revaccination with the two most prevalent serogroups occurred at an interval of 3-12 months. A reduction in footrot prevalence was most marked in the three flocks with one or two serogroups, with elimination of severe footrot within 12-28 months.

It took longer for footrot prevalence to drop in the 8 flocks with five or more serogroups at the start of the study, and elimination was not achieved for 4 out of 8 of these within the five-year study. This may have been in part due to an increase in prevalence in non-targeted serogroups, similar to the observation of serotype replacement in large scale pneumococcal multivalent vaccination programmes in people. In the latter example it has been demonstrated that vaccination results in a reduction in overall disease incidence within 5 years, whilst the incidence of disease caused by non-vaccine strains increases (Bender et al., 2008, Miller et al., 2011). In the study by Dhungyel et al. (2013) a decrease in footrot prevalence was observed on all 12 farms between first (pre-vaccination) and second inspections, a period that varied from 3-24 months.

Control of less virulent strains of footrot ('intermediate' footrot) was achieved using bivalent vaccines in three of four commercial flocks in Australia (Smith et al., 2021b). Footrot lesions disappeared before the trial started in the fourth flock and did not reappear during the seven months until the last flock inspection. No more than two serogroups were known to be present in the flocks at the start of the trial. Both the prevalence and severity of footrot lesions were reduced in the three flocks where footrot lesions were present at the time of first vaccination. However, there were no control groups and the reduction in footrot could have been due to the exceptionally dry weather.

A trial in four commercial flocks in Australia directly compared the efficacy of sequential bivalent vaccines, a multivalent pilus vaccine with a novel adjuvant formulation, and an oil adjuvant control (McPherson et al., 2021). Sheep were randomised to one of the three treatment groups in equal numbers within each flock and each received two doses of the

multivalent vaccine or control treatment four weeks apart, or four doses of the bivalent vaccine at 0, 30, 60 and 90 days. Cure rates and improvement rates, based on footrot lesion severity scores (Egerton and Roberts, 1971), were calculated at two, three and seven months after the first vaccine dose. There was not a consistent difference between the three treatment groups in either cure rate or improvement rate, and in one flock the cure rate was 100% in the control group that underwent monthly footbathing, compared to approximately 60% in the two vaccine groups. The expression of footrot could have been affected by the dry pasture conditions for much of the trial.

In conclusion, the use of targeted, bivalent footrot vaccines, in conjunction with the removal of sheep that have severe footrot, can be effective in the rapid elimination of the disease in flocks that have only 1-2 serogroups. In flocks that have multiple serogroups, a bivalent vaccine can still be a useful control measure for reducing footrot prevalence when used alongside treatment and culling, but the impact of this strategy is slower. Targeting only two serogroups within a more diverse population of *D. nodosus* serogroups can still have a measurable impact over a six month clinical trial but this is dependent upon climatic conditions.

1.3.5 Vaccine adjuvants and injection site reactions

One concern with footrot vaccines is that those with oil adjuvants cause injection site reactions or abscesses (Lambell, 1986, Mulvaney et al., 1984, Ross and Titterington, 1984). In a series of three trials, oil based adjuvants produced larger abscesses than alum-based aqueous vaccines and affected 50-100% of sheep per group (Ross and Titterington, 1984). Lambell (1986) reported injection site reactions in 93.5% of 200 ewes vaccinated

subcutaneously on the neck, which were not associated with a loss of production. In contrast, in a study by Stewart et al. (1985b), castrated males ("wethers") gained less weight than controls for six weeks following vaccination and showed breed related variation in the nature of injection site reactions. More recently, an autogenous vaccine containing four or five serogroups and using an alum adjuvant was compared against oil-adjuvanted Footvax® in two sheep flocks in Germany. The prevalence of footrot fell in both vaccine groups and control animals, but vaccine site reactions were observed 16 weeks post vaccination in 20.8% of sheep that received Footvax® compared with only 1.3% of sheep that received the autogenous vaccine (Ennen et al., 2009). In contrast, Hardi-Landerer et al. (2012) recorded injection site reactions in only 7/261 (2.7%) sheep in one flock, and no animals in a second flock of 338 sheep, four weeks after being vaccinated with Footvax®. The authors attributed the unexpectedly low number of reactions to a recent modification in the vaccine adjuvant made by the manufacturer.

The datasheet for Footvax® contraindicates vaccination within 4 weeks of shearing (NOAH, 2021), presumably due to the risk of injury caused by the presence of a swelling at the injection site. A vaccine that is as efficacious as Footvax®, without the localised reaction, would be advantageous. Therefore, the prevalence of these reactions in sheep receiving the bivalent vaccine was considered an important outcome in this study.

1.3.6 Alternative vaccines under development

Work is ongoing to identify an antigen that induces an immune response across all *D. nodosus* strains. A live, recombinant vaccine that secreted the *D. nodosus* basic protease failed to confer footrot protection in an artificial challenge study (Moore et al., 2001).

Sequencing of the *D. nodosus* genome has facilitated discovery of potential vaccine antigens in regions conserved between strains (Myers et al., 2007). Two of these antigens induced antibodies in mice during preliminary investigations, including a lysozyme inhibitor used for bacterial defence (Humbert et al., 2019). Improved adjuvants are another strategy for increasing the protective effect of vaccines (McPherson et al., 2021). Supplementary melatonin raised antibodies titres to specific fimbrial antigen in a serological study of sheep in the absence of disease challenge (Ramos et al., 2018). This was due to the stimulatory effect of melatonin on both B and T lymphocytes. A further option may be to try and modulate elements of the local inflammatory response, rather than relying on the humoral response, which would perhaps be advantageous in preventing earlier stages of footrot (Davenport et al., 2014). None of these alternatives are commercially available so there is a need to continue research into optimal vaccine strategies using the existing recombinant fimbrial vaccines.

1.4 Other causes of lameness in sheep flocks in the UK

1.4.1 Infectious causes of lameness in adult sheep

CODD is a common infectious cause of lameness (Dickins et al., 2016). Farmer surveys reported CODD in 37% of British flocks in 2018 (Lewis et al., 2021), 58% of flocks in England in 2013 (Dickins et al., 2016) and 35% of flocks in Wales in 2012 (Angell et al., 2014), with a within flock mean prevalence of approximately 2% in the same surveys. CODD lesions appear as focal areas of ulceration or proliferation and hair loss on the dorsal skin-horn junction, then progressively undermine the horn leading to avulsion of the hoof (Angell et al., 2015a). The process of horn separation is particularly painful and affected sheep have

higher locomotion scores, indicative of greater severity of lameness, than for footrot (Angell et al., 2015c, Angell et al., 2015a). CODD is a polymicrobial disease, which is consistently associated with the presence of *Treponema medium, Treponema phagedenis and Treponema pedis* (Staton et al., 2021, Sullivan et al., 2015). Although within flock prevalence of CODD is not as high as footrot, there is a strong association between CODD lesions and the presence of *D. nodosus* and *Fusobacterium necrophorum,* CODD and footrot lesions can occur on a foot concurrently, and footrot has recently been proposed as an initiating factor in the development of CODD lesions (Staton et al., 2021). The association between footrot and CODD lesions was demonstrated by a 32% efficacy of Footvax® against CODD in a group of lambs with concurrent footrot and CODD (Duncan et al., 2012). Cure rates of approximately 90% can be achieved by using two doses of amoxycillin (10mg/kg) to treat CODD (Staton et al., 2021), whereas the CODD associated *Treponeme* spp. have a lower *in vitro* susceptibility to oxytetracycline (Angell et al., 2015b), the most commonly prescribed antibiotic for lameness (Davies et al., 2017). Therefore, Identification of CODD in foot lesions of mixed aetiology is important for the selection of effective antimicrobial therapy. It should be noted that mg of antibiotic per population corrected unit (mg/PCU) is higher for two doses of amoxycillin than a single dose of oxytetracycline, and so lameness aetiology affects flock antibiotic usage.

In recent years, sporadic outbreaks of ulcerative lesions on the legs of sheep have been reported (APHA, 2019, Staton et al., 2020). The lesions are typically located between the coronary band and the carpus or tarsus and are associated with haemorrhage and granulation tissue. In the author's experience they are associated with localised cellulitis and

cause lameness when situated on the lower limb. The appearance of the lesions is similar to CODD when they are close to the coronary band but they are more commonly located on the lateral aspect of the foot or the heel and they do not underrun the hoof horn. The aetiology of the lesions is currently unknown but *Streptococcus dysgalactiae* has been isolated from swabs taken from the lesions and they respond readily to amoxycillin (APHA, 2019, Staton et al., 2020). The lesions will henceforth be referred to as 'ulcerative skin lesions'.

1.4.2 Common non-infectious causes of lameness

White line degeneration (WLD) or 'Shelly hoof' is the separation of the hoof wall from the underlying dermal laminae (Conington et al., 2010) and was reported in 59% of British flocks in 2018 (Lewis et al., 2021). WLD does not consistently result in lameness and there is not an association between flock WLD prevalence and lameness prevalence (Winter et al., 2015). However, lameness can occur secondarily to impaction of the lesion and an increased risk of bacterial penetration due to the deficit in the hoof structure (Conington et al., 2010). Within flock prevalence of WLD can range from 0-95% (Reeves et al., 2019) and lesions were present in 77% (1086/1418) of ewe examinations over 12 months in a longitudinal study of four flocks in England and Wales (Best et al., 2021a).

Granulomas are painful protrusions of granulation tissue from the laminae underlying the sole horn. They are commonly present in flocks and were reported by 47% of British farmers in 2018 (Lewis et al., 2021). Within flock prevalence of granulomas is low, with a range of 0-8% (Reeves et al., 2019) and a mean prevalence of <1% (Lewis et al., 2021), which means they do not have a significant impact on flock lameness prevalence (Winter et al., 2015).

Some commonality in risk factors has been shown between infectious and non-infectious causes of lameness, namely the use of footbathing and foot trimming (Reeves et al., 2019). It might be expected that the prevalence of non-infectious lameness reduces with 'best practice' control measures for infectious causes of lameness, but WLD still occurs at a high prevalence in flocks that do not use contraindicated management practices (Best et al., 2021a) and the relationship between infectious foot disease and granulomas has not been fully investigated.

1.5 Justification for a clinical trial to investigate serogroup specific footrot vaccines in English flocks

1.5.1 Antimicrobial use in the sheep industry

In recent years concerns have been raised over the overuse of antibiotics in the livestock sector and the implications this may have for the development of antimicrobial resistance (O'Neill, 2016). In 2017 the Responsible Use of Medicines in Agriculture Alliance (RUMA) task force reviewed antimicrobial use in each livestock sector in response to these concerns and set a target of 10% reduction in antibiotic use by 2020 for the sheep sector (RUMA, 2017). The task force recommended an increased uptake in the use of vaccination against footrot in recognition of lameness accounting for two thirds of antibiotic use in sheep (Davies et al., 2017). Sheep farmers were already being advised to vaccinate against footrot following the adoption of the 'Five point plan' for lameness control by the sheep industry in 2014 (AHDB, 2020, Clements and Stoye, 2014). The recommendation was repeated in a 2020 review containing new targets to cover the period 2021-2024 (RUMA, 2020) and recently published guidelines for vaccination in livestock listed footrot as a high priority disease against which

almost all adult sheep should be vaccinated (NOAH, 2022). The limited impact of Footvax® on lameness despite the endemic status of footrot in English flocks (Winter et al., 2015), and the potential for serogroup specific vaccines to reduce lameness prevalence and consequently antimicrobial use through increased efficacy and duration of action (sections 1.3.1 and 1.3.2), is justification for a clinical trial under commercial flock conditions in England.

1.5.2 Factors likely to influence the success of a targeted footrot strategy in sheep flocks in England

There are important environmental and pathogen differences between England and the countries that have successfully employed targeted serogroup vaccination. England does not have the predictable non-transmission period that enabled footrot to be eliminated from farms in parts of Australia (Bennett and Hickford, 2011, Green and George, 2008). Therefore, the aim of a targeted vaccination programme will be to achieve control and not elimination of footrot. It is likely that *D. nodosus* survives in the soil for longer in the absence of a prolonged period of hot, dry weather (Clifton et al., 2019). As a result, susceptible sheep are presented with a more persistent *D. nodosus* challenge, effectively giving a greater competitive advantage to serogroups not included in the vaccine. The impact of a bivalent vaccine was more rapid when a flock had fewer serogroups (Dhungyel et al., 2013). Monaghan et al. (2021) detected a median of five serogroups per flock in 24 flocks in England, with up to 45 feet investigated per flock. In a survey of 164 English flocks, with up to eight feet investigated per flock, between 0 and 6 serogroups were detected per flock with no regional pattern to their distribution, but an increased likelihood of detecting three

or more serogroups with a stocking density of >4 ewes/acre (Prosser et al., 2020). The most densely stocked areas of England, including within the South West region, are populated with approximately 1-2.5 sheep/acre across all land use (APHA, 2018).

Sheep movements between flocks that are not followed by a period of isolation from the main flock are a risk for lameness through the introduction of new strains of footrot and CODD (Angell et al., 2014, Best et al., 2020, Dickins et al., 2016, Lewis et al., 2021, Prosser et al., 2019, Winter et al., 2015). However, many sheep farmers do not implement key biosecurity measures. For example, 260/304 (85.5%) of farmers in England purchased sheep, 49.7% always quarantined incoming sheep for >3 weeks and 16.1% always quarantined sheep returning to the farm for >3 weeks (Lewis et al., 2021).

Finally, CODD is present in over 50% of flocks in England (Dickins et al., 2016) and non-infectious causes of lameness such as WLD can also have a high within flock prevalence (Best et al., 2021a, Conington et al., 2010, Reeves et al., 2019). Coinfection of a flock with a primary pathogen other than *D. nodosus* or a high prevalence of lameness due to a non-infectious aetiology could be expected to impede the ability of targeted footrot vaccination to reduce lameness prevalence.

1.6 Study design

1.6.1 Considerations in the design of the clinical trial

A field trial under commercial flock conditions was required to compare a targeted vaccination strategy to control footrot against the existing footrot vaccine in English sheep flocks. 'Best practice' lameness control combines vaccination with prompt treatment of lame

sheep and other control measures. At present, not all farmers in England follow best practice recommendations for the control of lameness in their flock; for example, approximately 50% of farmers in England treat lame ewes within three days and 17.4% always separate ewes from the flock at time of treatment, and 81% do not vaccinate their ewes against footrot (Lewis et al., 2021). By introducing vaccination as a single intervention to flocks that represent the 'average' farmer in terms of lameness control measures, namely treating lame ewes within one week, not routinely separating lame sheep and using a negative control group that are unvaccinated, the true impact of vaccination can be observed under commercial flock conditions. This approach also safeguards the welfare of the sheep by not allowing prolonged periods of lameness and progression of infectious lesions. The use of saline as a negative control allows comparison of antibiotic usage in unvaccinated and vaccinated sheep. Additionally, weekly observation of lame sheep and foot lesions allows the collection of incidence data and the duration of lameness cases, in contrast to vaccination trials that measure direct efficacy by the comparison of flock prevalence at one or more timepoints. This provides more epidemiological insight into the duration of protection provided by the two vaccines and any impact on recovery time following therapeutic treatment.

Environment, host and pathogen factors vary between farms and this affects the protective ability of a vaccine in each flock (Dohoo et al., 2014). *D. nodosus* serogroups are a key pathogen factor that varies between flocks (Prosser et al., 2020) and so treatment and control groups are required within each flock. Vaccine efficacy, the difference in disease occurrence between vaccinated and unvaccinated groups, is reduced by keeping vaccinated

and non-vaccinated animals together due to herd immunity (Dohoo et al., 2014). However, physical separation of treatment and control groups within farms would not guarantee equal exposure to all serogroups present in each flock, which would impact negatively on estimates of treatment effect. Furthermore, high predicted incidence rates for footrot and the persistence of *D. nodosus* in the environment would limit the impact of herd immunity on the negative control group. Finally, separating treatment groups could compromise the ability to blind the study due to the presence of injection site reactions. In conclusion, the aims of the clinical trial would be achieved by managing three treatment groups together within each flock, one vaccinated with the bivalent vaccine, a second group vaccinated with Footvax® and a third unvaccinated group.

1.6.2 Aims of the clinical trial

1) Investigate the effect of targeted bivalent serogroup vaccination on the incidence of lameness and lameness caused by footrot in sheep flocks with multiple *D. nodosus* serogroups, compared to the licensed multivalent vaccine (Footvax®; MSD), or no vaccination.
2) Quantify the difference in antibiotic use for lameness treatments between the treatment and control groups.
3) Measure the size and persistence of injection site reactions.
4) Investigate the effect of vaccinating against two of the most prevalent *D. nodosus* serogroups in a flock on the distribution of those serogroups on the feet of sheep with footrot during the anticipated period of immunity.

CHAPTER 2 SELECTION OF FLOCKS AND SEROGROUPS FOR INCLUSION IN A FLOCK-SPECIFIC BIVALENT FOOTROT VACCINE

This chapter describes the methods used to identify prospective flocks for the clinical trial, and the collection and analysis of interdigital skin swabs from those flocks. The aims of the analysis were: -

1) To establish the serogroup diversity in flocks screened for potential enrolment in the trial

2) To identify the two most prevalent serogroups in flocks enrolled in the clinical trial for inclusion in a custom vaccine

The serogroup diversity results and estimated prevalences of the serogroups in each flock are presented. The effectiveness of the sampling strategy is discussed based on these results.

2.1 Materials and methods

2.1.1 Screening of prospective flocks for enrolment in a clinical trial

Between December 2018 and July 2019, the investigator contacted sheep farmers who were known to them in North and Mid Devon to invite them to participate in the study. The flocks were required to fit the following criteria: -

- Estimated prevalence of lameness was at least 5%
- Footrot was the main cause of lameness

- A group of at least 180 breeding ewes could be kept separate from any other sheep/goats on the holding for the duration of the trial (excluding rams)
- The ewes would not be vaccinated with Footvax® within 6 months of the start of trial
- The farmer did not use moxidectin 1% preparations (either recently prior to trial or intended to in the future)
- The farmer was willing to commit to the trial requirements and be able to assist with catching sheep on a weekly basis
- Pre-enrolment screening demonstrated that multiple *D. nodosus* serogroups were present

A total of 17 farmers were contacted, and 12 flocks were visited between March and July 2019 to check that they met the criteria for enrolment in the study. At each flock visit, one or more groups of ewes and/or lambs were observed at pasture or in a collecting yard to estimate lameness prevalence. Sheep that had a locomotion score of >1 on a validated scoring system (Kaler et al., 2009) were caught and their feet were examined. To facilitate examination, they were either tipped onto their rump or turned in a turnover crate. The purpose of examination was to check that footrot was the main cause of lameness and to collect samples for serogroup identification.

Six of the flocks that were visited met the first six criteria for participation and were screened for serogroup diversity. A sheep was sampled for serogroup screening if at least one foot had any severity of SFR or an ID score of >1 on a 0-4 scale adapted from Moore et al. (2005). The initial aim was to collect an interdigital swab from at least one foot with footrot from at least 40 sheep in each flock. This was predicted to provide at least 25

samples with a *D. nodosus* load sufficiently high to identify the serogroup(s), which would give 95% confidence of detecting six serogroups (Prosser, 2020). There was at least one serogroup in > 80% of swabs in 48 samples from the first two flocks investigated. Consequently, the minimum number of sheep sampled in each subsequent flock was reduced from 40 to 35 to reduce the time taken to screen the samples. Sheep that had an ID score of >2 were sampled preferentially, because these lesions have a higher *D. nodosus* load than less severe lesions (Witcomb et al., 2014). When there were <35 lame sheep, return visits were made until 35 sheep had been sampled. Wherever possible, sheep were sampled from multiple management groups within a flock so that the serogroup diversity of the whole flock could be assessed.

2.1.2 Sampling technique and handling of samples

A single foot was sampled by wiping a sterile cotton swab along the centre of the interdigital skin five times, rotating the swab between wipes. The swab was then replaced in Amies transport medium with charcoal (Transystem™; Copan). If a sheep only had SFR lesions and not ID lesions, the swab was wiped five times along the underrun horn beneath the sole. In some cases, two or four diseased feet from the same sheep were sampled with the same swab. The protocol was adapted by wiping the swab five times along the interdigital skin, rotating the swab between feet by 180^{o} or 90^{o} respectively. The ID and SFR lesion scores, and any other foot lesions present, were recorded for the sampled feet. Swabs were kept in a cool box until either they were transferred to a -20^{o}C freezer within a few hours of collection or refrigerated at 4^{o}C and processed the following day. In 2020 on week 1 of the

trial, all four feet of all ewes with any severity of footrot were sampled using a single swab by wiping the interdigital skin five times and rotating the swab by 90^{o} between feet.

2.1.3 Sampling of flocks to identify the two most prevalent serogroups

Of the six flocks screened for diversity (Table 2-1), flock 4 voluntarily withdrew, footrot primarily affected the lambs and was very low in the ewes in flock 5, and in flock 6 the facilities for lambing meant that it was only possible to keep the absolute minimum number of ewes required for the study separate from the rest of the flock. The three flocks that were selected for enrolment into the study were re-visited between August and October 2019. Flock 3 had only been partially screened prior to this visit because it replaced the flock that voluntarily withdrew after enrolment. Samples were taken from 20-21 ewes with footrot during a single visit to each farm, using Hill et al. (2010) on sampling strategies for serogroup selection for a bivalent footrot vaccine. The ewes selected for sampling belonged to management groups within the flocks that were likely to be used in the clinical trial. Ewes were sampled if they had a minimum of one foot with ID score 3 or 4, and/or any degree of SFR. In flocks 1 and 2 only lame ewes were selected for sampling. In flock 3 all ewes in a group with a high prevalence of lameness were turned and examined until 20 ewes that met the footrot lesion criteria had been sampled.

2.1.4 Laboratory techniques

2.1.4.1 Sample pooling

Swabs were pooled using a technique developed for a previous study (Monaghan et al., 2021). After thawing, each swab was placed in a separate, labelled 1.5ml microcentrifuge

tube. The swab shaft was removed using separate scissors for each pool. The scissors had been sterilised by being baked for 24 hours at >190°C. After adding 300µl of phosphate buffered saline (PBS), tubes were vortexed for 5 seconds and centrifuged for 1 minute at 11000 x *g*. A collection column was prepared for each sample by placing a labelled 1.5ml microcentrifuge tube on top of a second microcentrifuge tube inside a 50ml falcon tube. The assembled column was secured in place by pushing a sterile 2ml syringe through a hole in the lid of the falcon tube into the labelled microcentrifuge tube. The syringe plunger was removed and the swab transferred to the syringe using sterile scissors, one pair per pool. Columns were centrifuged at 1600 x *g* for 8 minutes before transferring the contents of all microcentrifuge tubes to a single 2ml screw cap tube, along with the resuspended PBS from the original microcentrifuge tubes. The 2ml tubes for all pooled samples were centrifuged at 15,000 x *g* for 5 minutes, the supernatant was removed and the pellet was resuspended in 200µl of PBS. Pooled samples were stored at -20°C.

2.1.4.2 DNA extraction

A modified protocol using the NucleoSpin® Tissue Kit (Macherey-Nagel) was used for DNA extraction, based on that described by Muzafar et al. (2015). Individual swabs were placed in separate, labelled 2ml tubes and the swab shaft removed using separate, baked scissors for each swab. 400µl of T1 lysis buffer (NucleoSpin® Tissue Kit) and 40µl of Proteinase K (NucleoSpin® Tissue Kit) were added to each tube for both individual swabs and pooled samples, vortexed for 5 seconds and incubated at 56°C for 10 minutes in a heat block. After adding 400µl of B3 lysis buffer (NucleoSpin® Tissue Kit), tubes were vortexed for 5 seconds and incubated at 70°C for 5 minutes, then allowed to cool for 5 minutes before centrifuging

at 12000 x *g* to pellet any debris. The supernatant was transferred to a new 2ml tube containing 400µl of ethanol and centrifuged at 11000 x *g* for one minute. The supernatant was loaded into a spin column (NucleoSpin® Tissue Kit) and centrifuged at 11000 x *g* for one minute. Flow through was discarded, 500µl of BW wash buffer (NucleoSpin® Tissue Kit) was added and samples were centrifuged at 11000 x *g* for one minute. The flow through was discarded and 600µl of B5 wash buffer (NucleoSpin® Tissue Kit) was added. Samples were centrifuged twice more at 11000 x *g* for one minute, discarding flow through. Each spin column was placed in a 1.5ml microcentrifuge tube and 45µl of BE elution buffer (NucleoSpin® Tissue Kit), preheated to 70°C, was added. After 1-2 minutes the tubes were centrifuged at 11000 x *g* for one minute to elute the DNA. Samples were stored at -20°C.

2.1.4.3 Single serogroup PCR

Samples were tested for serogroups A to I by single serogroup PCR in a 25µl reaction containing 1µl template DNA, 12.5µl MyTaq™ Red Mix (Bioline), 0.5µM forward primer, 0.5µM reverse primer and 0.5mg/ml BSA. The primers used were those published by Dhungyel et al. (2002). A Nexus GSX1 (Eppendorf) was used for amplification, and the same amplification cycles as Dhungyel et al. (2002) used with microcentrifuge tubes. Water was included as a non-template control on every PCR plate. Positive controls from known strains were included for each serogroup (Table A- 1). PCR products underwent electrophoresis on 1% agarose gels prepared with SYBR™ Safe DNA Gel Stain (Invitrogen) to allow visualisation under ultraviolet light.

For flock serogroup diversity screening, once a serogroup was known to be present in a flock further samples were not tested for that serogroup because they were not used for

quantitative estimates of prevalence. All samples taken from ewes in the three enrolled flocks to estimate flock serogroup prevalence were tested individually for all *D. nodosus* serogroups A-I.

2.1.5 Calculation of confidence intervals for flock serogroup prevalence

Confidence intervals for the prevalence of each serogroup in the three flocks were calculated using the Exact binomial method with 95% confidence in R versions 3.5.2 and 4.0.2 (R Core Team, 2020) using the 'propCI' function in the *prevalence* package v. 0.4.0 (Devleesschauwer et al., 2015). The samples collected from ewes during the flock visits described in section 2.1.3 were used to estimate flock serogroup prevalence in 2019. The samples collected from ewes on week 1 of the trial, including any ewes subsequently withdrawn from the study, were used to estimate flock serogroup prevalence in 2020.

2.2 Results

2.2.1 Flock serogroup diversity

There were 115 individual swabs and 122 pooled swabs tested in six flocks (Table 2-1) and 1077 PCR tests to identify the presence of individual serogroups. Samples were processed in batches of 19-21 on a rolling basis to recruit farms as efficiently as possible. The first 50 samples for flock screening were processed individually so that the proportion of swabs with one or more serogroups could be calculated. Thereafter, groups of 4-6 swabs were pooled based on similar footrot lesion score. In flock 2, three pooled samples resulted in a weak positive or no serogroup identification so subsequent samples were processed individually. The median number of serogroups per flock was 4.5. There were additional serogroups

detected at a low frequency in flocks 1 and 2 in the swabs taken to estimate serogroup prevalence in flocks 1, 2 and 3 (Table 2-2).

Table 2-1 Number of individual and pooled interdigital swabs analysed by PCR for screening flock D. nodosus serogroup diversity, and the serogroups detected in each flock

Flock	Number of individual samples (number of unique sheep)	Number of pooled samples (total number of swabs pooled)	Serogroups detected
1	2	7 (33)	C, E, H, I
2	42 (35)	3 (13)	A, B, F, H
3[†]	21	5 (25)	B, C, F, H, I
4	0	8 (40)	A, B, C, G, H
5	27	2 (11)	A, H
6	23	0	B, D, E, G, H, I
Total	115 (108)	25 (122)	A, B, C, D, E, F, G, H, I

[†]Additional samples for serogroup diversity and prevalence were collected simultaneously

Table 2-2 Total number of swabs analysed for serogroups in 2019, individually or pooled, and the serogroups detected in the three flocks enrolled in the trial

Flock ID	Number of swabs	Serogroups
1	56	**A, B**, *C,* E, H, I
2	76	A, B, **D,** F, H
3	46	B, C, F, H, I

Serogroups only detected during serogroup diversity screening are shown in italics, serogroups only detected in samples taken for prevalence estimation are shown in bold

2.2.2 Estimation of the two most prevalent serogroups

There were 62 swabs analysed from flocks 1, 2 and 3 and a total of 558 individual serogroup PCR tests to identify the most prevalent serogroups. The two most prevalent serogroups in flock 1 were serogroups H and I (Figure 2-1, Table A- 2). In flocks 2 and 3, the most

frequently detected serogroup was H but it was not possible to estimate the two most prevalent serogroups with 95% confidence (Table A- 3, Table A- 4).

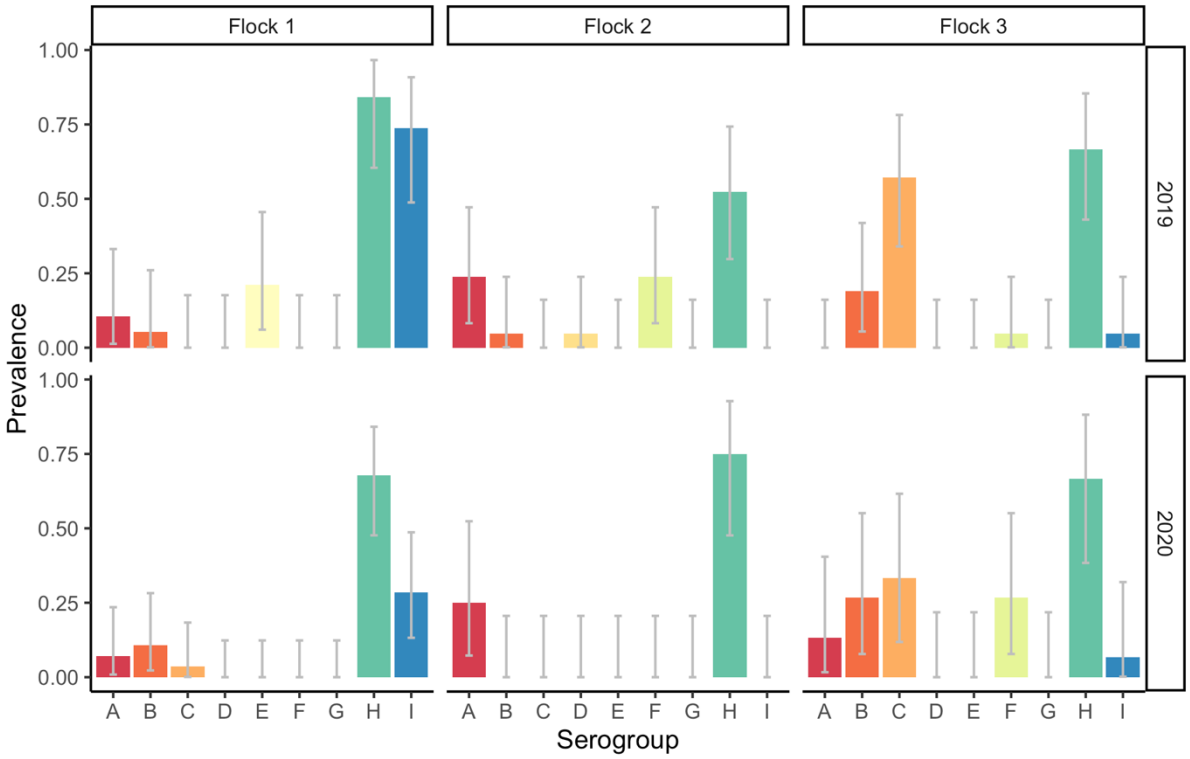

Figure 2-1 Serogroup prevalence in ewes with footrot lesions in 2019, and week 1 of the trial in 2020, for flocks 1,2 and 3 respectively. Error bars indicate 95% CI. n = number of swabs.

Prevalence estimates for flock 2 from all 56 swabs that were tested individually confirmed serogroup H as the most prevalent serogroup but it was still not possible to identify the second most prevalent serogroup (Table A- 5). Serogroup A and serogroup C were selected as the second serogroup in flocks 2 and 3 respectively because they were numerically the second most common serogroup in the flock, and additionally for flock 2, because serogroup A is more common than serogroup F in flocks nationally (Prosser et al., 2020).

Further sampling was planned for Spring 2020 to check for changes in serogroup prevalences prior to the commission to make the bivalent vaccine, but covid-19 lockdown restrictions prevented this from occurring. This resulted in a delay of 12 months between serogroup prevalence estimations and week 1 of the trial in all flocks. Additionally, all three flocks bought-in replacement ewes within two months of the start of the trial, which provided an opportunity for new serogroups to be introduced to the flocks. Despite the delay between sampling for serogroups and vaccination, and the introduction of new stock, the serogroups selected for the bivalent vaccines in 2019 remained the most frequently detected serogroups in 2020 (Figure 2-1). Serogroup H was still the most frequently detected serogroup in all three flocks in week 1 of the trial in 2020. Furthermore, serogroups I, A and C, in the bivalent vaccines for flocks 1, 2 and 3 respectively, were the second most frequently detected serogroups in those flocks in 2020. It was estimated with 95% certainty that at least one of the two most prevalent serogroups in footrot lesions at the start of the trial was present in the bivalent vaccines for flocks 1 and 2. In flock 3, it was not possible to predict with 95% certainty either of the two most prevalent serogroups due the greater diversity of serogroups in ewes with footrot and the low number of samples taken in week 1 (n=15). In general, the serogroups that were less prevalent in 2019 and 2020 were more variable in prevalence and diversity between 2019 and 2020.

In flock 1, there was no difference in the proportion of ewes with footrot lesions that had only serogroups in the bivalent vaccine between 2020 and 2019 (Table 2-3). In flock 2, a higher proportion of ewes with footrot lesions had only serogroups in the bivalent vaccine in 2020 than in 2019, and in flock 3 the proportion of ewes with serogroups in the bivalent

vaccine in week 1 was lower than in 2019. All serogroup combinations identified in week 1 of the trial are presented in Table A- 6.

Table 2-3 The percentage of ewes with footrot lesions and only bivalent vaccine serogroups detected out of all ewes with footrot lesions that had at least one serogroup detected

	Year	% (no. swabs)	95% CI
Flock 1			
	2019	57.1 (12/19)	34.0-78.1
	2020	60.7 (17/28)	40.6-78.5
Flock 2			
	2019	33.4 (7/21)	14.6-56.7
	2020	81.3 (13/16)	54.4-96.0
Flock 3			
	2019	65.0 (13/20)	40.8-84.6
	2020	20.0 (3/15)	4.3-48.1

2.3 Discussion

The three flocks that were enrolled in the clinical trial had a diversity of serogroups similar to the national flock (Prosser et al., 2020). The median number of serogroups of five per flock was consistent with the study by Monaghan et al. (2021) where a median of five serogroups per flock was detected after multiple interdigital skin swabs were analysed from 24 English flocks sampled on three occasions over 18 months. Serogroup H was the most frequently observed serogroup in all flocks at the start of the trial and there was no pattern to the presence or prevalence of other serogroups in the flocks. These observations are consistent with the results of previous surveys of British flocks (Thorley and Day, 1986) and English flocks (Prosser et al., 2020, Hindmarsh, 1985). Therefore, it is likely that the flocks that participated in the trial were not markedly different from other English flocks in terms of *D. nodosus* serogroup prevalence and diversity.

A bivalent vaccine is most effective when it contains the only two serogroups present in a flock (Dhungyel et al., 2013), however, there was a median of five serogroups per flock in this study and so the response to the bivalent vaccine was expected to vary between flocks depending on the prevalence and abundance of serogroups not in the vaccine. It was possible to identify at least one of the most prevalent serogroups in all three flocks in 2019 with 95% certainty by sampling 21 ewes. Increasing the number of samples for prevalence estimation to include all 56 swabs taken from flock 2 in 2019 led to the identification of serogroup H as most prevalent but did not enable determination of the second most common serogroup, a finding consistent with simulations carried out by Hill et al. (2010). Serogroup prevalence within the flocks, particularly the most dominant serogroups, remained relatively stable over 12 months despite the movement of sheep into the flocks and so it was not anticipated that the delay between testing and vaccinating would impact on the protective effect of the bivalent vaccine.

It was not possible to identify the serogroups present on some of the swabs taken in 2019 and week 1, 2020, although this did not exceed 21% in any flock in 2020. If the presence of serogroup M was the reason for non-identification of serogroups, this could result in a poorer clinical response to vaccination than expected (Dhungyel et al., 2015). However, in 2020 the highest *D. nodosus* load on any of the swabs with non-detected serogroups was only 431 copies/uL, below the sensitivity threshold for most of the serogroup PCRs and therefore it is likely that the non-identification of serogroups was due to the bacterial load being below the threshold.

2.4 Conclusions

The sampling strategy resulted in the bivalent vaccine containing the serogroup combination appropriate for the highest proportion of ewes in flocks 1 and 2. The diversity and prevalence of serogroups in flock 3 meant that a relatively low proportion of the flock were carrying only the serogroups contained in the bivalent vaccine, which might reduce the protective effective of the vaccine (Dhungyel et al., 2013).

CHAPTER 3 A RANDOMISED, DOUBLE BLINDED CONTROLLED TRIAL OF BIVALENT FLOCK-SPECIFIC FOOTROT VACCINES IN THREE COMMERCIAL FLOCKS

The protocol for the clinical trial is presented in the first part of this chapter. The following sections describe the materials and methods for laboratory and data analysis.

3.1 Clinical trial protocol

3.1.1 Purpose of the study

This was an exploratory trial into the use of targeted, bivalent serogroup vaccination for the prevention of footrot in sheep under commercial flock conditions in England. The trial was not intended to collect data to support an application for registration of the vaccine. The work was funded by BBSRC and AHDB who have no commercial interest in vaccine development.

3.1.2 Ethical approval

The study was approved by the University of Birmingham Animal Welfare and Ethical Review Body (19/08-07-3) and conducted under an Animal Treatment Certificate granted by the Veterinary Medicines Directorate (ATC 21761/0001).

3.1.3 Schedule of events

The timing of study procedures and observations are summarised in Figure 3-1.

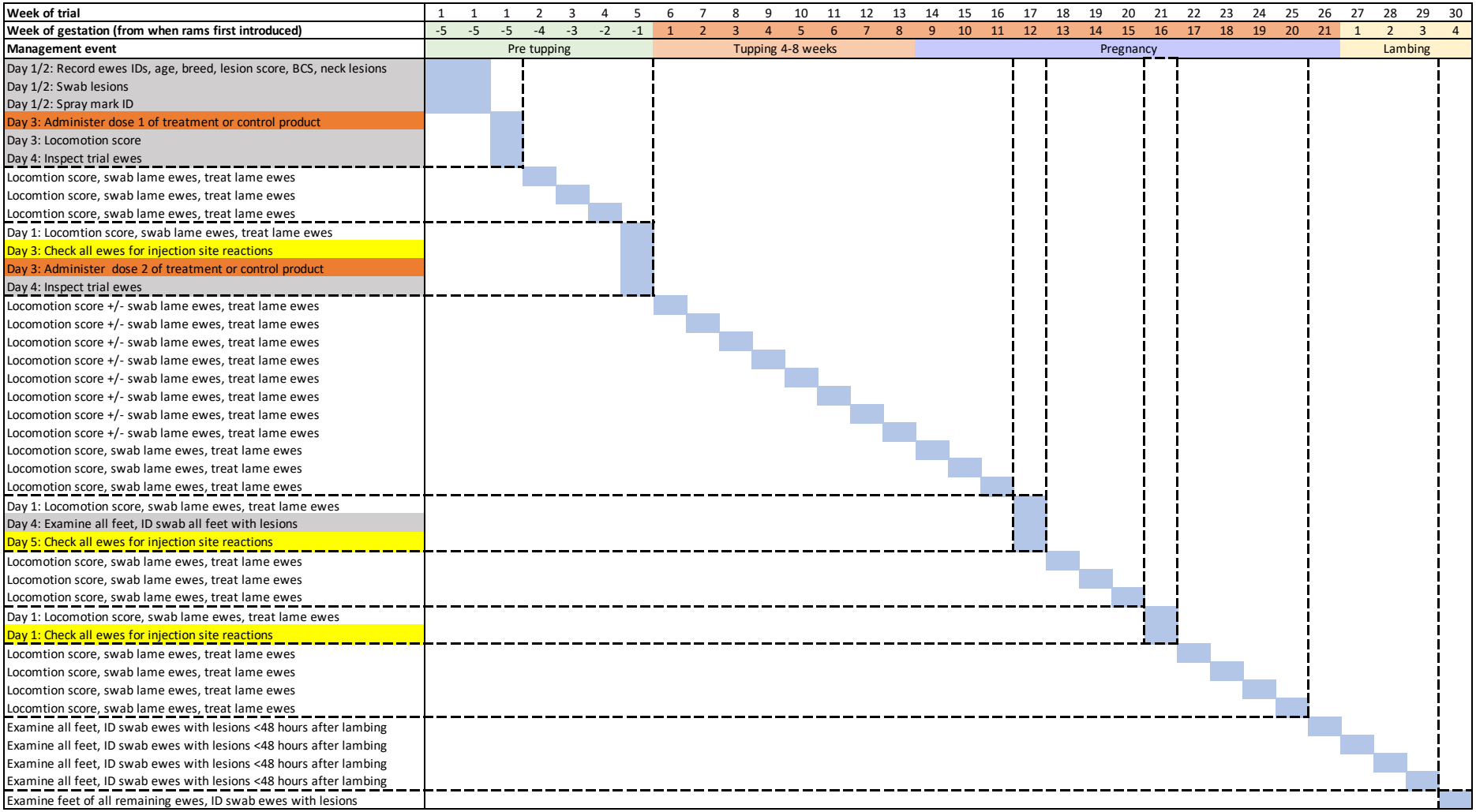

Figure 3-1 Schedule of treatments and observations

3.1.3.1 Administration of the treatment and control products

The first dose of treatment product was given on week 1. A second dose of treatment was administered exactly four weeks after the first dose.

3.1.3.2 Commencement of trial, monitoring period and trial termination

The trial lasted 7 months. It started approximately one month before the rams were introduced, which varied by flock and so the trial start dates were between 17th August and 30th September 2020. All sheep in the trial were monitored from week 1, the time of first treatment, until week 25. From week 25 onwards, ewes left the trial within 48 hours of parturition after a final inspection of their feet. This inspection was after parturition to avoid handling heavily pregnant ewes. All ewes that had not lambed by week 30 were examined at a final visit.

3.1.4 Study design

3.1.4.1 Overall design

The study was randomised and double-blinded, with two treatment products and a negative control, and conducted under field conditions.

3.1.4.2 Randomisation and blinding methods

On week 1, days 1 and 2, all trial ewes were examined by the investigator and, if they did not meet the criteria for exclusion (section 3.1.5.1), spray marked on both flanks with a unique number. The number was recorded against the ewe's unique ear tag number using an EID reader. Body condition score (BCS) (AHDB, 2019), age by dentition, breed, existing lumps on the neck, foot lesions and footrot and CODD severity were recorded. Data were entered into

an Excel spreadsheet (Microsoft Corporation, 2018). Footrot lesion severity per ewe was calculated using a weighted footrot score (FS). This was calculated as follows: ID and SFR scores on a foot (section 3.1.7.2) were summed then squared, the four scores from the four feet of a ewe were summed then divided by 4 to result in a score between 0 and 64. Ewes were allocated one of four footrot severity categories; 1 = no ID or SFR lesions, 2= 0<FS<2, 3= 2<FS<8, 4= FS>8.

Ewes were stratified into sub-groups by breed, BCS category (<2/2.5-3.5/>3.5), footrot lesion severity category (1-4, as described above) and CODD category (yes/no) by the investigator. An independent research assistant allocated each ewe within the strata to treatment group A, B or C in blocks of 3, or multiples of 3 based on group size, using the random generator 'sample' function in the R software (R Core Team, 2018). The randomly generated letter was then allocated in numerical order of ewe spray mark number within the strata and recorded on a hard copy of the list of ewes by the same research assistant.

The three treatment types were placed in sealed identical envelopes by the investigator. The independent research assistant was blind to the content of the envelopes and randomly labelled the envelopes A, B or C. This was repeated for each farm.

The research assistant unblinded themselves to the envelope contents immediately before the first treatment doses were administered on week 1, day 3, so that they could prepare the treatment doses. They recorded the code identities on the hard copy list of ewes with their treatment allocations. On both treatment days, the unblinded research assistant was informed of each ewe's identity as they were caught for injection. They checked the

treatment list, crossed the ewe off, and provided the appropriate treatment to the investigator, having first obscured the contents of the syringe and needle hub with a blank label. The investigator administered all treatments and disposed of the syringes and needles without separation to avoid the risk of seeing the residual contents in the syringe/needle hub.

The hard copy of the treatment lists, along with photograph images, were stored by the independent research assistant and a second member of the research group who was not involved in data collection or analysis.

Laboratory procedures and analyses were undertaken blinded to treatment identity. Treatment identities were unblinded to the investigator after exploratory data analysis of lameness, foot lesions and injection site reactions, and the statistical analysis of group randomisation, lameness and footrot in individual flocks. Survival analysis of lameness and footrot using the dataset for the combined flocks was performed unblinded because treatment aliases were different in the three flocks. It was also necessary to identify the positive and negative controls to use as the reference categories for treatment effect. Statistical analysis of injection site reactions with the two vaccine types was done after the treatments were unblinded.

3.1.4.3 Treatment groups

After treatment, trial ewes were mixed together regardless of treatment. Subdivision into management groups was permitted, for example for breeding and for feeding after

scanning. There was no way of controlling uneven distribution of treatment groups if ewes were divided by scanning result.

Ewes enrolled in the study were kept separate from ewes and lambs on the farm not enrolled in the study. Rams were untreated and mixed with study ewes during the mating period. The number and identity of the rams introduced to each mating group was recorded for each farm and the feet of all rams were lesion scored and swabbed before being introduced to the ewes. The ratio of rams to ewes varied between 1:13 and 1:196 and rams were moved between management groups.

Incidents of sheep escaping into or from the treatment groups were deemed inevitable and representative of field trial conditions. Nevertheless, these movements were documented as a deviation from protocol.

3.1.5 Animal selection and identification

The aim of the study was to test the protective effect of a novel vaccine strategy under the field conditions of commercial English sheep farms. Three lowland flocks in South West England consisting of crossbred ewes, mainly North Country Mules and Mules crossed with terminal sire breeds or Bluefaced Leicester, and used for producing lambs for meat, were selected to represent a large proportion of English sheep farms. The three flocks met the selection criteria outlined in chapter 2. Between 180 and 200 ewes were enrolled into the study from each farm. Ewes were a mixture of homebred and purchased replacements, were mixed ages and on average 70kg in bodyweight. Rams were not enrolled because they were not going to remain in the group for the full duration of the study.

All ewes had two ear tags with the flock identity and unique individual number, and at least one of these was electronic, in accordance with current legislation. The microchip failed in a small percentage of tags, and these were retained provided that the unique ewe ID was legible on at least one tag. If neither tag was legible the tag was replaced. If both ear tags were missing they were replaced, and the new unique ID recorded on a designated form against the spray mark number allocated to the ewe. Ewe EID numbers were cross referenced against the unique spray mark number before every individual examination or treatment.

3.1.5.1 Exclusion criteria and criteria for withdrawal after enrolment

Ewes were not eligible for enrolment under the following criteria.

1) A non-treatable foot condition such as a granuloma, or a chronic foot deformity such as historical CODD (score 5) accompanied by lameness on that foot
2) Systemic illness when first examined in week 1

Ewes were withdrawn from the trial if they developed a non-footrot condition that caused lameness, which did not show a clinical improvement after three consecutive weeks of treatment, or caused repeated lameness, and required off-protocol therapeutic treatment or a prolonged period for recovery. No living ewes that had been enrolled into the clinical trial left the farm holding for the duration of the trial.

3.1.6 Treatment and control products

3.1.6.1 Product details

The treatment products were the Custom R-Pilus vaccine, which contained *D. nodosus* serogroups H and I, H and A, or H and C for flocks 1, 2 and 3 respectively, and the multivalent vaccine Footvax®. Aqupharm no.1 infusion was used as the negative control treatment. Selected pharmaceutical particulars and details of marketing authorisation for the three treatments are provided in Appendix 4.

3.1.6.2 Storage of treatment and control products

The cold chain for the transport and storage of Custom R-Pilus vaccine was maintained and monitored from the point of manufacture until the day of use. The vaccine was shipped by air in an insulated container containing two thermo-dataloggers. On arrival in the UK the vaccine was transported to veterinary premises for storage in a monitored pharmaceutical fridge and the temperature data checked to ensure storage conditions had not been breached in transit. On the day of use, both the Custom R-Pilus and Footvax® packs were kept cool in an insulated box and removed in sufficient time to allow the vaccines to reach ambient temperature prior to injection. Vaccine containers that had been removed from the pharmaceutical fridge were disposed of if they had not been broached.

3.1.6.3 Dosing justification

The dosing regimen recommended by the manufacturer was used for the Custom R-Pilus vaccine, which was two 1ml doses given subcutaneously, 4 weeks apart. Footvax® is also licenced as a 1ml, subcutaneous dose. The datasheet for Footvax® has varying dosing

regimens for treatment or prevention of footrot allowing use of the product to be tailored to individual flock conditions. The datasheet for Footvax® states *'Prevention programme- Commence vaccination with a single dose of vaccine. Further doses of vaccine will be required according to the flock disease status and/or the climatic conditions. If, after 4 - 6 weeks significant levels of disease remain in the flock or climatic conditions conducive to footrot persist, administer a further dose'* (NOAH, 2021). A two dose regimen was selected to test the bivalent vaccine against the maximum achievable protection that can be gained from the multivalent vaccine in accordance with the datasheet. The same volume and dosing interval was used for all treatments to enable the blinding process.

3.1.6.4 Administration of vaccine and control treatments

The investigator was experienced in handling and injecting sheep. A separate sterile 2ml syringe and 18 gauge, 2.5cm needle was used to draw up and inject each individual dose of vaccine and negative control treatments. The first dose of treatment was injected as per datasheet *"2-3 inches behind the ear"* on the right side of the neck and the second dose on the left side of the neck (Figure 3-2). The standard operating procedure (SOP) for administering the vaccines and control treatments is in Appendix 5.

3.1.7 Assessment of the protective effect of the bivalent vaccine

3.1.7.1 Effects to be achieved for a protection to be claimed

1) A significantly lower incidence of lameness due to footrot in sheep vaccinated with the bivalent vaccine compared to the negative control treatment after full immunity from vaccination was anticipated.

2) The incidence of lameness due to footrot in sheep vaccinated with the bivalent vaccine to be equivalent, or lower than, the incidence of lameness due to footrot in sheep vaccinated with Footvax® after full immunity from vaccination was anticipated.
3) A significantly lower prevalence of injection site reactions in sheep vaccinated with the bivalent vaccine compared to Footvax® at each measurement time point.

Figure 3-2 Ewes were restrained by an assistant whilst the investigator administered the treatment doses subcutaneously 5-8cm behind the ear

Sample size was based on the survival probability for ewes experiencing at least one case of lameness with footrot during the trial. The 'ssizeCT.default' command in the *powerSurvEpi*

package v0.1.0 (Qui et al., 2021) was used for sample size calculation. An effect size (hazard ratio) of 2 was predicted between ewes in the bivalent vaccine treatment group and ewes in the saline treatment group and was deemed to be of clinical significance. Sheep in the bivalent vaccine treatment group were predicted to have a 15% probability of becoming lame with footrot, equivalent to 30 cases/100 ewes/year, and sheep in the saline treatment group were predicted to have a 30% probability of becoming lame with footrot, equivalent to 60 cases/100 ewes/year. To achieve 80% power of detecting a significant difference with α= 0.05, 157 sheep were required in each treatment group.

The sample size required for demonstrating a difference in the proportion of ewes with injection site reactions was calculated using epitools (Sergeant, 2018). To achieve 80% power of detecting a significant difference with α= 0.05, where 2% of the investigational treatment group and 10% of the positive control group have an injection site reaction, 162 ewes were required in each treatment group.

A small number of losses due to death or culling were anticipated. Also, occasional treatments with parenteral antibiotics for reasons other than lameness were expected to reduce the number weeks that ewes were 'at risk' of becoming lame, therefore a minimum of 60 ewes per treatment group per farm, giving 180 ewes per treatment overall, were enrolled.

3.1.7.2 Measurement and recording of clinical effects

All measurements were taken by the investigator.

1) Lameness presence and severity was measured in individual ewes using a validated seven-point scale for locomotion (Kaler et al., 2009) (Table 3-1).

Table 3-1 Locomotion scoring scale, reproduced from Kaler et al. (2009)

Score	0	1	2	3	4	5	6
Posture and locomotion							
Bears weight evenly on all four feet	■						
Uneven posture		■	■	■	■	■	
Short stride		■	■	■	■	■	
Noticeable flicking of head in time with short stride			■				
Excessive flicking of head in time with short stride				■	■		
Not weight bearing on affected limb when standing				■	■		
Discomfort when moving				■	■		
Not weight bearing on affected limb when moving					■		
Extreme difficulty rising						■	
Reluctant to move once standing						■	
More than one limb affected						■	
Will not stand or move							■

2) ID and SFR lesions on all feet were recorded using an objective scoring system adapted from Moore et al. (2005) (Table 3-2) and Buller and Eamens (2014) (Table 3-3) respectively.

Table 3-2 Interdigital dermatitis (ID) lesion classification, adapted from Moore et al. (2005)

Score	Description of lesion
0	Clean interdigital space with no dermatitis lesions
1	Slight interdigital dermatitis, partial loss of hair, slight redness but dry
2	Slight interdigital dermatitis (<10% of the interdigital area affected), partial/complete loss of hair, redness and/or grey erosive appearance to skin, +/-pasty scum.
3	Moderate interdigital dermatitis (10-50% of the interdigital area affected), partial/complete loss of hair, redness and/or grey erosive appearance to skin, +/- pasty scum.
4	Severe interdigital dermatitis (>50% of the interdigital area affected), partial/complete loss of hair, redness and/or grey erosive appearance to skin, +/- pasty scum.

Table 3-3 Severe footrot (SFR) lesion classification, adapted from Buller and Eamens (2014)

Score	Description of lesion
0	No inflammation or under-running of axial horn. No under-running of the wall or sole of the digit
1	Necrotising inflammation which involves part or all of the soft horn of the axial wall of the digit. No under-running of axial horn.
2	Underrunning of the hoof horn involving any degree of the axial, solar and heel horn but not extending to the abaxial wall
3	Underrunning of the hoof horn involving any degree of the axial, solar and heel horn with separation of the abaxial hoof wall
4	Chronically deformed foot, with separation of hoof horn but not an active lesion

In addition to footrot lesion scores, all feet were scored for CODD using the scoring scale of Angell et al. (2015a) (Table A- 10), and other foot lesions were recorded if present at all examinations.

3) The location and size of injection site reactions were recorded as per the SOP in Appendix 6.

3.1.8 Timing and frequency of study observations

3.1.8.1 Locomotion scores

From week 1 until week 25, all trial sheep were scored for locomotion every week. In week 1 they were scored within seven hours of receiving the treatment products. It was not possible to score them earlier because (1) the sheep required spray marks to be identified for scoring and (2) they were housed so their fleeces were dry before being injected to reduce the risk of injection site infections. From week 2, ewes were scored on the same day of the week in each flock. For most of the study the sheep were scored for locomotion whilst at grass. From week 18 and 16 the sheep were housed in flocks 1 and 2 respectively. They were then observed in their pens if there was sufficient space for unhindered and natural movement, or they were moved into a yard for observation.

3.1.8.2 Lesion scores

a. Whole flock examinations

   The feet of all enrolled sheep were examined and lesion scored on three occasions:

   1) Week 1 at enrolment
   2) Week 17 after scanning
   3) Within 48 hours of lambing, or on week 25 if lambing or abortion had already happened, or week 30 if the ewe had not yet lambed.

b. Lame sheep examinations

   Any sheep that scored >1 for locomotion during a weekly observation, or locomotion score 1 for the second consecutive week from week 3 was caught and examined. The exception was during weeks 6-9, 11 and 12 in flock 2 because the farmer did not

want his ewes gathered at these times because they were concerned that gathering and handling might be detrimental to flock fertility.

3.1.8.3 Injection site reactions

At start of the trial the necks of all sheep were examined and the size and position of any pre-existing masses present in the skin, subcutaneous tissue or muscle recorded as per SOP (Appendix 6). This examination was repeated on weeks 5, 17 and 21. The final examination was done before administration of the pre-lambing clostridial booster vaccination.

3.1.8.4 Body condition score and other key performance indicators

Ewe BCS was scored on week 1, week 17 and within 48 hours of lambing using a recognised BCS scale to half points (Table A- 11). Ewes that did not lamb or lambed/aborted before week 25 did not have their BCS measured at the end of the trial. The number of lambs born and the number of lambs alive were also recorded at the exit examinations. Pregnancy scanning results were recorded so that barren ewes could be identified for exit examination at the start of the lambing period.

3.1.9 Sampling method

At all examinations, every ewe with ID or SFR on at least one foot had the interdigital skin of all feet swabbed. Lame ewes without a visible infectious cause of lameness were also swabbed. Ewes were either restrained in a sitting position by the investigator or the farmer (Figure 3-3a) or restrained upside-down in a turnover crate (Figure 3-3b). All swabs were collected by the investigator who changed gloves after each ewe. A single cotton tipped swab was wiped along the interdigital skin of each foot five times, rotating the shaft by 90^{o}C

between feet. Healthy feet were swabbed first, and the remaining feet swabbed in order of increasing severity of ID then SFR score. The swab was placed in Amies transport medium with charcoal (Transystem™; Copan) and kept in a cool box prior to storage at -20°C on the day of collection. Swabs were labelled with the flock ID, ewe spray number and date

Figure 3-3 Technique for swabbing the interdigital skin with (a) the ewe restrained by the investigator and (b) the ewe restrained in a turnover crate

3.1.10 Animal management and housing

3.1.10.1 Containment and feeding

The flock owner was responsible for the feeding and routine management of the trial sheep following their normal farming practice. The trial sheep were housed on organic bedding material and grazed on forage pastures in accordance with standard commercial conditions. Details of the locations of groups of trial ewes were recorded on a weekly basis so that opportunities for indirect transfer of *D. nodosus* serogroups were documented.

3.1.10.2 Permissible and non-permissible concomitant veterinary care and therapy

During the trial the investigator was responsible for all lameness treatments and control measures. Ewes were not footbathed during the trial. Owners were instructed to contact the investigator if they had any concerns about a lame sheep between the weekly farm visits. On week 1, only noticeably lame ewes (locomotion score >2 - non-weightbearing at rest or during locomotion) or ewes with active CODD lesions were given therapeutic treatments. Treatments were given at the time of trial product administration, within 7 hours of being scored for locomotion so that baseline lameness measurements were not affected by the treatments. On subsequent weeks, only ewes that exceeded the threshold for lameness that were examined (as per section 3.1.8.2) were given therapeutic treatment. On all weeks therapeutic treatments were selected according to a defined protocol (Table 3-4). No foot trimming was performed except for the removal of a section of detached abaxial hoof wall to release impacted material in three cases of WLD.

No other vaccinations were given within two weeks of the trial commencing or until week 21. Concurrent routine flock treatments such as anthelmintics were permissible, except for moxidectin 1% injectable products. The owner had been advised in advance of the trial that the use of those products was contraindicated for the lifetime of the ewe. In addition, the private veterinary surgeon for the farm was also informed of this contraindication in a letter explaining the trial prior to commencement. Routine treatments were not administered on the same day as the treatment and control products. All other veterinary treatments were permitted. Details of all flock and individual treatments were recorded by the investigator at the weekly visits.

Table 3-4 Products, doses and protocols used for therapeutic treatment of foot conditions. The protocol for amoxycillin was used when concurrent foot lesions with different treatment indications occurred. Score is the maximum score on any foot of a ewe.

Condition	Score	Treatment
ID or SFR	All scores	20 mg/kg oxytetracycline (Alamycin LA 200mg/ml solution for injection; Norbrook) Topical tetracycline spray for 5 seconds on all feet (Cyclo spray, chlortetracycline HCL 2.45 % w/w; Decra)
Active CODD	1-4	15mg/ml amoxycillin (Betamox LA 150mg/ml; Norbrook; or Trymox 150mg/ml; Univet). Repeat dose after 48 hours given by farmer. Topical tetracycline spray for 5 seconds on all feet (Cyclo spray, chlortetracycline HCL 2.45 % w/w; Decra)
Ulcerative skin lesions	NA	15mg/ml amoxycillin (Betamox LA 150mg/ml; Norbrook; or Trymox 150mg/ml; Univet). Repeat dose after 48 hours given by farmer. Topical tetracycline spray for 5 seconds on lesion (Cyclo spray, chlortetracycline HCL 2.45 % w/w; Decra)
Granuloma	NA	15mg/ml amoxycillin (Betamox LA 150mg/ml; Norbrook; or Trymox 150mg/ml; Univet). Repeat dose after 48 hours given by farmer. Topical tetracycline spray for 5 seconds on lesion (Cyclo spray, chlortetracycline HCL 2.45 % w/w; Decra)
White line degeneration	NA	Remove any impacted material. Topical tetracycline spray for 5 seconds on all feet (Cyclo spray, chlortetracycline HCL 2.45 % w/w; Decra) If evidence of infection (inflamed laminae or heat and swelling of digit) 20 mg/kg oxytetracycline (Alamycin LA 200mg/ml solution for injection; Norbrook)
Hoof wall abscess	NA	15mg/ml amoxycillin (Betamox LA 150mg/ml; Norbrook; or Trymox 150mg/ml; Univet)
Pain in foot but no visible cause of lameness	0	Topical tetracycline spray for 5 seconds on all feet (Cyclo spray, chlortetracycline HCL 2.45 % w/w; Decra)
Other	NA	Dependent upon clinical findings

3.1.11 Adverse events

Study animals were observed by the investigator for a minimum of two hours after the last animal received a trial treatment. The investigator returned the following day to inspect the study animals in situ after both first and second doses were given. Thereafter the study animals were inspected a minimum of once a week by the investigator. Interim inspections were carried out by the owner in accordance with their typical routine. For the first three months, the owner was instructed to contact the investigator immediately if they observed any sick or dead sheep enrolled in the study so an examination or post-mortem examination could be performed by the investigator or the owner's private veterinary surgeon and reported to the VMD if a serious adverse event was suspected.

3.1.12 Recording and storage of data

Locomotion scores were recorded directly onto a spreadsheet (Google Sheets) against the individual spray number by use of a tablet. All other scheduled observations, group identity and any therapeutic treatments were recorded on the EID reader. Ad hoc records and details of group locations were kept on designated forms and stored in a ring bound file for each farm, which were kept by the investigator in a locked cabinet. Digital images of all paper records were taken on completion of each form.

3.2 Laboratory analysis

3.2.1 Randomisation and blinding procedure for interdigital swabs

The criteria and procedure for collecting interdigital skin swabs are described in section 3.1.9. All swabs were stored at -20^{o}C until processing. A researcher randomised and allocated a code to all swabs collected during the trial using the RAND and RANK functions in Microsoft Excel (Microsoft Corporation, 2018). The same researcher obscured the identity of the swabs that were selected for DNA extraction by applying a label displaying the code. The investigator was blinded to the flock, date and ewe identity for all swabs taken on week 1('Entry'), 17 ('Mid'), trial exit examination and any other samples taken from lame ewes after week 25 but before lambing ('interim'), and unblinded to the flock identity for swabs taken on all other weeks. The investigator was unblinded to flock, ewe and date details for data analysis purposes after laboratory analyses were complete.

3.2.2 Selection of samples for D. nodosus quantification

D. nodosus was quantified for all swabs taken from ewes with visible footrot lesions throughout the trial, and for all swabs taken from ewes on week 1, 17, trial exit and interim examinations, regardless of lesion status. *D. nodosus* was also quantified on all remaining swabs taken from the purchased ewe group in flock 1, and a further 22 swabs from ewes without visible footrot lesions selected at random from any stage of the trial.

3.2.3 D. nodosus quantification

DNA was extracted from individual swabs as described in 2.1.4.2 and the *D. nodosus* load in each sample was quantified using the *AprV2/AprB2* qPCR method from Frosth et al. (2015),

with an additional 2-minute 50°C incubation step at the start of the amplification cycle, and the PCR mix modified to include only 1µl template DNA. Primers and probes were sourced from Life Technologies Ltd. (Thermo Fisher Scientific Inc.) and a QuantStudio5 instrument (Thermo Fisher Scientific Inc.) was used for all qPCR cycles. The VCS 1703A *D. nodosus* strain was used for the *aprV2* standard and the C305 *D. nodosus* strain for the *aprB2* standard, both sourced from Australia. *AprV2* and *AprB2* standard curves were run in duplicate from 10^6 to 10^1 on every plate but to increase the accuracy of quantity estimates, one set of standard curves with R^2 >0.995 was selected for each genotype and applied across all the qPCR plates. Water was included as a non-template control. Samples were considered positive for *D. nodosus* if either the *AprV2* or *AprB2* signal crossed the threshold for a positive result in all three sample replicates. The number of *AprV2* and *AprB2* copies was combined to give total *D. nodosus* load in each sample.

3.2.4 Selection of samples for serogroup identification

Samples with >10 copies of *D. nodosus* underwent individual serogroup PCR testing using the method described in 2.1.4.3, with the modification that blank samples from DNA extraction were not used as negative controls because all samples had undergone qPCR. All samples collected on week 1, week 17, interim weeks and on the final examination were tested for serogroups A to I by singleplex PCR. Samples from all other weeks were tested for serogroup H and the second bivalent vaccine included (BVI) serogroup specific to the flock of origin, as illustrated in Figure 3-4.

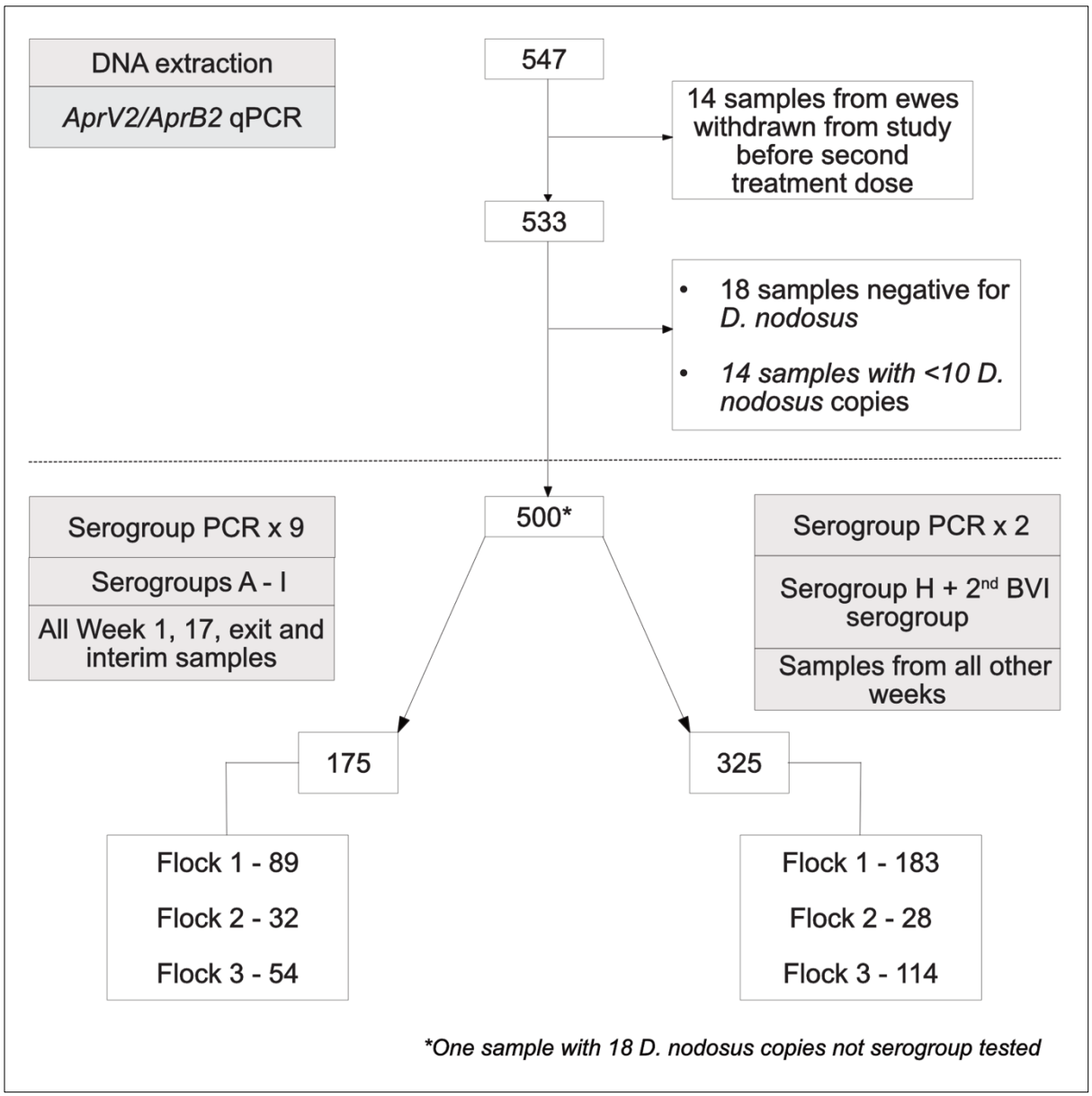

Figure 3-4 Number of samples that underwent AprV2/AprB2 qPCR and serogroup PCR tests. PCRs were run individually for each serogroup. BVI = bivalent vaccine included.

3.3 Data analysis

3.3.1 Preparation of data

3.3.1.1 General preparation of data

The data files were downloaded daily from the EID reader into Farm IT 3000 (Border software, Welshpool, UK) then exported as text files into an Excel spreadsheet (Microsoft Corporation, 2018) and inspected for errors and missing values. Locomotion scores were copied from Google sheets onto the same spreadsheet and checked for errors and missing values. All further data preparation was done in R version 4.1.0 (R Core Team, 2021) through the RStudio interface (v1.4, RStudio Team, 2020), using the 'dplyr' package v.1.0.7 (Wickham et al., 2021). Ewes that were repeatedly lame from the start of the trial were withdrawn from the study at week 5 before they received their second treatment dose. All observations for these ewes were removed from the dataset before analysis of locomotion scores and foot lesions. Data for ewes that were withdrawn from the trial on subsequent weeks were retained until the week of withdrawal to prevent bias. Ewes were withdrawn either because they were repeatedly or chronically lame and responded poorly to the treatment protocol, or due to death (Table A- 15).

3.3.1.2 Categorisation of variables

Some of the BCS at first vaccination, Age and Breed categories contained too few observations to be meaningful in the statistical models and so were merged for some of the flocks, as shown in Table 3-5. The number of ewes in the original categories is presented in Appendix **8**. An additional variable, 'origin', was created for the whole dataset to indicate

whether ewes had recently been bought-in from another flock ('Purchased') or had been on farm for at least 12 months ('Existing').

Table 3-5 Original (unshaded) and combined (shaded) categories for BCS, Breed and Age. The combined categories were used for variable selection in the statistical models. NC Mule = North Country Mule.

<table>
<tr><td></td><td colspan="6">Variable</td></tr>
<tr><td>Flock</td><td colspan="2">BCS category</td><td colspan="2">Breed</td><td colspan="2">Age</td></tr>
<tr><td rowspan="3">1</td><td><2.5</td><td><2.5</td><td colspan="2" rowspan="3"></td><td colspan="2" rowspan="3"></td></tr>
<tr><td>2.5-3.5</td><td rowspan="2">2.5-3.5</td></tr>
<tr><td>>3.5</td></tr>
<tr><td rowspan="4">2</td><td><2.5</td><td><2.5</td><td colspan="2" rowspan="4"></td><td>4-tooth</td><td rowspan="2">4/6T</td></tr>
<tr><td>2.5-3.5</td><td rowspan="3">2.5-3.5</td><td>6-tooth</td></tr>
<tr><td>>3.5</td><td>Full mouth</td><td rowspan="2">FM</td></tr>
<tr><td colspan="2"></td><td>Broken mouth</td></tr>
<tr><td rowspan="6">3</td><td colspan="2" rowspan="6"></td><td>NC Mule</td><td>NC Mule</td><td>Ewe lamb</td><td rowspan="2">Under 18 months</td></tr>
<tr><td>Exmoor Mule</td><td>Exmoor Mule</td><td>2-tooth</td></tr>
<tr><td>Charollais X</td><td rowspan="4">Whitefaced</td><td>4-tooth</td><td rowspan="4">Mature</td></tr>
<tr><td>Suffolk X</td><td>Full mouth</td></tr>
<tr><td rowspan="2">Texel X</td><td rowspan="2">Broken mouth</td></tr>
<tr><td></td></tr>
</table>

For analysis of the three flocks in a combined dataset, all ewes were either categorised as 'under 18 months' or 'mature' for Age. There were five Breed categories: Bluefaced Leicester, North Country Mules, Exmoor Mules, Suffolk X and Whitefaced. The Whitefaced group was derived from the remainder of the crossbreeds in flocks 1 and 3, which were predominantly Texel X mules.

An additional variable was created to combine the ID and SFR lesion scores into a single scoring system that enables comparison with the 0-5 point modified Egerton footrot score (MEFS) system (Buller and Eamens, 2014). MEFS score 2 describes necrotising inflammation

of the interdigital skin which involves the axial wall horn. In 23/142 ewe observations over the whole trial (12 in flock 1, 3 in flock 2 and 8 in flock 3) SFR score 1 was the most severe score but there was no concurrent ID on the same foot, for which there is no equivalent MEFS score; these observations were allocated a MEFS score of 2.

Table 3-6 ID and SFR lesion scores used in the trial (section 3.1.7.2) and the equivalent MEFS score (Buller and Eamens, 2014)

ID/SFR score	MEFS score
ID 1-4	1
SFR 1	2
SFR 2	3
SFR 3	4
SFR 4	5

3.3.1.3 Preparation of data for survival analysis

For survival analysis of lameness, the dataset was arranged in calendar time format. Each observation represented the period at risk for one ewe, starting with the week number that the ewe was first 'at risk' and ending with the week number that either a lameness event or censoring occurred. Due to the definition of being 'at risk' of becoming lame, each observation was discontinuous in time, ewes being absent from the risk set for a minimum of one week of the trial following the onset of lameness. The dataset for survival analysis of footrot was arranged in the same way, with the risk period for every ewe observation ending in a footrot event or censoring. Additionally, ewes were temporarily absent from the risk set following parenteral antibiotic treatment for a reason other than footrot. Ewe deaths in non-lame ewes were indicated by censoring. All ewes that were still present and not lame on week 25 of the trial were censored.

3.3.1.4 Preparation of litter size and change in BCS data

Litter sizes for all lambs born before week 30, ewes that had scanned empty, and ewes that had lambed or aborted prior to week 30 of the trial, were included in the dataset for analysis of litter size and number of living lambs by treatment group, provided they had not been excluded during the trial. The BCS of ewes that lambed between week 25 and 30 of the trial were included in the dataset for flocks 1 and 2. In flock 3, due to the lambing period starting earlier than expected, the BCS of the ewes that lambed between weeks 24 and 29 were included. The BCS of ewes that had been scanned empty was recorded within the same time interval.

3.3.1.5 Preparation of injection site reaction data

Injection site reactions were removed from the dataset if a lesion had been recorded in the same position on week 1 of the trial. Lesions recorded on the neck were removed from the dataset if they were not found at the site of injection. Lesion sizes were allocated to small (1-20mm) and medium (21-50mm) categories for exploratory data analysis but combined into a present/absent category for the binomial model.

3.3.2 Exploratory data analysis

The *ggplot2* package v.3.3.5 (Wickham, 2016) was used for general data visualisation. The weekly prevalence of lameness was plotted by flock and treatment group. Lameness prevalence was plotted against origin of sheep (flock 1), age (flock 2, flock 3), breed (flock 2, flock 3) and BCS at first vaccination category (all flocks). The same procedure was repeated for the weekly prevalence of lame sheep with footrot. The percentage of each treatment

group with an injection site reaction was plotted for the variables breed, presence of fleece and side of injection.

Kaplan Meier survival curves, cumulative hazard plots and complementary log-log plots of survival probability were used to visualise the hazard ratio between variable categories and inspect the data for signs of non-proportionality. Plots were created using the 'survfit' function in the *survival* package v.3.3-1 (Therneau, 2021), selecting the 'log-log' transformation for the 95% confidence interval, and the 'ggsurvplot' command in the *survminer* package v.0.4.9 (Kassambara et al., 2021).

3.3.3 Statistical analyses

All statistical analyses were performed in R version 4.1.0 (R Core Team, 2021) using the Rstudio v1.4 interface (RStudio Team, 2020).

3.3.3.1 Assessment of randomisation of ewes to treatment groups

Randomisation to treatment group within each strata was evaluated for each flock by Pearson's Chi-squared test using the *arsenal* package v3.6.3 (Heinzen et al., 2021). Differences between treatment groups in the number of lame sheep on week 1 of the trial were evaluated for each flock using Fisher's Exact test. In both cases, a p value of ≤ 0.05 was considered significant.

3.3.3.2 Binomial mixed effect models for weekly lameness prevalence and footrot prevalence

Binomial mixed effect models were constructed for each flock separately to investigate associations between lameness prevalence and prevalence of footrot by treatment group

whilst adjusting for confounding variables. Analysis was performed on a restricted dataset (week 7-25), when full immunity was expected to have been achieved from the second dose of vaccine (Amend et al., 2021). The dependent variable was the presence or absence of lameness. Two-level mixed effect models were used to account for clustering of ewes within flocks and repeated weekly observations of each ewe. Additional variables and plausible interactions were investigated individually. Variables that resulted in a p value of ≤ 0.05 on likelihood ratio test (LRT) and a lower Akaike information criterion (AIC) value and interaction terms that had a statistically significant effect were included in a multivariable model. A backwards elimination procedure was used to retain only variables that improved model fit on LRT (Dohoo et al., 2014). The models were constructed using the 'glmer' function in the lme4 package v.1.1, using the adaptive Gauss-Hermite quadrature method of integration and the 'bobyqa' optimizer (Bates et al., 2015). The *jtools* package (Long, 2020) v.2.1.4 was used to view the model results.

3.3.3.3 Binomial mixed effect model for the duration of lameness cases

A binomial mixed effect model was constructed for the combined flock data after the treatment identities were unblinded. Analysis was performed on the same restricted dataset (week 7-25) as the lameness prevalence binomial models. Additionally, cases of unknown duration, that is cases of lameness that started on week 25 or had not resolved by week 25, were removed from the dataset to minimise bias. The dependent variable was whether a case of lameness lasted one week or longer than one week, because the majority of cases lasted only one week and the true length of longer cases would have been biased by the exclusion of ewes that were non-responsive to treatment. Ewe was included as a random

variable to account for repeated observations in some ewes. All variables of interest and potential biological significance were included in the model. The model was constructed using the 'glmer' function in the lme4 package v.1.1 (Bates et al., 2015), as described in section 3.3.3.2.

3.3.3.4 Binomial mixed effect model for injection site reactions

A binomial mixed effect model was constructed for the combined flock data after the treatment identities were unblinded and the saline treatment group was removed from the dataset. The dependent variable was the presence or absence of a lesion at the injection site that measured >0mm. A two-level mixed effect model was used to account for clustering of ewes within flocks and repeated observations of each ewe. Treatment, breed, presence of fleece at time of observation, side of injection and flock were introduced to the model individually as fixed effects and retained if they met the conditions described in section 3.3.3.2. The model was constructed using the 'glmer' function in the lme4 package v.1.1 (Bates et al., 2015), as described in section 3.3.3.2.

3.3.3.5 Cox Proportional Hazard models and Frailty models

Survival analysis was performed on a restricted dataset (week 6-25), which only considered new lameness cases and new footrot cases occurring on week 7 or later, when full immunity was expected to have been achieved from the second dose of vaccine (Amend et al., 2021). For each flock, Cox proportional hazards (PH) and frailty models were constructed separately for lameness events and footrot events to estimate the hazard ratio between treatment groups. The Cox PH models estimated treatment differences in time to first event only. The frailty models included all events and included a random (frailty) effect for ewe to account

individual heterogeneity and the lack of independence between repeated events in individual sheep (Ullah et al., 2014, Box-Steffensmeier and De Boef, 2006). The 'coxph' command in the *survival* package v.3.3-1 (Therneau, 2021) was used to construct the Cox PH models. The Cox PH model was extended with a frailty term using the 'coxme' command in the *coxme* package v.2.2-16 (Therneau, 2020). In all models, the treatment group with the most events over the whole trial period, or the treatment group that had the least relationship with time over the whole trial period where there were similar numbers of cases between treatment groups, was set as the reference category. Variables and biologically plausible interaction terms were investigated as described in section 3.3.3.2, with variable selection based on a p value of $\leq$ 0.05 on LRT. In some cases, variables significantly improved model fit for recurrent events but not for single events within a flock, in which case the variable was included in both models for comparison. The 'cox.zph' command in the *survival* package (Therneau, 2021) and the 'ggcoxzph' function in the *survminer* package (Kassambara et al., 2021) were used to check the proportional hazards assumption by means of the score (goodness of fit) test, and to observe the shape of any non-proportionality in plots of the scaled Schoenfeld residuals against time for each covariate, specifying 'identity' for the time transformation option.

3.3.3.6 Number of antibiotic treatments used for treatment of lameness or footrot

Pearson's chi-squared test was used to compare the three treatment groups for the proportion of ewe-weeks when a treatment for lameness was given. The test was performed using the prop.test command in R (R Core Team, 2021). All three treatment groups were included in the initial test and pairwise comparisons with the saline group made if a

significant difference was demonstrated. A p value of $\leq$ 0.05 was considered significant. The same method was used to compare the proportion of ewe-weeks when a treatment for footrot was given between treatment groups.

3.3.3.7 Differences in litter size and change in BCS between treatment groups

The Kruskal-Wallis rank sum test was used to test for differences between treatment groups for litter size, number of living lambs per ewe at exit examination, and units of change in BCS between week 1 and 17, week 17 and lambing, and week 1 and lambing. Tests were performed separately for each flock. A p value of $\leq$ 0.05 was considered significant.

CHAPTER 4 THE PREVALENCE AND INCIDENCE OF LAMENESS AND FOOT DISEASES, AND THE IMPACT OF FOOTROT VACCINATION ON THE CONTROL OF LAMENESS AND FOOTROT IN THREE COMMERCIAL SHEEP FLOCKS

This chapter provides the results of the trial stratification and randomisation procedures and further details about the sheep that were enrolled, examined and withdrawn from the trial. The case definitions for lameness and footrot are described, then descriptive results are presented for the three flocks for the prevalence and incidence of lameness and footrot in lame ewes, and the prevalence of other infectious and non-infectious lesions in lame ewes. Model results for the impact of vaccination on lameness and footrot prevalence are presented for the three flocks separately. Finally, the results of time-to-event analysis on the impact of vaccination are presented for all three flocks and for each flock separately for four scenarios: -

- Probability of survival without a case of lameness
- Relative risk of experiencing a case of lameness when recurrent events are considered
- Probability of survival without a case of lameness with footrot
- Relative risk of experiencing a case of lameness with footrot when recurrent events are considered

4.1 Enrolment, treatment group allocation and withdrawal of ewes

4.1.1 Randomisation of ewes within treatment groups

The randomisation of ewes to each treatment group following stratification for breed, BCS group, footrot score and presence/absence of CODD was evaluated. There were no significant differences between the proportion of ewes in each treatment group within any of the strata in any flock (Appendix **8**). Additionally, there were no significant differences in ewe age between treatment groups in any of the flocks.

4.1.2 Mixing of sheep within flocks

In flock 1, the purchased ewes, which comprised all the Bluefaced Leicester cross ewes and the single Dorset cross ewe, were managed separately for the entire trial, with the exception of occasional movements of individuals into the main flock and out again. Ewes in the purchased group were distributed evenly between treatment groups. Other divisions of the flock into sub-groups were based on BCS at the start of the trial. BCS was a stratification measure and so this ensured ewes in all three treatment groups were sub-grouped evenly.

The ewes in flock 2 remained as a single group until scanning when they were split into two adjacent groups in a single shed based on scanning results. Ewes were evenly distributed between the treatment groups in the two pens (chi-squared test $p = 0.91$ and $p = 0.94$).

In flock 3, the ewes were divided into two management groups based on BCS between week 1 and 5 of the trial. From week 5 of the trial until after scanning the flock was divided into two breed groups for mating, the Whitefaced and the Exmoor mules in one group and the

North Country mules in the second group. After scanning (week 17) the flock was kept together with some lame ewes kept in a separate group closer to the handling facilities.

Opportunities for cross infection of *D. nodosus* serogroups between management groups occurred weekly throughout the trial in flock 1 and up until week 17 in flock 3 because the groups were sequentially gathered in the same pen and race facilities for examination on these farms, facilities that were not cleaned for the duration of the trial. This is a known risk for transmission of *D. nodosus* (Whittington, 1995).

4.1.3 Withdrawal of ewes from the trial

Five ewes were excluded from the dataset in flock 1, two ewes from flock 2 and four ewes from flock 3 due to persistent lameness from the start of the trial. A further ewe was excluded from flock 2 because she received the incorrect treatment. There were 11 further withdrawals from flock 1, two withdrawals from flock 2 and seven withdrawals from flock 3. Most withdrawals occurred in the later weeks of the trial and were not evenly distributed between treatment groups (Table 4-1). The reasons for individual ewe withdrawals are presented in Table A- 15. The number of ewes present in each treatment group at key events during the trial is presented in the trial profiles in Figure 4-1 - Figure 4-3.

Table 4-1 Number and percentage of ewe - weeks lost following withdrawal due to death or chronic lameness between week 7 and 25 of the trial by treatment group

	Custom R-Pilus	Footvax®	Saline
Flock 1	22/954 (2.3%)	7/1024 (0.7%)	42/1042 (4.0%)
Flock 2	0	0	6/1154 (0.5%)
Flock 3	0	20/1167 (1.7%)	11/1134 (1.0%)

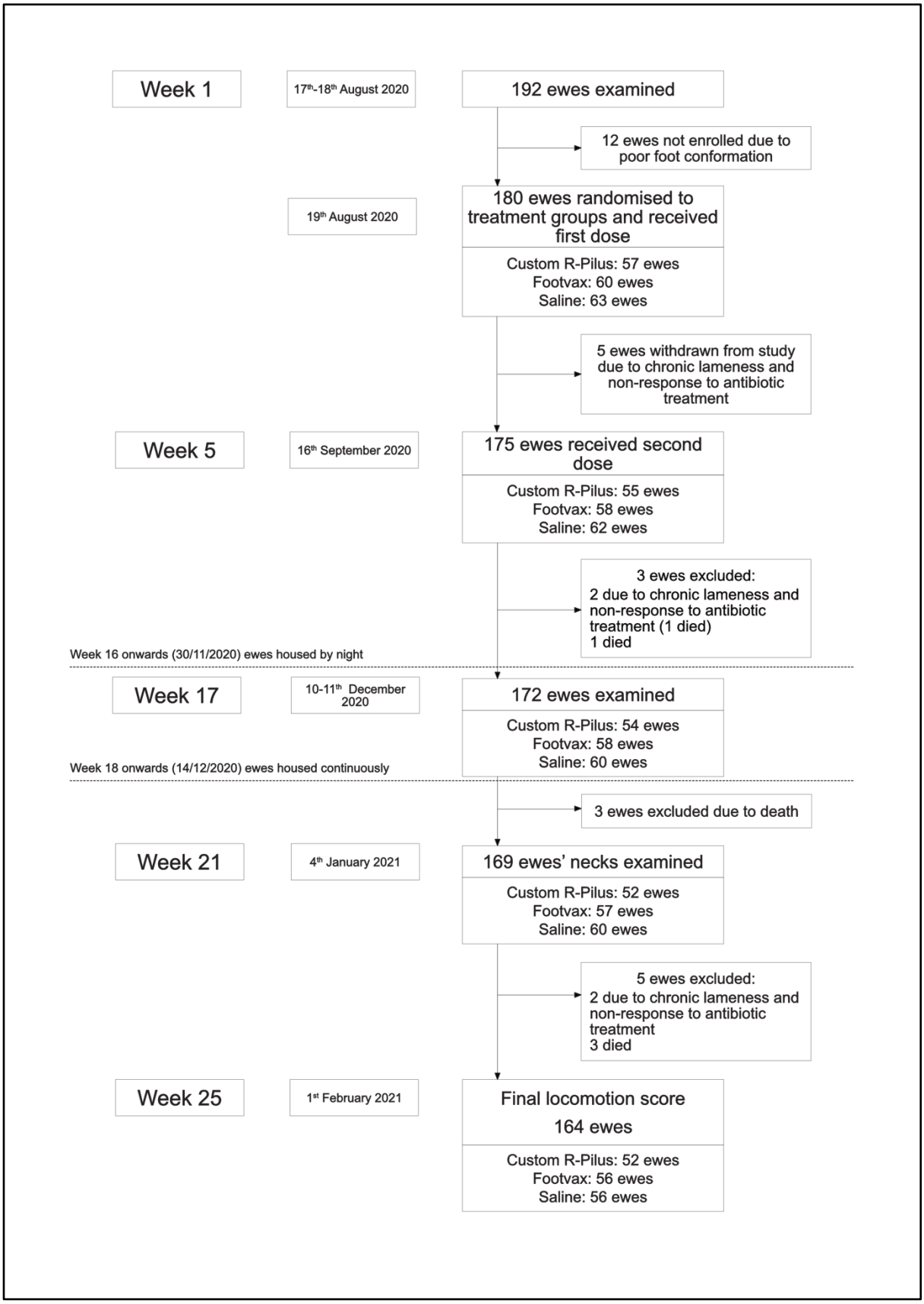

Figure 4-1 Trial profile for ewes enrolled in Flock 1

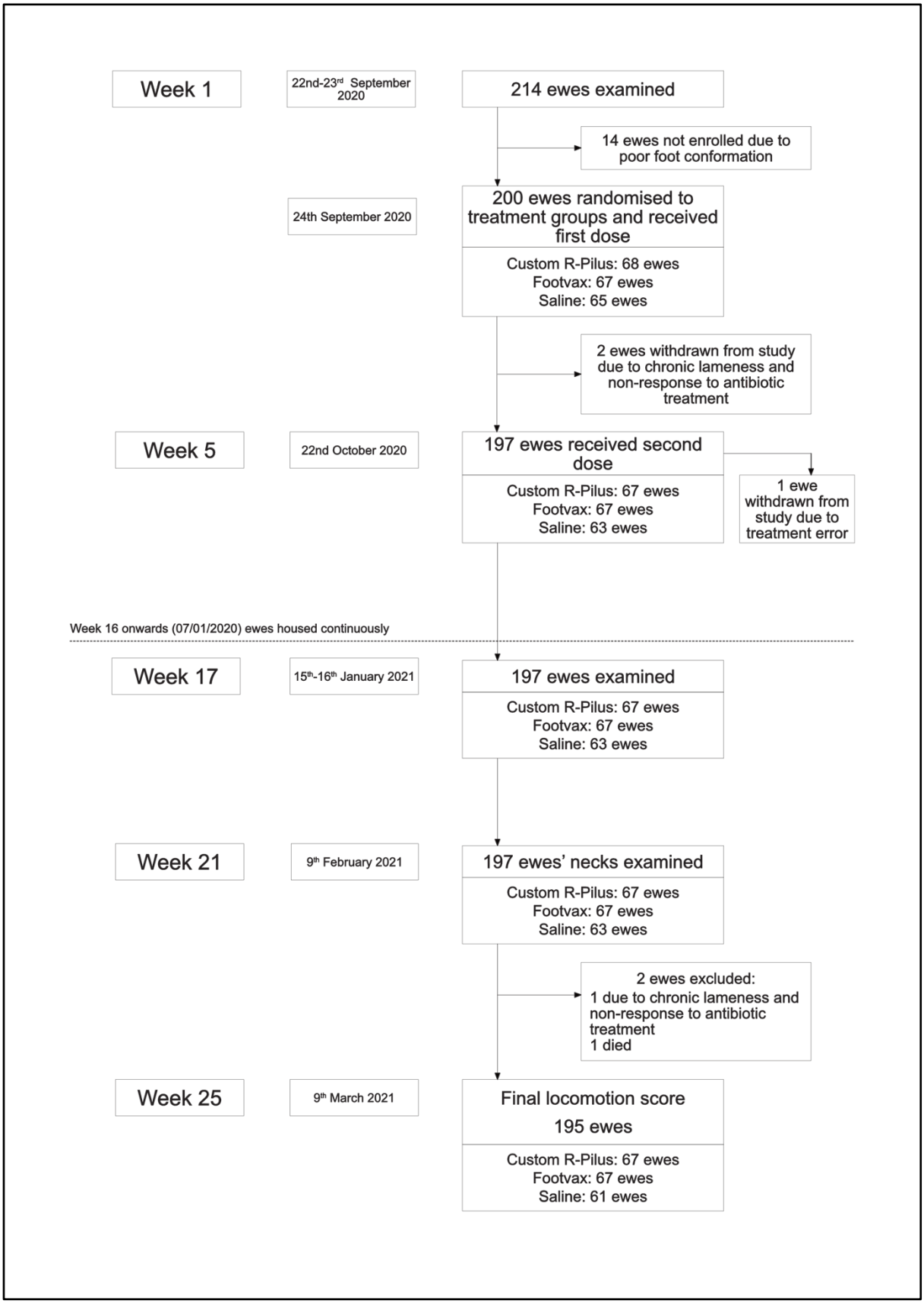

Figure 4-2 Trial profile for ewes enrolled in Flock 2

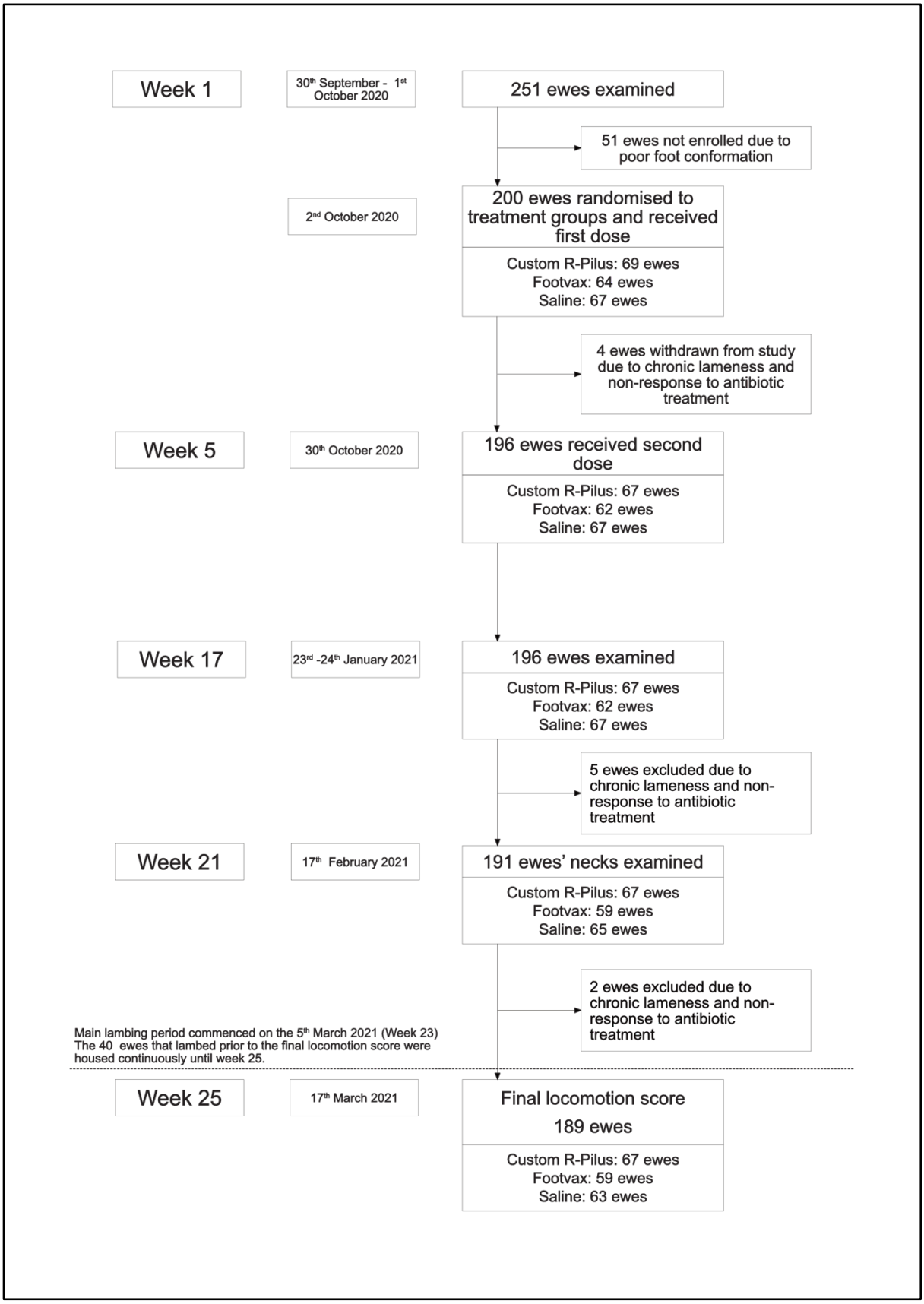

Figure 4-3 Trial profile for ewes enrolled in Flock 3

4.2 Definitions of lameness and footrot events

4.2.1 Denominators for calculating lameness prevalence and incidence

Lameness prevalence and incidence were calculated separately for the three flocks. The number of ewes observed each week was the denominator for the weekly prevalence of lameness. The number of ewes at risk of becoming lame each week was the denominator for incidence of lameness. Ewes were not considered at risk of becoming lame on week 1 because their lameness status in week 0 was unknown. Ewes were at risk of becoming lame after week 1 if they met the following conditions:

- LS =1 for consecutive weeks since week 1
- LS = 0 in week (x-1)
- LS = 1 in week (x-1) and LS = 0 in week (x-2)
- Week = 2 and not treated with systemic antibiotics in week 1

Ewes were considered at risk of becoming lame if they had received a dose of parenteral antibiotics for a condition other than lameness in week (x-1). The number of ewes at risk of becoming lame by flock is presented in Table A- 16. The lowest proportion of ewes eligible to become lame in any treatment group on any week from week 7 onwards was 76.9% in the Saline group in flock 3 on week 21.

4.2.2 Numerator for calculating lameness prevalence and incidence

A ewe was defined as lame if she scored >1 for locomotion. The number of ewes that were lame was the numerator for lameness prevalence. The number of ewes at-risk of becoming lame in week x that were lame in week x was the numerator for the incidence of lameness.

The same numerators were used for the statistical models. In the context of survival analysis, when a ewe had a new lameness incidence, it is referred to as a lameness event. In all other contexts a new lameness incidence is referred to as a lameness case.

4.2.3 Denominators for calculating the prevalence of foot lesions and incidence of footrot

The number of ewes scored for locomotion in each treatment group for each week of the trial was used as the denominator for calculating the weekly prevalence of foot lesions in lame ewes. The number of ewes at risk of becoming lame *with a new footrot lesion* in each week was used as the denominator for calculating footrot incidence in lame ewes. Ewes were not considered at risk of developing footrot on week 1 because the duration of existing footrot lesions was unknown. Ewes were at risk of a new case of footrot in week (x>1) if they met the following conditions:

- At risk of a new lameness case as described in Section 4.2.1, or
- They met the conditions for a new lameness case on week i and consecutively scored LS>0, but were not treated with parenteral antibiotics, between weeks i and x
- Not treated with parenteral antibiotics on week (x-1) for a reason unrelated to lameness

The number of ewes at risk of becoming lame with footrot each week is presented in Table A- 17. The lowest proportion of ewes eligible to develop footrot in any week in any treatment group from week 7 onwards was 86.6% in the Custom vaccine group in flock 3 in week 21. The total number of ewe-weeks not at risk of footrot following parenteral

antibiotic treatment for a reason other than footrot varied by flock but not between treatment groups within flock (Table 4-2). Only 5, 1 and 2 ewe-weeks were removed from the risk set after antibiotic treatments for reasons other than lameness in flocks 1,2 and 3 respectively. Two observations of footrot in flock 1, both in the Custom R-Pilus treatment group, and three observations in flock 3, one in each treatment group, were not defined as new cases because they occurred on the week following parenteral antibiotic treatment for another cause of lameness. No cases of footrot occurred after parenteral antibiotic treatment for another cause of lameness in flock 2.

Table 4-2 Number of ewe-weeks not at risk of developing footrot due to parenteral antibiotic treatment for a reason other than footrot, expressed as a percentage of all ewe-weeks in trial between week 7 and 25 for each treatment group

	Custom R-Pilus	Footvax®	Saline
Flock 1	9/1023 (0.8%)	7/1095 (0.6%)	14/1136 (1.3%)
Flock 2	4/938 (0.4%)	4/938 (0.4%)	3/1186 (0.3%)
Flock 3	26/1273 (2.0%)	32/1158 (2.8%)	34/1256 (2.9%)

4.2.4 Numerators for calculating the prevalence of foot lesions and the incidence of footrot

Most new cases of footrot in lame sheep were acute and had not progressed as far as underrunning the sole horn because lame ewes were treated within a week of onset of lameness. Therefore, most footrot lesions scored 1-4 on the ID lesion score scale and <2 on the SFR lesion score scale (3.1.7.2), equivalent to a maximum MEFS score of 2 (Table 3-6) (Appendix 11), and after week 3 no more than one ewe per treatment group scored SFR>1. Consequently, lesion scores were not used to differentiate severity of footrot.

The numerator for the weekly prevalence of footrot was the number of ewes with LS>1 that had an ID or SFR lesion score of >0 on at least one foot. The weekly prevalence of CODD was calculated using the number of ewes with LS>1 that had a CODD lesion of score 1-4 on at least one foot. The weekly prevalence of other foot lesions was also calculated based on the presence of lesions on any foot in ewes scoring LS>1.

The numerator for the weekly incidence of footrot was the same as for weekly prevalence but with these additional conditions:

- At risk of footrot as described in section 4.2.3
- Any lesions of score SFR>1 were not pre-existing
- *D. nodosus* positive ID swab

The same numerators were used for the statistical models. Any subsequent reference to a case of footrot or a footrot 'event' describes a ewe that was both lame and had footrot. The term footrot 'event' has been used to describe a new footrot incidence in the context of survival analysis, whilst in all other contexts the term 'case' has been used to describe an incidence of footrot.

4.2.5 Calculation of incidence rates for lameness and footrot

The lameness incidence rate and footrot incidence rate for each flock were calculated for weeks 2-25 of the trial. The total number of new lameness or footrot cases and the incidence rates (cases per 100 ewe-weeks) were calculated for the three treatment groups in each flock for the period between week 2 and 7 before full immunity from the two doses of

vaccine was anticipated, and for the period between week 7 and 25 when full immunity was expected.

4.3 Prevalence of lameness, footrot and other foot lesions

4.3.1 Number of foot observations

Over the entire trial, there were 805, 676 and 973 ewe examinations for foot lesions in flocks 1, 2 and 3 respectively. In flocks 1 and 3, 49% of ewe examinations were of lame ewes, compared to only 21% in flock 2, where fewer ewes were lame (Table A- 18). In flock 1, two lame ewes were released without examination in error, both on week 3. In flock 3, one lame ewe was released without examination in error on week 25. There were 48/310 (15.5%), 34/102 (33.3%) and 62/388 (16.0%) lame ewes with no visible cause of lameness in flocks 1, 2 and 3 respectively.

4.3.2 Prevalence of lameness and footrot by treatment group on week 1 of trial

Lame ewes were not evenly distributed between treatment groups within flocks on week 1 (Table 4-3). Lameness prevalence (LS of 2 or higher) varied by 14.4% by treatment group in flock 3 (Fisher's exact test, p=0.053). Locomotion scores were likely to have been exacerbated by handling prior to scoring, a practice that only occurred on week 1. The prevalence of ID, SFR and all FR lesions in lame ewes on week 1 of the trial was not significantly different between treatment groups in any of the flocks (Appendix **8**).

Table 4-3 Lameness prevalence for each treatment group on week 1 of the trial

	Custom R-Pilus	Footvax®	Saline	p value‡
Flock 1	5/55 (9.1%)	9/58 (15.5%)	6/62 (9.7 %)	0.53
Flock 2	7/67 (10.4%)	4/67 (5.8%)	3/63 (4.8%)	0.48
Flock 3	8/67 (11.9%)	4/62 (6.5%)	14/67 (20.9%)	0.053

‡ Fisher's exact test

On week 1, ewes that had footrot lesions were only treated with antibiotics if they were lame when their feet were examined or they had CODD. In the ewes that were untreated, a new case after week 1 was defined as a new footrot lesion on a different limb, or a new footrot lesion on the previously affected limb only if an interim examination had recorded the absence of the original lesion. 4/13 ewes that had untreated footrot on week 1 in flock 1 had new footrot cases after week 6 of the trial, 3/12 in flock 2 and 2/4 in flock 3. Ewes with footrot lesions on week 1 that received treatment did not result in lesions being mis-classified as new cases of footrot in any of the flocks during the trial.

4.3.3 The prevalence of lameness and foot diseases in lame ewes over the trial

The results presented in this section revealed contrasting lameness trends and responses to the clinical trial interventions for the three flocks. The results can be largely explained by the differences in aetiology of lameness between the flocks.

4.3.3.1 Weekly lameness prevalence

4.3.3.1.1 Weekly lameness prevalence by flock

In flocks 1 and 3, weekly lameness prevalence varied, but the overall trend was for a decrease in lameness prevalence in flock 1, whereas in flock 3 the initial decrease was

followed by a return to prevalence levels at the start of the trial (Figure 4-4). In flock 3, the weekly variation in lameness prevalence was greater after week 11 (mid-November). The geometric mean weekly prevalence of lameness, excluding weeks 1-6, was 6.2% (SD 3.1%) in flock 1 and 7.3% (SD 3.6%) in flock 3, compared to only 2.0% (SD 1.3%) in flock 2.

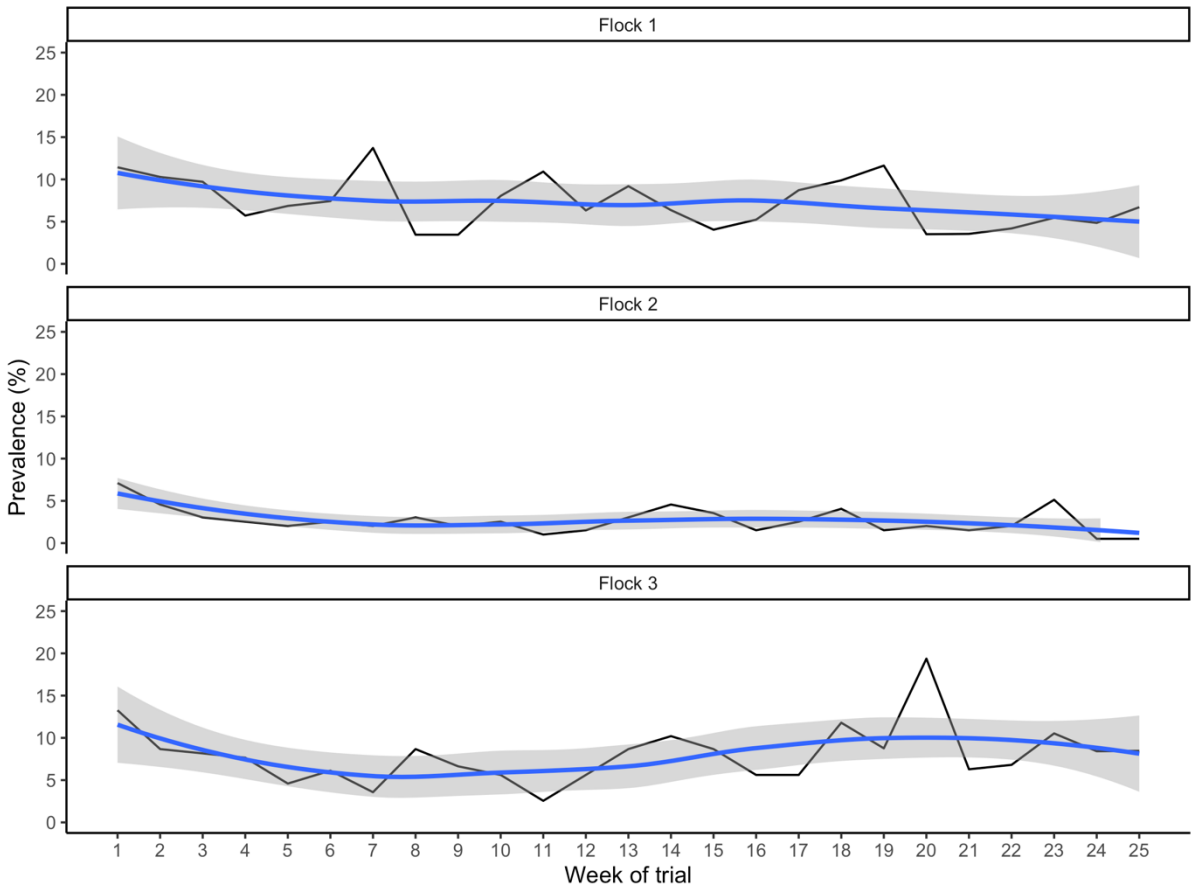

Figure 4-4 Weekly prevalence of lameness by flock. The black line shows the true prevalence, the blue line is fitted using Loess regression for which the 95% CI is shaded in grey.

4.3.3.1.2 Weekly lameness prevalence by flock and treatment group

In flock 1, the variation in weekly lameness prevalence for the flock was across the three treatment groups. There was an overall trend for a decrease in weekly prevalence in the Footvax® group, and the Custom R-Pilus vaccine group until week 20 when it started to increase (Figure 4-5). Lameness prevalence remained low across all three treatment groups in flock 2 (Figure 4-6). In flock 3, the three treatment groups had the same trend as for the flock, but true prevalence on a weekly basis varied between the groups (Figure 4-7).

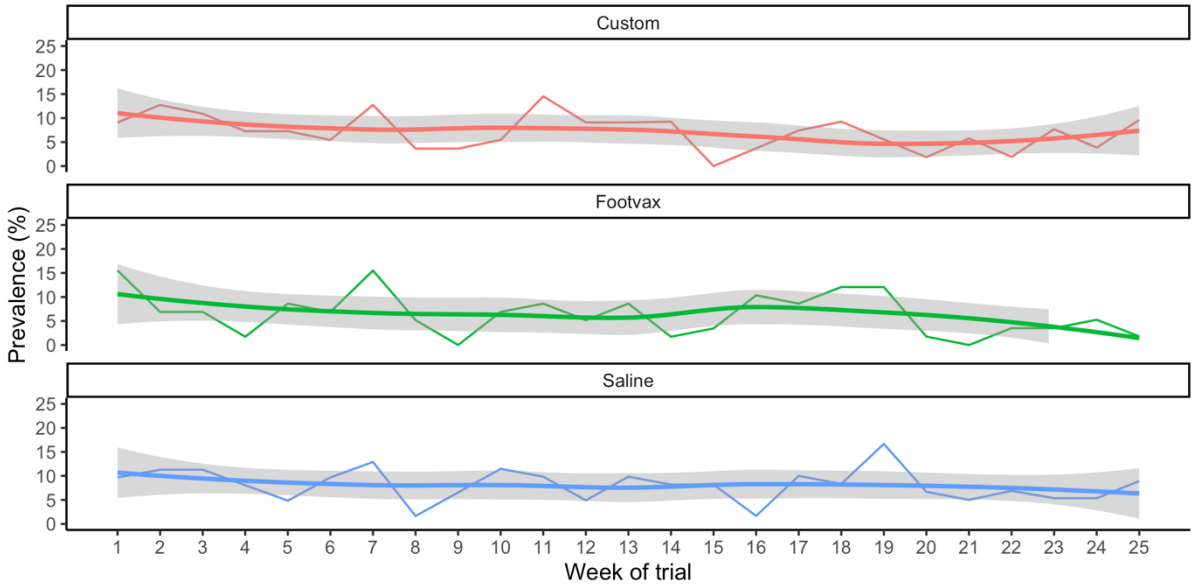

Figure 4-5 Weekly prevalence of lameness by treatment group in flock 1. The thin line shows the true prevalence, the thick line is fitted using Loess regression for which the 95% CI is shaded in grey.

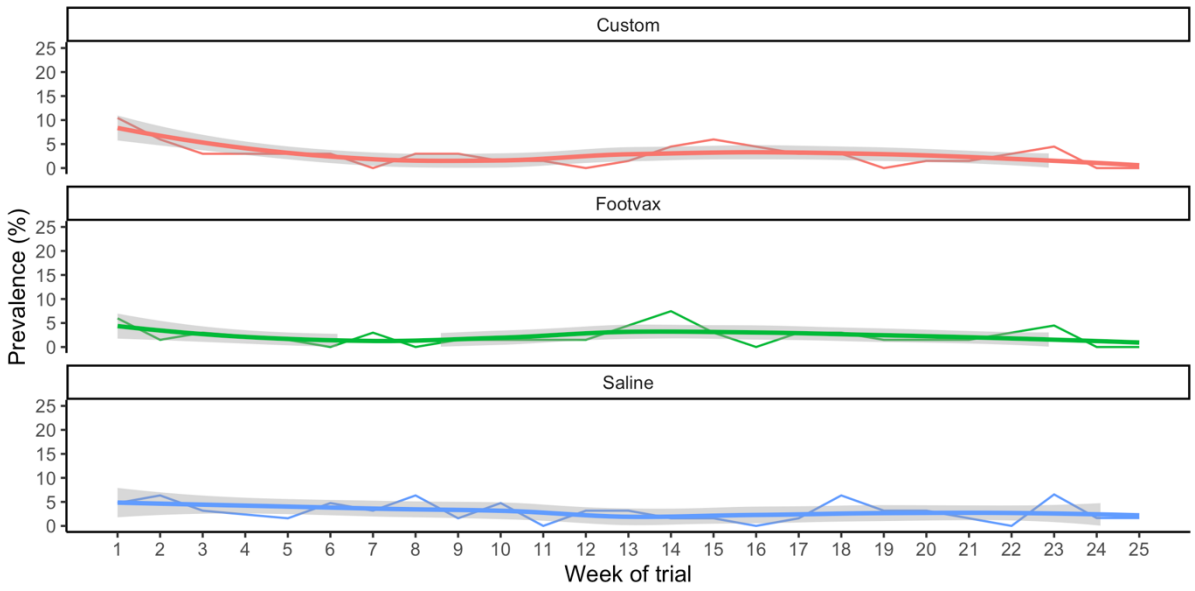

Figure 4-6 Weekly prevalence of lameness by treatment group in flock 2. The thin line shows the true prevalence, the thick line is fitted using Loess regression for which the 95% CI is shaded in grey.

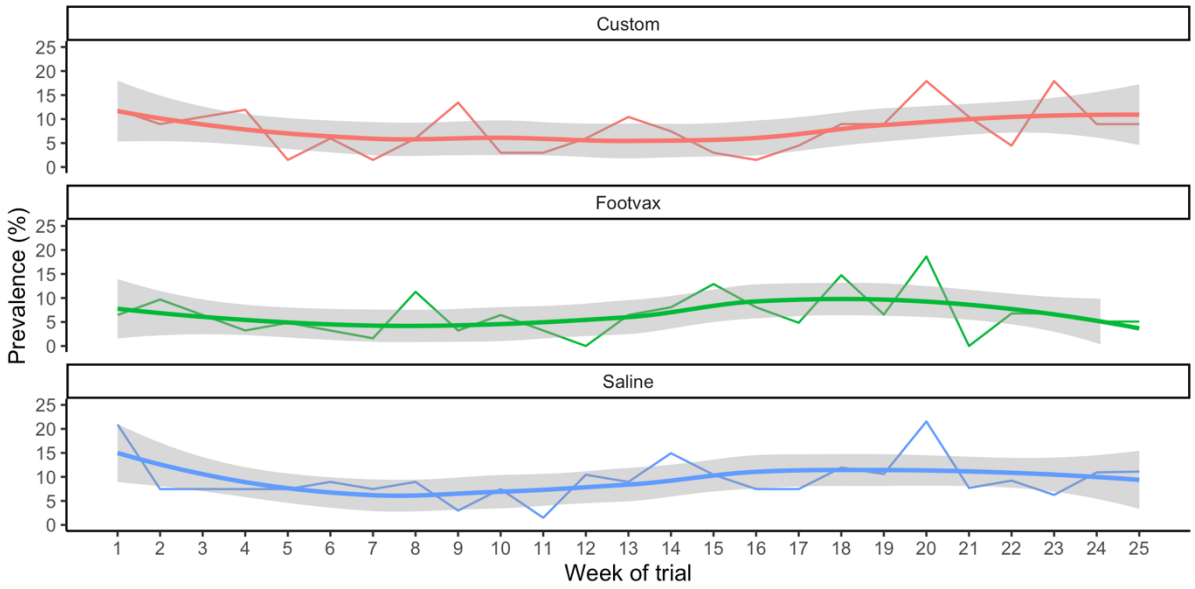

Figure 4-7 Weekly prevalence of lameness by treatment group in flock 3. The thin line shows the true prevalence, the thick line is fitted using Loess regression for which the 95% CI is shaded in grey.

4.3.3.1.3 Weekly lameness prevalence by flock and other variables

In flock 1, Purchased ewes had a higher weekly lameness prevalence and much greater variance in lameness prevalence than Existing ewes for most of the trial (Figure 4-8). Lameness prevalence was similar in both groups from week 20 when the groups were housed. Weekly lameness prevalence did not vary with BCS at first vaccination. All ewes in flock 2 were the same breed and origin category, and age and BCS category at first vaccination was not associated with weekly lameness prevalence. In flock 3, the Purchased ewe group was comprised of all the Exmoor Mules. Weekly lameness prevalence was lower in the Whitefaced crossbreeds than in the Mule types, and the numerically small Exmoor Mule group was disproportionately affected when lameness prevalence peaked in week 20 (Figure 4-9). There was no difference in prevalence of lameness between groups for the variables BCS at first vaccination and age.

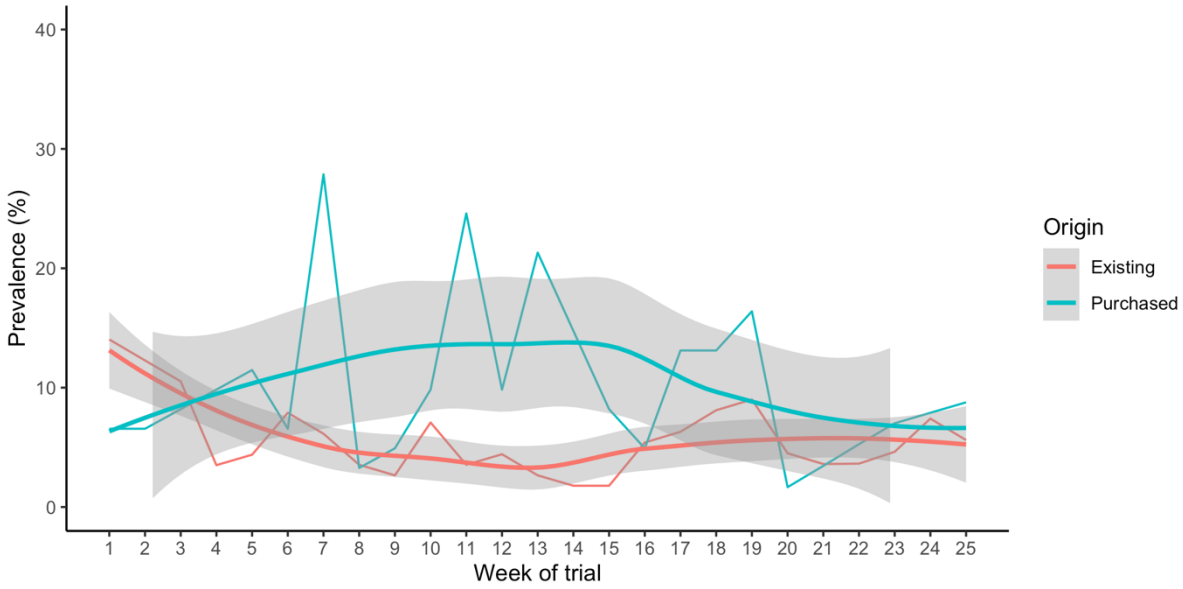

Figure 4-8 Weekly prevalence of lameness by origin category in flock 1. The thin lines show the true prevalence, the thick lines are fitted using Loess regression for which 95% CIs are shaded in grey.

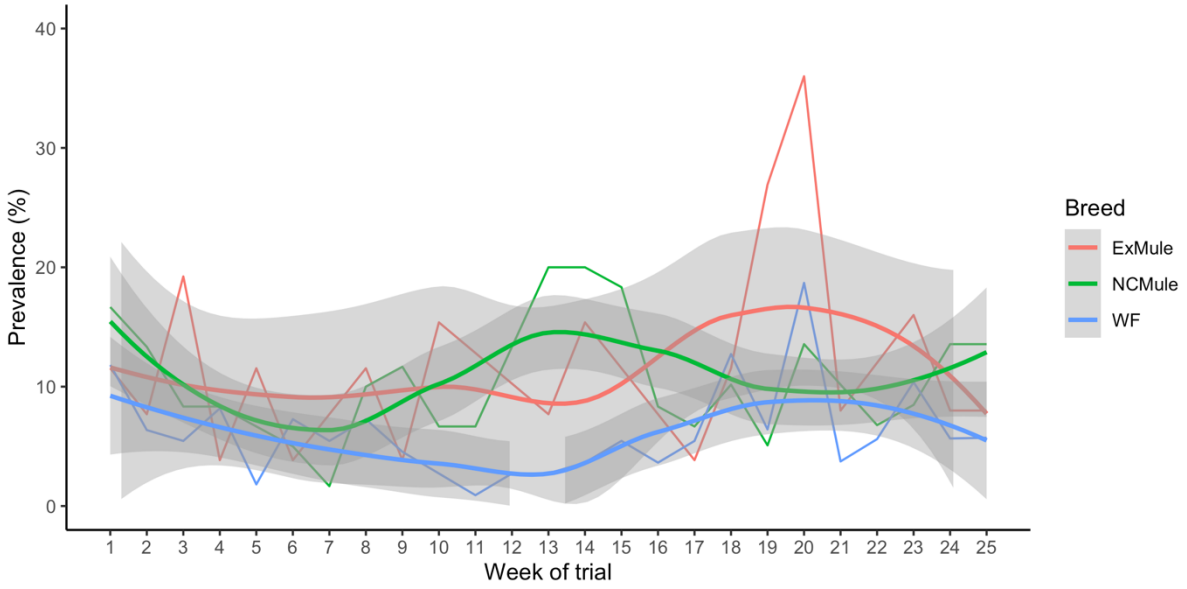

Figure 4-9 Weekly prevalence of lameness by breed category in flock 3. The thin lines show the true prevalence, the thick lines are fitted using Loess regression for which 95% CIs are shaded in grey. ExMule = Exmoor Mule, NCMule = North Country Mule, WF = Whitefaced.

4.3.3.1.4 Binomial mixed effect models for lameness prevalence

Binomial mixed effect regression models (Dohoo et al., 2014) were constructed for each flock to investigate the effect of treatment and other variables on lameness prevalence between week 7 and 25 of the trial. The variables tested were origin (flock 1), age (flock 2, flock 3), breed (flock 3), BCS category at first vaccination, and lame in week 1 (all flocks). These variables were selected because of their potential for having a biological effect on the prevalence of lameness; breed due to genetic variation in immune response, age due to age-related immunity and variation in historical footrot vaccination status, BCS category to test if poor body condition had a detrimental effect on immune response to vaccination. Origin was tested instead of age and breed in flock 1 due to confounding between these variables and the risk of overfitting the model. Purchased ewes comprised a small proportion of flock

3 and were confounded with breed, therefore origin was not tested. There was no variation in breed and origin in flock 2 ewes. 'Lame in week 1' was tested because the duration of lameness was unknown for ewes lame at the start of the trial and ewes that had been lame for some weeks might have been at a higher risk of being lame later in the trial. In flock 1 treatment and origin were combined to create six categories, to facilitate interpretation of an interaction between these two variables.

Table 4-4 Binomial mixed effects regression model for lameness prevalence between week 7 and 25 of the trial in flock 1

Variable	No.	%	Odds ratio	Lower 95% CI	Upper 95% CI
Fixed effects					
Week	3254		0.97	0.95	1.00
Cos(Week)	3254		**1.49**	**1.22**	**1.82**
Treatment origin					
Existing Saline	722	22	Ref		
Existing Custom	672	21	**0.40**	**0.21**	**0.76**
Existing Footvax®	721	22	0.71	0.41	1.25
Purchased Saline	414	13	1.61	0.91	2.87
Purchased Custom	351	11	2.27	1.27	4.06
Purchased Footvax®	374	11	1.21	0.65	2.24
Random effects					
Ewe	175	Variance = 0.49		ICC = 0.13	

No. and % represent the number and percentage of observations in each category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Odds ratios are statistically significant when the CI does not include unity and are indicated in bold.

Weekly lameness prevalence did not change significantly after week 7 in flocks 1 and 2 (Table 4-4, Table 4-5) and in flock 3 weekly lameness prevalence increased (Table 4-6). The only beneficial effect of treatment on weekly prevalence was observed in the Custom R-Pilus vaccinated ewes in flock 1, but this only occurred in the Existing ewes (Table 4-4). The only other variables that had a significant effect on lameness prevalence were breed and lame on week 1 in flock 3 (Table 4-6).

Table 4-5 Binomial mixed effects regression model for lameness prevalence between week 7 and 25 of the trial in flock 2

Variable	No.	%	Odds ratio	Lower 95% CI	Upper 95% CI
Fixed effects					
Week	3736		0.99	0.95	1.03
Treatment					
Saline	1190	32	Ref		
Custom R-Pilus	1273	34	0.87	0.45	1.70
Footvax®	1273	34	0.91	0.47	1.76
Random effects					
Ewe	197	Variance = 1.14		ICC = 0.26	

No. and % represent the number and percentage of observations in each category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Odds ratios are statistically significant when the CI does not include unity and are indicated in bold.

Table 4-6 Binomial mixed effects regression model for lameness prevalence between week 7 and 25 of the trial in flock 3

Variable	No.	%	Odds ratio	Lower 95% CI	Upper 95% CI
Fixed effects					
Week	3687		**1.04**	**1.02**	**1.07**
Treatment					
Saline	1256	34	Ref		
Custom R-Pilus	1273	36	0.82	0.56	1.20
Footvax®	1158	31	0.74	0.50	1.20
Breed					
Whitefaced cross	2067	56	Ref		
Exmoor Mule	488	13	**1.91**	**1.20**	**3.06**
North Country Mule	1132	31	**1.94**	**1.36**	**2.75**
Lame on 1st week					
No	2700	73	Ref		
Yes	987	27	**1.44**	**1.01**	**2.05**
Random effects					
Ewe	196	Variance = 0.70		ICC = 0.13	

No. and % represent the number and percentage of observations in each category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Odds ratios are statistically significant when the CI does not include unity and are indicated in bold.

4.3.3.2 Weekly prevalence of footrot

4.3.3.2.1 Weekly prevalence of footrot by flock

In flocks 1 and 2, the trend in weekly prevalence of footrot followed the trend in weekly prevalence of lameness, whereas in flock 3 the prevalence of footrot was consistently low in the second half of the trial whilst the prevalence of lameness increased. Flock 1 had the highest geometric mean weekly prevalence of footrot at 3.3% (SD = 2.1%) between weeks 7 and 25, compared to 1.1% (SD=1.0%) in flock 2 and 1.6% (SD=1.5%) in flock 3.

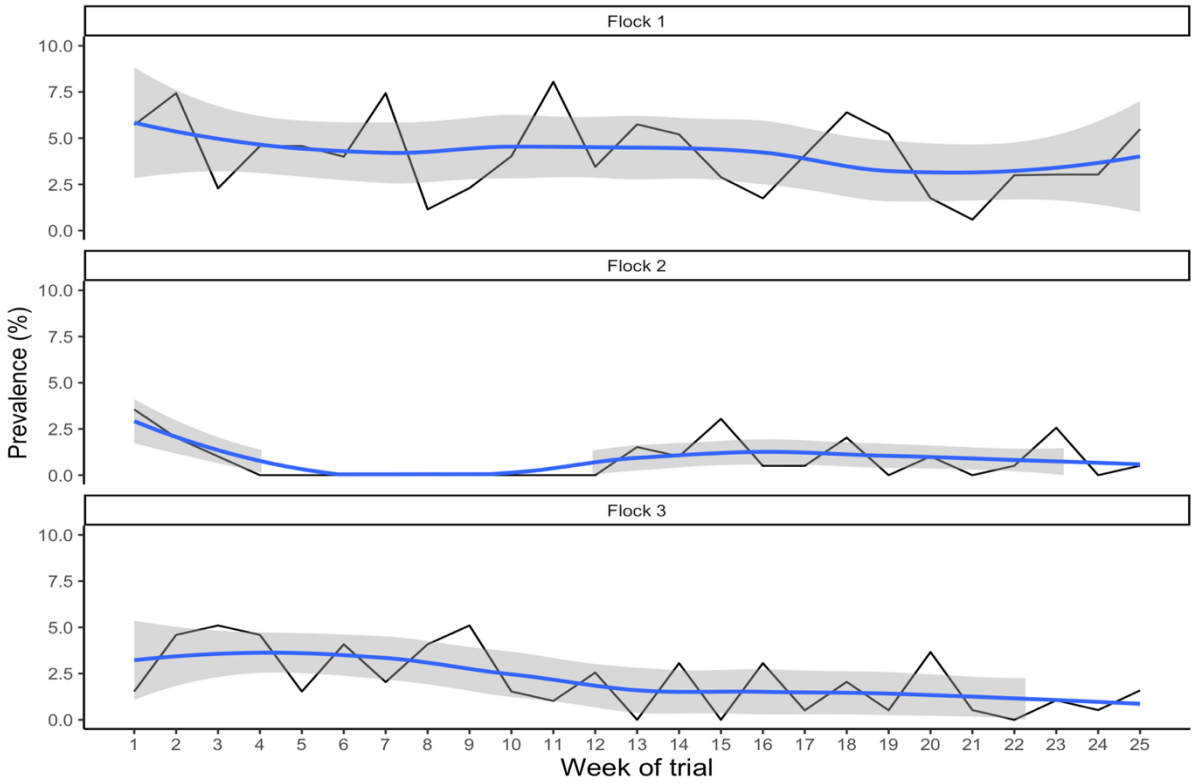

Figure 4-10 Weekly prevalence of footrot by flock. The black line shows the true prevalence, the blue line is fitted using Loess regression for which the 95% CI is shaded in grey. Ewes were not examined in weeks 6-9, 11 and 12 in flock 2.

4.3.3.2.2 Weekly prevalence of footrot by flock and treatment group

In flock 1, the variation seen in the weekly prevalence of footrot for the flock was also seen across the three treatment groups (Figure 4-11). Furthermore, weekly prevalence of footrot in each treatment group followed the same trends as for weekly prevalence of lameness in the treatment groups. There were no notable differences between the treatment groups in trends in weekly prevalence of footrot in flock 2 (Figure 4-12). In flock 3, although weekly prevalence of footrot was higher in the Custom vaccinated group during the first half of the trial, the same trend in prevalence was seen across the three treatment groups (Figure 4-13).

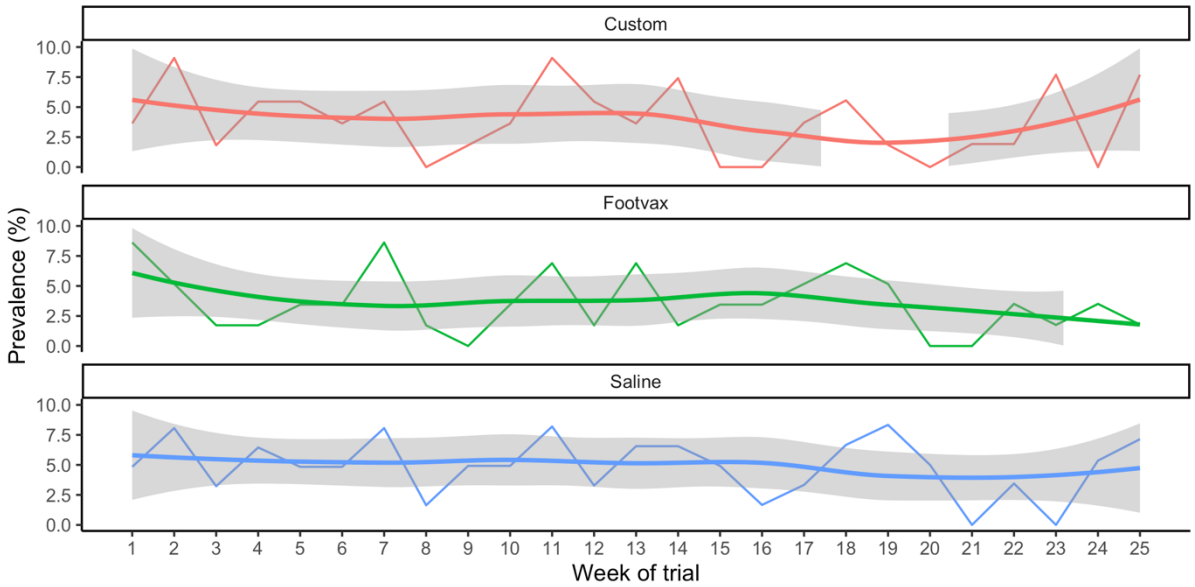

*Figure 4-11 Weekly prevalence of foot*rot *by treatment group in flock 1. The thin line shows the true prevalence, the thick line is fitted using Loess regression for which the 95% CI is shaded in grey.*

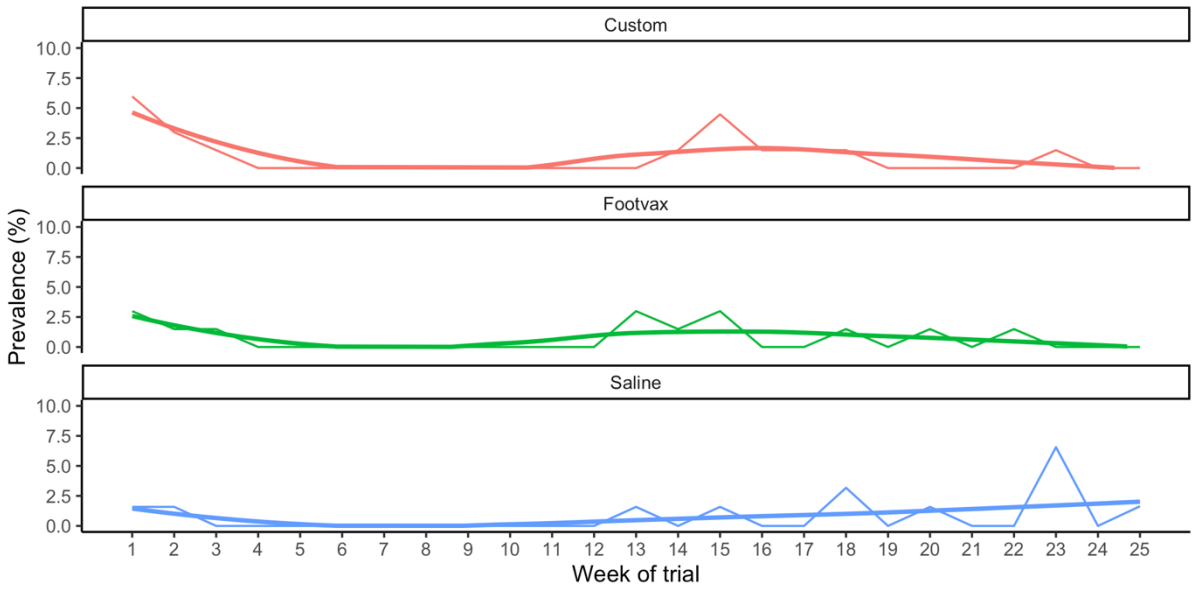

Figure 4-12 Weekly prevalence of footrot by treatment group in flock 2. The thin line shows the true prevalence, the thick line is fitted using Loess regression for which the 95% CI is shaded in grey. Ewes were not examined in weeks 6-9, 11 and 12 in flock 2.

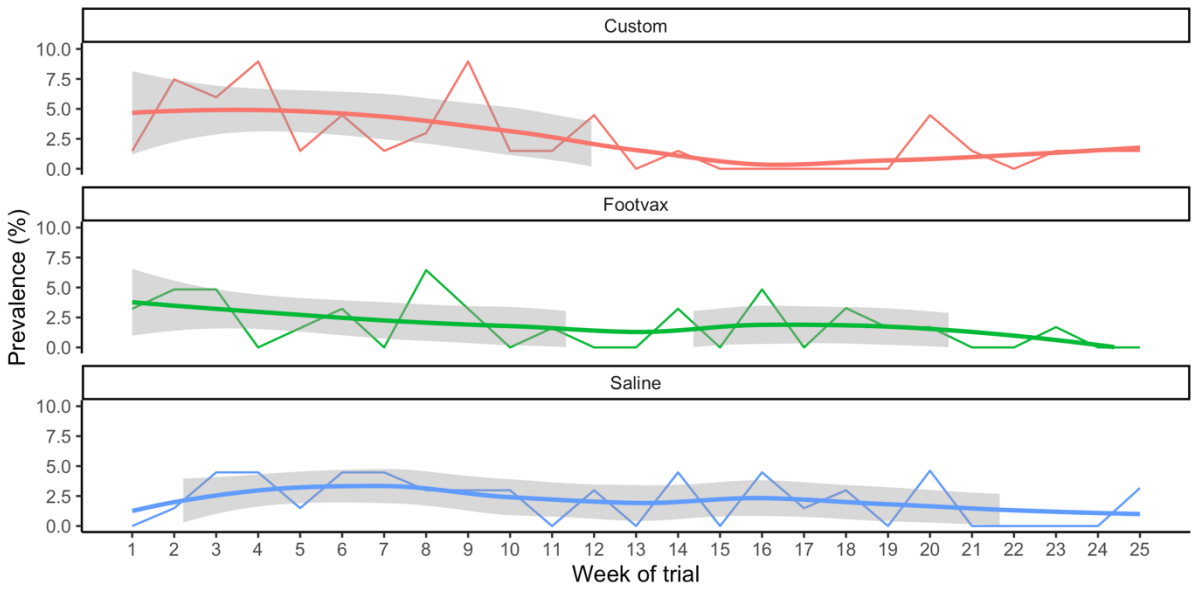

Figure 4-13 Weekly prevalence of footrot by treatment group in flock 3. The thin line shows the true prevalence, the thick line is fitted using Loess regression for which the 95% CI is shaded in grey.

4.3.3.2.3 Weekly prevalence of footrot by flock and other variables

In flock 1, the two ewe origin categories showed different trends for weekly prevalence of footrot as they did for weekly lameness prevalence (Figure 4-14). Footrot prevalence varied more in the Purchased group, which had a geometric mean of 7.1% (SD=5.3%), compared to 1.5% (SD=2.0%) in the Existing group. Footrot in Existing ewes did not exceed one case per week between week 7 and 17, after which there was a gradual increase in prevalence of footrot that coincided with being housed. Weekly prevalence of footrot did not vary between the two BCS at first vaccination categories.

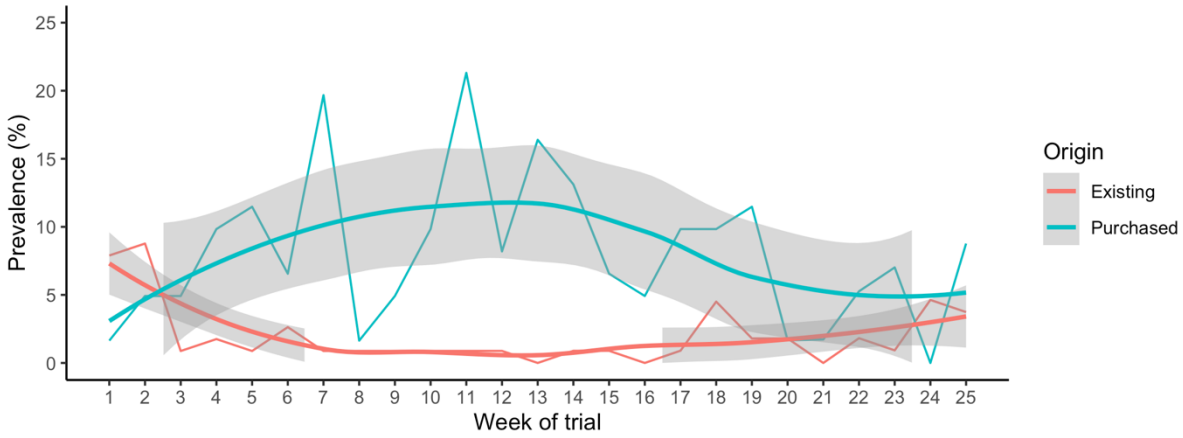

Figure 4-14 Weekly prevalence of footrot by origin category in flock 1. The thin lines show the true prevalence, the thick lines are fitted using Loess regression for which 95% CIs are shaded in grey.

In flock 2, Age and BCS category at first vaccination did not show any associations with weekly prevalence of footrot. In flock 3, in contrast to weekly lameness prevalence, prevalence of footrot in the Exmoor Mules remained low after week 8 (Figure 4-15). The North Country Mules were the most affected by footrot after this time, as they were for

lameness. No difference was seen between groups for the variables BCS at first vaccination and age.

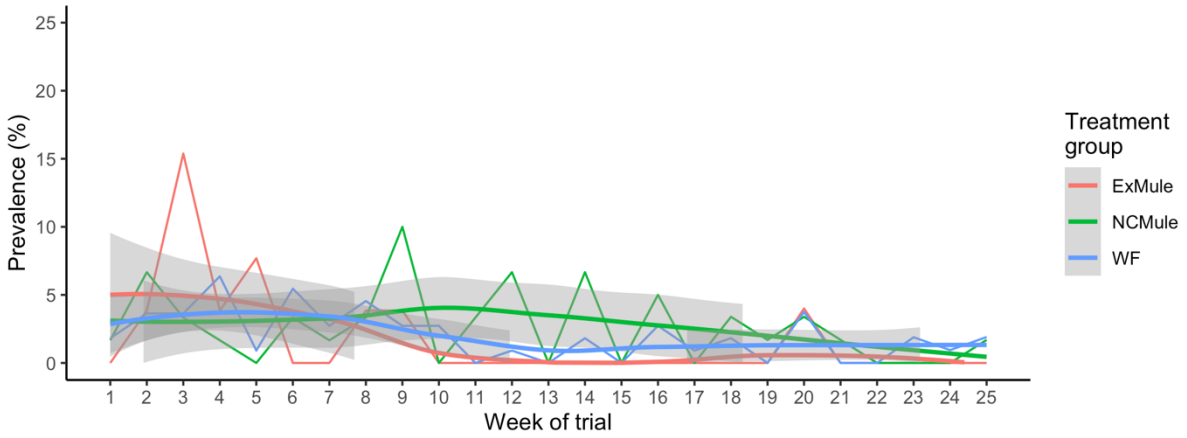

Figure 4-15 Weekly prevalence of footrot by breed category in flock 3. The thin lines show the true prevalence, the thick lines are fitted using Loess regression for which 95% CIs are shaded in grey. ExMule = Exmoor Mule, NCMule = North Country Mule, WF = Whitefaced.

4.3.3.2.4 Binomial mixed effect models for prevalence of footrot

Binomial mixed effect regression models were constructed for each flock to estimate the trend in footrot prevalence between week 7 and 25 of the trial, the effect of treatment group, and any influential variables. The variables tested were origin (flock 1), age (flock 2, flock 3), breed (flock 3), BCS category at first vaccination, lame in week 1, and footrot lesions in week 1 (all flocks). The reasons for the selection of these variables, excluding footrot lesions in week 1, are detailed in section 4.3.3.1.4. 'Footrot lesions in week 1' was tested because the duration of footrot lesions found in week 1 was unknown, and footrot lesions of a prolonged duration might have predisposed to a recurrence of footrot later in the trial. Treatment and origin in flock 1 were combined to create six categories, to facilitate interpretation of an interaction between these two variables.

The prevalence of footrot did not change after week 7 in flocks 1 and 2 (Table 4-7, Table 4-8). The prevalence of footrot decreased in flock 3 (Table 4-9) in contrast to the increase in lameness prevalence observed in this flock. The only treatment effect observed was in the Existing ewes that were vaccinated with Custom R-Pilus, who were at significantly lower odds of having footrot (Table 4-7). None of the other variables influenced footrot prevalence.

Table 4-7 Binomial mixed effects regression model for prevalence of footrot between week 7 and 25 of the trial in flock 1

Variable	No.	%	Odds ratio	Lower 95% CI	Upper 95% CI
Fixed effects					
Week	3254		0.97	0.94	1.01
Cos(Week)	3254		**1.59**	**1.22**	**2.07**
Treatment origin					
Existing Saline	722	22	Ref		
Existing Custom	672	21	**0.06**	**0.01**	**0.47**
Existing Footvax®	721	22	0.70	0.33	1.50
Purchased Saline	414	13	4.11	2.25	7.51
Purchased Custom	351	11	4.62	2.50	8.51
Purchased Footvax®	374	11	3.11	1.64	5.91
Random effects					
Ewe	175	Variance = 0.06		ICC = 0.02	

No. and % represent the number and percentage of observations in each category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Odds ratios are statistically significant when the CI does not include unity and are indicated in bold.

Table 4-8 Binomial mixed effects regression model for prevalence of footrot between week 7 and 25 of the trial in flock 2

Variable	No.	%	Odds ratio	Lower 95% CI	Upper 95% CI
Fixed effects					
Week	3328		3.36	0.51	22.04
Week^2	3328		8.26×10^{-139}	0.00	1.41×10^{63}
Week^3	3328		1.5×10^{77}	9.07×10^{-32}	2.49×10^{185}
Week^4	3328		5.60×10^{-36}	1.13×10^{-84}	2.77×10^{13}
Treatment					
Saline	1064	32	Ref		
Custom R-Pilus	1139	34	0.74	0.29	1.90
Footvax®	1139	34	0.74	0.29	1.90
Random effects					
Ewe	197	Variance = 0.00		ICC = 0.00	

No. and % represent the number and percentage of observations in each category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Odds ratios are statistically significant when the CI does not include unity and are indicated in bold.

Table 4-9 Binomial mixed effects regression model for prevalence of footrot between week 7 and 25 of the trial in flock 3

Variable	No.	%	Odds ratio	Lower 95% CI	Upper 95% CI
Fixed effects					
Week	3686		**0.93**	**0.89**	**0.98**
Treatment					
Saline	1256	34	Ref		
Custom R-Pilus	1273	36	0.86	0.47	1.57
Footvax®	1157	31	0.73	0.38	1.38
Breed					
Whitefaced cross	2066	56	Ref		
Exmoor Mule	488	13	0.39	0.11	1.31
North Country Mule	1132	31	1.68	0.99	2.84
Random effects					
Ewe	196	Variance = 0.17		ICC = 0.13	

No. and % represent the number and percentage of observations in each category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Odds ratios are statistically significant when the CI does not include unity and are indicated in bold.

4.3.3.3 Weekly prevalence of other foot lesions

4.3.3.3.1 Weekly prevalence of active CODD and ulcerative lesions in lame ewes

Cases of CODD occurred sporadically throughout the trial in flocks 1 and 2 and remained <2% in prevalence after existing lesions in week 1 had been treated (Figure 4-16, Figure 4-17).

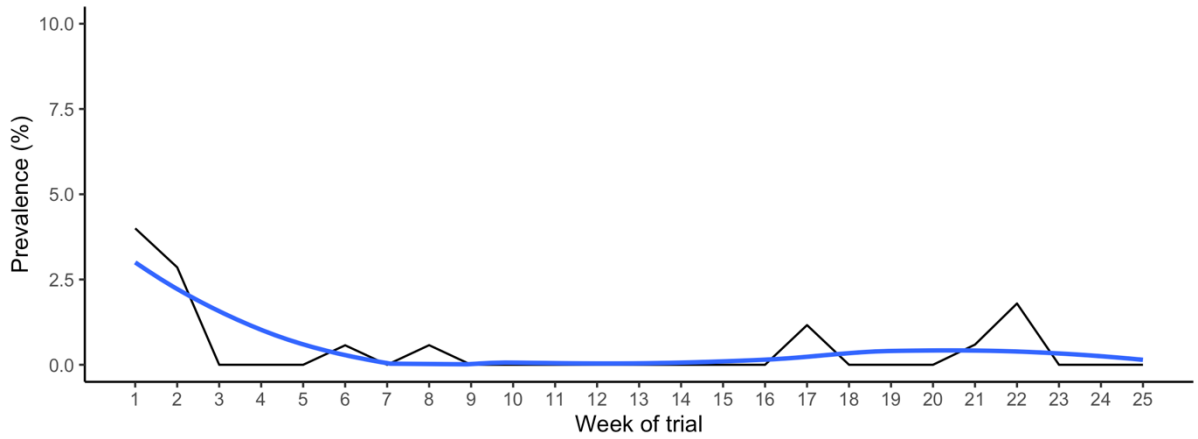

Figure 4-16 Weekly prevalence of active CODD lesions (score 1-4) in lame ewes in flock 1. The black line shows the true prevalence, the blue line is fitted using Loess regression.

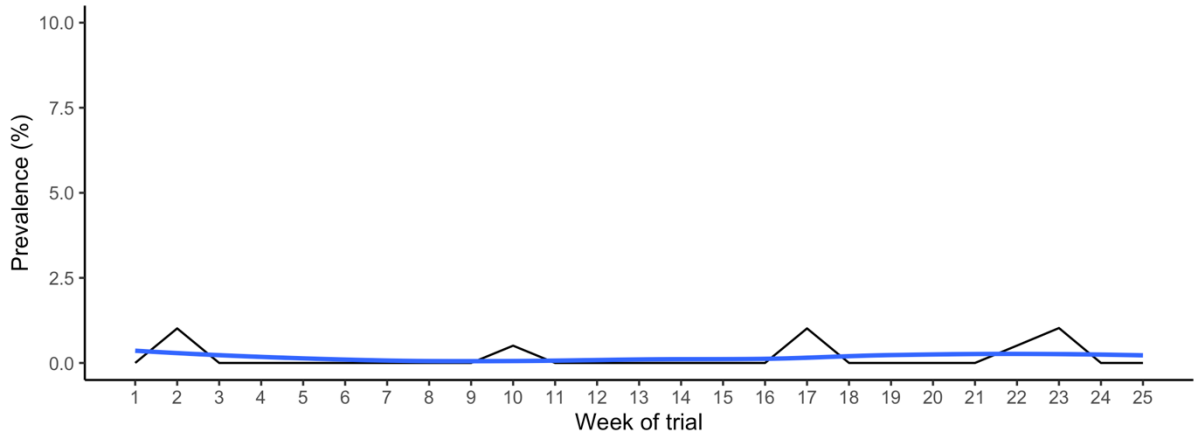

Figure 4-17 Weekly prevalence of active CODD lesions (score 1-4) in lame ewes in flock 2. The black line shows the true prevalence, the blue line is fitted using Loess regression. Ewes were not examined in weeks 6-9, 11 and 12.

Prevalence of CODD in lame ewes was also low in flock 3, except for two peaks at weeks 13-14 and week 20, neither of which exceed 5.2% (Figure 4-18). The weekly prevalence of ulcerative skin lesions peaked at the same time as active CODD lesions during weeks 13-15, but prevalence did not exceed 3.1% (Figure 4-19). Ulcerative skin lesions were observed once in flock 1 and did not occur in flock 2.

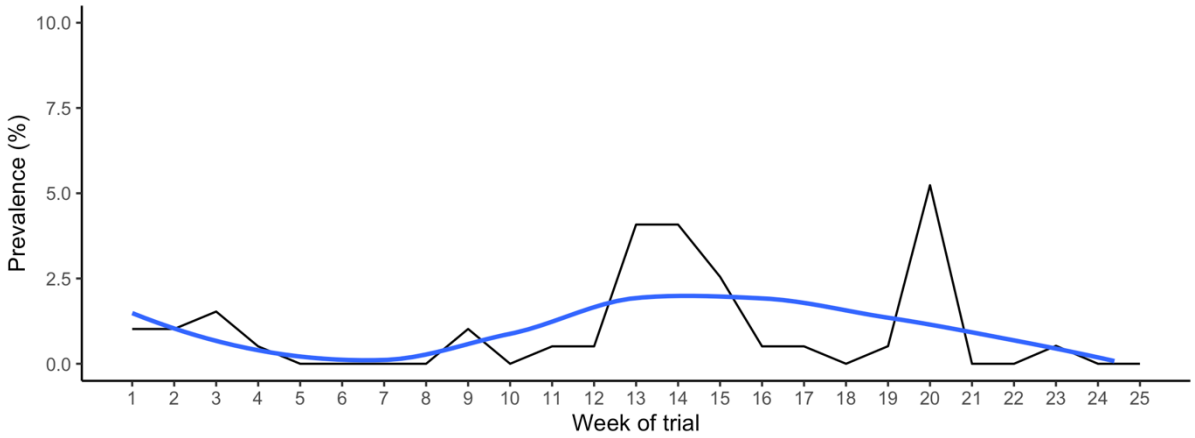

Figure 4-18 Weekly prevalence of active CODD lesions (score 1-4) in lame ewes in flock 3. The black line shows the true prevalence, the blue line is fitted using Loess regression.

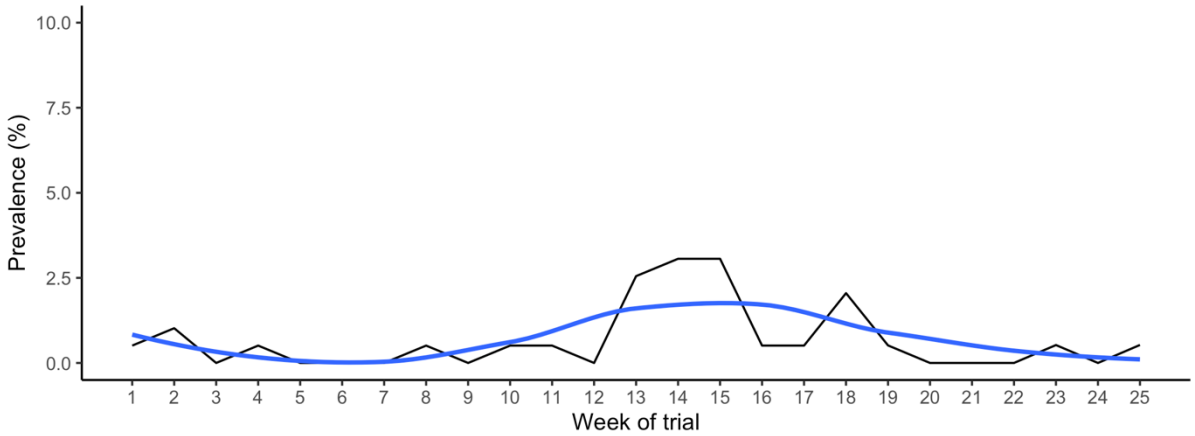

Figure 4-19 Weekly prevalence of ulcerative skin lesions in lame ewes in flock 3. The black line shows the true prevalence, the blue line is fitted using Loess regression.

After active CODD lesions present in week 1 had been treated, all new cases of CODD were detected at an early stage in the disease before extensive hoof separation had occurred (scores 1-2) (Figure 4-20), except for one saline treated ewe in flock 1 who was lame for two consecutive weeks.

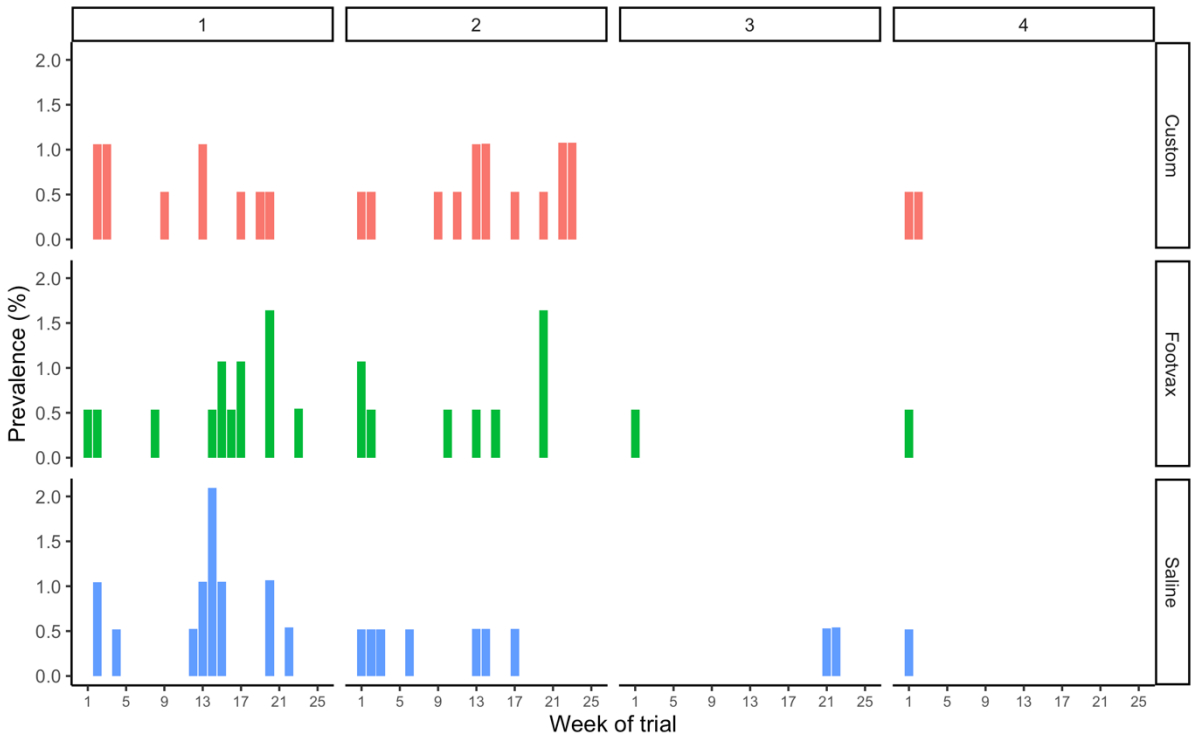

Figure 4-20 The weekly prevalence of active CODD lesions in lame sheep by treatment group and severity score for the combined flocks

4.3.3.3.2 Weekly prevalence of hoof horn lesions in lame ewes

The weekly prevalence of hoof horn lesions in lame ewes remained low throughout the trial in flocks 1 and 2 and did not exceed 3.5% and 2.5% in the respective flocks. Notably, the prevalence remained at its lowest after housing day and night in week 19 in flock 1 and week 17 in flock 2 (Figure 4-21, Figure 4-22). In contrast, there was more variation in the

prevalence of hoof horn lesions in flock 3 and prevalence increased during the second half of the trial, when hoof horn damage was the most commonly observed lesion in lame sheep (Figure 4-23).

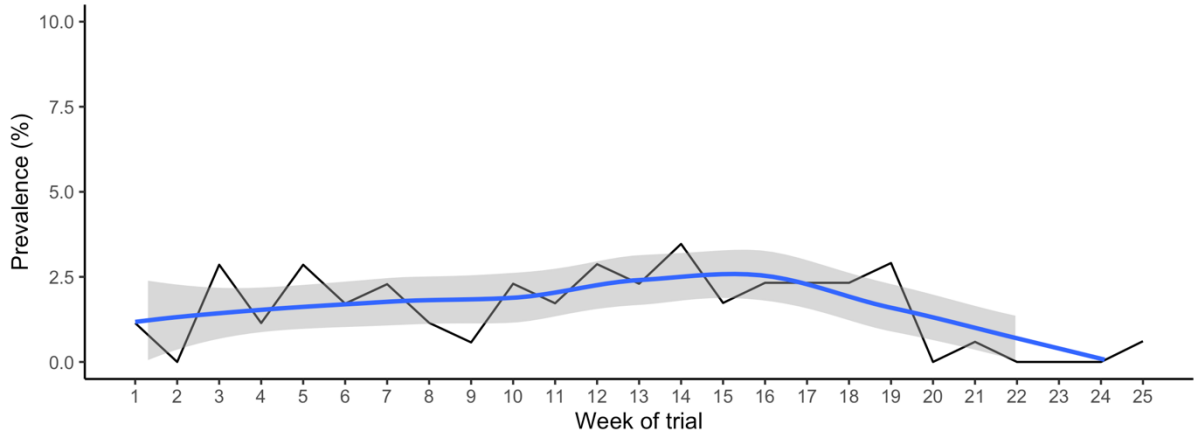

Figure 4-21 Weekly prevalence of hoof horn lesions in lame ewes in flock 1. The black line shows the true prevalence, the blue line is fitted using Loess regression for which the 95% CI is shaded in grey.

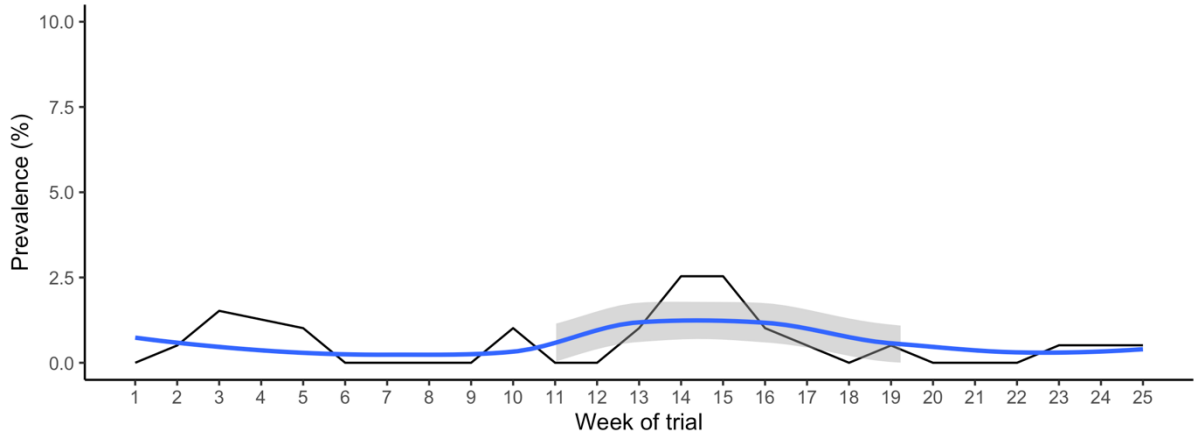

Figure 4-22 Weekly prevalence of hoof horn lesions in lame ewes in flock 2. The black line shows the true prevalence, the blue line is fitted using Loess regression for which the 95% CI is shaded in grey. Ewes were not examined in weeks 6-9, 11 and 12.

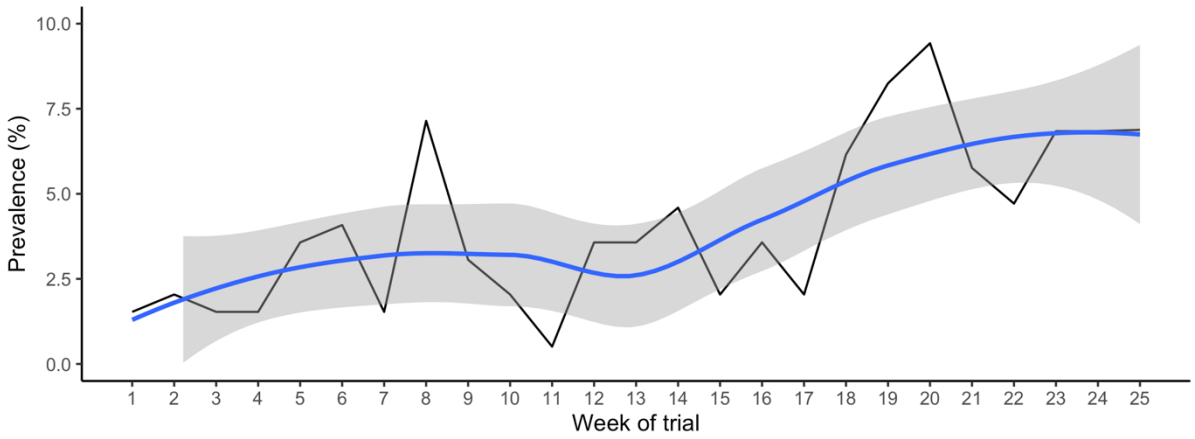

Figure 4-23 Weekly prevalence of hoof horn lesions in lame ewes in flock 3. The black line shows the true prevalence, the blue line is fitted using Loess regression for which the 95% CI is shaded in grey.

4.4 Incidence of lameness and footrot over the trial

4.4.1 Incidence of lameness by flock and treatment group

Lameness incidence rates for the whole trial were 6.6 cases/100 ewe-weeks for both flocks 1 and 3, and 1.9 cases/100 ewe-weeks in flock 2. Incidence of lameness in the Footvax® group was lower than in the Custom R-Pilus and saline groups in all three flocks prior to week 7 (Table 4-10). The difference in incidence rates between vaccinated groups was smaller after week 7 (Table 4-11). Plots of weekly lameness incidence by treatment group showed very similar trends to weekly prevalence rates by treatment group for all flocks and are presented in Appendix 13.

Table 4-10 Number of new lameness cases and lameness incidence by treatment group and flock between week 2 and 6 of the trial

	Number of new cases	No. ewe-weeks at risk	Incidence (cases/100 ewe-weeks)
Flock 1			
Custom R-Pilus	22	247	8.9
Footvax®	13	262	5.0
Saline	21	278	7.6
Total	56	787	7.2
Flock 2			
Custom R-Pilus	6	320	1.9
Footvax®	3	327	0.9
Saline	8	304	2.6
Total	17	951	1.8
Flock 3			
Custom R-Pilus	22	301	7.3
Footvax®	14	288	4.9
Saline	19	294	6.5
Total	55	883	6.2

Table 4-11 Number of new lameness cases and lameness incidence for each treatment group in each flock between week 7 and 25 of the trial

	Number of new cases	No. ewe-weeks at risk	Incidence (cases/100 ewe-weeks)
Flock 1			
Custom R-Pilus	53	954	5.6
Footvax®	60	1024	5.9
Saline	81	1042	7.8
Total	194	3020	6.4
Flock 2			
Custom R-Pilus	24	1243	1.9
Footvax®	23	1245	1.8
Saline	21	1154	1.8
Total	68	3642	1.9
Flock 3			
Custom R-Pilus	76	1167	6.5
Footvax®	63	1073	5.9
Saline	88	1134	7.8
Total	227	3374	6.7

4.4.2 Incidence of footrot by flock and treatment group

The incidence rate for footrot for the whole trial was 3.9 cases/100 ewe-weeks in flock 1, 2.1 cases/100 ewe-weeks in flock 3, and 0.7 cases/100 ewe-weeks in flock 2. Incidence rates between weeks 2 and 6 are presented in Table 4-12 and for weeks 7-25 in Table 4-13. Incidence of footrot was very low in flock 2 and only exceeded 1 case/100 ewes/week in the saline group. Trends in weekly footrot incidence were similar between treatment groups within the flocks (Appendix 13) and comparable to the trends in weekly footrot prevalence.

Table 4-12 Number of new footrot cases and footrot incidence for each treatment group in each flock between week 2 and 6 of the trial

	Number of new cases	No. ewe-weeks at risk	Incidence (cases/100 ewe-weeks)
Flock 1			
Custom R-Pilus	13	251	5.2
Footvax®	7	266	2.6
Saline	13	279	4.7
Total	33	796	4.1
Flock 2			
Custom R-Pilus	1	255	0.4
Footvax®	1	262	0.4
Saline	0	248	0
Total	2	765	0.3
Flock 3			
Custom R-Pilus	17	304	5.6
Footvax®	8	289	2.8
Saline	9	300	3.0
Total	34	893	3.8

Table 4-13 Number of new footrot cases and footrot incidence for each treatment group in each flock between week 7 and 25 of the trial

	Number of new cases	No. ewe-weeks at risk	Incidence (cases/100 ewe-weeks)
Flock 1			
Custom R-Pilus	33	977	3.8
Footvax®	35	1046	3.5
Saline	51	1062	4.8
Total	119	3085	3.9
Flock 2			
Custom R-Pilus	7	926	0.8
Footvax®	6	926	0.6
Saline	10	862	1.2
Total	23	2714	0.8
Flock 3			
Custom R-Pilus	18	1210	1.5
Footvax®	16	1103	1.5
Saline	22	1186	1.9
Total	56	3499	1.6

4.5 Distribution of repeat lameness and footrot cases

4.5.1 Number of cases of lameness per ewe between week 7 and 25

The number of cases of lameness per ewe in each treatment group is shown in Figure 4-24 and Table A- 23. The number of weeks that the ewe was at risk of becoming lame was not accounted for when comparing the number of lameness cases, and this varied due to persistent lameness or withdrawal from the trial (Table 4-1). Repeat cases of lameness occurred frequently in flocks 1 and 3 with no notable differences between treatment groups.

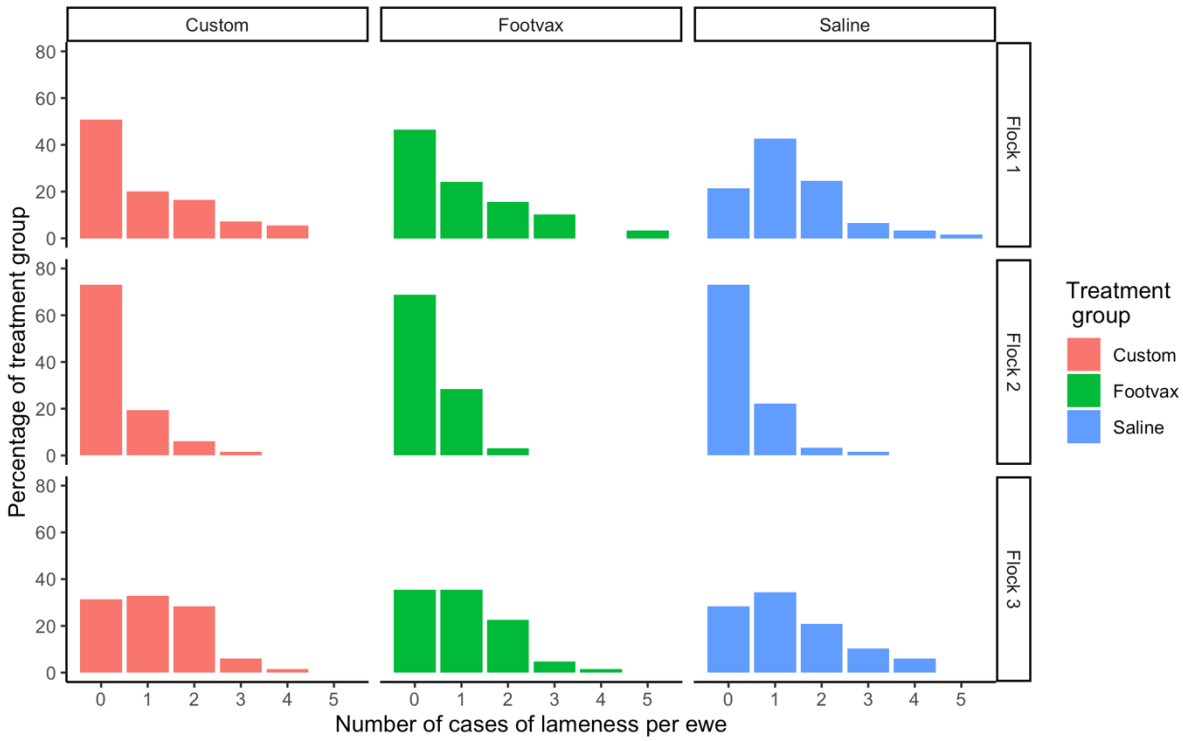

Figure 4-24 The number of cases of lameness per ewe between week 7 and 25 of the trial as a percentage of each treatment group

In flock 1, the distribution of repeat lameness cases in the two origin categories was different to that of the combined flock because most repeat cases occurred in the Purchased ewes (Figure 4-25). There was also a difference between the origin categories in the percentage of ewes that did not become lame in each treatment group, most notably the protective effect of the Custom R-Pilus vaccine in the Existing ewes and Footvax® in the Purchased ewes.

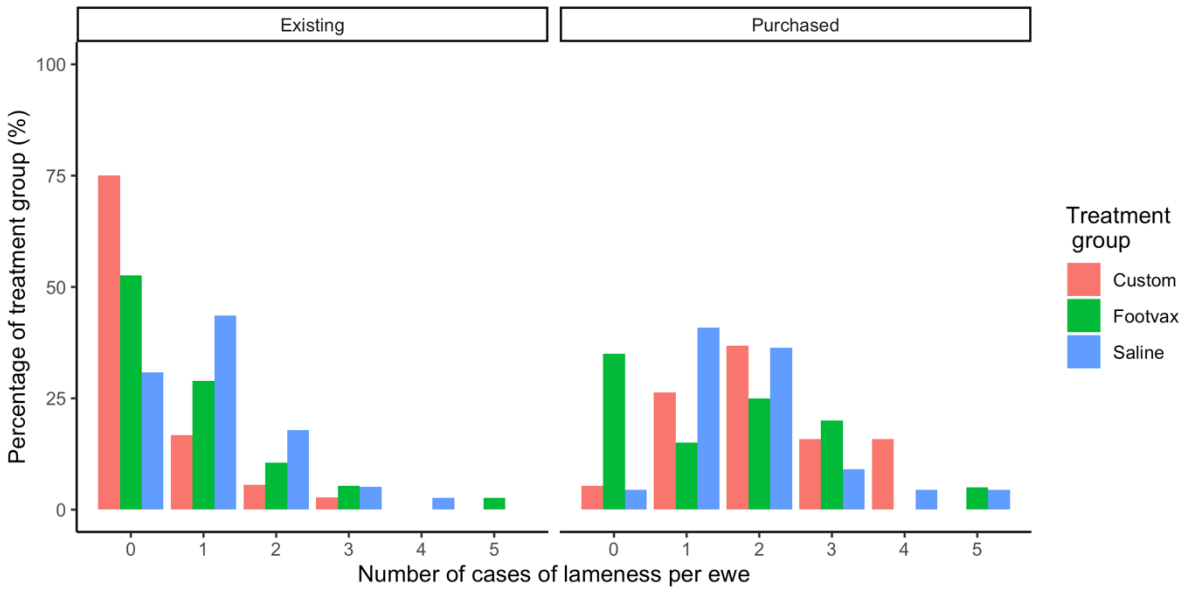

Figure 4-25 The number of cases of lameness per ewe between week 7 and 25 of the trial as a percentage of each treatment group by origin category in flock 1

4.5.2 Number of cases of footrot per ewe between week 7 and 25

The number of cases of footrot per ewe in each treatment group is shown in Figure 4-26 and Table A- 24. Ewes in flock 1 had up to four cases of footrot, but only two ewes in the Existing category had repeat cases (Figure 4-27). No repeat cases occurred in flock 2 and only one repeat case occurred in each treatment group in flock 3.

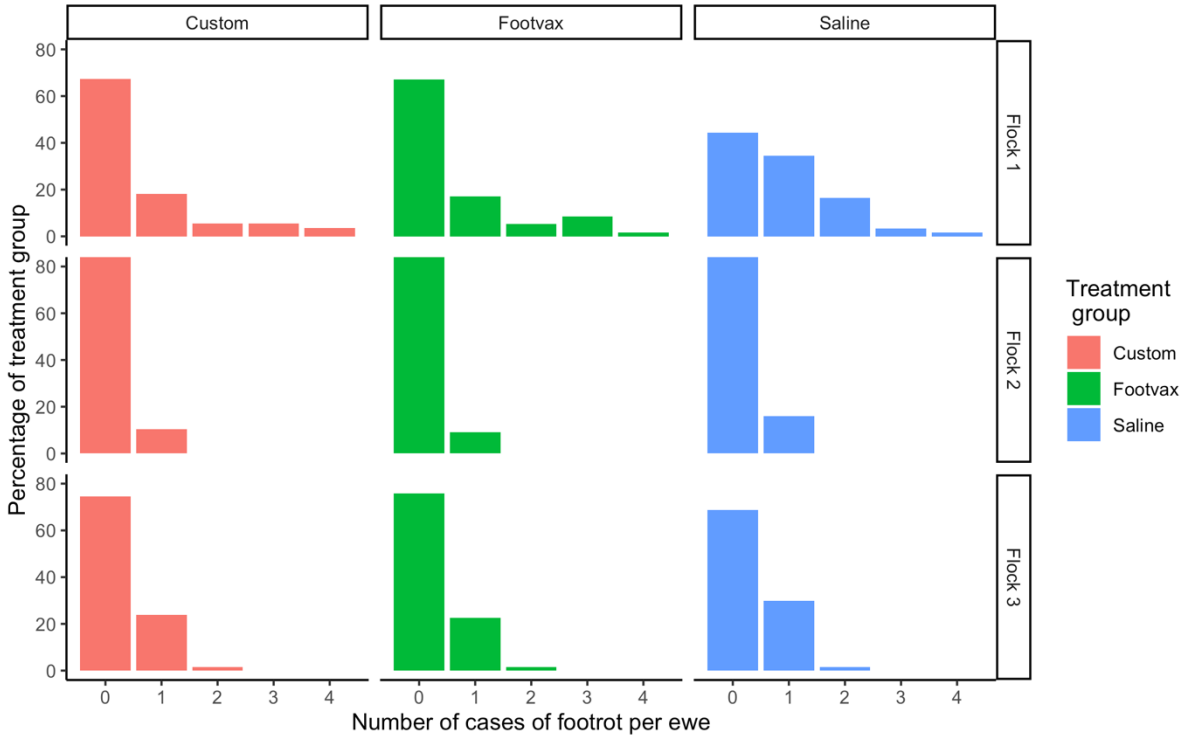

Figure 4-26 The number of cases of footrot per ewe between week 7 and 25 of the trial as a percentage of each treatment group

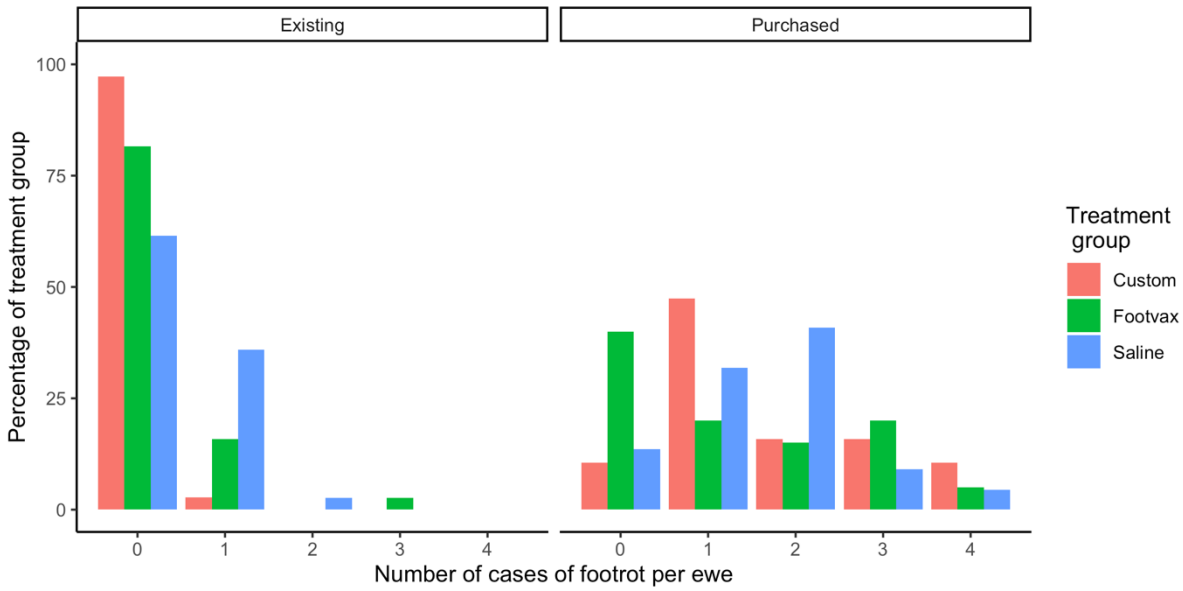

Figure 4-27 The number of cases of footrot per ewe between week 7 and 25 of the trial as a percentage of each treatment group by origin category in flock 1

4.6 Survival analysis for first and recurrent lameness and footrot events

The relationship between survival time, treatment, and other potential predictors for both lameness and footrot events was examined visually before the construction of separate survival models for each flock. First, KM and cumulative hazard (CH) plots were used to investigate changes in hazard rate in the treatment groups for lameness events over the whole trial duration (week 1-25). The truncated dataset (week 6-25) was then used to produce KM plots, CH plots and plots of the complementary log-log of survival probability, which were used to observe the hazard ratios between categories for the following variables: treatment, origin (flock 1), age (flock 2, flock 3), breed (flock 3), and for all flocks BCS group at first vaccination, a binary variable for having a lameness event at least once between week 2 and 6 ('Previously lame'), and a binary variable for being lame on the first week of the trial ('Lame on 1st week '). The same plots were produced for footrot events to observe the relationship between categories for the following variables: treatment, origin (flock 1), age (flock 2, flock 3), breed (flock 3), and for all flocks BCS group at first vaccination, a binary variable for having a footrot event at least once between week 2 and 6 ('Previous FR'), a binary variable for being lame on the first week of the trial ('Lame on 1st week'), and a binary variable for having a footrot lesion on the first week of the trial ('FR on 1st week'). These variables were selected for testing in the models based on their biological potential to influence the occurrence of lameness and footrot events, as previously described in sections 4.3.3.1.4 and 4.3.3.2.4. Additionally, the 'Previously lame' and 'Previous FR' variables were tested because some ewes appeared to have a predisposition to repeated lameness and footrot events respectively (Figure 4-25, Figure 4-26) and some of these occurred before

week 7. Ewes lame on week 1 were not confounded with ewes that had footrot lesions on week 1.

Although all models for individual flocks were constructed blinded to treatment identity, in all cases the number of events was highest in the saline treatment group and therefore this was the reference treatment group for all individual flock models. After the treatment identities were unblinded, the truncated datasets (weeks 6-25) for the flocks were combined. Survival probabilities and cumulative hazards were plotted, and frailty models were constructed, to demonstrate the overall impact of treatment for first and all cases of lameness and footrot. Both categories of the variables Previously lame, Lame on Week 1, Previous Footrot and FR on Week 1 were present in all flocks and were tested in construction of the combined flock models as previously described for the individual flock models. The results for the combined flock models are presented first as an overview before the results of the detailed, individual flock analyses are presented.

4.6.1 The impact of vaccination on time to first lameness event

4.6.1.1 All flocks

Survival functions for time to first lameness event are presented by flock and treatment group for the combined flocks in Figure 4-28 and Figure 4-29 respectively.

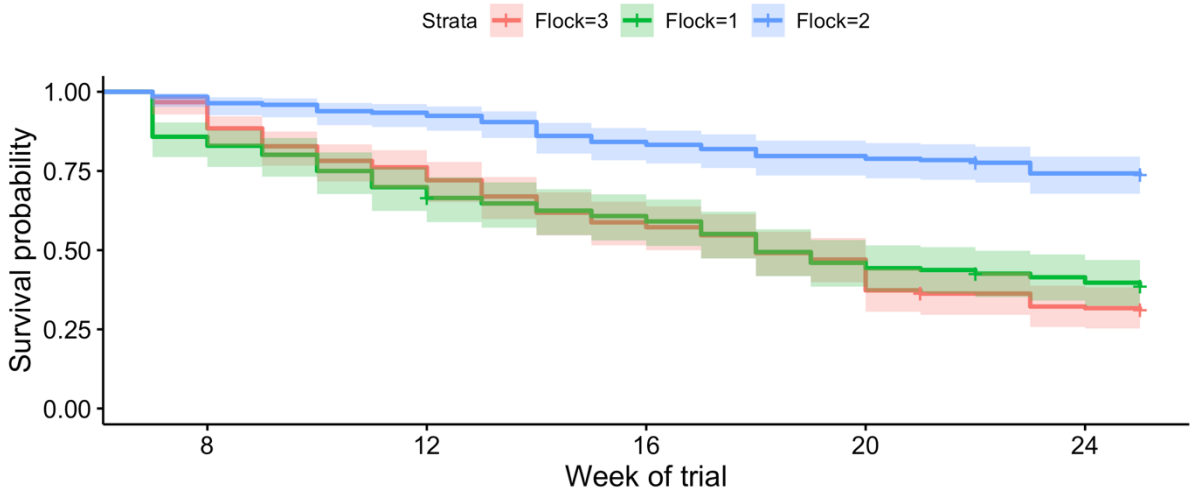

Figure 4-28 KM plot for time to first lameness event after week 6 of the trial by flock (shaded areas represent the 95% CIs)

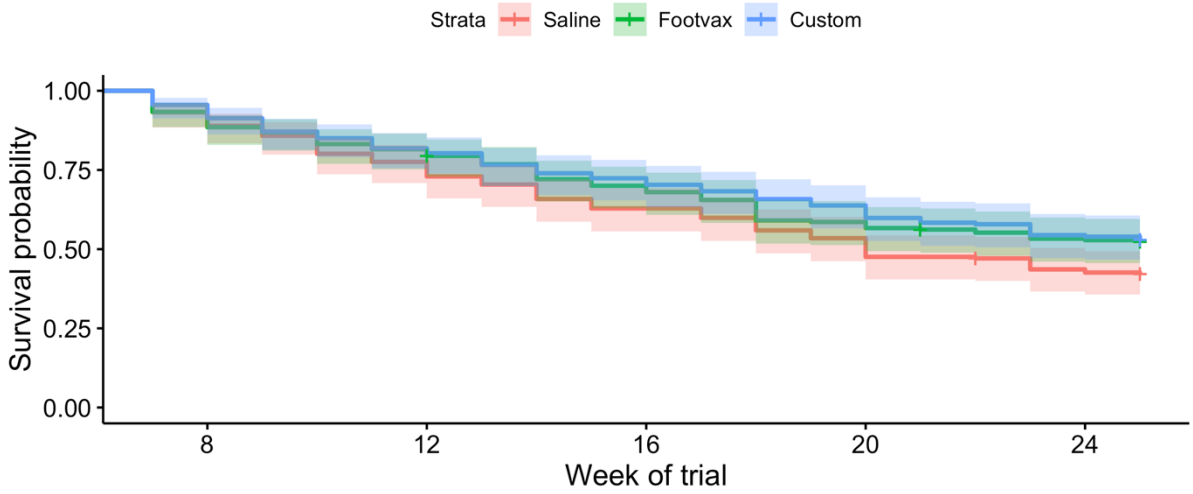

Figure 4-29 KM plot for time to first lameness event after week 6 of the trial by treatment group in all flocks (shaded areas represent the 95% CIs)

Table 4-14 Frailty model for time to first lameness event after week 6 of the trial in all flocks, reference treatment = saline

Variable	No.	%	HR	SE	p value
Treatment					
Saline	113	55	Ref		
Footvax®	92	44	0.77	0.14	0.07
Custom R-Pilus	91	45	**0.70**	**0.14**	**0.01**
Flock					
3	134	68	Ref		
1	106	61	0.98	0.13	0.87
2	56	23	**0.29**	**0.16**	**<0.01**
Previously lame					
No	136	43	Ref		
Yes	160	54	**1.38**	**0.12**	**<0.01**
Random effect					
Flock	Variance = 1.54×10^{-5}				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p \leq 0.05$ and are indicated in bold.

Ewes vaccinated with Custom R-Pilus were at a 30% lower risk of ever becoming lame than saline treated ewes (Table 4-14), but there was not a significant difference between Footvax® and Custom R-Pilus treated ewes (Table A- 25). The score test for non-proportionality was non-significant for all variables.

4.6.1.2 Flock 1

In flock 1, a change in the survival probability rate for the first lameness event was evident at week 7 in the Custom R-Pilus vaccinated ewes (Figure 4-30).

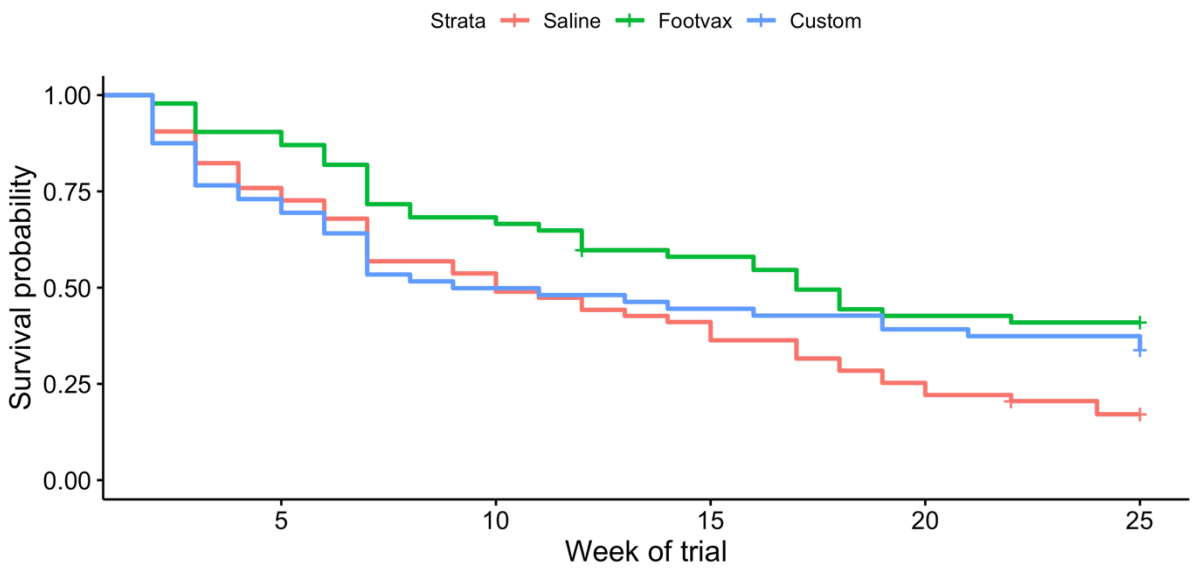

Figure 4-30 KM plot for time to first lameness event by treatment group over all weeks of the trial in flock 1

The KM plot for the treatment groups in flock 1 indicated that both vaccines were protective compared to saline (Figure 4-31). Ewes in the Footvax® group were more likely to become lame than the Custom R-Pilus vaccinated ewes in the first few weeks after housing in week 17. Survival probability in the Purchased ewes was much lower than in the Existing ewes (Figure 4-32) and stratification by both origin and treatment group showed evidence of non-proportionality and an interaction between origin and the two vaccine groups (Figure 4-33).

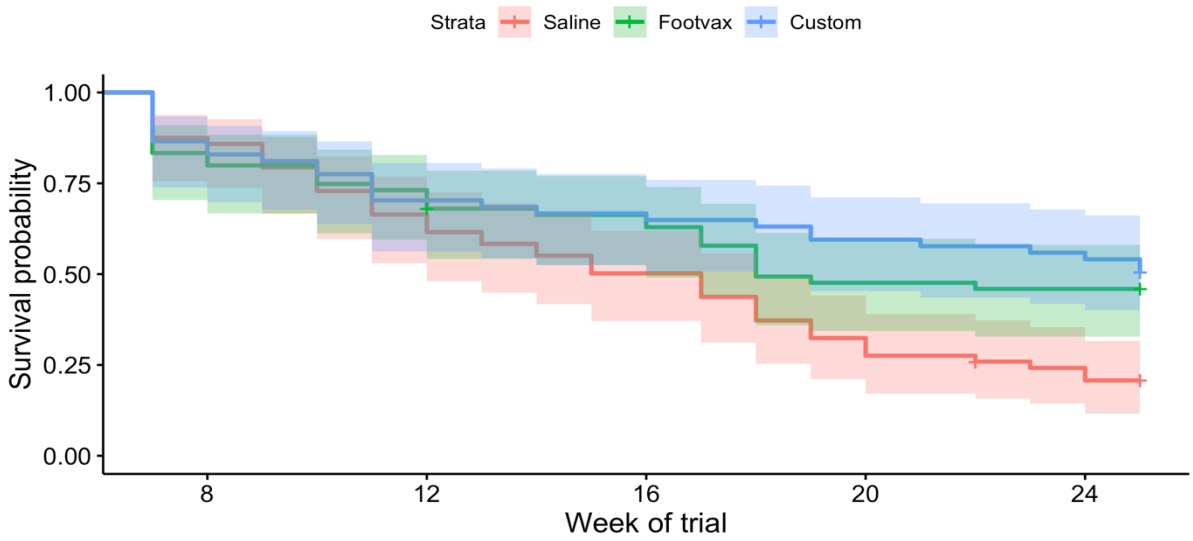

Figure 4-31 KM plot for time to first lameness event after week 6 of the trial by treatment group in flock 1 (shaded area represents the 95% CIs)

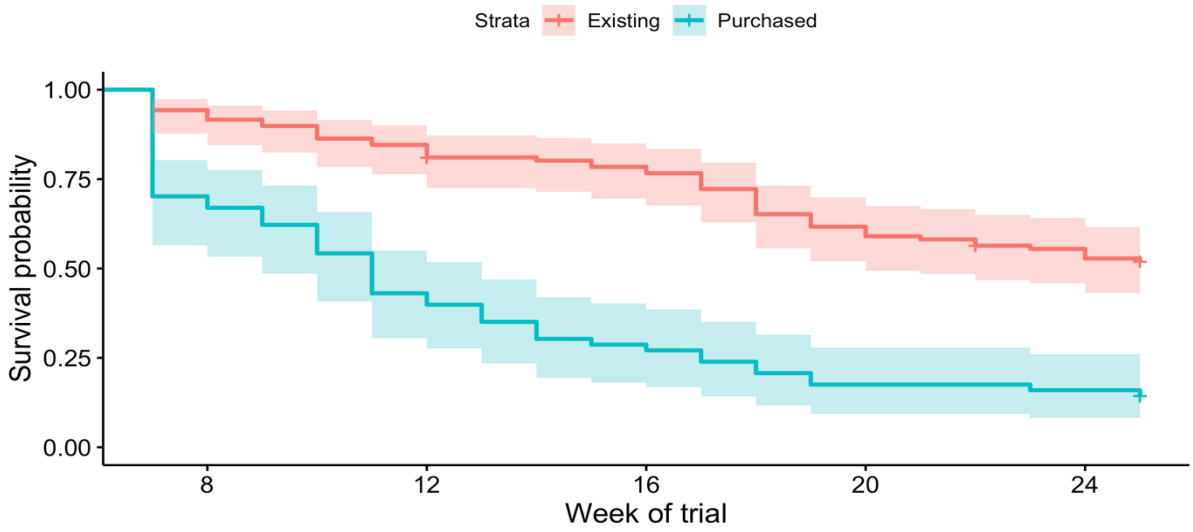

Figure 4-32 KM plot for time to first lameness event after week 6 of the trial by origin in flock 1 (shaded area represents the 95% CIs)

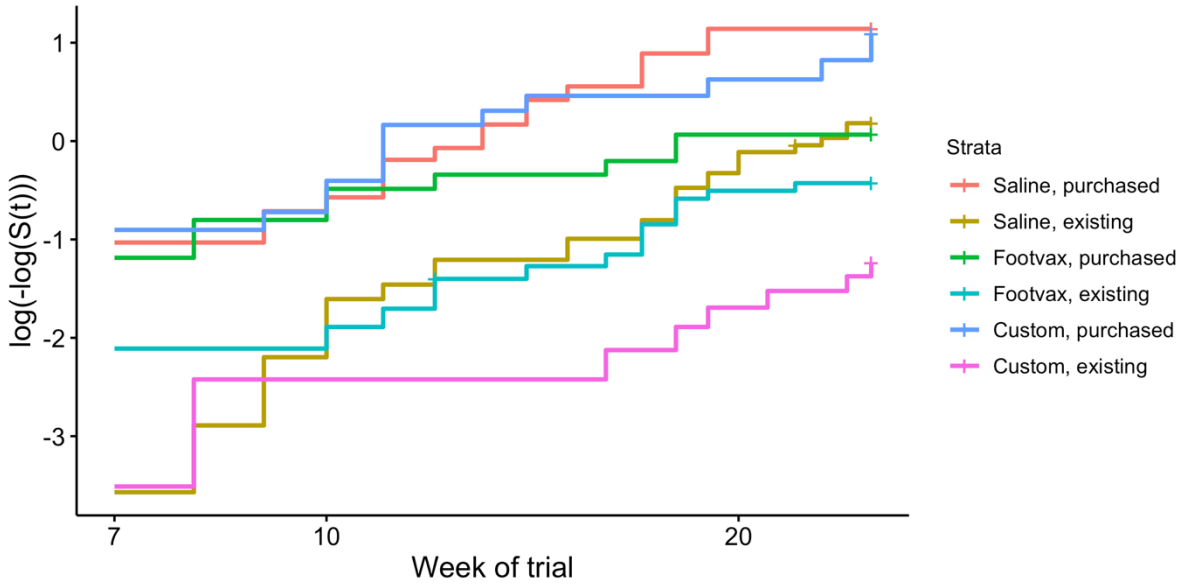

Figure 4-33 Complementary log-log of the survival probability for the first lameness event after week 6 of the trial with stratification by treatment and origin in flock 1

There was a small difference in survival probability for the Previously lame variable (not shown). The results of the Cox PH model for the first lameness event in flock 1 are presented in Table 4-15. The global score test for the model was not significant (p = 0.11) although there was evidence of a non-linear relationship over time in the treatment and origin variables, but not for the interaction term. Purchased ewes that had received Footvax® were at a 58% lower risk of ever becoming lame than those that had received saline. There was no difference in survival probability between Purchased ewes that had received the Custom R-Pilus vaccine and those that had received saline. Existing ewes that had received the Custom R-Pilus vaccine were at a 73% lower risk of ever becoming lame compared to those that received saline. There was not a significant difference in risk of ever becoming lame between Footvax® and saline treated Existing ewes. To facilitate interpretation of the treatment

interaction in the two origin categories, further models were constructed separately for these categories. The results are presented in Appendix 16. The results from both models were consistent with the combined model, and both models had a non-significant result in the score test for non-proportionality.

Table 4-15 Cox PH model for time to first lameness event after week 6 of the trial in flock 1

Variable	No.	%	Coef.	HR	Lower 95% CI	Upper 95% CI
Treatment						
Saline	48	79	Ref			
Footvax®	31	53	-0.86	**0.42**	**0.21**	**0.86**
Custom R-Pilus	27	49	-0.15	0.86	0.45	1.62
Origin						
Purchased	52	85	Ref			
Existing	54	47	-1.10	**0.33**	**0.19**	**0.60**
*Treatment*Origin*						
Saline Existing	27	69	Ref			
Footvax® Existing	18	46	0.42	1.53	0.60	3.83
Custom Existing	9	25	-1.17	**0.31**	**0.12**	**0.83**
Previously lame						
No	65	61	Ref			
Yes	41	67	0.22	1.25	0.83	1.87
Concordance = 0.72 (se = 0.024)						

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when the CI does not include unity and are indicated in bold.

4.6.1.3 Flock 2

The survival probability functions for time to first lameness event in flock 2 did not indicate a treatment effect either over all weeks of the trial or after week 6 (Figure 4-34). There was a small and proportional difference in hazard between ewes that had been previously lame.

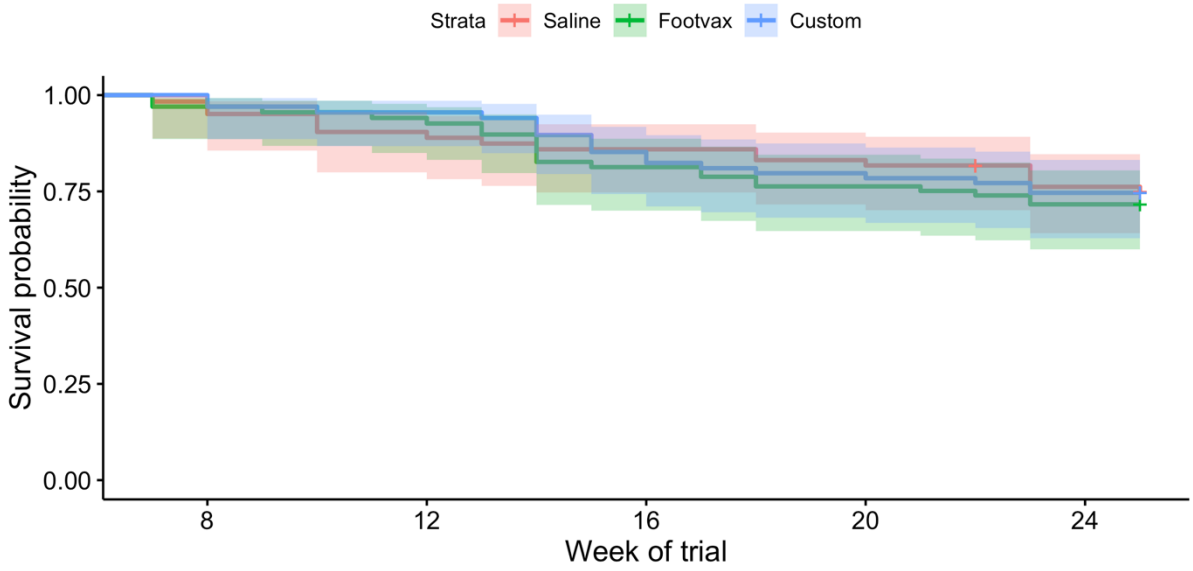

Figure 4-34 KM plot for time to first lameness event after week 6 of the trial by treatment group in flock 2 (shaded areas represent the 95% CIs).

The Cox PH model for the time to first lameness case did not show a treatment effect and was not improved by the variables BCS group, age, Previously lame or Lame on week 1 (Table 4-16).

Table 4-16 Cox PH model for time to first lameness event after week 6 of the trial in flock 2

Variable	No.	%	HR	Lower 95% CI	Upper 95% CI
Treatment					
Saline	17	22	Ref		
Footvax	21	24	1.16	0.61	2.19
Custom R-Pilus	18	23	1.01	0.52	1.95
Concordance = 0.52 (se = 0.037)					
No. represents the number of events in each category and % represents the events as percentage of all observations in the category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when the CI does not include unity and are indicated in bold.					

4.6.1.4 Flock 3

The survival probability functions for time to first lameness event in flock 3 did not indicate a treatment effect (Figure 4-35) but stratification of breed by treatment group showed a treatment effect in the Whitefaced crossbreed ewes (Figure 4-36). The two mule categories were combined because the lameness hazard was similar between the two groups and the Exmoor mule group was relatively small (n= 25). There was also a small difference in survival probability between categories for the age and Previously lame variables, but breed was the only variable that had a significant effect on time to first lameness event in the Cox PH model (Table 4-17).

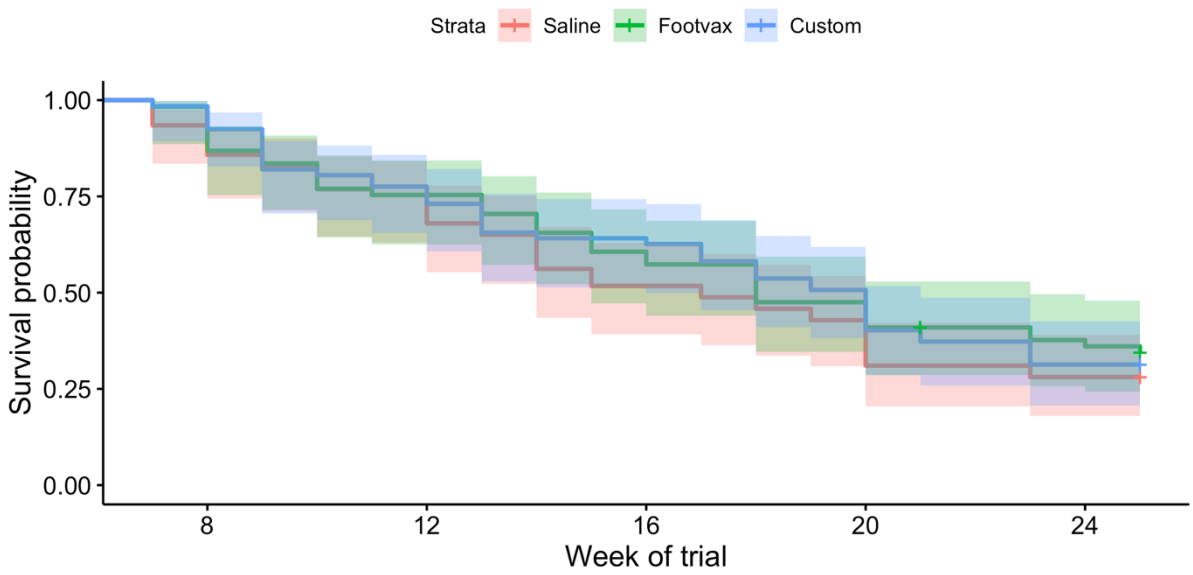

Figure 4-35 KM plot for time to first lameness event after week 6 of the trial by treatment group in flock 3 (shaded areas represent the 95% CIs)

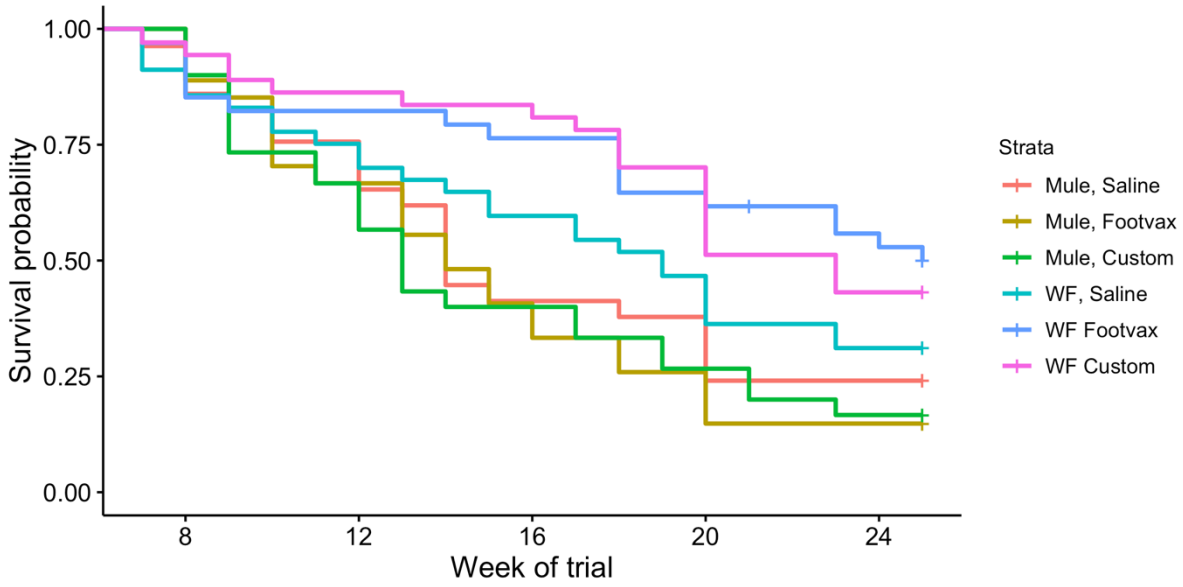

Figure 4-36 KM plot for time to first lameness event after week 6 of the trial in flock 3 stratified by breed and treatment group. Mule = North Country or Exmoor Mule, WF = Whitefaced.

Table 4-17 Cox PH model for time to first lameness event after week 6 of the trial in flock 3

Variable	No.	%	HR	Lower 95% CI	Upper 95% CI
Treatment					
Saline	48	72	Ref		
Footvax®	40	65	0.87	0.57	1.33
Custom R-Pilus	46	71	0.91	0.60	1.36
Breed					
Mule type	70	81	Ref		
Whitefaced cross	64	58	**0.49**	0.35	0.69
Concordance = 0.61 (se = 0.027)					

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when the CI does not include unity and are indicated in bold.

There were no significant interactions between the breed categories and treatment categories. Whitefaced ewes were at half the risk of experiencing at least one lameness

event than the mule type ewes. The score test for non-proportionality for both variables in the model was non-significant.

4.6.2 The impact of vaccination on the relative risk of all lameness events

4.6.2.1 All flocks

The hazard for all lameness cases was comparable between flocks 1 and 3, as for survival to the first case of lameness (Figure 4-37). However, it was necessary to stratify the model for all lameness cases by flock to deal with non-proportionality in the flock variable. Custom R-Pilus vaccinated ewes were at a significantly lower hazard of becoming lame than saline treated ewes when all events were included in the model, but risk of lameness was only reduced by 20%, which is of limited biological significance (Figure 4-38, Table 4-18). There was no significant difference in hazard of lameness between the Footvax and Custom R-Pilus vaccinated ewes (Table A- 26).

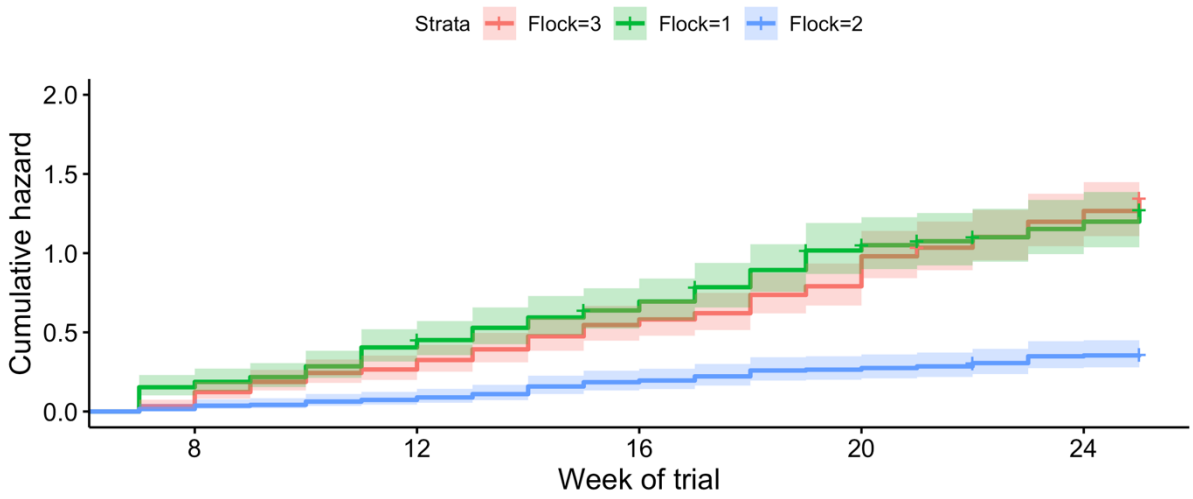

Figure 4-37 Cumulative hazard for all lameness events by flock after week 6 of the trial (shaded areas indicate 95% CIs)

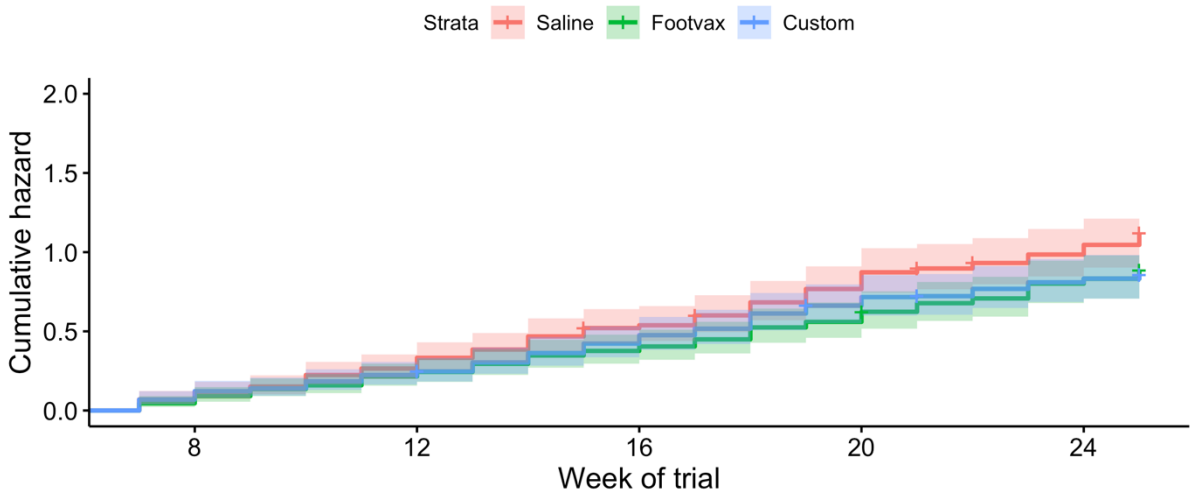

Figure 4-38 Cumulative hazard for all lameness events by treatment group after week 6 of the trial in all flocks (shaded areas indicate 95% CIs)

Table 4-18 Frailty model for all lameness events after week 6 of the trial in all flocks, stratified by flock

Variable	No.	%	HR	SE	p value
Treatment					
Saline	190	54	**Ref**		
Footvax®	146	48	0.80	0.11	0.05
Custom R-Pilus	153	46	**0.79**	**0.11**	**0.04**
Previously lame					
No	214	45	Ref		
Yes	275	53	**1.42**	**0.10**	**<0.01**
Random effect					
Flock	Variance < 0.01				
Ewe	Variance = 0.09				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p < 0.05$ and are indicated in bold.

4.6.2.2 Flock 1

In flock 1, the KM plot of all lameness cases over all weeks of the trial showed a decrease in cumulative hazard rate for the Custom R-Pilus group mid-trial (Figure 4-39). The difference between the vaccinated and saline treatment groups was smaller when all lameness cases were included in the dataset than for the first case of lameness only (Figure 4-40). There was a difference in the hazard of lameness with ewe origin, but a treatment effect was only observed in the Custom vaccinated Existing ewes (Figure 4-41).

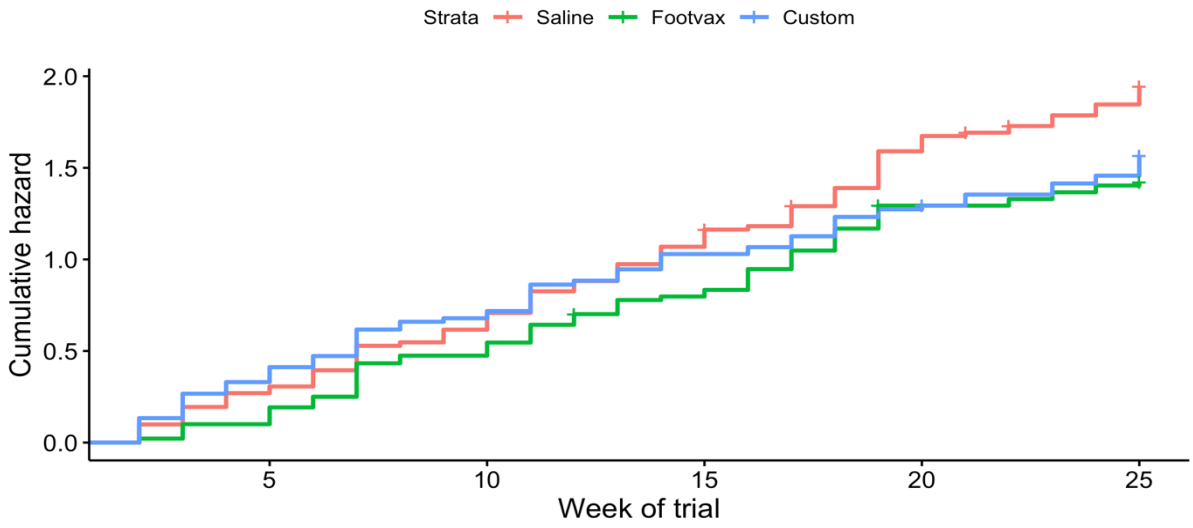

Figure 4-39 Cumulative hazard for all lameness events by treatment group over all weeks of the trial in flock 1

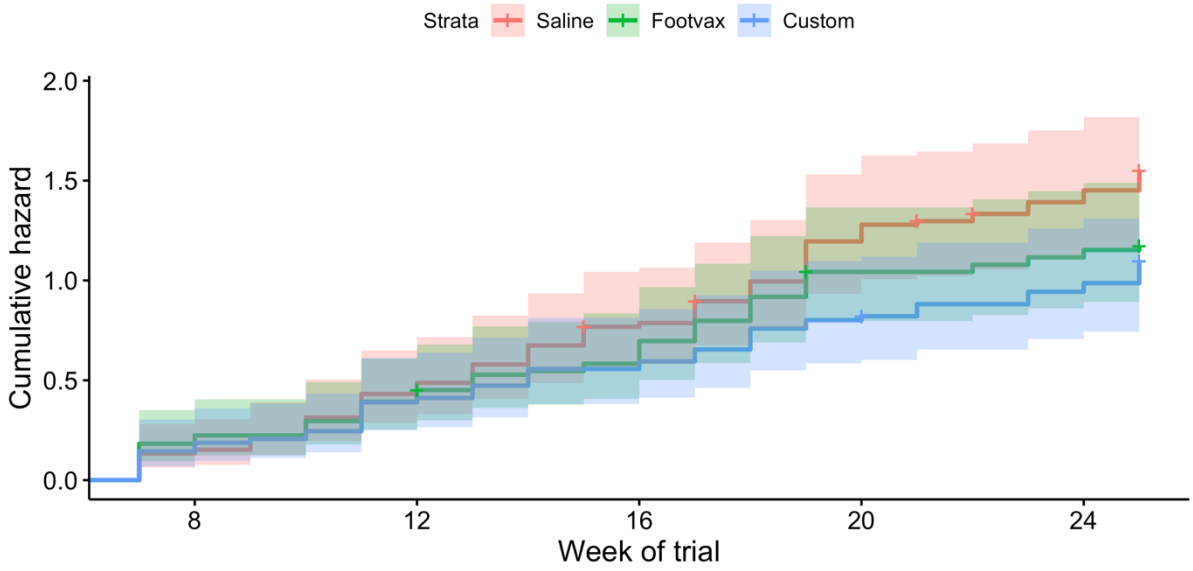

Figure 4-40 Cumulative hazard for all lameness events after week 6 of the trial by treatment group in flock 1 (shaded area represents the 95% CIs)

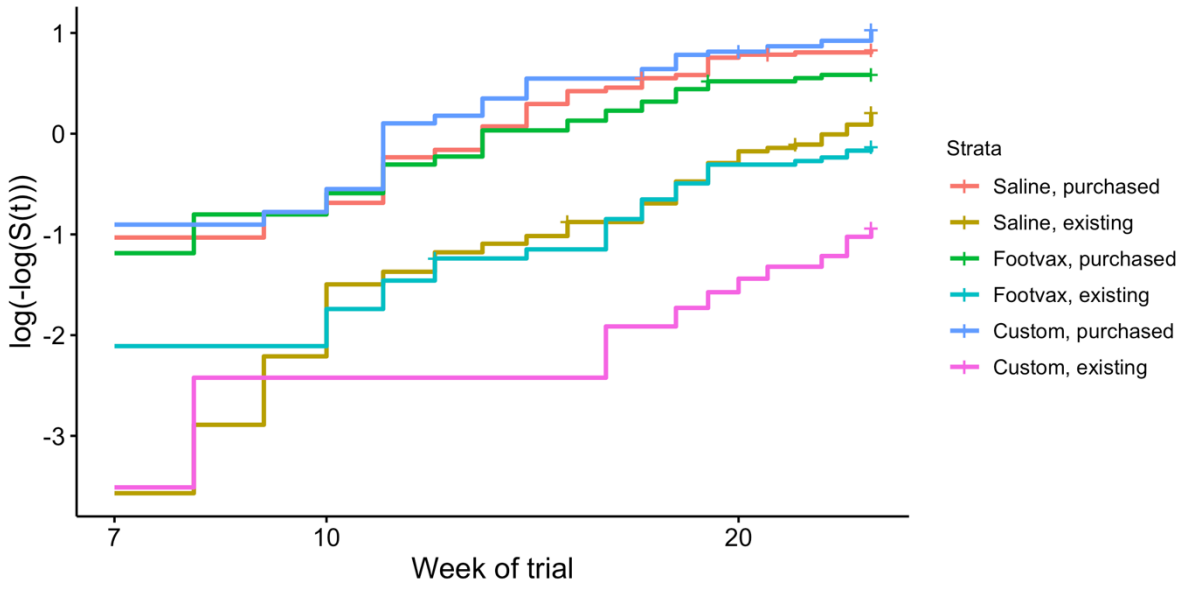

Figure 4-41 Complementary log-log of the survival probability for all lameness events after week 6 of the trial with stratification by treatment and origin in flock 1

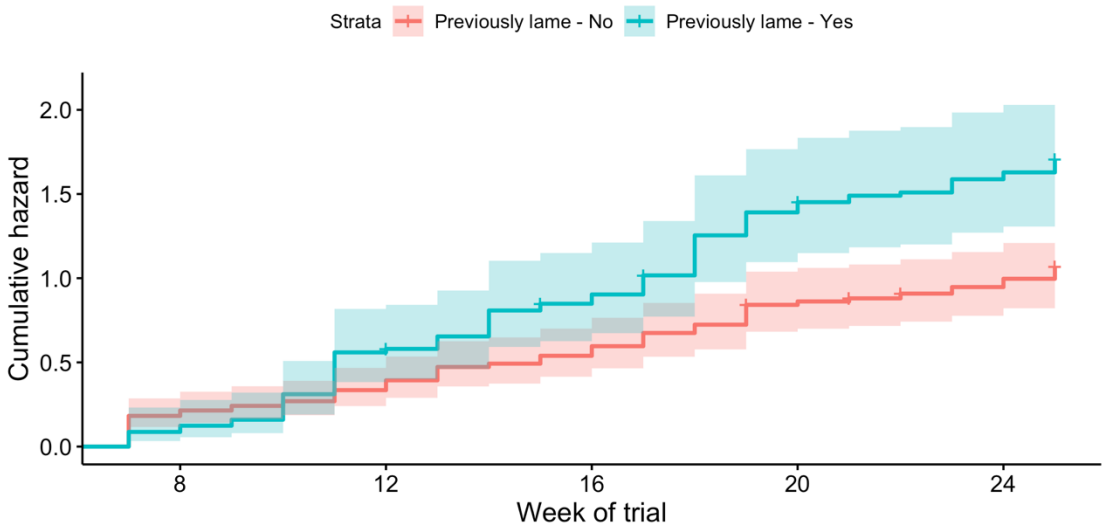

Figure 4-42 Cumulative hazard for all lameness events after week 6 of the trial in flock 1 for the Previously lame variable (shaded areas represent the 95% CIs)

Plots of the Lame 1st and BCS group at first treatment variables did not show any difference in hazard ratio between categories. There was a difference in hazard ratio for the Previously lame categories (Figure 4-42) and being previously lame was a significant predictor of becoming lame again in the final model (Table 4-19). Existing ewes that had received Custom R-Pilus had a 68% lower risk of having a lameness event than the saline treated ewes, whereas there was no significant protective effect of Footvax® compared to saline in these ewes. Neither vaccine was significantly better at preventing lameness events compared to saline in the Purchased ewes.

Table 4-19 Frailty model for all lameness events after week 6 of the trial in flock 1

Variable	No.	%	Coef.	HR	SE	p value
Treatment						
Saline	81	53	Ref			
Footvax®	60	52	-0.15	0.86	0.29	0.61
Custom R-Pilus	53	55	0.16	1.17	0.27	0.57
Origin						
Purchased	110	67	Ref			
Existing	84	47	-0.61	**0.55**	**0.26**	**0.02**
*Treatment*Origin*						
Saline Existing	41	58	Ref			
Footvax® Existing	30	46	-0.17	0.84	0.39	0.66
Custom Existing	13	30	-1.30	**0.27**	**0.44**	**<0.01**
Previously lame						
No	110	53	Ref			
Yes	84	61	0.48	**1.61**	**0.17**	**<0.01**
Random effect						
Ewe	Variance = 0.25					

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p \leq 0.05$ and are indicated in bold.

The relationship between the scaled Schoenfeld residuals and time was close to monotone for the treatment origin interaction variable, and for the treatment variable until week 20, but the PH assumption was not met for the origin variable and the score test for the global model was significant. The results of separate models for the two origin categories were consistent with the results of the flock model (Appendix 16). The separate models did not violate the proportional hazards assumption.

4.6.2.3 Flock 2

Cumulative hazard plots by treatment group over all weeks of the trial, treatment group after week 6 (Figure 4-43), age, BCS group and Lame on week 1 showed no differences in hazard between categories.

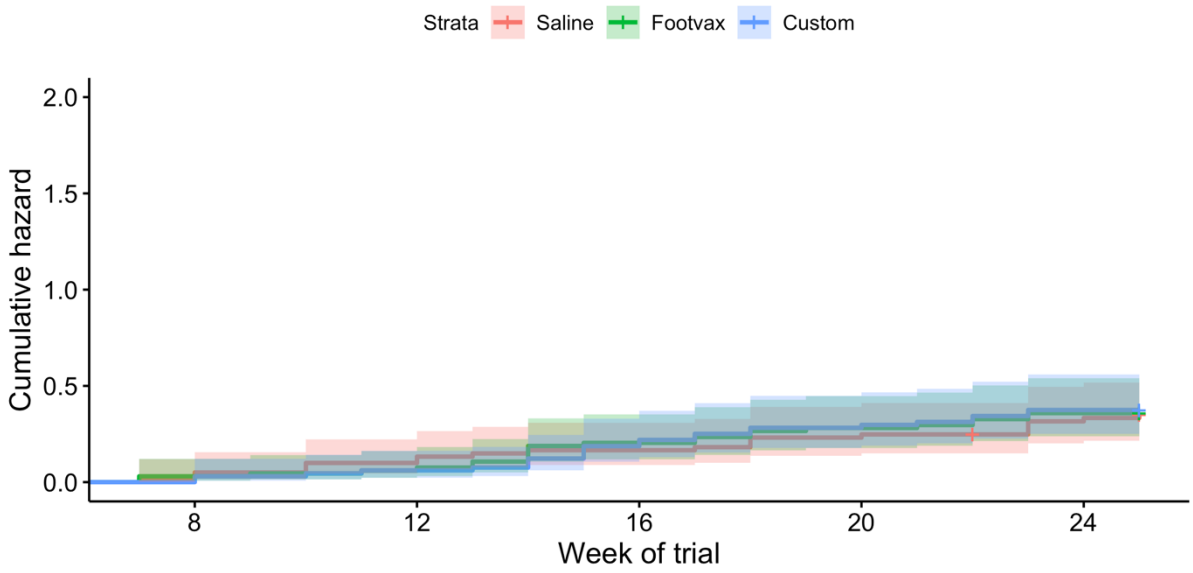

Figure 4-43 Cumulative hazard for all lameness events after week 6 of the trial by treatment group in flock 2 (shaded areas represent the 95% CIs)

A small and proportional difference between categories for the Previously lame variable was observed, but no variables were significant in the final model (Table A- 31).

4.6.2.4 Flock 3

The Custom R-Pilus treatment group did not have a constant cumulative hazard rate in flock 3 (Figure 4-44). No treatment effect was observed for all lameness events after week 6 (Figure 4-45) but the cumulative hazard differed between the Mule types and the vaccinated Whitefaced crossbreeds (Figure 4-46).

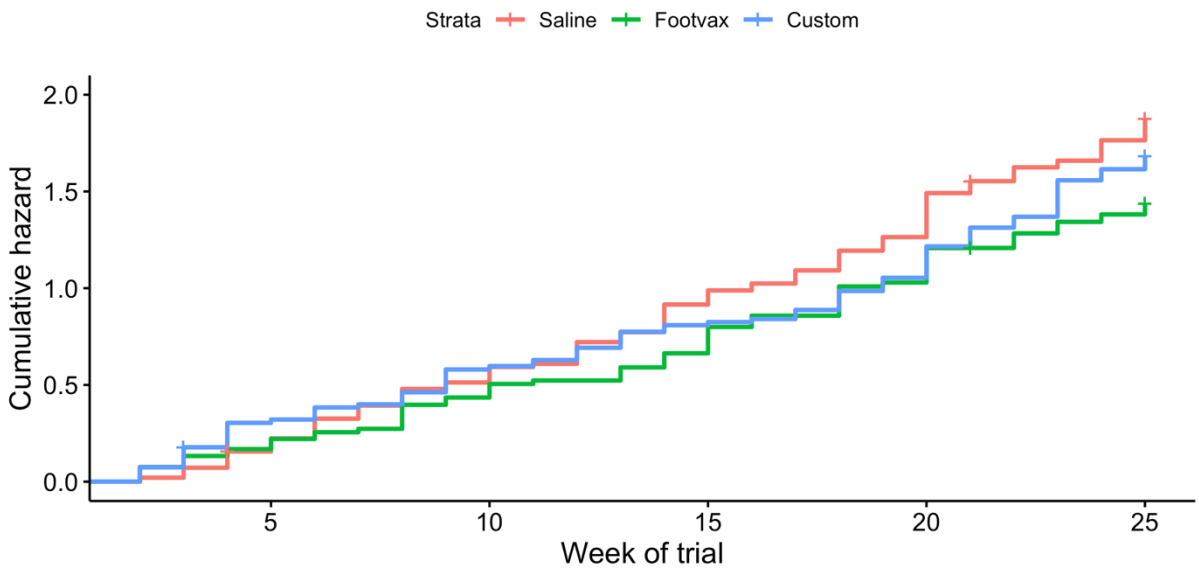

Figure 4-44 Cumulative hazard for all lameness events by treatment group over all weeks of the trial in flock 3

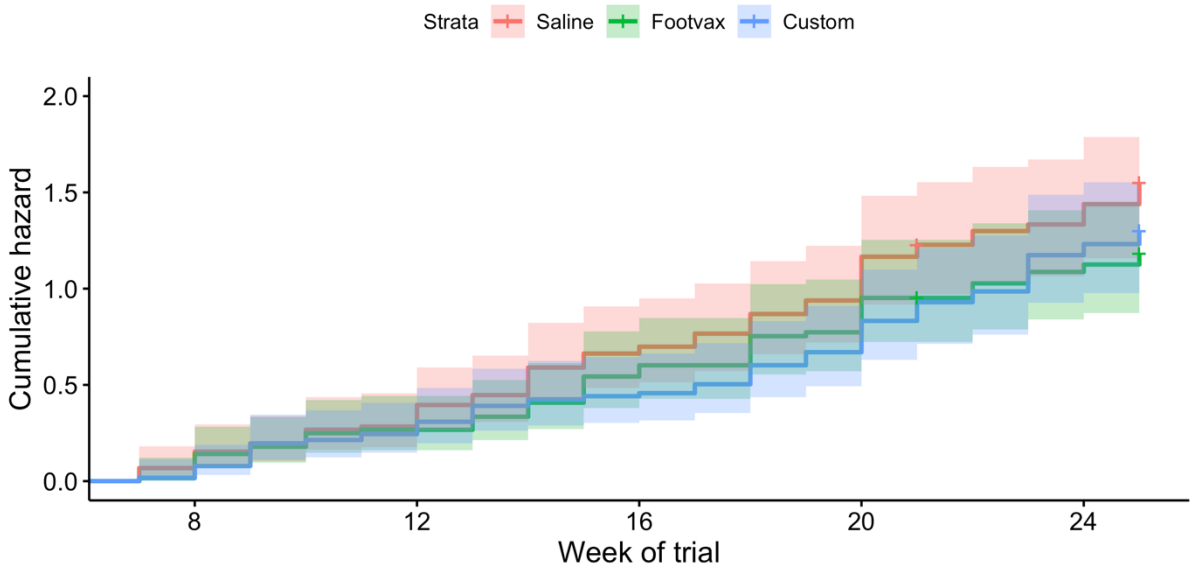

Figure 4-45 Cumulative hazard for all lameness events after week 6 of the trial by treatment group in flock 3 (shaded areas represent the 95% CIs)

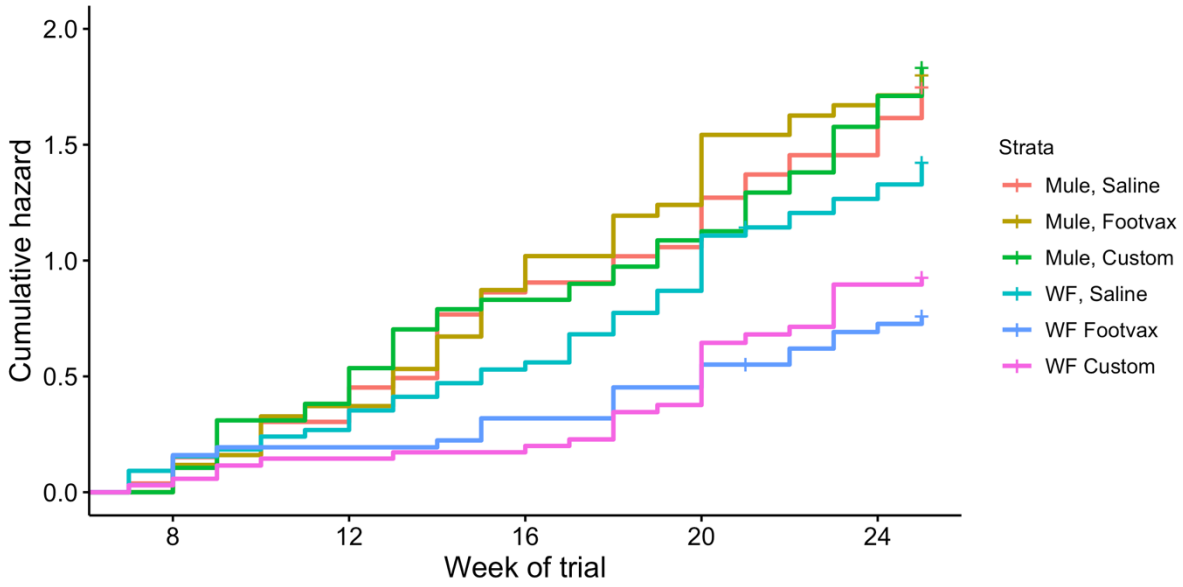

Figure 4-46 Cumulative hazard for all lameness events after week 6 of the trial stratified by breed and treatment group in flock 3

Breed was the only variable with a significant effect on the hazard of lameness in the final frailty model and there was no interaction between breed and treatment group (Table 4-20). The score test for both variables was non-significant.

Table 4-20 Frailty model for all lameness events after week 6 of the trial in flock 3

Variable	No.	%	HR	SE
Treatment				
Saline	88	63	Ref	
Footvax®	63	55	0.75	0.18
Custom R-Pilus	76	56	0.82	0.17
Breed				
Mule type	128	66	Ref	
Whitefaced cross	99	51	**0.58**	**0.15**
Random effect				
Ewe	Variance = 0.15			

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p \leq 0.05$ and are indicated in bold.

4.6.3 The impact of vaccination on time to first footrot event

4.6.3.1 All flocks

Survival probability for time to first footrot event differed between the three flocks (Figure 4-47) in contrast to that for first lameness event, for which flocks 1 and 3 had equivalent hazard ratios with flock 2 (Figure 4-28). Both vaccines were statistically and biologically protective against ewes ever getting footrot compared to saline (Table 4-21), but there was no difference in effect between the two vaccines (Appendix 18). Schoenfeld residuals for treatment coefficients tended to increase with time but score tests for the variables and global model were all non-significant.

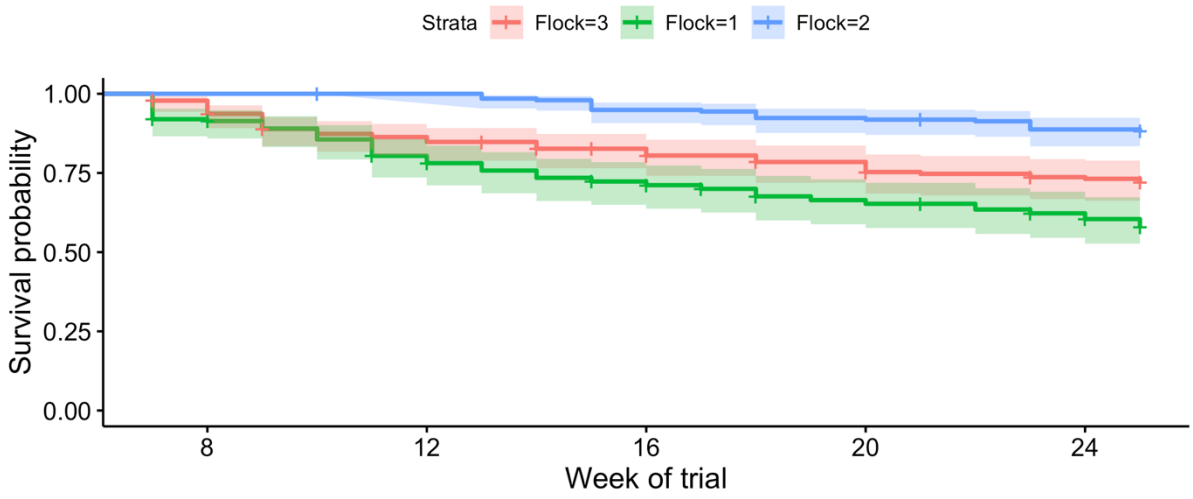

Figure 4-47 KM plot for time to first footrot event after week 6 of the trial by flock (shaded areas represent the 95% CIs)

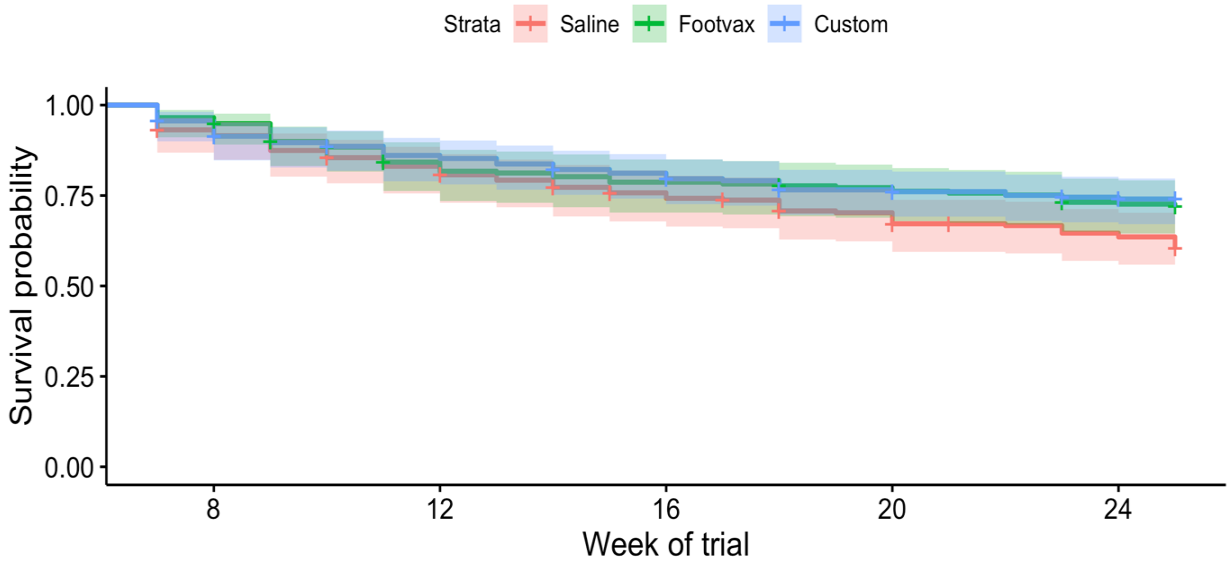

Figure 4-48 KM plot for time to first footrot event after week 6 of the trial by treatment group (shaded areas represent the 95% CIs)

Table 4-21 Frailty model for time to first footrot event after week 6 of the trial in all flocks

Variable	No.	%	HR	SE	p value
Treatment					
Saline	65	23			
Footvax®	40	15	**0.57**	**0.20**	**<0.01**
Custom R-Pilus	42	14	**0.59**	**0.20**	**0.01**
Flock					
3	53	21			
1	71	37	**1.84**	**0.19**	**<0.01**
2	23	6	0.70	0.26	0.17
Previous FR					
No	91	18			
Yes	56	27	**1.76**	**0.17**	**<0.01**
Random effect					
Flock	Variance = 1.62×10^{-5}				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p \leq 0.05$ and are indicated in bold.

4.6.3.2 Flock 1

The KM plot for all trial weeks showed the same change in the survival rate for time to first footrot event in the Custom R-Pilus vaccinated group at week 7 as had been observed for time to first lameness event (Figure 4-49). From week 6 onwards, the survival functions for time to first footrot event indicated that both vaccines were equally protective compared to saline (Figure 4-50). However, as for survival to first lameness event, the effect of Custom R-Pilus and Footvax® varied with ewe origin category, and there was evidence of non-proportionality between some of the strata (Figure 4-51).

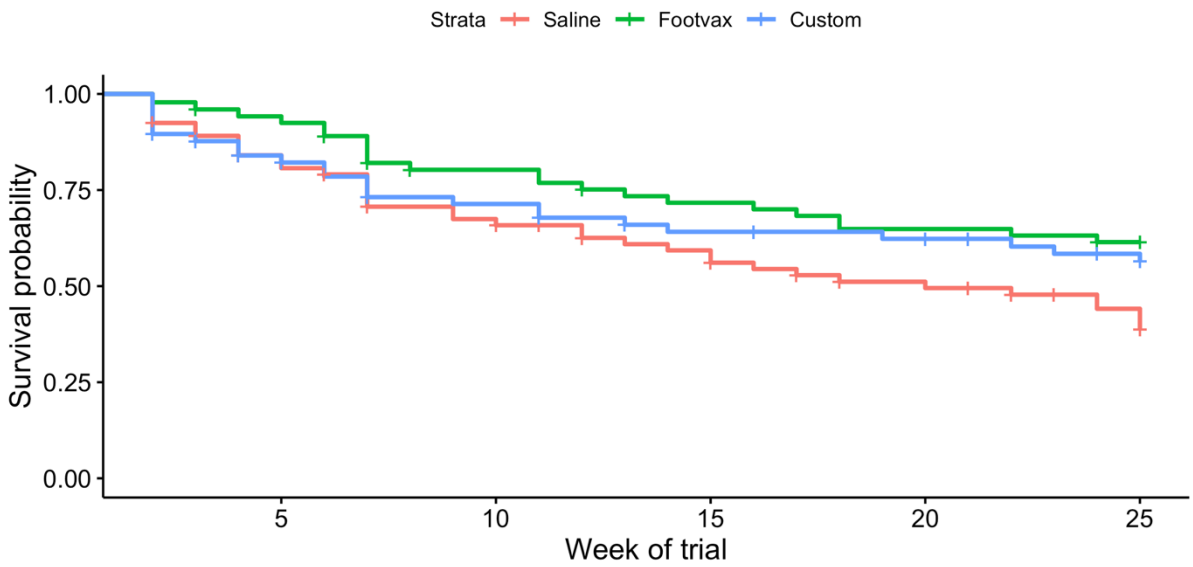

Figure 4-49 Survival probability for first footrot event by treatment group over all weeks of the trial in flock 1

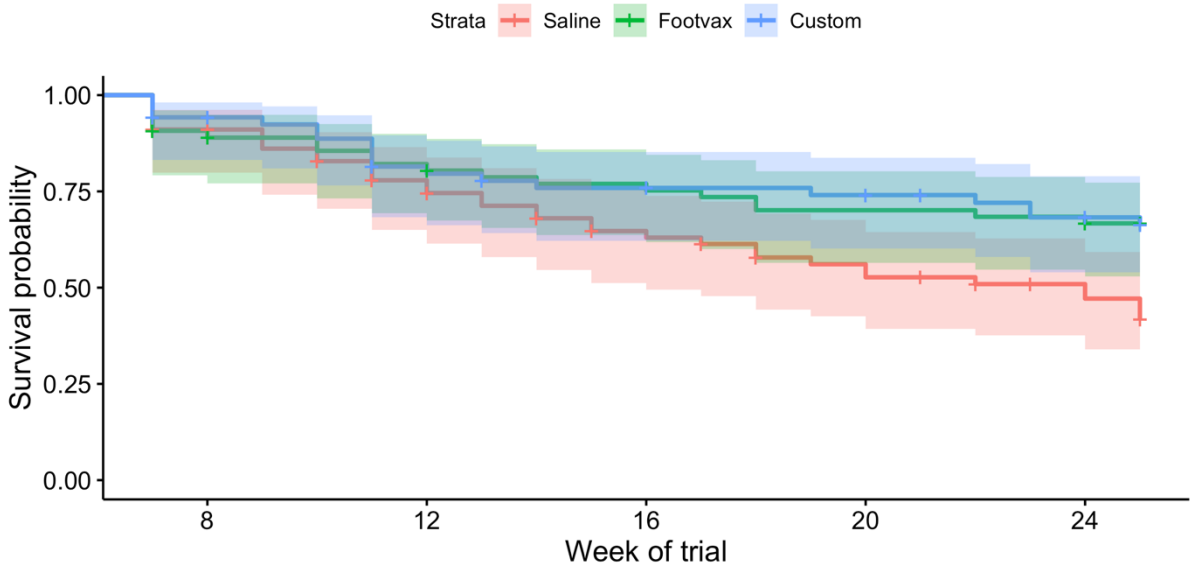

Figure 4-50 Survival probability for first footrot event after week 6 of the trial by treatment group in flock 1 (shaded area represents the 95% CIs)

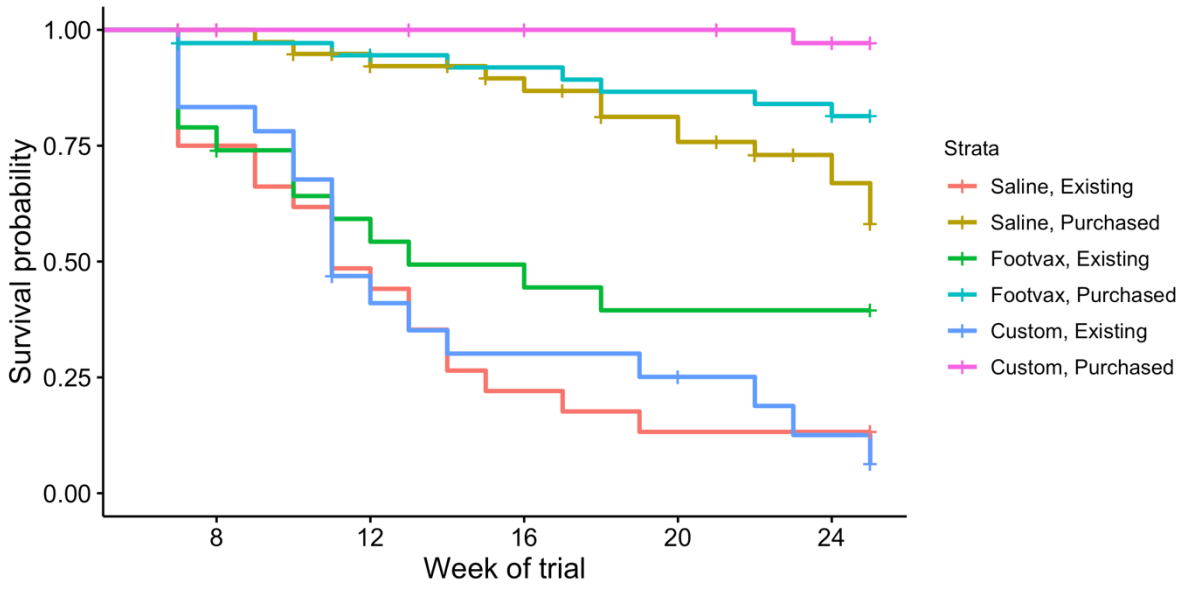

Figure 4-51 Survival probability for first footrot event after week 6 of the trial by treatment group and origin in flock 1

KM plots of the BCS at first vaccination, FR on week 1 and Previous FR variables showed differences in survival between categories, but only FR on week 1 was significant in the final model (Table 4-22). Neither vaccine resulted in a significant reduction in the proportion of Purchased ewes that experienced at least one footrot event compared to saline. Existing ewes in the Custom vaccine group had a 94% lower risk of developing footrot compared to saline treated ewes.

Table 4-22 Cox PH model for time to first footrot event after week 6 of the trial in flock 1

Variable	No.	%	Coef.	HR	Lower 95% CI	Upper 95% CI
Treatment						
Saline	34	49	Ref			
Footvax®	19	30	-0.68	0.51	0.24	1.08
Custom R-Pilus	18	30	-0.08	0.92	0.47	1.81
Origin						
Purchased	48	75	Ref			
Existing	23	18	-1.75	**0.17**	**0.09**	**0.35**
*Treatment*Origin*						
Saline Existing	15	31	Ref			
Footvax® Existing	7	17	-0.16	0.85	0.27	2.73
Custom Existing	1	2	-2.66	**0.07**	**<0.01**	**0.59**
FR on week 1						
No	58	35	Ref			
Yes	13	45	0.90	**2.46**	**1.26**	**4.82**
Lame on week 1						
No	62	36	Ref			
Yes	9	38	0.19	1.21	0.55	2.65
Previous FR event						
No	46	31	Ref			
Yes	25	53	0.30	1.35	0.79	2.30
Concordance = 0.83 (se = 0.019)						

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when the CI does not include unity and are indicated in bold.

The treatment group and origin variables both showed a relationship with week of trial and the global score test was p=0.05, indicating that the PH assumption was not met. The PH assumption was met in separate models for the two origin categories. There was no treatment effect in Purchased ewes, consistent with the flock model (Table A- 34). Existing ewes in both vaccination groups were at a lower risk of footrot compared to saline treated ewes, and ewes that were lame on week 1 were at a higher risk of subsequent footrot than ewes that were not lame on week 1 (Table A- 35).

4.6.3.3 Flock 2

Survival functions for the treatment groups over all weeks and for all variables in the truncated dataset were comparable. There were only two footrot events before week 9 and the dataset was left truncated to start at week 9 due to the censored period from week 6-9 when no lame ewes were examined. The Cox PH model for the time to first footrot event did not show a treatment effect and was not improved by any of the other variables (Table 4-23).

Table 4-23 Cox PH model for time to first footrot event after week 6 of the trial in flock 2

Variable	No.	%	HR	Lower 95% CI	Upper 95% CI
Treatment					
Saline	10	9	Ref		
Footvax®	6	4	0.57	0.21	1.56
Custom R-Pilus	7	5	0.66	0.25	1.74
Concordance = 0.56 (se = 0.058)					

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when the CI does not include unity and are indicated in bold.

4.6.3.4 Flock 3

Over all weeks of the trial, survival probability was higher in the Footvax® treated group and survival rate for both vaccine groups increased after week 9 compared to saline (Figure 4-52). There was no difference in treatment effect after week 6 (Figure 4-53) but in contrast to the survival probability for lameness events, Exmoor mules were at a significantly lower risk of footrot than the North Country mules (Figure 4-54, Table 4-24).

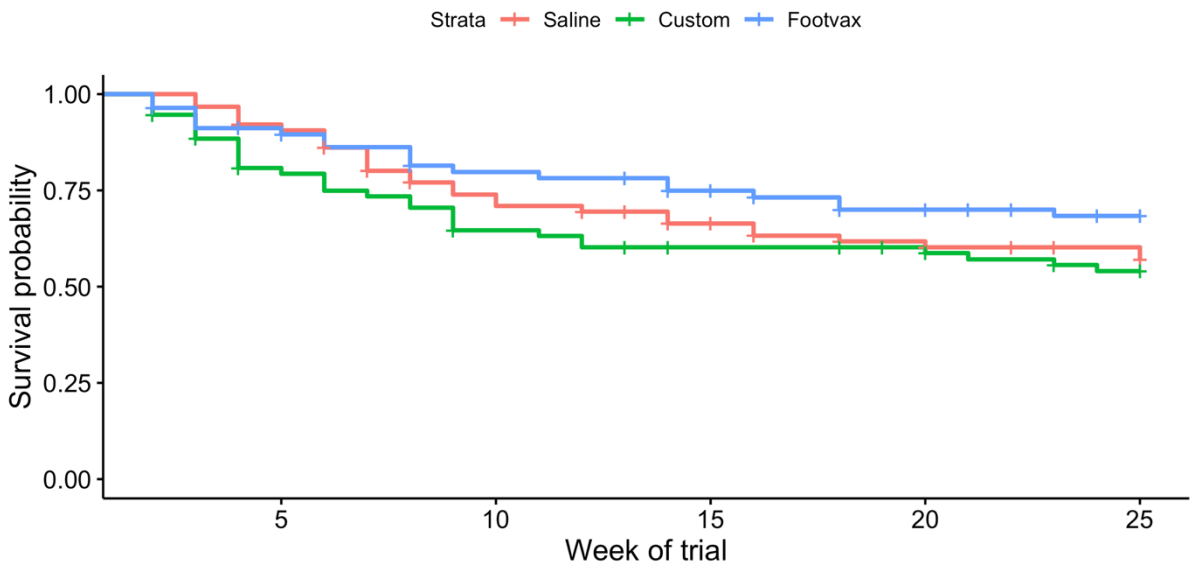

Figure 4-52 KM plot for time to first footrot event by treatment group over all weeks of the trial in flock 3

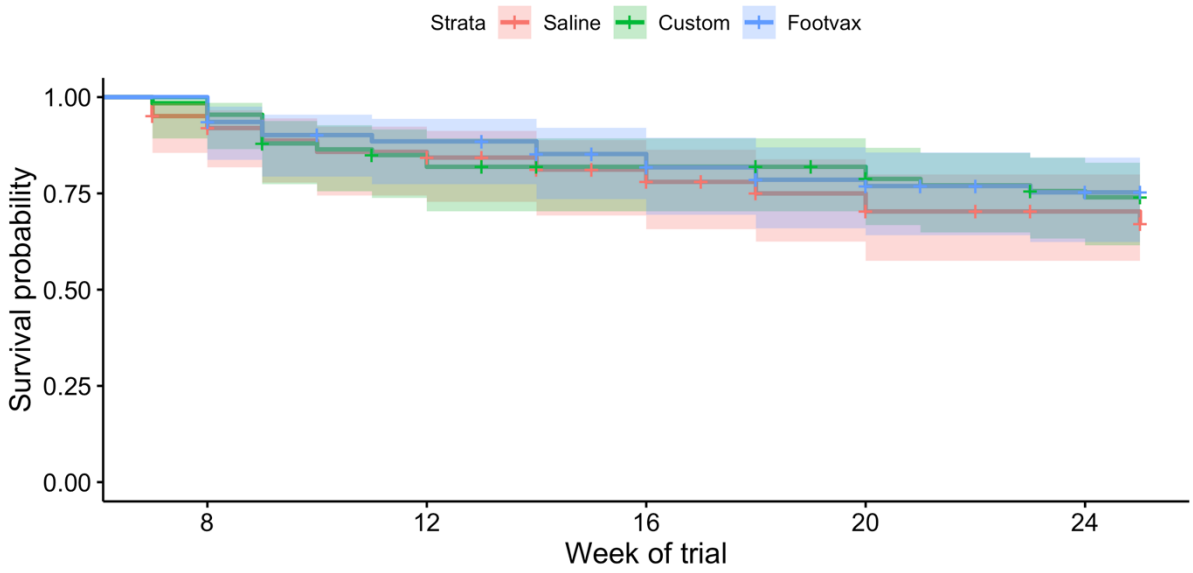

Figure 4-53 Survival probability for first footrot event after week 6 of the trial by treatment group in flock 3 (shaded areas represent the 95% CIs)

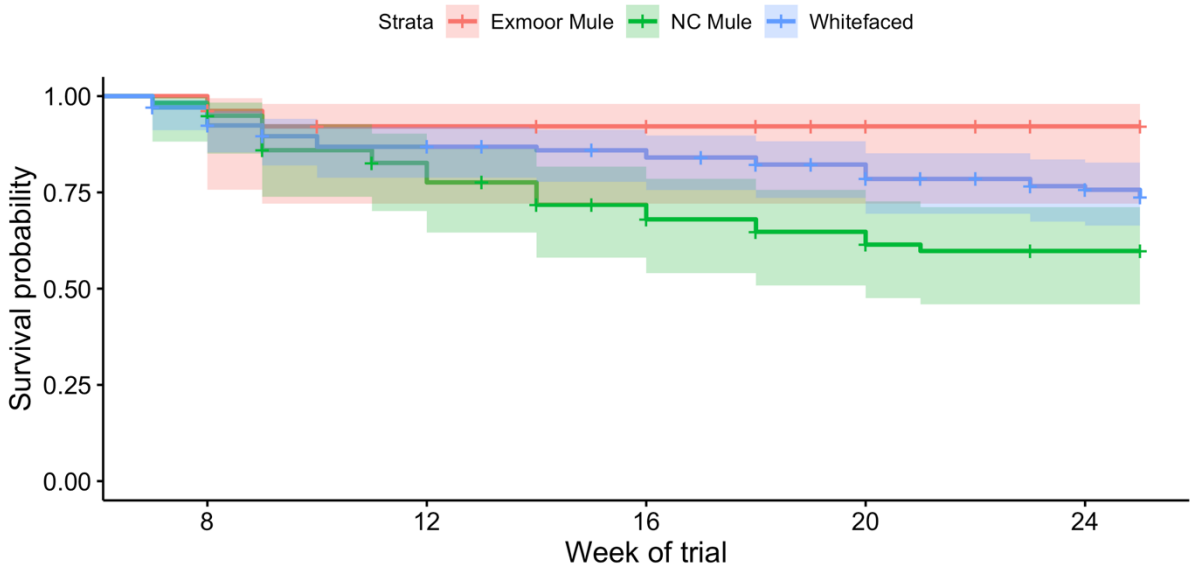

Figure 4-54 KM plot for time to first footrot event after week 6 of the trial by breed category in flock 3 (shaded areas represent the 95% CIs)

Table 4-24 Cox PH model for time to first footrot event after week 6 of the trial in flock 3

Variable	No.	%	HR	Lower 95% CI	Upper 95% CI
Treatment					
Saline	21	40	Ref		
Footvax®	15	28	0.72	0.37	1.40
Custom R-Pilus	17	32	0.75	0.40	1.42
Breed					
NC mule	23	43	Ref		
Exmoor mule	2	4	**0.17**	0.04	0.71
Whitefaced cross	28	53	0.60	0.34	1.04
Concordance = 0.62 (se = 0.037)					

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when the CI does not include unity and are indicated in bold.

4.6.4 The impact of vaccination on the relative risk of all footrot events

4.6.4.1 All flocks

Cumulative hazard plots for all footrot events by flock and treatment group are presented in Figure 4-55 and Figure 4-56 respectively. Flock and Previous footrot event were the most influential variables for a ewe to experience a footrot event in the combined dataset. Both vaccine groups had around a 30% lower risk of having a footrot event compared to saline treated ewes (Table 4-25). All variables were non-significant on the score test for proportionality.

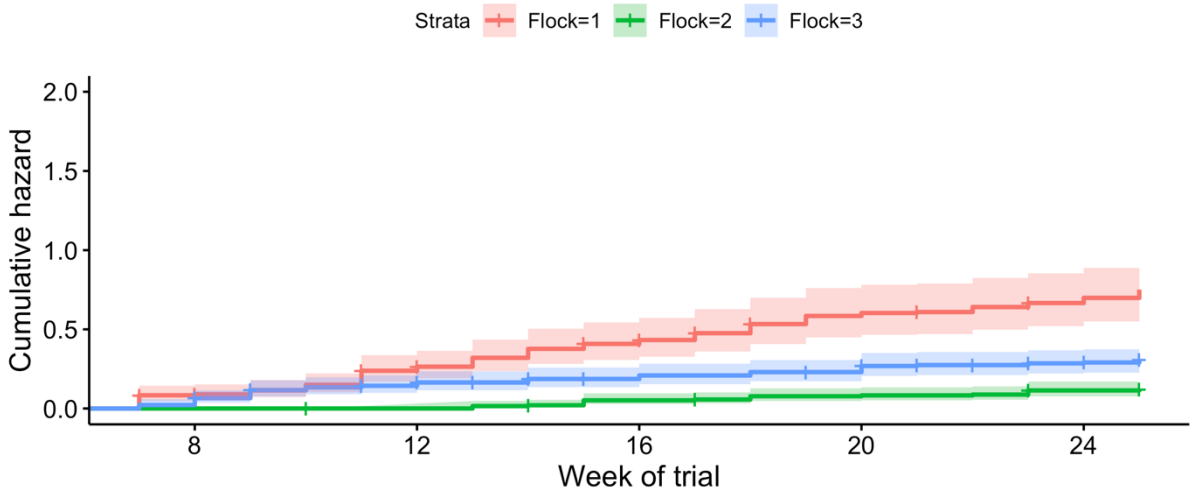

Figure 4-55 Cumulative hazard for all footrot events by flock after week 6 of the trial (shaded areas indicate 95% CIs)

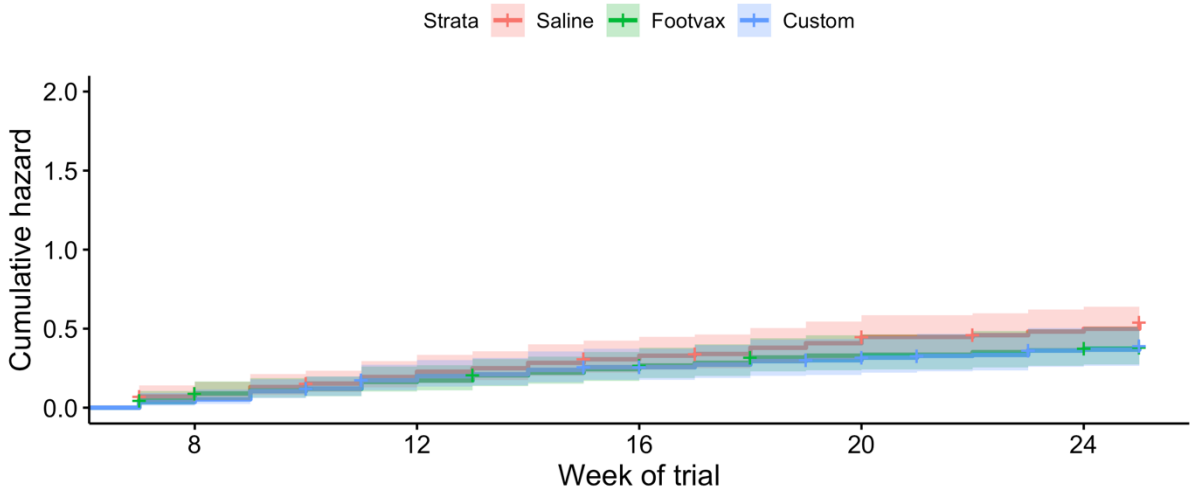

Figure 4-56 Cumulative hazard for all footrot events by treatment group after week 6 of the trial in all flocks (shaded areas indicate 95% CIs)

Table 4-25 Frailty model for all footrot events after week 6 of the trial in all flocks

Variable	No.	%	HR	SE	p value
Treatment					
Saline	83	23	Ref		
Footvax®	57	17	**0.69**	**0.18**	**0.04**
Custom R-Pilus	58	17	**0.70**	**0.18**	**0.04**
Flock					
3	56	18	Ref		
1	119	39	**2.81**	**0.17**	**<0.01**
2	23	5	0.68	0.26	0.14
Previous footrot					
No	117	17	Ref		
Yes	81	24	**1.92**	**0.16**	**<0.01**
Random effect					
Flock	Variance < 0.01				
Ewe	Variance = 0.14				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p < 0.05$ and are indicated in bold.

4.6.4.2 Flock 1

The same change in cumulative hazard rate was seen in the Custom R-Pilus treatment group after week 11 as was seen for all lameness events, with the hazard of footrot initially equivalent to saline treated ewes but approaching that of Footvax® treated ewes by the end of the trial (Figure 4-57). The CH plot by treatment groups in the truncated dataset did not show a difference between the two vaccine groups (Figure 4-58), but a difference in hazard between ewe origin categories was evident (Figure 4-59, Table 4-26), as it had been for lameness events and time to first footrot event. Only one footrot event occurred in the Custom-R-Pilus, Existing ewe group, which resulted in a hazard ratio of 0.06 compared to saline treated Existing ewes. No other treatment effects were observed. Non-proportionality was present between the origin categories and this resulted in a significant score test for the

model. The results from separate models for the two origin categories were consistent with the full model and had non-significant score tests for non-proportionality (Table A- 36, Table A- 37).

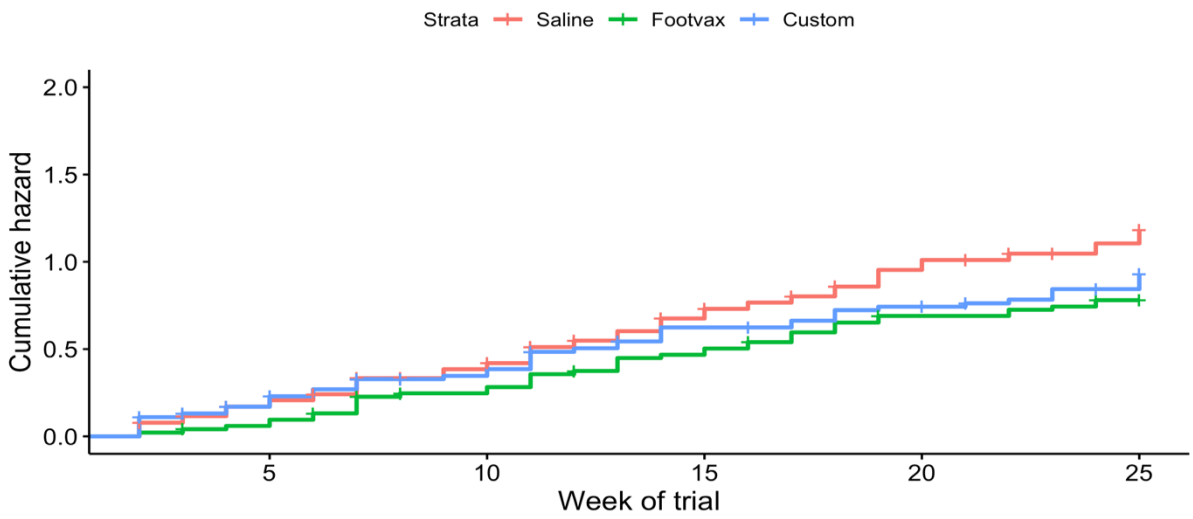

Figure 4-57 Cumulative hazard for all footrot events by treatment group over all weeks of the trial in flock 1

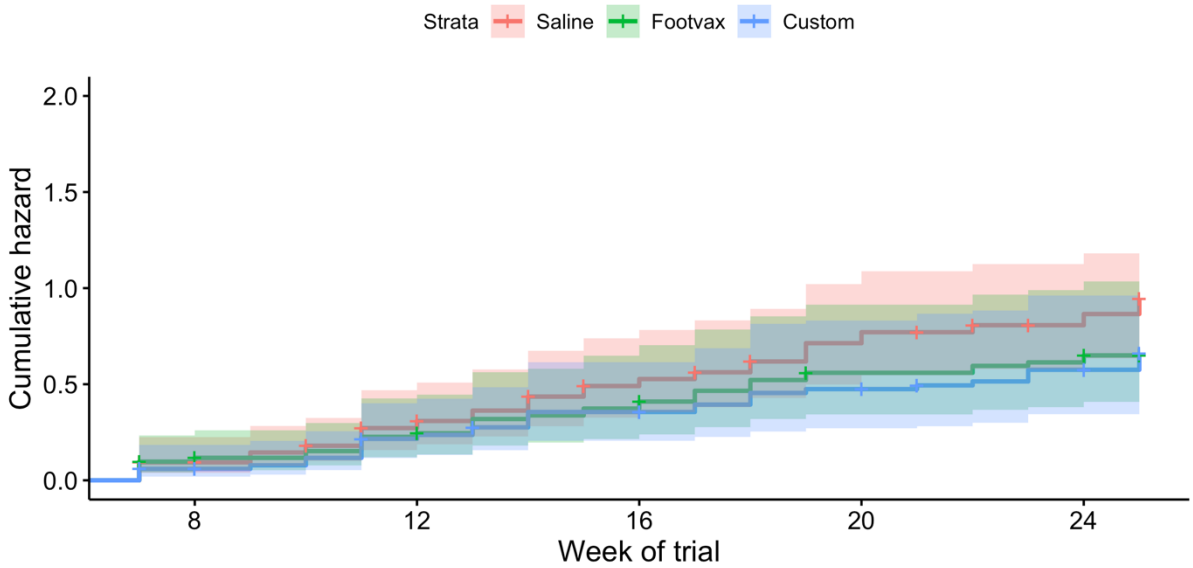

Figure 4-58 Cumulative hazard for all footrot events by treatment group after week 6 of the trial in flock 1 (shaded area represents the 95% CIs)

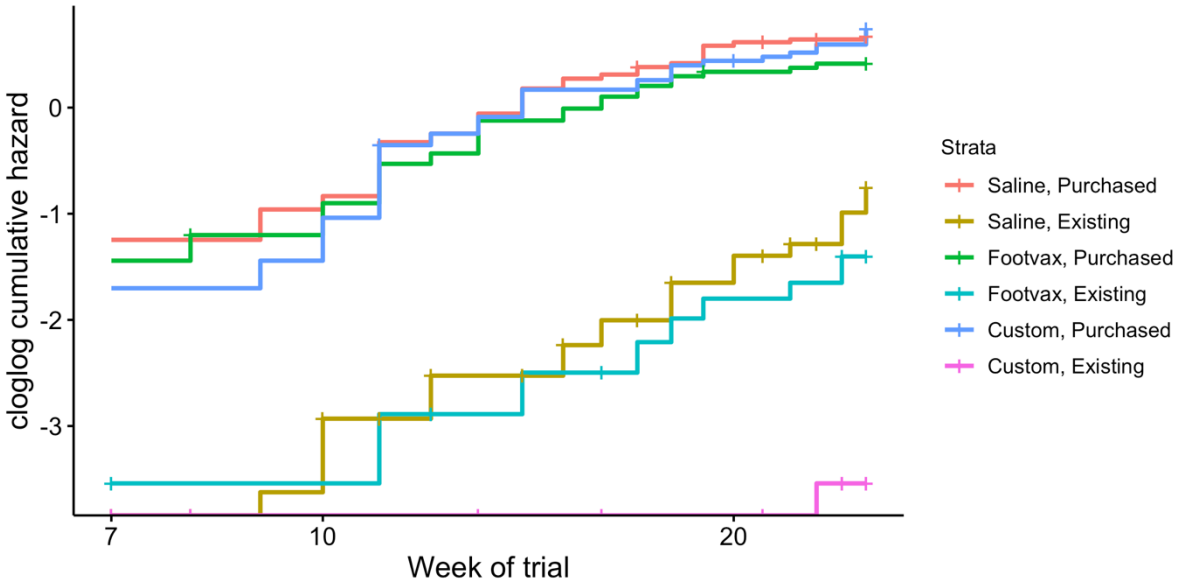

Figure 4-59 Complementary log-log of the survival probability for all footrot events after week 6 of the trial with stratification by treatment and origin in flock 1

Table 4-26 Frailty model for all footrot events after week 6 of the trial in flock 1

Variable	No.	%	Coef.	HR	SE	p value
Treatment						
Saline	51	44	Ref			
Footvax®	35	36	-0.12	0.89	0.28	0.67
Custom R-Pilus	33	37	0.06	1.07	0.26	0.83
Origin						
Purchased	93	61	Ref			
Existing	26	17	-1.43	**0.24**	**0.32**	**<0.01**
*Treatment*Origin*						
Saline Existing	16	28	Ref			
Footvax®Existing	9	18	-0.49	0.61	0.51	0.33
Custom Existing	1	98	-2.74	**0.06**	**1.07**	**0.01**
FR on week 1						
No	102	40	Ref			
Yes	17	40	0.42	1.52	0.31	0.18
Lame on week 1						
No	106	40	Ref			
Yes	36	13	0.07	1.07	0.36	0.86
Previous FR event						
No	70	34	Ref			
Yes	49	52	0.54	**1.71**	**0.22**	**0.01**
Random effect						
Ewe	Variance = 0.09					

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p \leq 0.05$ and are indicated in bold

4.6.4.3 Flock 2

The cumulative hazard plot for all footrot events by treatment group after week 9 is presented in Figure 4-60. There were no repeated footrot events in the truncated dataset, therefore the distribution of footrot events was the same as for the dataset for time to first footrot event with no significant differences between treatment groups. Accordingly, the model was not improved by the inclusion of ewe as a frailty term or any other variables.

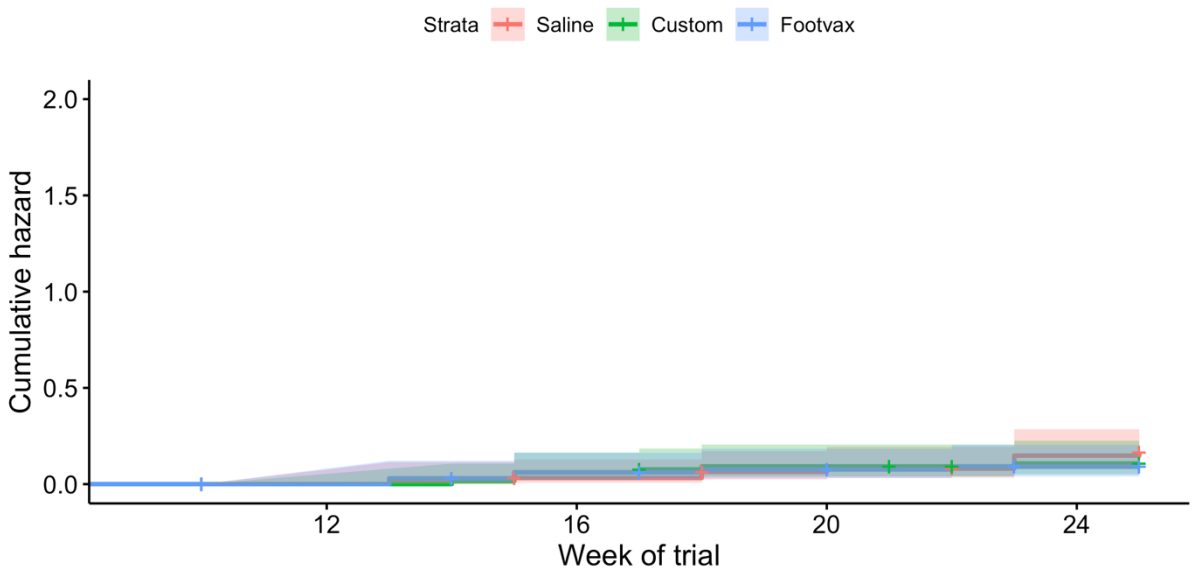

Figure 4-60 Cumulative hazard for all footrot events by treatment group after week 6 of the trial in flock 2 (shaded area represents the 95% CIs)

4.6.4.4 Flock 3

For all footrot cases over all weeks of the trial, cumulative hazard reduced after week 9 in the two vaccine groups, as had been observed in survival rate for time to first footrot event (Figure 4-61). After week 6, there was no difference between the treatment groups (Figure 4-62). The CH plot of footrot cases by breed showed a difference in hazard between breeds and this was seen in the final model results (Table 4-27). There was no interaction between breed and treatment group and the variables in the model met the proportional hazards assumption.

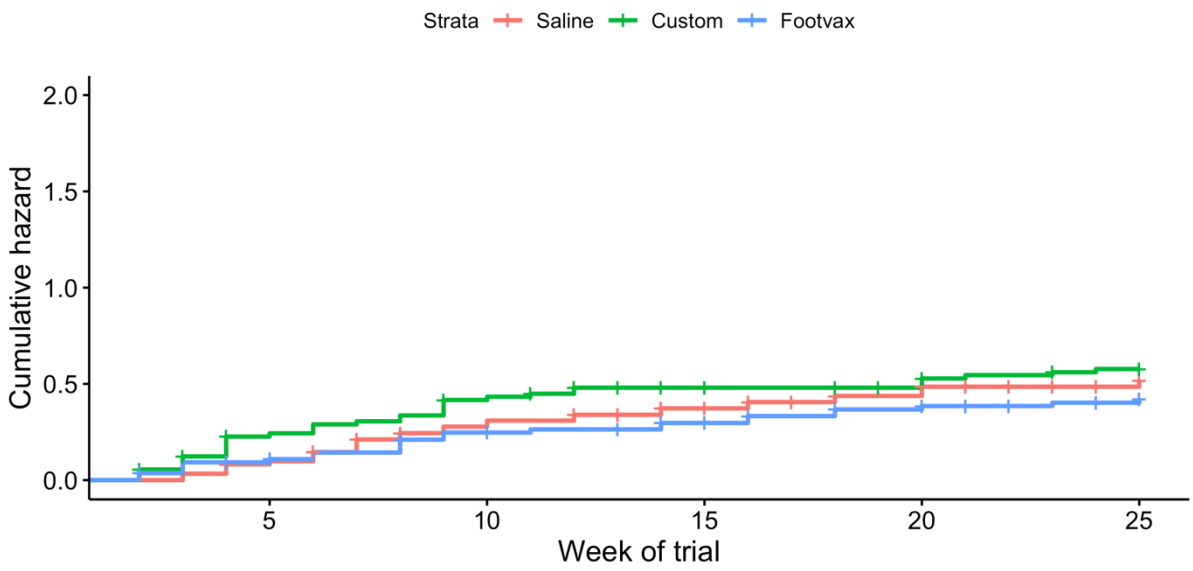

Figure 4-61 Cumulative hazard for all footrot events by treatment group over all weeks of the trial in flock 3

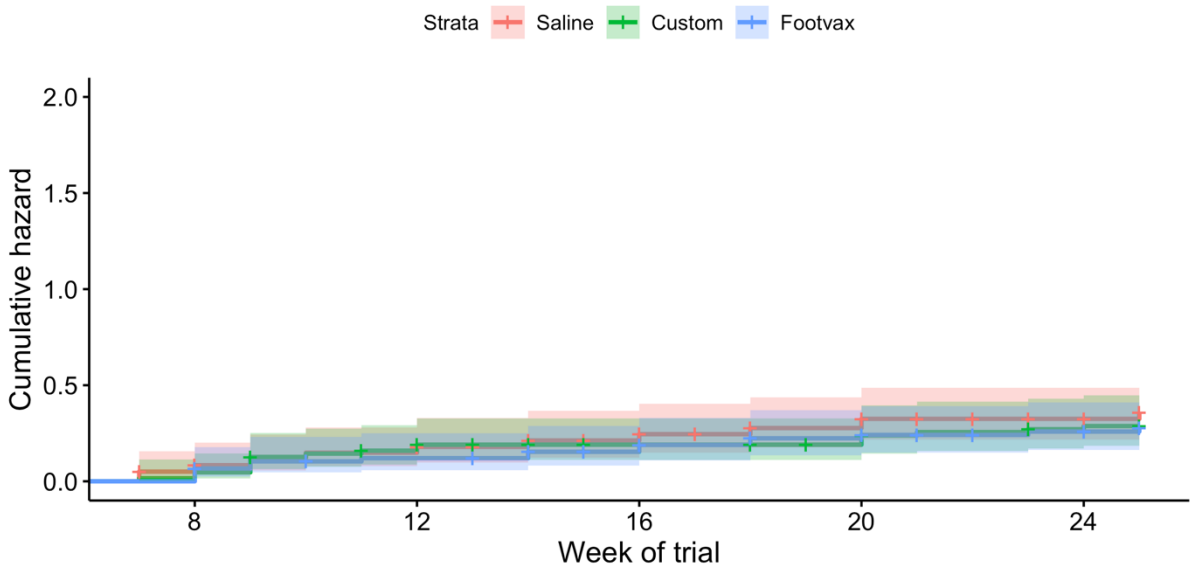

Figure 4-62 Cumulative hazard for all footrot events by treatment group after week 6 of the trial in flock 3 (shaded area represents the 95% CIs)

Table 4-27 Frailty model for all footrot events after week 6 of the trial in flock 3

Variable	No.	%	HR	SE	p value
Treatment					
Saline	22	20	Ref		
Footvax®	16	17	0.77	0.33	0.44
Custom R-Pilus	18	16	0.79	0.32	0.45
Breed					
NC mule	24	21	Ref		
Exmoor mule	3	7	**0.28**	**0.61**	**0.04**
Whitefaced cross	29	18	0.64	0.28	0.10
Random effect					
Ewe	Variance = 4.0×10^{-4}				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p \leq 0.05$ and are indicated in bold

CHAPTER 5 THE IMPACT OF BIVALENT AND MULTIVALENT FOOTROT VACCINES ON ANTIBIOTIC USAGE FOR TREATMENT OF LAMENESS, EWE PRODUCTIVITY, AND FREQUENCY OF ADVERSE EVENTS

In this chapter, the results of the effects of vaccination and weekly therapeutic treatments on the duration of cases of lameness and footrot, and ewe productivity, are presented. The number of antibiotic treatments and standardised quantity of antibiotic that was used to control lameness is compared between the three treatment groups. The final section covers the results of monitoring for adverse events, mainly focussing on injection site reactions.

5.1 Materials and methods

5.1.1 The duration of cases of lameness and footrot

The duration of lameness cases was calculated for each flock, and for the three treatment groups within the flocks, for all new cases between week 7 and 24 for which the case duration was known. The case was defined as resolved if the locomotion score had returned to 0. New cases on week 25 were excluded because the ewes were not scored for locomotion on week 26. The duration of cases of footrot was also calculated for each flock, and treatment groups within flocks, over the same period. A case of footrot was defined as resolved if the locomotion score had returned to 0 or the ewe remained lame but footrot lesions were no longer present. All new cases on week 24 had resolved by week 25. It should be noted that in flock 2, lame ewes were not caught on week 6-9, 11 or 12. A binomial model was built to test for differences between the treatment groups and flocks in the duration of lameness cases. The most common causes of lameness, grouped into footrot,

active CODD, ulcerative skin lesions, granulomas, non-infectious hoof wall lesions, and other lesions, were included in the model because lesion type was expected to influence lameness duration. These causes of lameness were categorised as 'present' if they were observed on at least one week of a single case of lameness.

5.1.2 Number of antibiotic treatments and standardised quantity of parenteral antibiotics used to treat lameness and footrot

Lame ewes with infectious lesions of the foot (or leg) were treated with parenteral antibiotics according to a pre-defined protocol (Table 3-4). Lame ewes with footrot that did not have any other infectious foot lesions were treated with parenteral and topical oxytetracycline. Lame ewes with concurrent infectious lesions such as CODD, ulcerative skin lesions and granulomas were treated with parenteral amoxycillin and topical oxytetracycline. Antibiotic treatments were administered weekly to lame ewes only, until any infectious lesion resolved. Lame ewes that no longer had visibly infected lesions did not receive further doses of parenteral antibiotics. A single injection of parenteral oxytetracycline was defined as one treatment. Two doses of parenteral amoxycillin given 48 hours apart was defined as one treatment. The number of antibiotic treatments given as a proportion of all ewe-weeks when a treatment could have been given was compared between the three treatment groups for the trial period from week 7 -25 inclusive. In flock 2, where lame ewes were not caught in weeks 6-9, 11 and 12, the dataset was truncated to include week 10 onwards. In addition, the quantity of antibiotic per standardised unit of sheep weight (mg/kg) was calculated for each flock and treatment group. These quantities were calculated separately for all lameness treatments and for treatments given to sheep

lame with footrot. The standardised unit of sheep weight was 75kg, which is the population corrected unit (PCU) used for antibiotic use monitoring at a national level (VMD, 2016). However, the mg/PCU metric is a measure of annual antibiotic use and it was not possible to extrapolate annual use from the limited duration of this study, therefore the mean weekly mg/kg is presented for the 19 week period in flocks 1 and 3, and the 14 week period in flock 2, and is presented for purposes of comparison with other commercial flocks only.

5.2 Duration of lameness and footrot cases by treatment group

5.2.1 Number of weeks consecutively lame per lameness case

The duration of lameness cases varied between the flocks, which reflected differences in the underlying cause of lameness and response to treatment. In flock 1, 163/183 (89.1%) of lameness cases were one week in duration, compared to 52/67 (77.6%) in flock 2 and 160/214 (74.8%) in flock 3. In flock 2, two lame ewes were not caught on week 12 and were treated on week 13 on their second consecutive week of lameness.

The distribution of the duration of lameness cases by treatment group is presented in Figure 5-1. The most variation between treatment groups occurred in flock 1 where 79% of cases in the Custom R-Pilus group lasted only one week compared to 93% in the Footvax® group and 92% in the saline group. A difference in case duration between treatment groups is potentially important because the number of antibiotic treatments used per treatment group depended upon both number of cases and duration of cases. However, not all lameness cases required treatment with parenteral antibiotics.

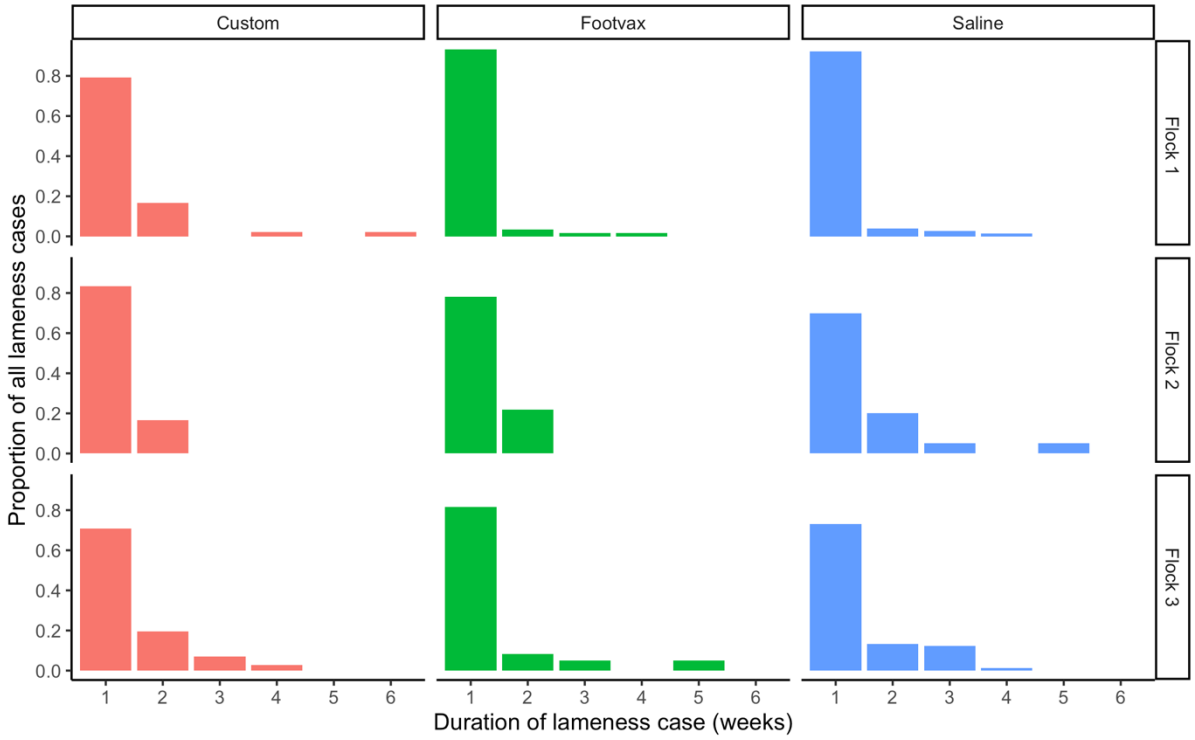

Figure 5-1 The distribution of the duration of cases of lameness within each treatment group

When the presumptive cause of lameness was accounted for in the binomial model for lameness duration, no difference was seen between the three treatment groups or flocks (Table 5-1). Cases of lameness in which active CODD, granulomas, non-infectious hoof wall lesions and other (uncategorised) lesions were present were significantly more likely to last longer than one week.

Table 5-1 Binomial mixed effects regression model for lameness cases of one week in duration for all cases of known duration between week 7 and 24 of the trial

Variable	No.	%	Odds ratio	Lower 95% CI	Upper 95% CI
Fixed effects					
Treatment					
Saline	32	38	Ref		
Custom R-Pilus	32	38	0.91	0.47	1.73
Footvax®	20	24	1.50	0.74	3.03
Flock					
3	53	63	Ref		
1	20	24	1.47	1.71	3.03
2	11	13	0.65	0.27	1.53
Foot/leg lesions					
Footrot – absent	52	62	Ref		
Footrot – present	32	38	0.62	0.34	1.14
Active CODD - absent	64	76	Ref		
Active CODD – present	20	24	**0.18**	**0.08**	**0.44**
Ulcerative skin lesions – absent	78	93	Ref		
Ulcerative skin lesions – present	6	7	0.65	0.21	2.01
Granuloma – absent	77	92	Ref		
Granuloma – present	7	8	**0.11**	**0.03**	**0.48**
Hoof wall lesion - absent	30	36	Ref		
Hoof wall lesion - present	54	64	**0.22**	**0.11**	**0.44**
Other lesion – absent	63	75	Ref		
Other lesion - present	21	25	**0.26**	**0.12**	**0.56**
Random effects					
Ewe			Variance = 0.21		ICC = 0.06

No. and % represent the number and percentage of lameness cases lasting >1 week in each category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Odds ratios are statistically significant when the CI does not include unity and are indicated in bold.

5.2.2 Number of weeks consecutively lame per case of footrot

A case of footrot lasted one week in 95.5% (106/111), 95.5% (21/22) and 90.6% (48/53 of new incidences in flocks 1,2 and 3 respectively and there was little variation between treatment groups (Figure 5-2).

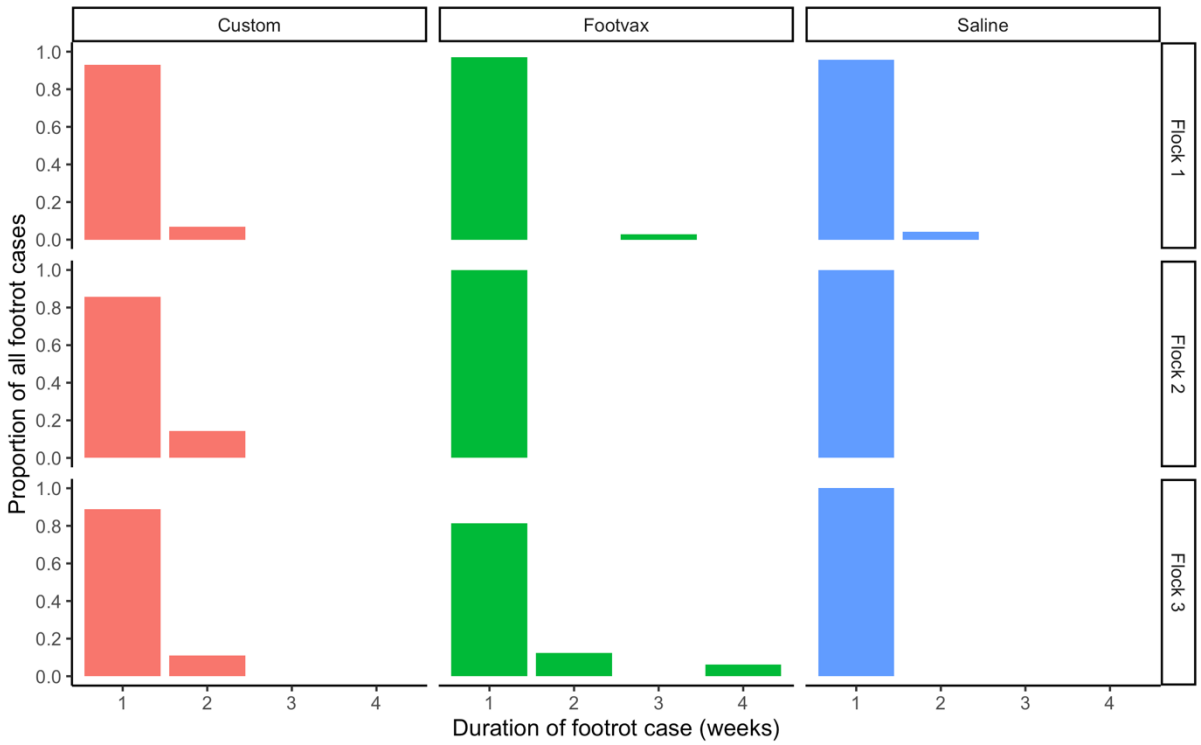

Figure 5-2 The distribution of case durations for footrot within each treatment group

The slightly higher percentage of cases with a duration of >1 week in flock 3 was explained by a higher occurrence of concurrent foot lesions, which is consistent with the results of the binomial model for lameness duration (Table 5-1) and also apparent in the higher percentage of cases of footrot that received parenteral amoxycillin instead of oxytetracycline in this flock (Table 5-2).

Table 5-2 The number (%) of antibiotic treatments for footrot by type of antibiotic between weeks 7 and 25 of the trial in each flock

	Parenteral + topical oxytetracycline	Parenteral amoxycillin + topical oxytetracycline	Topical oxytetracycline	Total
Flock 1	124 (94.7%)	5 (3.8%)	2 (1.5%)	131
Flock 2	24 (100%)	0	0	24
Flock 3	52 (78.8%)	13 (19.7%)	1 (1.5%)	66

5.3 Number of antibiotic treatments used in each treatment group

5.3.1 Number of parenteral antibiotic treatments used to treat lameness

Flock 1 required the most antibiotic treatments per ewe-week at risk, and ewes in the saline treatment group received >50% more treatments than ewes in either vaccine group (Table 5-3). In flock 2 there was little difference in the number of antibiotic treatments given between treatment groups. In flock 3, ewes in the saline group received 26% more antibiotic treatments than ewes in the Custom R-Pilus vaccine group, and 11% more antibiotic treatments than ewes that had been vaccinated with Footvax®. Although the number of antibiotic treatments was higher in flock 1, flock 3 had the highest quantity of antibiotic use on a standardised mg/kg basis due to the mixed aetiology of infectious lameness and consequent requirement for more doses of amoxycillin. Across the combined flocks, significantly more antibiotic treatments were given for lameness to the unvaccinated ewes than to the vaccinated ewes; ewes in the saline group received 36% more treatments than in the Custom R-Pilus vaccine group ($p= 0.02$) and 30% more treatments than in the Footvax® group ($p=0.05$).

Table 5-3 Number of parenteral antibiotic treatments and standardised quantity of parenteral antibiotic used for treatment of lameness by flock and treatment group between weeks 7 and 25 of the trial in flocks 1 and 3, and between weeks 10 and 25 in flock 2.

Treatment group	Number of treatments	Number of ewe-weeks	Treatments per 100 ewe-weeks at risk	Mean weekly antibiotic quantity (mg/kg)
Flock 1				
Custom R-Pilus	42	1023	4.11	0.88
Footvax®	44	1095	4.02	0.86
Saline	69	1136	6.07	1.30
Total	155	3254	4.76	1.02
Flock 2				
Custom R-Pilus	12	938	1.30	0.33
Footvax®	12	938	1.30	0.33
Saline	12	875	1.37	0.36
Total	36	2751	1.31	0.34
Flock 3				
Custom R-Pilus	50	1273	3.93	1.01
Footvax®	52	1158	4.49	1.15
Saline	62	1254	4.94	1.27
Total	164	3685	4.34	1.14
Combined flocks				
Custom R-Pilus	104	3234	3.22	0.77
Footvax®	108	3191	3.38	0.81
Saline	143	3265	4.38	1.04
Total	355	9690	3.66	0.87

5.3.2 Number of parenteral antibiotic treatments used to treat footrot

All weekly treatments for footrot in ewes with locomotion scores of >0 were included in the calculation of the number of parenteral antibiotics used to treat footrot. There were three incidences where ewes were scored with footrot lesions but only treated topically (Table 5-4), two of which occurred on the last week of observation and the third following parenteral treatment for CODD on the previous week. Trends in the number of weekly antibiotic treatments used for footrot followed the trends for whole flock prevalence and incidence of footrot in the three flocks (Chapter 4, Appendix 13). In flock 1, the overall number of antibiotic treatments per 100 ewe-weeks at risk was 4.06 for footrot (Table 5-4) compared to 4.76 for all lameness cases (Table 5-3). Approximately 40% more antibiotic treatments were used for footrot in unvaccinated ewes than in vaccinated ewes, although this was not a statistically significant difference ($p=0.13$). The number of antibiotic treatments for footrot in flock 2 was comparable between treatment groups, as it was for treatment of lameness cases. The most difference between the number of antibiotic treatments used for lame ewes, and ewes with footrot, was observed in flock 3. In this flock, only 43% of all antibiotic treatments were used for footrot, and <2 antibiotic treatments per 100 ewe-weeks were used for footrot in the two vaccine treatment groups (Table 5-4). Overall, the number of antibiotic treatments given for footrot was not significantly different between the three treatment groups ($p=0.06$), but the number of antibiotic treatments used in the Custom R-Pilus group approached being significantly fewer than in the saline group ($p=0.05$).

Table 5-4 Number of parenteral antibiotic treatments and standardised quantity of parenteral antibiotic used for treatment of footrot in lame sheep by flock and treatment group between weeks 7 and 25 of the trial in flocks 1 and 3, and between weeks 10 and 25 in flock 2.

Treatment group	Number of treatments	Number of ewe-weeks at risk	Treatments per 100 ewe-weeks at risk	Mean weekly antibiotic quantity (mg/kg)
Flock 1				
Custom R-Pilus	36	1023	3.52	0.71
Footvax®	39	1095	3.56	0.72
Saline	57	1136	5.02	1.02
Total	132	3254	4.06	0.82
Flock 2				
Custom R-Pilus	8	938	0.85	0.20
Footvax®	8	938	0.85	0.20
Saline	10	875	1.14	0.27
Total	26	2751	0.95	0.22
Flock 3				
Custom R-Pilus	23	1273	1.81	0.40
Footvax®	20	1158	1.73	0.38
Saline	26	1254	2.07	0.46
Total	69	3685	1.87	0.42
Combined flocks				
Custom R-Pilus	67	3234	2.07	0.44
Footvax®	67	3191	2.10	0.44
Saline	93	3265	2.85	0.60
Total	227	9690	2.34	0.49

5.4 The effect of treatment group on ewe productivity

5.4.1 Exclusions from the dataset for analysis of ewe productivity

In flock 1, the BCS of four ewes were excluded from the dataset because they lambed or aborted before week 25 of the trial, and 21 ewes were excluded for BCS and litter size because they had been scanned as pregnant but not yet lambed by week 30 of the trial. In flock 2, only 3 ewes were excluded because they had not yet lambed by week 30. In flock 3, the BCS of 13 ewes that lambed between week 15 and week 24 of the trial were not recorded. A further 6 ewes did not lamb before week 30 and were excluded.

5.4.2 Litter size and number of living lambs at trial exit

The distribution of litter size and number of living lambs per ewe at the exit examination was similar between the treatment groups in all three flocks (Appendix 20). There was no difference in litter size between treatment groups in any of the flocks (Flock 1, $p = 0.97$; Flock 2, $p = 0.78$; Flock 3, $p = 0.61$), nor any difference in number of living lambs between treatment groups (Flock 1, $p = 0.53$; Flock 2, $p = 0.95$; Flock 3, $p = 0.48$).

5.4.3 Change in BCS between week 1, week 17 and lambing

The distribution of change in BCS units between weeks 1 and 17, week 17 and lambing, and week 1 and lambing are shown in Appendix 21 . There were no significant differences between the three treatment groups in change in BCS score between pre-tupping (week 1), scanning (week 17) and lambing.

5.5 Adverse events in vaccinated ewes

5.5.1 Severe adverse events

No severe adverse events were recorded in any of the flocks.

5.5.2 Injection site reactions

In all flocks, injection site reactions in Footvax® treated ewes were mainly in the larger size category at 4 weeks after vaccination (Figure 5-3). Across the combined flocks, 87% (57/65) of all reactions in Footvax® treated ewes were 21-50mm at 4 weeks compared to 23% (13/57) at 12 weeks after vaccination. Purulent discharge from the injection site was observed up to 16 weeks after vaccination and affected 14 ewes vaccinated with Footvax® and 3 ewes vaccinated with the Custom R-Pilus vaccine.

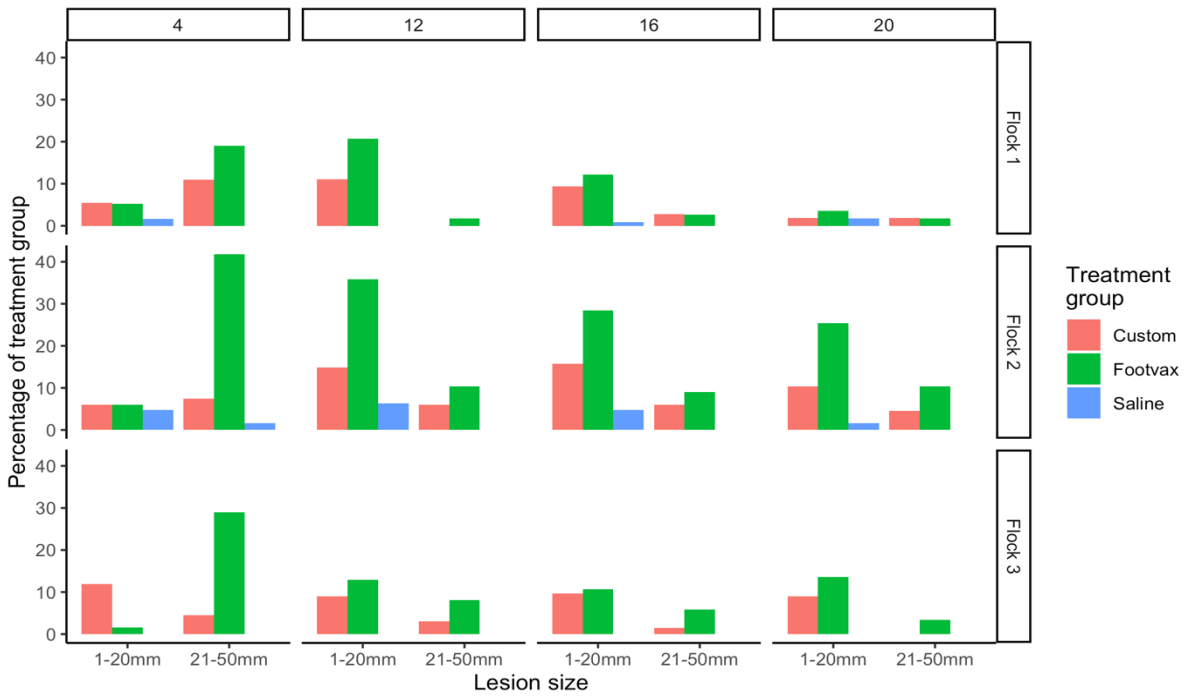

Figure 5-3 Percentage of treatment group affected by injection site reactions in each flock by size of reaction and number of weeks since vaccination

Table 5-5 Binomial mixed effects regression model for presence of injection site reactions in all flocks

Variable	No.	%	Odds ratio	Lower 95% CI	Upper 95% CI
Fixed effects					
Weeks since vaccination	1864		**0.91**	**0.88**	**0.95**
Treatment					
Footvax®	935	50%	Ref		
Custom R-Pilus	945	50%	**0.34**	**0.19**	**0.60**
Fleece					
Not shorn	1125	60%	Ref		
Shorn	745	40%	**1.89**	**1.10**	**3.23**
Side of injection					
Right	1128	60%	Ref		
Left	752	40%	1.24	0.92	1.69
Breed					
North Country Mule	870	46%	Ref		
Bluefaced Leicester	195	10%	2.45	0.31	19.17
Exmoor Mule	85	5%	**5.70**	**1.17**	**27.86**
Suffolk cross	290	15%	2.55	0.36	18.22
Whitefaced cross	440	23%	1.9	0.58	6.22
Flock					
2	670	36%	Ref		
1	565	30%	0.16	0.02	1.05
3	645	34%	**0.31**	**0.10**	**0.97**
Random effects					
Ewe	376	Variance = 4.97		ICC = 0.602	

No. and % represent the number and percentage of observations in each category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Odds ratios are statistically significant when the CI does not include unity and are indicated in bold.

All variables were significant when fitted in univariable models but breed and side of injection did not improve the fit of the multivariable model. Injection site reactions were significantly less likely to occur in ewes vaccinated with the Custom R-Pilus vaccine than ewes vaccinated with Footvax®, and presence of fleece was influential in the ability to detect the lesions (Table 5-5).

5.6 Discussion

This chapter presented the analysis of the impact of vaccination on indirect effects that are consequential to a reduction in lameness and footrot cases. These outcomes focus on ewe productivity and social responsibility instead of the direct adverse effect of case numbers on ewe welfare. The results of the analysis of adverse effects, which can have a direct effect on both ewe welfare and productivity, were also reported in this chapter.

The duration of lameness cases was analysed to gain a greater understanding of the relationship between lesion type and the number of weeks a ewe might be under treatment for lameness, because this could influence the number of antibiotic treatments used in each treatment group if lameness associated lesions were not distributed equally between the three treatment groups. Cure rates for footrot of over 90% were observed in all three flocks, consistent with previous studies (Kaler et al., 2011, Kaler et al., 2010a, Wassink et al., 2010b). Therefore, given that all lame ewes with footrot were administered effective therapeutic treatment within one week of becoming lame, it was anticipated that no difference would be seen in lameness duration between the three treatment groups. Modelling confirmed that there were no differences between treatment groups after accounting for the varying causes of lameness. Current industry recommendations are that Footvax® is a 'core' vaccine that should be used in almost all flocks in England, without consideration towards the cause of lameness in individual flocks (NOAH, 2022). It was therefore reassuring to demonstrate that once the three flocks were combined, the number of antibiotic doses was reduced by 23% with the use of Footvax® compared to unvaccinated ewes, an amount that would certainly be of industry significance if reproduced at a national level. However, the difference in number of antibiotic treatments used between ewes vaccinated with Footvax® and unvaccinated ewes was most

evident in flock 1, which suggests that there would be a greater benefit to sheep farmers if Footvax® use was targeted in flocks where footrot was the main driver of lameness.

No difference was observed between the treatment groups for change in BCS, litter size and number of living lambs born. These measures of ewe productivity can be adversely affected by untreated lameness (Wassink et al., 2010b), but a difference between treatment groups was not anticipated in this study, despite a difference in the number of lameness cases, due to the weekly therapeutic intervention in lame ewes.

Finally, it is important that pharmaceutical interventions do not have inadvertent side effects that have a negative impact on sheep welfare and productivity. No serious adverse events occurred in any treatment group during this trial. Fewer injection site reactions were expected in the Custom R-Pilus vaccinated ewes than in the Footvax® treatment group based on information supplied by the manufacturer of Custom R-Pilus after extensive use of the vaccine in Australian flocks. In this trial, injection site reactions were shown to be larger, last longer and were more frequently associated with purulent discharge in the Footvax® treatment group. The implications of this finding are discussed further in chapter 7.

CHAPTER 6 THE EFFECT OF BIVALENT FLOCK-SPECIFIC VACCINATION ON THE DIVERSITY OF *D. NODOSUS* SEROGROUPS ON EWES WITH FOOTROT LESIONS

In this chapter, the results of the serogroup analysis of interdigital swabs are presented. The hypothesis was that the serogroups of *D. nodosus* on the feet of ewes vaccinated with the Custom R-Pilus vaccine would be perturbed. That is, swabs taken from footrot lesions on these ewes would detect the two targeted serogroups with decreasing frequency as the trial progressed. Serogroup diversity was predicted to remain unchanged on Footvax® and saline treated ewes based on previous evidence (Prosser et al., 2020, Smith et al., 2017).

6.1.1 Association between footrot lesions and presence of D. nodosus

D. nodosus was present on almost all ewes sampled in flocks 1 and 3, regardless of footrot status (Table 6-1). Only one sample was negative for *D. nodosus* out of 21 swabs taken from purchased ewes without footrot lesions in flock 1. Furthermore, there was no notable difference in the distribution of the *AprV2* and *AprB2* genotypes between swabs taken from ewes with and without visible footrot lesions (Table 6-2). The variation in *AprV2/AprB2* genotype prevalence between the flocks was the same as that reported in a previous survey of sheep flocks with clinical footrot in England (Monaghan et al., 2021). The range of *D. nodosus* loads on all *D. nodosus* positive swabs is shown for each ID severity score in Figure 6-1. An increase in *D. nodosus* load was seen with increasing ID lesion score, particularly in flocks 1 and 3 from which substantially more swabs were analysed (Figure 3-4). Most notably, *D. nodosus load* was consistently lower in swabs taken from ewes without ID lesions

than those with visible ID lesions. In flock 3, this trend is more apparent once the *AprB2* positive swabs are removed from the dataset (Figure 6-2)

Table 6-1 Number (%) of all 533 swabs that were positive for D. nodosus by AprV2/AprB2 qPCR by flock and lesion status

	Footrot lesion status		
Flock	Present	Absent	Total
1	248/254 (97.6%)	31/32 (97.0%)	279/286 (97.6%)
2	60/64 (93.8%)	4/8 (50.0%)	64/72 (88.9%)
3	155/157 (98.7%)	17/18 (94.4%)	172/175(98.3%)
Total	463/475 (97.5%)	52/58(89.7%)	515/533 (96.6%)

Footrot lesion positive = one or more foot with an ID or SFR lesion score of one or more

Table 6-2 Number (percentage) of AprV2 and AprB2 positive swabs in each flock by footrot lesion status

	APR genotypes			
Footrot status	*AprV2* only	*AprB2* only	*AprV2 + AprB2*	Total
Flock 1				
Present	246 (99.2%)	0	2 (0.8%)	248 (88.9%)
Absent	31 (100%)	0	0	31 (11.1%)
Total	277 (99.3%)	0	2 (0.7%)	279
Flock 2				
Present	50 (83.3%)	0	10 (16.7%)	60 (93.8%)
Absent	4 (7.4%)	0	0	4 (6.2%)
Total	54 (84.3%)	0	10 (15.6%)	64
Flock 3				
Present	57 (36.8%)	5 (3.2%)	93 (60.0%)	155 (90.1%)
Absent	6 (35.3%)	0	11 (64.7%)	17 (9.9%)
Total	63 (36.6%)	5 (2.9%)	104 (60.5%)	172
All flocks				
Present	353 (76.2%)	5 (1.1%)	105 (22.7%)	463 (89.9%)
Absent	41 (78.8%)	0	11 (21.2%)	52 (10.1%)
Total	394 (76.5%)	5 (1.0%)	116 (22.5%)	515

Footrot lesion positive = one or more foot with an ID or SFR lesion score of one or more

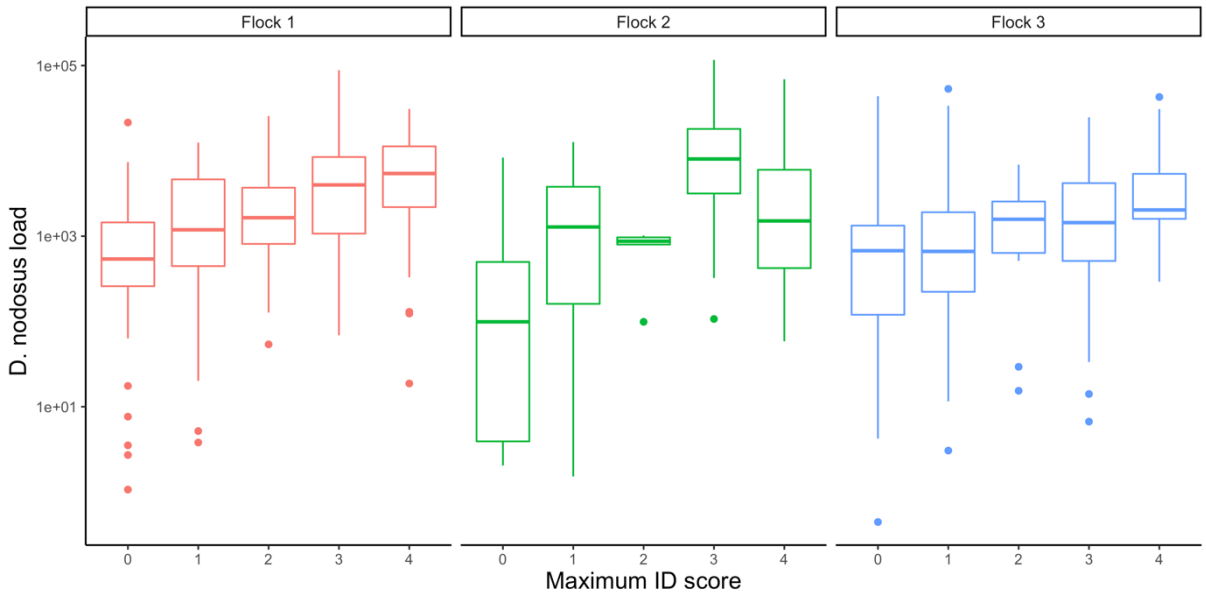

Figure 6-1 Boxplot of D. nodosus load on individual swabs by maximum ID score for any concurrent SFR score for each flock. Outliers are intervals beyond 1.5 x the interquartile range (IQR).

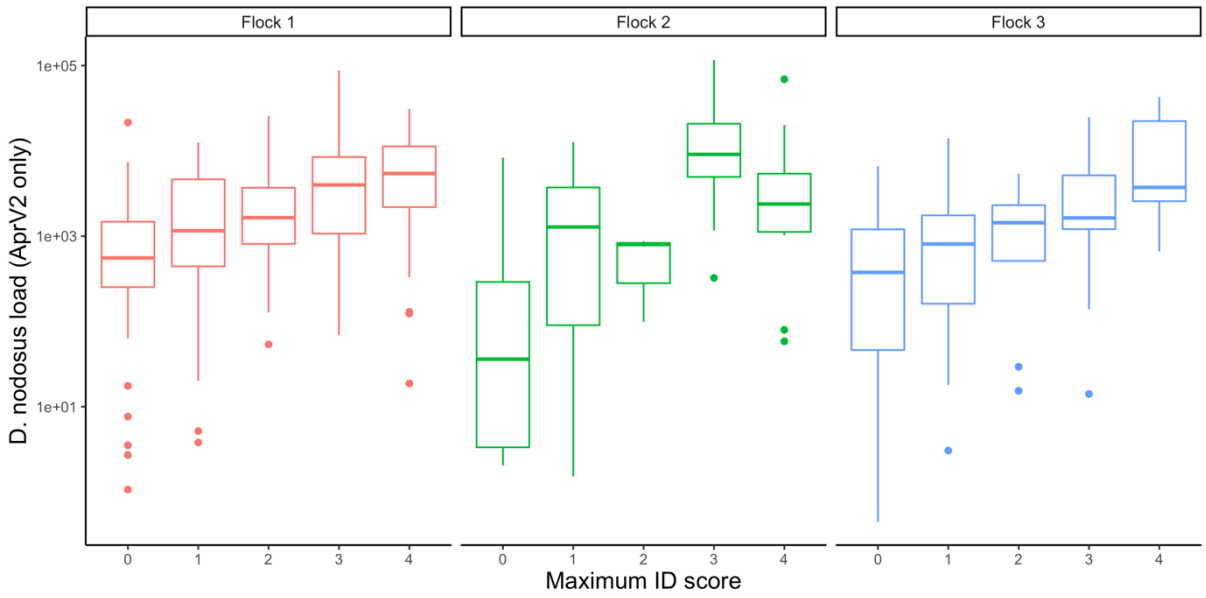

Figure 6-2 Boxplot of D. nodosus load for individual swabs carrying the AprV2 genotype only by maximum ID score for any concurrent SFR score for each flock. Outliers are intervals beyond 1.5 x the interquartile range (IQR).

6.1.2 Serogroup detection and relationship with D. nodosus load and Apr genotype

No serogroup was identified in 25 (14.3%) of the 175 *D. nodosus* positive samples that were tested for all nine serogroups (Table 6-3). Nine (5.2%) of these samples had a *D. nodosus* load of less than 80, the minimum load at which any serogroup was detected across all samples (Table A- 39). The median number of serogroups per sample was one but up to four serogroups were detected on samples from saline treated ewes. The absence of footrot lesions did not prevent multiple serogroups being detected on a swab (Table 6-3). Serogroups D, E and G were not detected in any samples.

In flock 3, serogroup C was detected in one sample that was positive for *AprB2* only and serogroup C was more prevalent in samples positive for *AprB2* and *AprV2* in which at least one serogroup was identified (51/63, 80.9%) than *AprV2* only positive samples (3/35, 8.6%). Serogroup C was not detected in isolation on any of the samples that were tested for all nine serogroups. The potential difference in phenotypic expression of footrot between the *AprB2* and *AprV2* genotypes (Frosth et al., 2015, Monaghan et al., 2021) means it is possible that other more virulent strains of a different serogroup were responsible for the footrot lesions observed on feet where serogroup C was detected, and this could have implications for the efficacy of the bivalent vaccine, which contained serogroup C.

Table 6-3 Distribution of number of serogroups detected per swab (% of total samples) in samples tested for all nine serogroups by flock and footrot lesion status

	Number of serogroups					
FR status	0	1	2	3	4	Total
Flock 1						
Present	10 (11.9%)	29 (34.5%)	27 (32.1%)	17 (20.2%)	1 (1.2%)	84
Absent	0	2 (40.0%)	1 (20%)	2 (40.0%)	0	5
Total	10 (11.2%)	31 (34.8%)	28 (31.5%)	19 (21.3%)	1 (1.1%)	89
Flock 2						
Present	3 (9.7%)	21 (67.7%)	6 (19.4%)	1 (3.2%)	0	31
Absent	1 (100%)	0	0	0	0	1
Total	4 (12.5%)	21 (65.6%)	6 (18.8%)	1 (3.1%)	0	32
Flock 3						
Present	9 (19.6%)	13 (28.3%)	12 (26.1%)	10 (21.7%)	2 (4.3%)	46
Absent	2 (25.0%))	2 (25.0%)	3 (37.5%)	0	1 (12.5%)	8
Total	11 (20.4%)	15 (27.8%)	15 (27.8%)	10 (18.5%)	3 (5.6%)	54
All flocks						
Present	22 (13.7%)	63 (39.1%)	45 (34.4%)	28 (17.4%)	3 (1.9%)	161
Absent	3 (21.4%)	4 (28.6%)	4 (28.6%)	2 (14.3%)	1 (7.1%)	14
Total	25 (14.3%)	67 (38.3%)	49 (28.0%)	30(17.1%)	4 (2.3%)	175

Footrot lesion positive = one or more foot with an ID or SFR lesion score of one or more

6.1.3 Serogroup prevalence and diversity by treatment group on week 1, 17 and exit examinations

On week 1 and week 17, all ewes within each flock were examined within a 32-hour period. For the final ('Exit') examination, all ewes within a flock were examined within 48 hours of lambing over a period of 28, 24 and 29 days for flocks 1, 2 and 3 respectively. All ewes that were present on weeks 1 and 17 were included in the denominator for the prevalence of serogroups in these weeks, whereas all ewes remaining in the trial at week 25 were included in the denominator for prevalence of serogroups at 'exit' examinations. The numerator at all timepoints was the number of times a serogroup was detected in ewes with an ID or SFR lesion score of >0 on at least one foot.

The prevalence and severity of footrot lesions on week 1 and 17 when all ewes were examined is shown in Figure 6-3 and provides context for the serogroup results that follow. In all three flocks, a greater reduction in prevalence and/or severity of footrot lesions between week 1 and 17 was observed in the vaccinated ewes compared to the unvaccinated ewes.

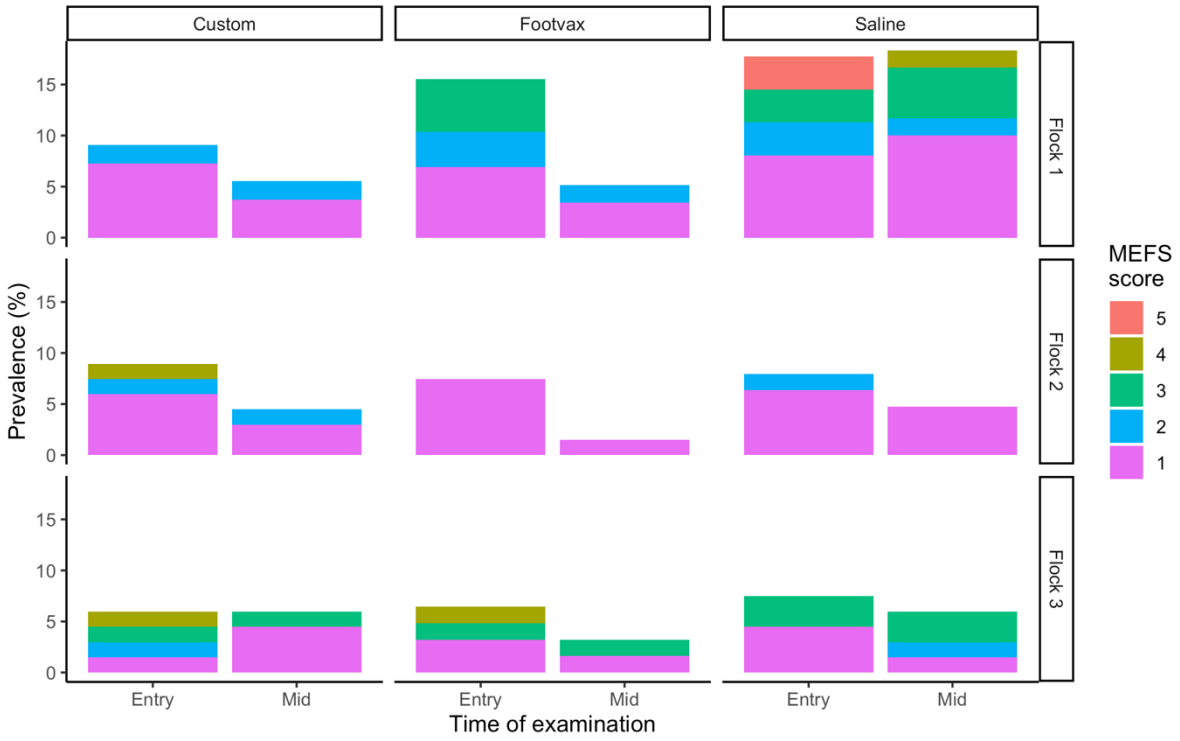

Figure 6-3 The prevalence of ewes with footrot lesions by lesion severity (MEFS score) for each flock, treatment group and timepoint. A MEFS score of 1 indicates ID lesions only (Table 3-6).

Serogroup presence and prevalence varied between treatment groups in all three flocks on week 1 (Figure 6-4). The variation could be attributed to the relatively low number of footrot diseased ewes with *D. nodosus* positive swabs in each treatment group, the low prevalence of some serogroups and the detection of multiple serogroups on some swabs. In flock 1, the prevalence of most serogroups mid trial was lower in the two vaccinated groups compared

to the saline treated group, most notably for serogroup H. The decrease in serogroup prevalence seen in the vaccinated groups between trial entry and mid trial was mainly attributable to fewer footrot lesions in ewes in the Existing origin category (Figure 6-5). In contrast, ewes in the Purchased origin category had a greater prevalence of serogroups mid trial than at the start across all treatment groups (Figure 6-5). The high prevalence of serogroup H in saline treated ewes in flock 1 was due to the high proportion of Purchased ewes on which it was detected. Serogroup diversity was lower in vaccinated ewes mid trial, and although four serogroups were present in Custom R-Pilus vaccinated ewes compared to three in the Footvax® group, the BVI serogroup I was absent in this group and remained at a low prevalence at the trial exit compared to the Footvax® and saline treated groups (Figure 6-4). By the end of the trial serogroup H was the most dominant serogroup in both vaccinated groups.

Serogroup prevalence and diversity in flock 2 was low in all three treatment groups throughout the trial and changes in serogroup prevalence by treatment were not observed (Figure 6-4). The prevalence of footrot lesions was also low in flock 3 at the start and mid points of the trial, but the saline group had consistently higher serogroup diversity. It is noteworthy that serogroup H was not detected in the two vaccinated groups at the mid trial or exit examinations, and that two non BVI serogroups, F and B, became the most prevalent in the Custom R-Pilus and Footvax® treated groups respectively.

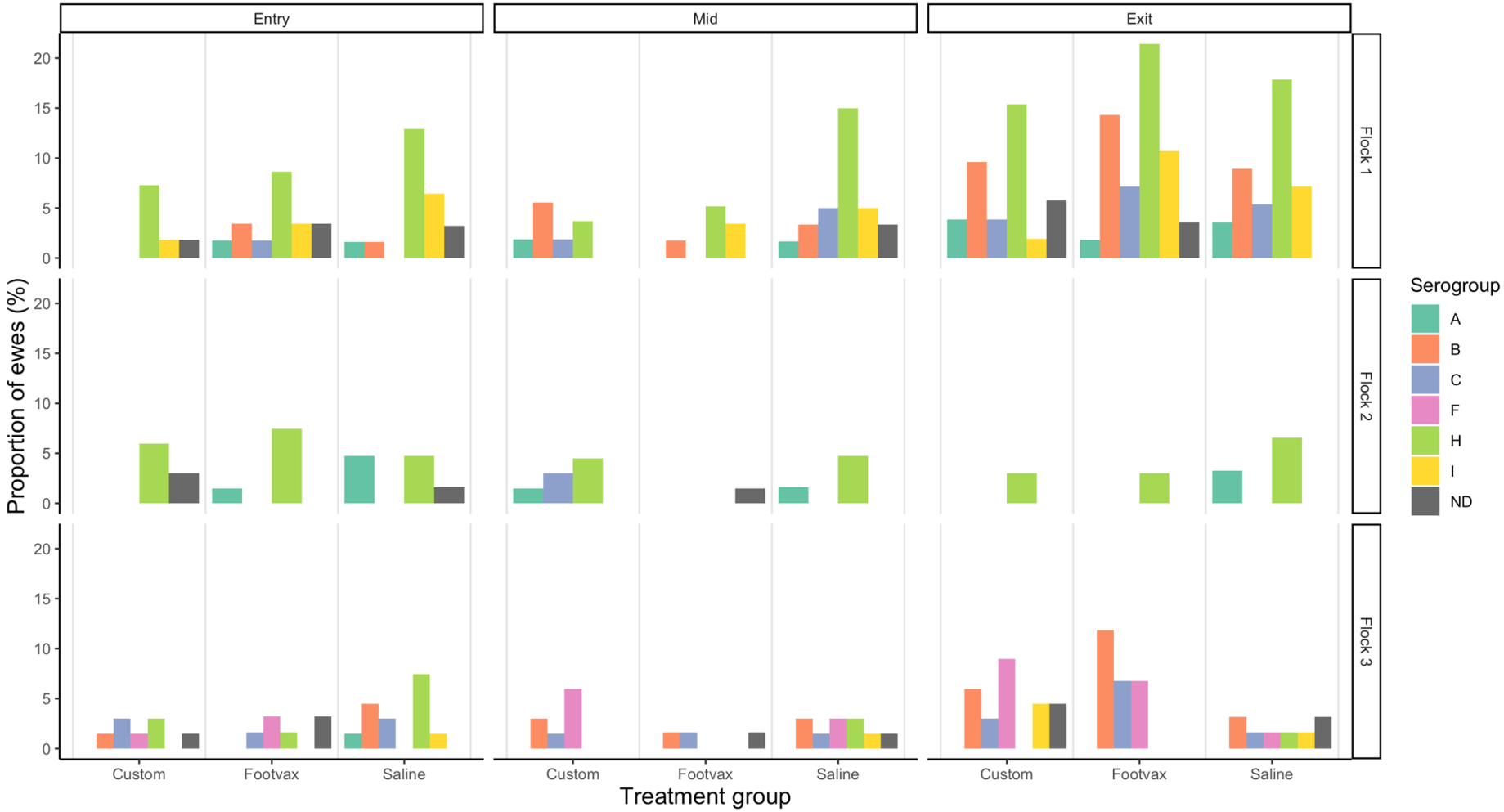

Figure 6-4 Serogroups detected in all ewes with footrot as a proportion of all ewes in each treatment group for each flock at entry, mid-point and exit from the trial. ND =. No serogroup detected on D. nodosus positive sample

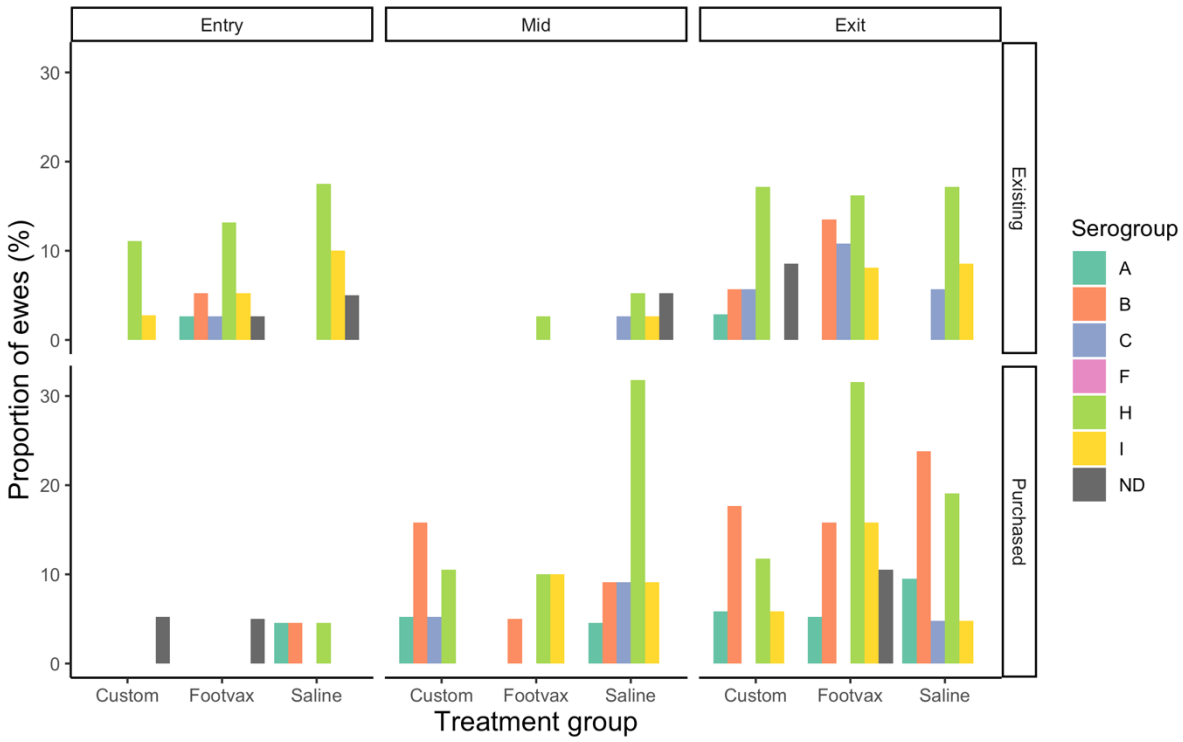

Figure 6-5 Serogroups identified in ewes with footrot in flock 1 as a proportion of those at risk by origin and treatment group at entry, mid-point and exit from the trial. ND = No serogroup detected on D. nodosus positive sample

The number of ewes with footrot lesions in each treatment group at the three whole flock examinations is presented in Table 6-4 with the percentage of ewes with at least one BVI serogroup on the interdigital swab. The BVI serogroups did not disappear from the feet of ewes vaccinated with Custom R-Pilus vaccine in any of the flocks.

Table 6-4 Number of ewes with footrot lesions (% of D. nodosus positive ewes in treatment group) and BVI serogroups detected on interdigital swabs at whole flock examinations. N = no. ewes in the treatment group at risk of having footrot lesions.

Number of BVI serogroups	Treatment Group		
	Custom R-Pilus	Footvax®	Saline
Flock 1			
Entry	N = 55	N = 58	N = 62
0	1 (20.0%)	3 (33.3%)	3 (27.3%)
1-2	4 (80.0%)	6 (66.7%)	8 (72.7%)
Mid	N = 54	N = 58	N = 60
0	1 (33.3%)	0	2 (18.2%)
1-2	2 (66.7%)	3 (100%))	9 (81.8%)
Exit	N=52	N = 56	N = 56
0	5 (38.5%)	4 (22.2%)	1 (9.1%)
1-2	8 (61.5%)	14 (77.8%)	10 (90.9%)
Flock 2			
Entry	N = 67	N = 67	N = 63
0	2 (33.3%)	0	1 (20%)
1-2	4 (66.7%)	5 (100%)	4 (80.0%)
Mid	N = 67	N = 67	N = 63
0	0	1 (100%)	0
1-2	3 (100%)	0	3 (100%)
Exit	N = 67	N = 67	N = 61
0	0	0	0
1-2	2 (100%)	2 (100%)	5 (100%)
Flock 3			
Entry	N = 67	N=62	N = 67
0	2 (50.0%)	2 (50.0%)	0
1-2	2 (50.0%)	2 (50.0%)	5 (100%)
Mid	N = 67	N=62	N = 67
0	3 (75.0%)	1 (50.0%)	2 (50%)
1-2	1 (25.0%)	1 (50.0%)	2 (50%)
Exit	N = 67	N = 59	N = 63
0	8(80.0%)	4 (50.0%)	4 (66.7%%)
1-2	2 (20.0%)	4 (50.0%)	2 (33.3%)

6.1.4 The presence of serogroups in the bivalent vaccine in sheep lame with footrot at weekly examinations

A sheep with footrot was defined as score >1 for locomotion and score >0 for ID and/or SFR on at least one foot, as previously described in section 3.1.7.2. A total of 308 samples from sheep lame with footrot were only tested for BVI serogroups: 171 from flock 1, 34 from flock 2 and 104 from flock 3.

In all three flocks, at least one of the two targeted serogroups was present throughout the trial in ewes with footrot that had received the Custom R-Pilus vaccine. In flock 1, the most prevalent serogroup, H, persisted in all three treatment groups, whilst serogroup I became uncommon in the Custom R-Pilus treatment group from mid trial onwards (Figure 6-6). The persistence of serogroup H was most evident in the Purchased ewe group. In the Existing ewe group, serogroup H was intermittently detected from footrot cases in the Footvax® and saline treated groups throughout the trial (Figure 6-7). In contrast, there was only a single case of footrot after week 6 in the Custom R-Pilus vaccinated group, which indicated that the vaccine was efficacious because ongoing exposure to serogroups H and I was known to have occurred.

Cases of footrot were intermittent across all three treatment groups in flock 2, but serogroup H was detected in all weeks when footrot occurred in ewes in both vaccine groups (Figure 6-8). Serogroup A was consistently detected throughout the trial in saline treated ewes only.

Cases of footrot in flock 3 were intermittent across all treatment groups from approximately halfway through the trial (Figure 6-9). In contrast to flocks 1 and 2, serogroup H was not present in ewes with footrot throughout the trial, whereas serogroup C, which was less prevalent than serogroup H at the start of the trial, persisted in the Custom R-Pilus vaccinated group throughout the trial. The only occurrence of serogroup H after week 21 was its novel detection in a saline treated ewe in week 28 and there were 31 samples eligible for serogroup testing between these two timepoints. It is possible that serogroup H had been reintroduced to the flock in week 26 when, for ease of management, the farmer moved the few remaining non-trial ewes that hadn't yet lambed into the trial group.

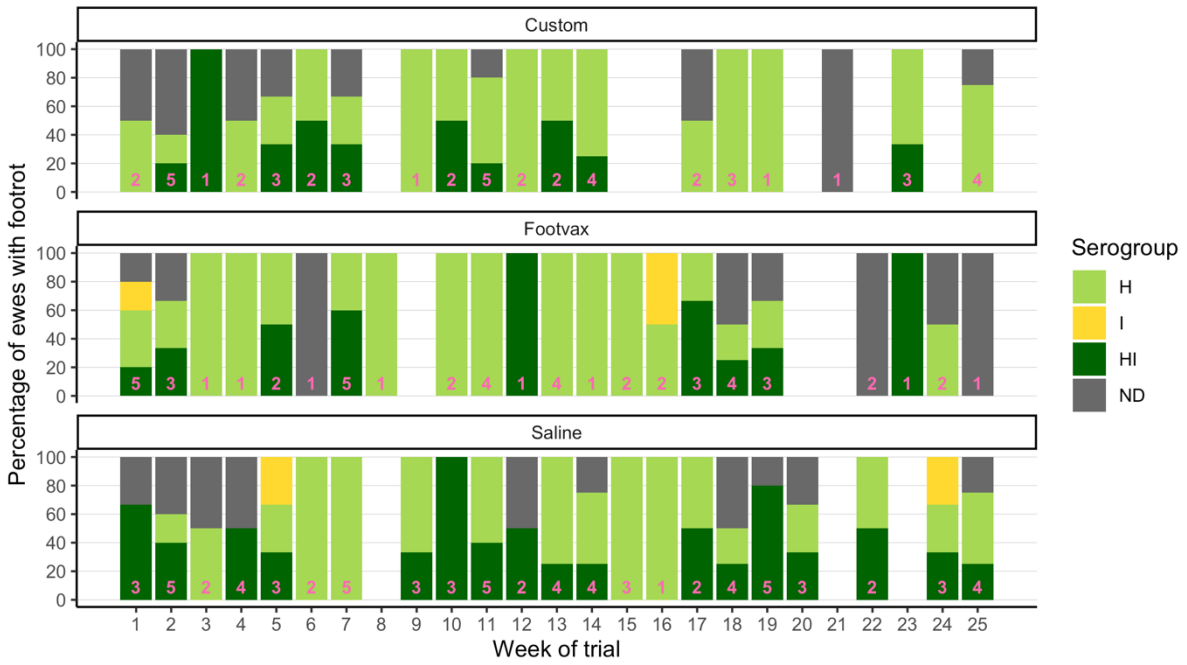

Figure 6-6 Relative percentage of ewes lame with footrot with serogroup H, I, both or neither BVI serogroup (ND) by treatment group in flock 1 (number in column = denominator)

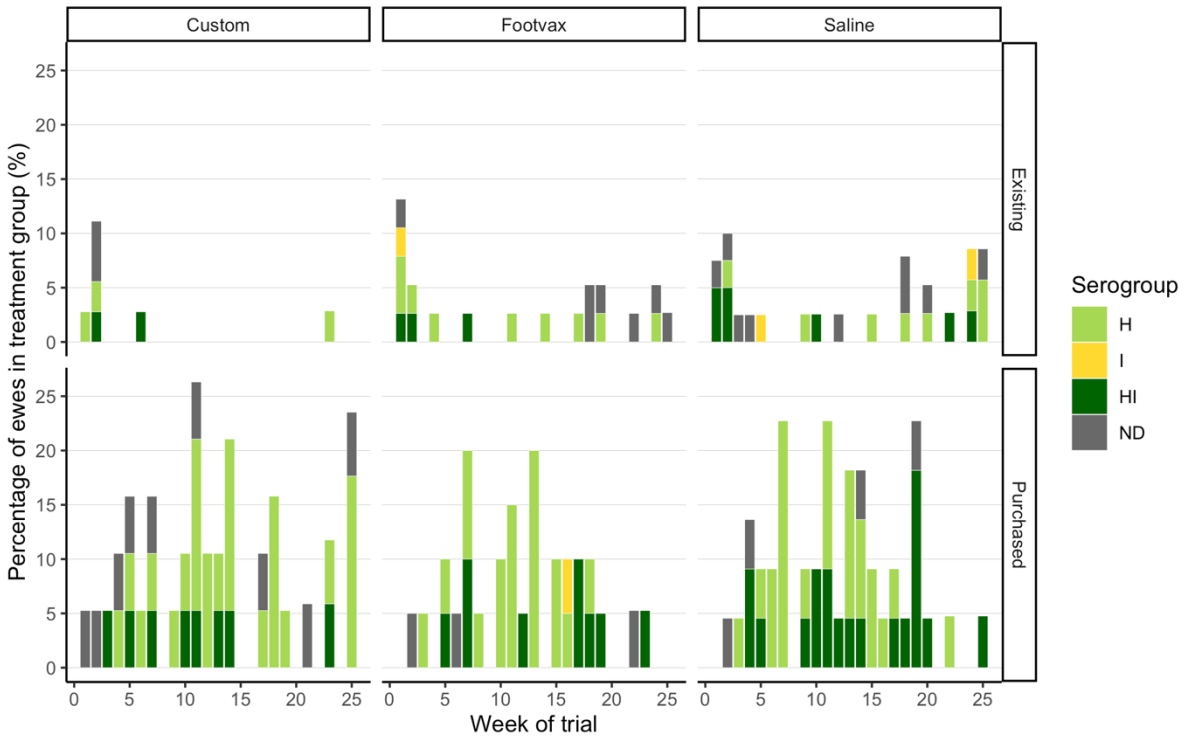

Figure 6-7 Relative percentage of all ewes lame with footrot with serogroup H, I, both or neither BVI serogroup (ND) by treatment group and origin in flock 1

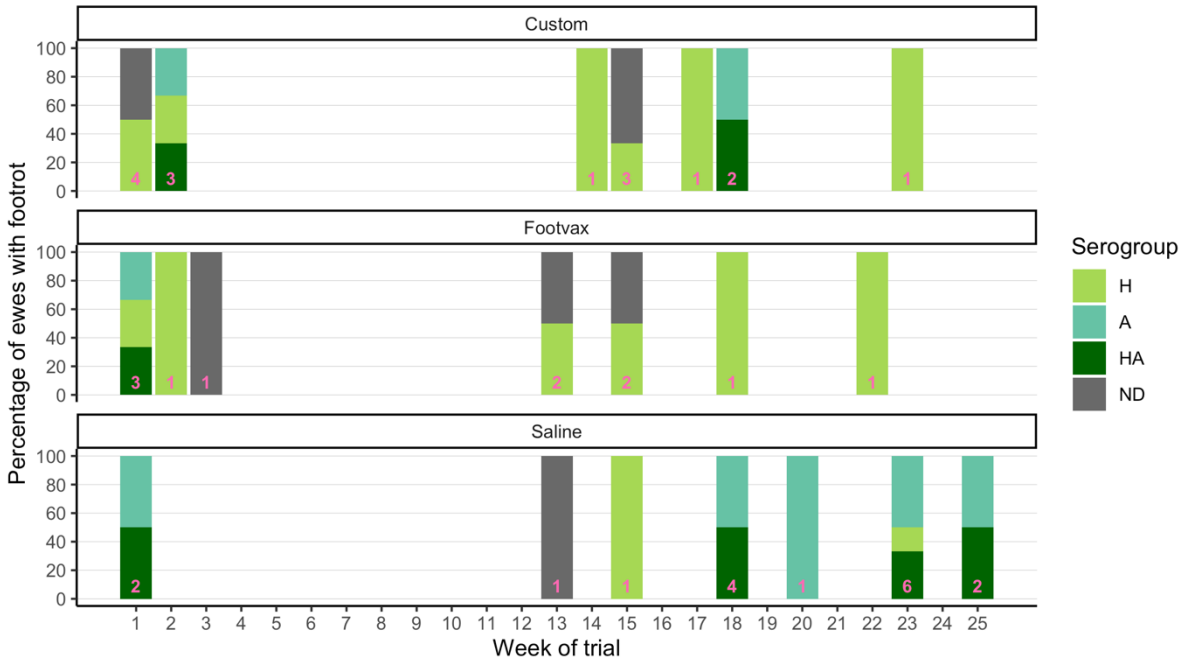

Figure 6-8 Relative percentage of ewes lame with footrot with serogroup H, A, both or neither BVI serogroup (ND) by treatment group in flock 2 (number in column = denominator)

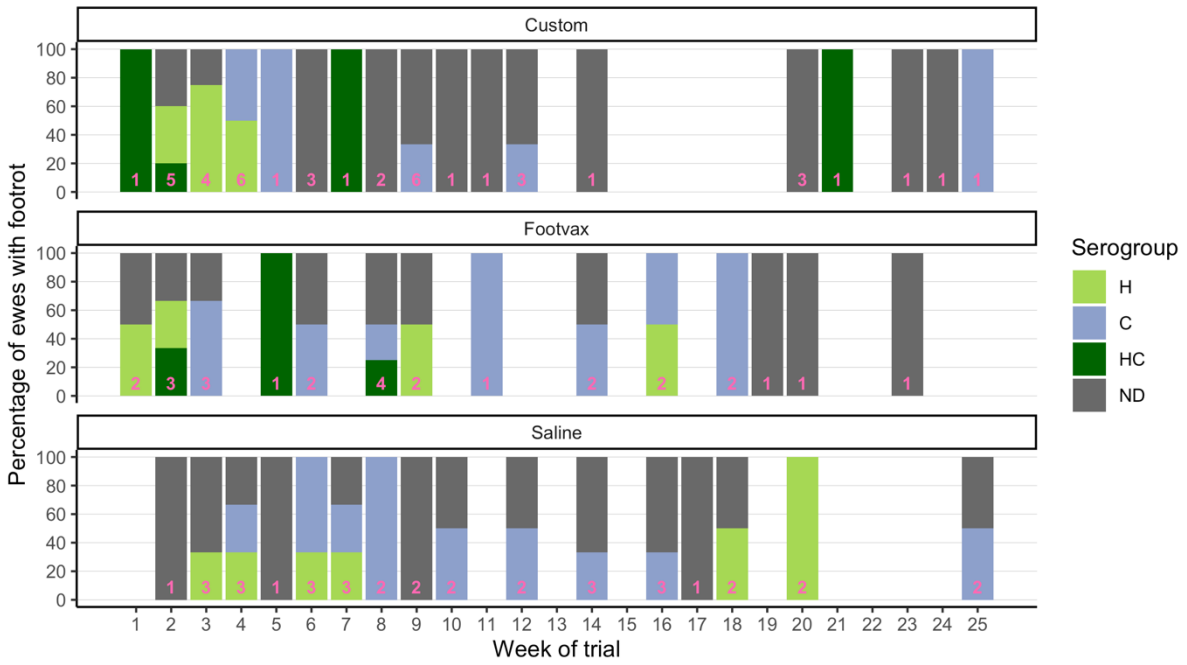

Figure 6-9 Relative percentage of ewes lame with footrot with serogroup H, C, both or neither BVI serogroup (ND) by treatment group in flock 3 (number in column = denominator)

6.2 Discussion

The aim of sampling footrot lesions throughout the trial was to detect any changes in the prevalence of the serogroups targeted by the bivalent vaccines compared to those which were not targeted. The hypothesis was that perturbation would occur in clinical footrot cases so that the targeted serogroups would be detected with decreasing frequency, possibly to be replaced by the non BVI serogroups. A perturbation effect was not observed in the results generated by the trial because the small number of footrot cases in the vaccinated ewes in flock 1, and in flocks 2 and 3 generally, prevented the interpretation of differences in serogroup prevalences between the treatment groups with any certainty. Additionally, swabs taken at the exit examination of ewes could not be directly compared to

the week 1 and 17 examinations because the exit swabs were not taken at a single timepoint and the post-vaccination duration of immunity was unknown.

Furthermore, there were limitations to the interpretation of the serogroup data caused by the study design. Equal exposure to serogroups between treatment groups was facilitated by allowing the ewes to mix together. BVI serogroups were detected in Custom-R Pilus vaccinated ewes that were lame with footrot in all three flocks throughout the trial. However, it was not possible to test the relative quantity of individual serogroups when multiple serogroups were present on a single swab, and therefore interpret the importance of individual serogroups in causing clinical disease. The detection of BVI serogroups on the feet of Custom R-Pilus vaccinated ewes could have been due to surface contamination of bacteria from the environment that they shared with non-vaccinated ewes. Indeed, it was evident from the results a random selection of lame ewes without footrot lesions that *D. nodosus* was usually present on the interdigital skin but at a lower bacterial load than on the feet of ewes clinically affected by footrot. Therefore, cases of footrot in Custom R-Pilus vaccinated ewes in which BVI serogroups were detected could have been caused by non BVI serogroups. A trial in which treatment groups remain separate and there is not the opportunity for indirect transmission of serogroups between groups would be necessary to detect serogroup perturbation with confidence. Such a trial would be strengthened further by lasting beyond the anticipated duration of immunity from the primary vaccination course and involving whole flock examinations with testing of swabs for all serogroups on multiple occasions.

CHAPTER 7 DISCUSSION, CONCLUSION, APPLICATION OF FINDINGS AND FUTURE RESEARCH

7.1 Key outcomes from the clinical trial

7.1.1 The effect of targeted bivalent serogroup vaccination on the incidence of lameness and footrot in commercial flocks

The trial demonstrated that targeted bivalent vaccination for footrot was an effective strategy for reducing the proportion of the flock that experienced lameness with footrot under commercial sheep farming conditions. The existing multivalent vaccine, Footvax®, was equally protective as targeted vaccination, both strategies reducing the number of ewes affected by footrot by approximately 40% (Table 4-21). Targeted bivalent vaccination was also effective in reducing the incidence of lameness due to all causes, including footrot, compared to the saline control and performed better than Footvax® when compared to the saline control (Table 4-18). However, the response to vaccination was heterogenous between flocks and within sub-populations of the flocks, which limits the generalisability of the study findings.

7.1.2 The prevalence and persistence of injection site reactions in Custom R-Pilus and Footvax® treated ewes

Injection site reactions were largest in ewes vaccinated with Footvax®, which were at three times the odds of developing a reaction than ewes vaccinated with the Custom R-Pilus vaccine. No injection site reactions were >50mm at any measurement timepoint, and most

were <20mm by 12 weeks, but injection site reactions persisted to at least 20 weeks after vaccination in both vaccine groups.

7.2 General discussion and limitations of the study

The main objective of the clinical trial was to investigate how protective targeted serogroup vaccines are for the control of lameness, and lameness with footrot, in commercial flock conditions alongside existing treatment and management measures. There was a flock specific response to intervention because the bivalent vaccine only had a significant effect when the prevalence of footrot was high. Consequently, targeted vaccination was protective in flock 1, where footrot was the most common cause of lameness, and the mean prevalence of lameness was high at 6.2%. In contrast, there was no overall benefit from either the bivalent or multivalent vaccine in flock 3, which had a higher lameness prevalence (7.3%) than flock 1, but in which lameness was caused by multiple infectious and non-infectious conditions (section 4.3.3.3). For comparison, surveys of British farmers for the 2017- 2018 season and UK farmers for the 2018-2019 winter period both reported a prevalence of lameness in ewes of >5% in approximately 20% of flocks (Best et al., 2020, Lewis et al., 2021). In both surveys approximately 40% of flocks had a prevalence of lameness of <2%, below the prevalence in flock 2, in which vaccination had no effect. This means that the percentage of flocks in which the vaccine is likely to have a significant impact is relatively small. Furthermore, it is not known how generalisable the results are from flock 3, which had a high prevalence of lameness. In the 2018 survey by Lewis et al. (2021), footrot was still the most common cause of lameness, with ID and SFR reported in 87.8% and 75.3% of flocks respectively. However, mean flock lameness prevalence has fallen over

the last two decades with the adoption of better control measures for footrot (Prosser et al., 2019, Winter et al., 2015), and it is possible that causes of lameness other than footrot are responsible for a greater proportion of lameness cases, as seen in flock 3.

Multiple serogroups were detected in all the flocks when they were sampled in 2019, yet it was possible to identify at least one of the most dominant serogroups in all three flocks by sampling 21 diseased ewes. Consequently, it is likely that 21 swabs from sheep with footrot are sufficient to select two serogroups for an effective flock specific bivalent vaccine, provided that the flock is managed as a single epidemiological unit. Many more samples would be required to increase the accuracy of the prevalence estimates, increasing the cost of serogroup screening without improving the expected response to targeted vaccination.

The bivalent vaccine was as protective as the multivalent vaccine for reducing the incidence of footrot when there were multiple serogroups in a flock. The bivalent vaccine was apparently most protective in flock 1, the only flock in which the two most prevalent serogroups were identified in 2019. However, flock 1 also had the highest prevalence of footrot and this is likely to have been more influential in the response to vaccination than the number of serogroups present in a flock. Footrot incidence was equivalent between treatment groups in flock 2 in which cases of footrot almost exclusively carried the bivalent vaccine serogroups.

The difference in responses to the bivalent and multivalent vaccines in the two sub-populations of flock 1 was an unexpected and interesting finding that warrants further investigation. The incidence of lameness was approximately 2.5 times as high in the

Purchased ewes as the Existing ewes, and the incidence of footrot was approximately seven times as high. The Purchased ewes in flock 1, were all the same age, sourced from a single flock, and included an unknown number of paternal half-siblings. A better understanding of the factors that caused the difference in response might enable more targeted, cost effective use of each vaccine.

One hypothesis for the lack of protection provided by the bivalent vaccine in Purchased ewes is that they were immunologically naïve to the other serogroups present on the farm and it was these serogroups that caused lameness in the Purchased ewes. Serogroup H, contained in the bivalent vaccine, was detected in most of the footrot cases in Custom-R Pilus vaccinated Purchased ewes. Indeed, the bivalent vaccine serogroups did not disappear from the feet of ewes with footrot that had received the Custom R-Pilus vaccine in any of the flocks, but it was not possible to differentiate the presence of serogroups involved in the pathogenesis of the footrot from contaminants because ewes from all treatment groups were kept together. In general, there were insufficient samples from footrot cases to investigate changes in serogroup distribution with any certainty, this was in part because vaccination reduced the number of footrot cases and because of the low prevalence of footrot in two flocks.

Footvax® prevented lameness in more than 50% of Purchased ewes, yet certain individuals were repeatedly lame with footrot, suggesting that the vaccine was not protective at all in these ewes. The repeated cases of footrot that affected some Purchased ewes might have been due to differences in immune response between individuals, both to natural infection with footrot and to vaccination (Amend et al., 2021, Raadsma et al., 1999, Raadsma et al.,

2018). Variation in the strength of immune response might have been less likely in the Existing ewes because 105/114 ewes were over 3.5 years old and the farmer might have culled some ewes with repeated episodes of lameness previously, although they did not have a strict culling policy for lameness. However, ewes with chronic foot deformities that were at a higher risk of recurrent footrot (Kaler et al., 2010b) were excluded from the current trial.

Footvax® was more effective at preventing footrot in the Existing ewes than in the Purchased ewes and fewer Existing ewes were repeatedly lame. The Existing ewes had been vaccinated with Footvax® previously but not for at least 18 months. Schwartzkoff et al. (1993b) reported that sheep vaccinated with a recombinant fimbrial vaccine containing serogroups A-I for a third time 52 weeks after two vaccinations given four weeks apart had a much higher agglutinating antibody titre, indicating a better response to the third dose of vaccine. Both vaccines in this study contained recombinant fimbrial protein and both could have induced an amnestic response to previous vaccination with Footvax®, and the stronger response to the bivalent vaccine might be expected because of reduced antigenic competition compared to Footvax® (Schwartzkoff et al., 1993a). Unfortunately, the purchased ewes were different crossbreeds to the main flock in both flocks 1 and 3. The result was age and previous vaccination status were confounded with breed and origin in flock 1, and so the individual attribution of these variables to the contrasting responses to the vaccines in flock 1 is unknown.

Previous studies have reported a lower prevalence of lameness in flocks that have used Footvax® for 5 years or more than in flocks that have used Footvax® for less than a year or

are unvaccinated (Best et al., 2020, Lewis et al., 2021, Prosser et al., 2019). The current study supports two possible explanations for this: The first is that the amnestic response to vaccination results in cumulative levels of protective antibody and therefore a stronger immune response with each year of vaccination. The second is that flocks that combine vaccination and culling ewes that are repeatedly lame to control footrot will select ewes that have a strong immune response to Footvax®. This is supported by evidence that farmers using Footvax® are more likely to cull repeatedly lame sheep than farms that do not vaccinate against footrot (Best et al., 2020). Selecting replacement ewes from those that never have footrot will also increase the percentage of the flock resistant to footrot, although the ability to identify susceptible ewes in a self-replacing flock is diminished as the consequent decrease in environmental exposure to *D nodosus* starts to drive lower expression of footrot across the flock (Nieuwhof et al., 2009).

The agricultural sector is encouraging farmers to increase the use of vaccines to improve animal health and welfare and consequently to improve 'One Health' and the sustainability of antibiotic use across animals and people (NOAH, 2022). In flock 1 there was approximately 33% less antibiotic used in vaccinated compared to unvaccinated ewes. If this result is generalisable it is significant to the sheep industry. Lameness accounted for 65.5% of antibiotic usage in the most recent survey of British sheep flocks (Davies et al., 2017) so reducing the amount of antibiotic needed to treat lameness would have a large impact on overall usage in the sheep sector. However, the mean prevalence of lameness in flock 1 was 6.2%, compared to a mean of 3.5% across English flocks in 2013 (Winter et al., 2015) and, more recently, only 1.4% in British flocks (Lewis et al., 2021). This makes flock 1

representative of a relatively small proportion of the flocks nationally, although farmers tend to slightly underestimate lameness prevalence in their flocks (King and Green, 2011). In the other two flocks, in which footrot prevalence was lower, the difference in antibiotic usage between vaccinated and unvaccinated groups was smaller, to the extent that there was no significant difference between groups when flock incidence of footrot was approximately one case per 100 ewe-weeks. These findings highlight the need for a thorough understanding of the cause and severity of lameness problems in individual flocks before introducing vaccination as part of a lameness control programme. This approach contradicts recently published guidelines for vaccination in livestock that advises the use of Footvax® as a core vaccine in all adult sheep in flocks that purchase replacement breeding stock (NOAH, 2022).

The percentage of ewes vaccinated with Footvax® with injection site reactions at the final measurement on week 20 was 20.2%, comparable with Ennen et al. (2009). The percentage of Custom R-Pilus vaccinated ewes with injection site reactions was 9.7% at week 20 and only 2.2% of ewes had reactions that were >20mm, compared to 5.5% of ewes that were vaccinated with Footvax®. All ewes were vaccinated with sterile needles by a single researcher and were housed before treatment to minimise the inadvertent introduction of bacterial contaminants from wet skin, therefore most injection site reactions were likely to be type IV hypersensitivity reactions (Tizard, 2019). The higher risk of injury to sheep during shearing and the additional losses from carcass trimming in sheep with injection site abscesses due to Footvax® are arguments in favour of using the Custom R-Pilus vaccine. Although careful and correct use of the vaccine would reduce losses from carcass trimming

(Eppleston and Windsor, 2007), incorrect administration of vaccines is widespread among UK sheep farmers. For instance, in a recent survey only 26.1% of farmers identified the correct location to inject a subcutaneously administered vaccine (Hall et al., 2022) Additionally, the potential for injury to the farmer whilst injecting the vaccine is an important consideration because the potential consequences are severe from self-injection with Footvax® (NOAH, 2021). Custom R-Pilus, whilst not innocuous, does not carry such severe warnings for the user on the material safety datasheet as that for Footvax®.

7.3 Application of the trial outcomes and direction for future research

The study demonstrated that the bivalent vaccine could contribute to control of footrot in English sheep flocks, together with other management practices. The overall performance of the bivalent vaccine was marginally better than Footvax® over the relatively short period of the trial. Further investigation is required to establish whether serial bivalent vaccination with different serogroups, which is used in elimination programmes (Dhungyel and Whittington, 2010), would result in a further reduction in incidence of footrot.

In this study, ewes from all treatment groups were kept together to expose all sheep to all *D. nodosus* serogroups throughout the trial. Mixed groups also enabled the double blinded trial design to minimise bias and made participation from farmers feasible because farmers were unable to farm three groups of sheep separately for 6 months. Further assessment of the impact of bivalent vaccination in a flock with multiple serogroups requires a longer trial so that the serogroups can be sequentially targeted. A next study could be a before and after (step wedge) clinical trial with whole group vaccination to investigate herd immunity,

changes in serogroup prevalence and to estimate the impact of whole flock vaccination on antibiotic usage.

Targeted, bivalent footrot vaccination is currently a costly alternative to Footvax®. The vaccines are not licensed or manufactured in the UK and targeted vaccination requires PCR testing of foot swabs, which is not commercially available in the UK. Licensing, import and laboratory costs along with increased handling of the flock to sample and vaccinate add significant expense to the vaccination strategy, which does not make it realistic as a long-term control measure. Furthermore, in the current study, survival curves showed that protection from Footvax® commenced after the first injection in flock 1, including in Purchased ewes that had not been vaccinated previously. A single dose of Footvax® given before high risk periods might prove more cost effective than an initial course of two injections, in cost of product and time taken to handle the flock. A long term intervention study that combines vaccination with Footvax® with culling of non-responders and selection of vaccine responders as replacements would provide a better understanding of the heritable response to footrot vaccination. Comparison of these interventions in partially and fully self-replacing flocks would provide valuable insight into the practical application of this strategy for the control of footrot.

7.4 Conclusion

Targeted bivalent vaccination against footrot was effective in reducing the incidence of lameness in a commercial flock in which lameness was mainly caused by footrot. Fewer, smaller injection site reactions occurred in sheep vaccinated with the Custom R-Pilus vaccine compared to Footvax®. There was no clinically significant difference in performance

between the bivalent and multivalent vaccines in the reduction of footrot incidence. The study demonstrated the diversity in aetiology and prevalence of both infectious and non-infectious causes of lameness in commercial flocks in England, and the need for vaccination recommendations to be tailored to individual flocks. The generalisation of lameness control recommendations to include vaccination based on lameness prevalence alone could result in unnecessary costs to the farmer.

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Appendix 1 Source of *D. nodosus* strains for use as positive controls for serogroup PCR

Table A- 1 Source of D. nodosus strains for use as positive controls for serogroup PCR

Serogroup	Strain and clone ID	Source
A	3LR 50.1	UK - Clifton et al. (2019)
B	12 20.3	UK -Muzafar et al. (2015)
C	C305 50.1	Australia
D	20 20.1	UK - Muzafar et al. (2015)
E	E ME 50.1	UK – University of Bristol
F	JIR3568 20.3	Australia
G	VCS ME 20.2	Australia
H	EDH 20.5	UK - Smith et al. (2017)
I	EDI 75.1	UK - Smith et al. (2017)

Appendix 2 Confidence intervals for serogroup prevalence estimations in 2019

Table A- 2 Prevalence and 95% confidence intervals for D. nodosus serogroups in ewes with footrot in flock 1 in 2019

Serogroup	Prevalence	Lower limit	Upper limit
H	0.76	0.53	0.92
I	0.67	0.43	0.85
E	0.19	0.05	0.42
A	0.10	0.01	0.30
B	0.05	0.001	0.24

Table A- 3 Prevalence and 95% confidence intervals for D. nodosus serogroups in ewes with footrot in flock 2 in 2019

Serogroup	Prevalence	Lower limit	Upper limit
H	0.52	0.30	0.74
A, F	0.24	0.08	0.47
B, D	0.05	0.001	0.24

Table A- 4 Prevalence and 95% confidence intervals for D. nodosus serogroups in ewes with footrot in flock 3 in 2019

Serogroup	Prevalence	Lower limit	Upper limit
H	0.70	0.46	0.88
C	0.60	0.36	0.81
B	0.20	0.06	0.44
F, I	0.05	0.001	0.25

Table A- 5 Prevalence and 95% confidence intervals for D. nodosus serogroups in all individually sampled ewes and lambs with footrot in flock 2 in 2019

Serogroup	Prevalence	Lower limit	Upper limit
H	0.63	0.49	0.75
A	0.32	0.20	0.46
F	0.27	0.16	0.40
B	0.23	0.13	0.36
D	0.02	0.0004	0.10

Appendix 3 Serogroup combinations detected on interdigital swabs in 2020

Table A- 6 Serogroup combinations detected on interdigital swabs taken from ewes with footrot on week 1 of the trial

Flock 1		Flock 2		Flock 3	
Serogroups	Frequency	Serogroups	Frequency	Serogroups	Frequency
H	10	H	9	H	2
AHI	1	AH	3	CH	1
BH	1	A	1	BCH	3
CH	1			ABHI	1
HI	6			HF	2
I	1			CF	1
B	1			F	1
AB	1				
None detected	6	None detected	3	None detected	3
Total	28	Total	16	Total	15

Appendix 4 Details of treatment and control products

Table A- 7 Composition, authorisation and pharmaceutical particulars for the first treatment product

Product Name	Custom R-Pilus footrot vaccine		
Manufacturer	Treidlia Biovet		
Country	Australia		
MA/Vm number	NA- minor use exemption		
Species	Sheep		
Pharmaceutical Form	Emulsion for injection		
Withdrawal period	28 days		
Storage	2-8^{o}C		
Packaging	10 x 20 dose packs for each farm. Total of 600 doses.		
Qualitative composition	Recombinant pilus vaccine containing pili from two *D. nodosus* serogroups		
	Flock 1 H +I	Flock 2 H+A	Flock 3 H+C
Batch protocol/certificate	Provided to the VMD before the start of the trial		

Table A- 8 Composition, authorisation and pharmaceutical particulars for the second treatment product (NOAH, 2021)

Product Name	Footvax
MA holder	MSD
Country	UK
MA/Vm number	01708/4553
Species	Sheep
Pharmaceutical Form	Emulsion for injection
Qualitative and quantitative composition	Each dose (1 ml) contains 10 µg pili of each of *Dichelobacter nodosus* serotypes A, B1, B2, C, D, E, F, G and H and 5 x 10^{8} cells of *D. nodosus* serotype I. 60% Light mineral oil NF and 4.5% Manide oleate are added as adjuvants. 0.015 % Thiomersal is added as a preservative.
Withdrawal period	Nil
Storage	2-8^{o}C
Batch numbers and expiry date	2243A/008 expiry 31/10/2020

Table A- 9 Composition, authorisation and pharmaceutical particulars for the negative control product (NOAH, 2016)

Product Name	Aqupharm No.1 Infusion
MA holder	Animalcare Limited
Country	UK
MA/Vm number	10347/4038
Species	Cats, Cattle, Dogs, Goats, Horses and other equidae, Pigs, Rabbits, Sheep
Pharmaceutical Form	Clear, colourless particle free solution.
Qualitative and quantitative composition	Each ml contains 9mg Sodium Chloride
Withdrawal period	Nil
Storage	500 ml bags do not require any special storage conditions.
Batch numbers and expiry date	19040852 expiry 03/2022

Appendix 5 SOP for administration of investigational products and control products

1) Use only 20-25ml dose containers for the trial product and positive control vaccine to avoid wastage. Broached containers must be used on the day of broaching.
2) Gather ewes into secure holding area. If rain is forecast on day of planned vaccination or the day before, ewes will be housed overnight before the day of vaccination.
3) Remove one container of each vaccine from cold storage and allow to warm up to ambient temperature.
4) Assistant to catch ewe and restrain securely, holding the ewe against a solid wall or hurdle and immobilising the head.
5) Scan ear tag with EID reader to ensure ewe has not already been treated. Double check eartag number against spray mark to confirm identity.
6) Trained personnel responsible for drawing up vaccine will check ewe ID against allocated treatment and pass the concealed dose of treatment to the investigator.
7) Inject under the skin of the right side of neck on Week 1 (Dose 1) and left side of the neck on Week 5 (Dose 2), approximately 2-3" behind the ear (Figure A-1).
8) Release ewe into post vaccination pen and observe for signs of injury or distress.
9) Dispose of used needle and syringe in pharmaceutical bin without separating or replacing needle cap.
10) Dispose of empty packaging and unused product in pharmaceutical bin, recording the details of wasted product on a sheet to be returned to the licensed waste contractor with the bin.

11) Once all ewes have been vaccinated and have been checked again for signs of injury and distress, release to pen or field.

12) Observe ewes for adverse reactions for a further two hours after the last treatment dose administration.

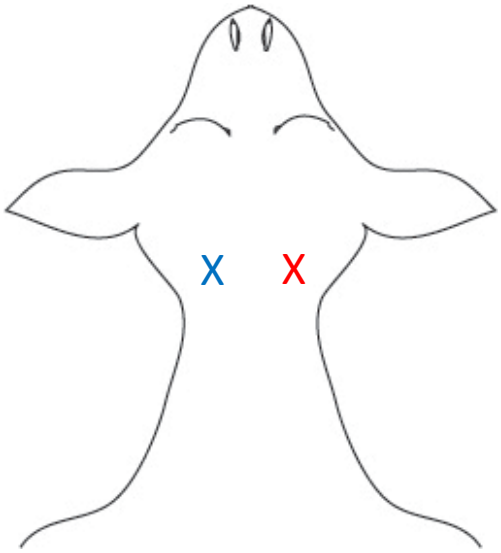

Figure A- 1 Site of injection for vaccines on Week 1 (X) and Week 5 (X)

Appendix 6 SOP for measurement and recording of injection site reactions

1) Assistant securely restrains the sheep in a standing position in the race.
2) Secure the swelling by gathering any slack skin beneath it with one hand. Use Vernier callipers to measure across the widest part of the swelling in mm. Several measurements may be required to ascertain the measurement.
3) Use the EID reader to record the measurement against the individual ear tag number along with the location of the swelling using the three-letter code based on the position on the neck (Figure A- 2).
4) Record if the lesion is discharging.

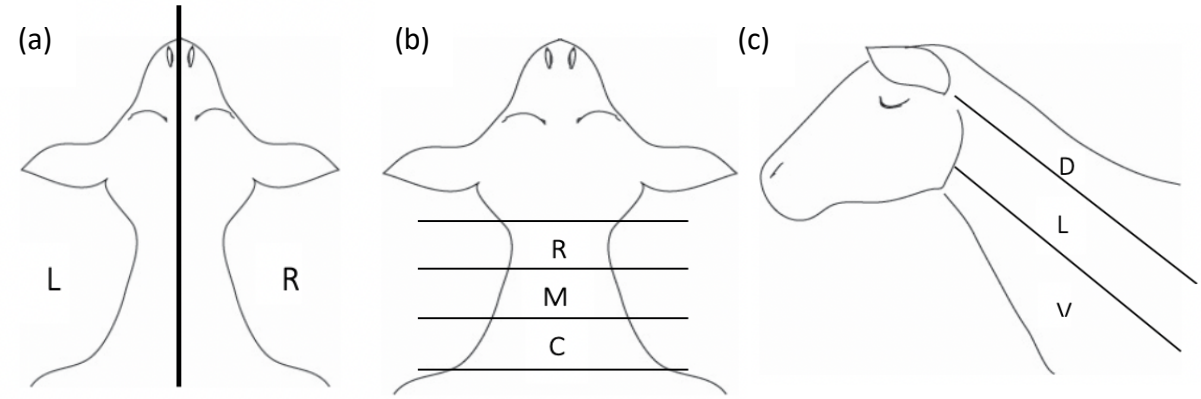

Figure A- 2 Code for recording the site of injection reactions (a) Left (L) or right (R); (b) Rostral (R), Mid (M) or Caudal (C); (c) Dorsal (D), Lateral (L) or Ventral (V).

Appendix 7 Other scoring scales used to record observations during the trial

Table A- 10 Contagious Ovine Digital Dermatitis (CODD) scoring scale reproduced from Angell et al. (2015a)

Score	Description of lesion
0	No CODD lesions
1	Erosion/ulceration with or without alopecia specifically at the level of the dorsal coronary band
2	Erosion/ulceration of the skin at the coronary band with partial (<50%) underrunning of the hoof horn dorsally, abaxially and tending towards circumferential underrunning
3	Erosion/ulceration of the skin at the coronary band with 50-100% underrunning of the hoof horn with possible hoof horn avulsion.
4	Healing foot with the horn beginning to regrow but an active lesion still present
5	Healed foot, often with deformation of the regrown horn

Table A- 11 Body condition scoring scale reproduced from AHDB (2019)

Score	Description of body condition
1	The spinous and transverse processes are prominent and sharp. The fingers can be pushed easily below the transverse bone and each process can be felt. The loin is thin with no fat cover.
2	The spinous processes are prominent but smooth, individual processes being felt only as corrugations. The transverse processes are smooth and rounded, but it is still possible to press fingers underneath. The loin muscle is a moderate depth but with little fat cover.
3	The spinous processes are smooth and rounded; the bone is only felt with pressure. The transverse processes are also smooth and well-covered; hard pressure is required with the fingers to find the ends. The loin muscle is full and with moderate fat cover.
4	The spinous processes are only detectable as a line. The ends of the transverse processes cannot be felt. The loin muscles are full and rounded and have a thick covering of fat.
5	The spinous and transverse processes cannot be detected even with pressure; there is a dimple in the fat layers where the processes should be. The loin muscles are very full and covered with very thick fat.

Appendix 8 **Stratification of treatment groups**

Table A- 12 Stratification of treatment groups by breed, BCS, footrot score, CODD presence and age for flock 1

	Treatment group				
	Footvax (N=58)	Saline (N=62)	Custom R-Pilus (N=55)	Total (N=175)	p value
Breed					0.946†
Bluefaced Leicester cross	20 (34.5%)	21 (33.9%)	19 (34.5%)	60 (34.4%)	
Charollais cross	8 (12.1%)	9 (14.5%)	7 (12.7%)	23 (13.1%)	
Clun cross	1 (1.7%)	1 (1.6 %)	1 (1.8%)	3 (1.7%)	
Suffolk cross	29 (50.0%)	30 (48.4%)	28 (50.9%)	87 (49.7%)	
Dorset cross	0 (0.0%)	1 (1.6%)	0 (0.0%)	1 (0.6%)	
Texel cross	1 (1.7%)	0 (0.0%)	0 (0.0%)	1 (0.6%)	
BCS					0.682†
<2.5	10 (17.2%)	9 (14.5%)	7 (12.7%)	26 (14.9%)	
2.5-3.5	48 (82.8%)	52 (83.9%)	48 (87.3%)	148 (84.6%)	
>3.5	0 (0.0%)	1 (1.6%)	0 (0.0%)	1 (0.6%)	
Footrot score group					0.816†
1	49 (84.5%)	51 (82.3%)	50 (90.9%)	150 (85.7%)	
2	4 (6.9%)	5 (8.1%)	3 (5.5%)	12 (6.9%)	
3	4 (6.9%)	4 (6.5%)	2 (3.6%)	10 (5.7%)	
4	1 (1.7%)	2 (3.2%)	0 (0.0%)	3 (1.7%)	
CODD (score 1-5)					0.758†
Yes	7 (12.1%)	5 (8.1%)	6 (10.9%)	18 (10.3%)	
No	51 (87.9%)	57 (91.9%)	49 (89.1%)	157 (89.7%)	
Age					0.731†
2T	24 (41.4%)	23 (37.1%)	20 (36.4%)	67 (38.3%)	
4T	2	1	0	3	

	(3.4%)	(1.6%)	(0.0%)	(1.7%)
6T	5 (8.6%)	9 (14.5%)	4 (7.3%)	18 (10.3%)
FM	25 (43.1%)	25 (40.3%)	27 (49.1%)	77 (44.0%)
BM	2 (3.4%)	4 (6.5%)	4 (7.3%)	10 (5.7%)

† Pearson's Chi-squared test

Table A- 13 Stratification of treatment groups by breed, BCS, footrot score, CODD presence and age for flock 2

	Treatment group			Total	
	Custom R-Pilus (N=67)	Footvax (N=67)	Saline (N=63)	(N=197)	p value
Breed					0.922†
North Country Mule	67 (100.0%)	67 (100.0%)	63 (100.0%)	197 (100.0%)	
BCS					1.000†
<2.5	13 (19.4%)	12 (17.9%)	12 (19.0%)	37 (18.8%)	
2.5-3.5	53 (79.1%)	54 (80.6%)	50 (79.4%)	157 (79.7%)	
>3.5	1 (1.5%)	1 (1.5%)	1 (1.6%)	3 (1.5%)	
Footrot score					0.724†
1	61 (91.0%)	62 (92.5%)	58 (92.1%)	181 (91.9%)	
2	3 (4.5%)	4 (6.0%)	2 (3.2%)	9 (4.6%)	
3	2 (3.0%)	1 (1.5%)	3 (4.8%)	6 (3.0%)	
4	1 (1.5%)	0 (0.0%)	0 (0.0%)	1 (0.5%)	
CODD					0.377†
Yes	0 (0.0%)	1 (1.5%)	0 (0.0%)	1(0.5%)	
No	67 (100.0%)	66 (98.5%)	63 (100.0%)	196 (99.5%)	
Age					0.201†
4T	7 (10.4%)	5 (7.5%)	1 (1.6%)	13 (6.6%)	
6T	27 (40.3%)	23 (34.3%)	33 (52.4%)	83 (42.1%)	
Full Mouth	31 (46.3%)	34 (50.7%)	26 (41.3%)	91 (46.2%)	
Broken Mouth	2 (3.0%)	5 (7.5%)	3 (4.8%)	10 (5.1%)	

† Pearson's Chi-squared test

Table A- 14 Stratification of treatment groups by breed, BCS, footrot score, CODD presence and age for flock 3

	Treatment group			Total	
	Custom R-Pilus (N= 67)	Saline (N= 67)	Footvax (N= 62)	(N= 196)	p value
Breed					0.933[†]
Charollais cross	2 (3.0%)	2 (3.0%)	1 (1.6%)	5 (2.6%)	
Exmoor Mule	9 (13.4%)	9 (13.4%)	8 (12.9%)	26 (13.3%)	
North Country Mule	21 (31.3%)	20 (29.9%)	19 (30.6%)	60 (30.6%)	
Suffolk Cross	0 (0.0%)	1 (1.5%)	0 (0.0%)	1 (0.5%)	
Texel cross	34 (50.7%)	35 (52.2%)	34 (54.8%)	103 (52.6%)	
BCS					0.994[†]
<2.5	24 (35.8%)	22 (32.8%)	21 (33.9%)	67 (34.2%)	
2.5-3.5	34 (50.7%)	35 (52.2%)	33 (53.2%)	102 (52.0%)	
>3.5	9 (13.4%)	10 (14.9%)	8 (12.9%)	27 (13.8%)	
Footrot score					0.750[†]
1	63 (94.0%)	62 (92.5%)	58 (93.5%)	183 (93.4%)	
2	1 (1.5%)	1 (1.5%)	2 (3.2%)	4 (2.0%)	
3	2 (3.0%)	4 (6.0%)	2 (3.2%)	8 (4.1%)	
4	1 (1.5%)	0 (0.0%)	0 (0.0%)	1 (0.5%)	
CODD					0.792[†]
Yes	1 (1.5%)	2 (3.0%)	2 (3.2%)	5 (2.6%)	
No	66 (98.5%)	65 (97.0%)	60 (96.8%)	191 (97.4%)	
Age					0.258[†]
Ewe lamb	0 (0.0%)	0 (0.0%)	1 (1.6%)	1 (0.5%)	
2T	26 (38.8%)	33 (49.3%)	25 (40.3%)	84 (42.9%)	
4T	0 (0.0%)	1 (1.5%)	4 (6.5%)	5 (2.6%)	
6T	4 (6.0%)	4 (6.0%)	3 (4.8%)	11 (5.6%)	
Full mouth	35 (52.2%)	25 (37.3%)	28 (45.2%)	88 (44.9%)	

Broken Mouth	2 (3.0%)	4 (6.0%)	1 (1.6%)	7 (3.6%)

† Pearson's Chi-squared test

Appendix 9 Withdrawal of ewes from the trial

Table A- 15 Reason for withdrawal of ewes from the trial before week 25 in all three flocks

Ewe number	Treatment group	Week of exclusion	No. of weeks lame (loco score >1)	Max. no. of continuous weeks lame	Reason for exclusion
Flock 1					
80	Saline	7	5	2	Repeatedly lame with non-infectious lesions affecting multiple feet.
164	Custom R-Pilus	13	7	6	Chronically lame with swollen left stifle. Died.
72	Saline	15	4	1	Died
56	Footvax	19	2	1	Died
44	Custom R-Pilus	20	4	2	Died
37	Custom R-Pilus	20	6	2	Died
168	Saline	21	8	2	Repeatedly lame with granuloma
54	Saline	21	5	1	Died
63	Saline	22	3	3	Repeatedly lame with granuloma
119	Saline	22	0	0	Died
64	Footvax	24	4	2	Repeatedly lame. Healed CODD lesion.
Flock 2					
82	Saline	21	9	5	Repeatedly lame, cause of pain not visible.
35	Saline	23	0	0	Died
Flock 3					
148	Footvax	17	5	5	Chronic, non-infectious lameness and slow response to treatment.
49	Saline	18	10	4	Repeatedly lame. Mixture of infectious and non-infectious foot lesions.
130	Footvax	19	7	6	Chronically lame with underrun horn, non-responsive to antibiotics.
169	Footvax	19	10	5	Repeatedly lame. Hoof wall lesion or cause of pain not visible.
175	Saline	19	6	3	Repeatedly lame. Hoof wall lesions and granuloma.
154	Saline	22	3	3	Chronic lameness due to hock trauma.
84	Saline	24	6	3	Chronic lameness due to swollen hock.

Appendix 10 Number of ewes at risk of becoming lame or developing footrot

Table A- 16 The mean (SD) and range of number of ewes (percentage of the treatment group) at risk of becoming lame for each week of the trial excluding week 1

	Custom R-Pilus	Footvax	Saline
Flock 1			
Mean (SD)	50 (2) (92.5%)	54 (3) (92.8%)	55 (2) (91.3%)
Minimum	47 (85.5%)	46 (79.3%)	50 (83.3%)
Maximum	54 (100%)	58 (100%)	60 (98.4%)
Flock 2			
Mean (SD)	65 (2) (97.0%)	65 (1) (97.6%)	61 (1) (96.9%)
Minimum	60 (90.0%)	62 (92.5%)	57 (93.4%)
Maximum	67 (100%)	67 (100%)	63 (100%)
Flock 3			
Mean (SD)	61 (4) (91.2%)	57 (3) (92.4%)	59 (4) (89.6%)
Minimum	54 (80.6%)	48 (81.4%)	49 (73.1%)
Maximum	66 (98.5%)	62 (100%)	66 (98.5%)

Table A- 17 The mean (SD) and range of number of ewes (percentage of the treatment group) at risk of developing footrot for each week of the trial excluding week 1

	Custom R-Pilus	Footvax	Saline
Flock 1			
Mean (SD)	51 (2) (94.7%)	55 (2) (94.8%)	56 (2) (92.8%)
Minimum	48 (87.2%)	46 (79.3%)	53 (85.5%)
Maximum	54 (100%)	58 (100%)	60 (98.4%)
Flock 2			
Mean (SD)	66 (2) (97.8%)	66 (1) (98.5%)	62 (2) (98.3%)
Minimum	60 (90.0%)	63 (94.0%)	57 (93.4%)
Maximum	67 (100%)	67 (100%)	63 (100%)
Flock 3			
Mean (SD)	63 (3) (94.2%)	58 (3) (94.8%)	62 (3) (93.7%)
Minimum	56 (83.6%)	52 (88.1%)	49 (73.1%)
Maximum	67 (100%)	62 (100%)	67 (100%)

Appendix 11 Number of foot observations throughout the trial and summary of footrot scores in lame ewes

Table A- 18 Number of foot observations at ewe level throughout the whole trial, during weeks 1-25 concurrent with locomotion scoring (% of all observations), and in lame ewes between weeks 1 and 25 (% of all observations between weeks 1 and 25)

	Flock 1	Flock 2	Flock 3
Total number of foot observations	805	676	973
Number of foot observations in ewes scored for locomotion	636 (79%)	481 (71%)	784 (81%)
No of foot observations in lame (LS >1) ewes	310 (49%)	102 (21%)	388 (49%)

Table A- 19 The number of observations in lame ewes for each ID lesion score and the total number (%) of observations in which ID was present on at least one foot

	ID lesion score					
	0	1	2	3	4	Total ID 1-4 (% lame ewes)
Flock 1	151	43	31	44	41	159 (51.3%)
Flock 2	68	8	3	14	9	34 (33.3%)
Flock 3	309	33	14	24	8	79 (20.4%)

Table A- 20 The number of observations in lame ewes for each SFR lesion score and the total number (%) of observations in which SFR was present on at least one foot

	SFR lesion score					
	0	1	2	3	4	Total SFR 1-4 (% lame ewes)
Flock 1	226	64	16	4	0	84 (27.1%)
Flock 2	83	15	2	2	0	19 (18.6%)
Flock 3	334	25	23	6	0	54 (16.2%)

Table A- 21 The number of observations in lame ewes for each MEFS lesion score, the number (%) of FR observations that did not extend to the sole horn (MEFS scores 1-2), and the total number (%) of observations in which FR was present on at least one foot

	MEFS lesion score						
	0	1	2	3	4	MEFS 1-2 (% of MEFS >0)	Total FR (% of lame ewes)
Flock 1	132	94	64	16	4	158 (88.8%)	178 (57.4%)
Flock 2	63	20	15	2	2	35 (89.7%)	39 (38.2%)
Flock 3	282	52	25	23	6	77 (72.6%)	106 (27.3%)

Appendix 12 ID, SFR and FR prevalence in lame ewes for each treatment group on week 1 of the trial

Table A- 22 ID, SFR and FR prevalence in lame ewes for each treatment group on week 1 of the trial

	Custom	Footvax®	Saline	p value‡
Lame and ID (score 1-4)				
Flock 1	2/55 (3.6%)	5/58 (8.6%)	3/62 (4.8 %)	0.61
Flock 2	4/67 (6.0%)	2/67 3.0%)	1/63 (1.6%)	0.51
Flock 3	1/67 (1.49%)	2/62 (3.2%)	0/67	0.32
Lame and SFR (score 1-4)				
Flock 1	1/55 (1.8%)	4/58 (6.9%)	3/62 (4.8%)	0.49
Flock 2	2/67 (3.0%)	0/67	1/63 (1.6%)	0.54
Flock 3	1/67 (1.5%)	1/62 (1.6%)	0/67	0.77
Lame and FR (any severity)				
Flock 1	2/55 (3.6%)	5/58 (8.6%)	3/62 (4.8%)	0.61
Flock 2	4/67 (6.0%)	2/67 (3.0)	1/63 (1.6%)	0.37
Flock 3	1/67 (1.5%)	2/62 (3.2%)	0/67	0.32

‡ Fisher's exact test

Appendix 13 Weekly incidence plots for lameness and footrot

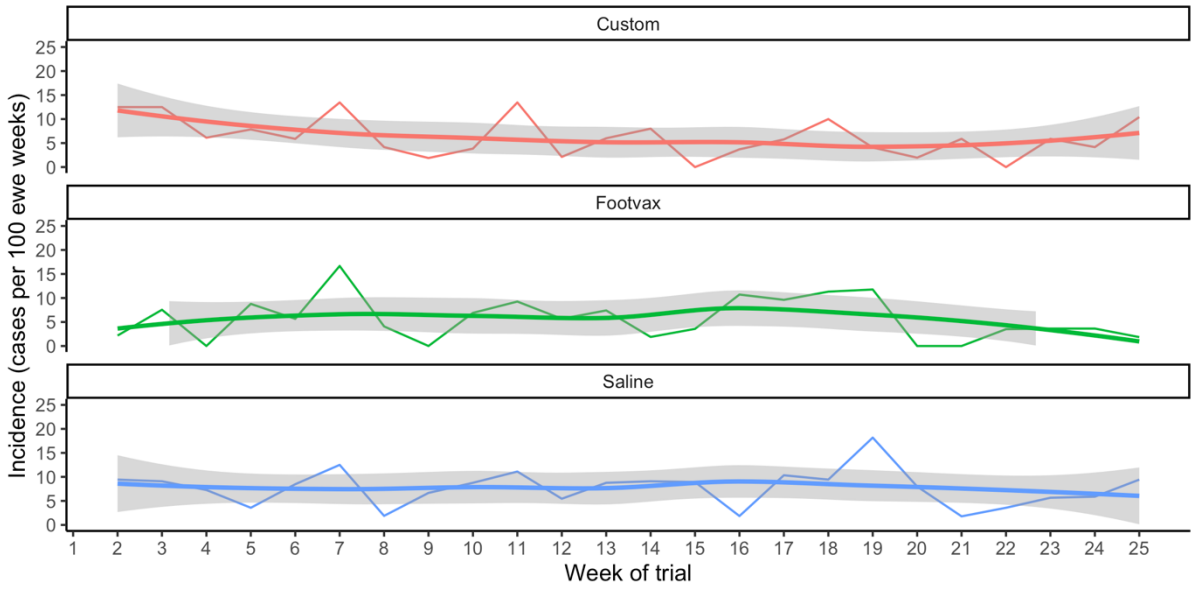

Figure A- 3 Weekly incidence of lameness by treatment group in flock 1. The thin line shows the true incidence, the thick line is fitted using Loess regression for which the 95% CI is shaded in grey.

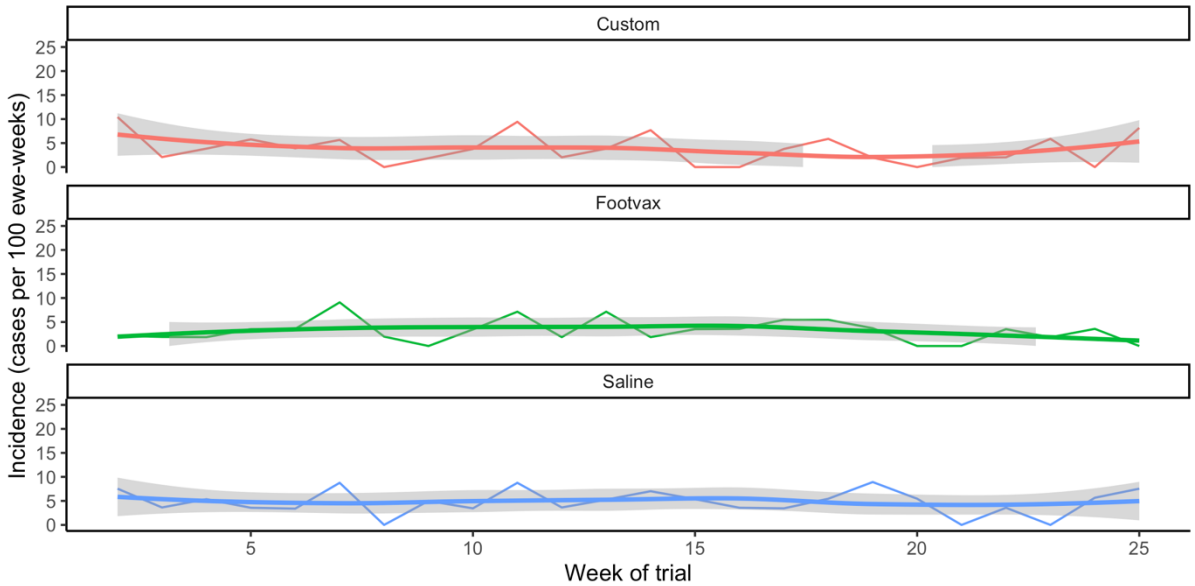

Figure A- 4 Weekly incidence of footrot by treatment group in flock 1. The thin line shows the true incidence, the thick line is fitted using Loess regression for which the 95% CI is shaded in grey.

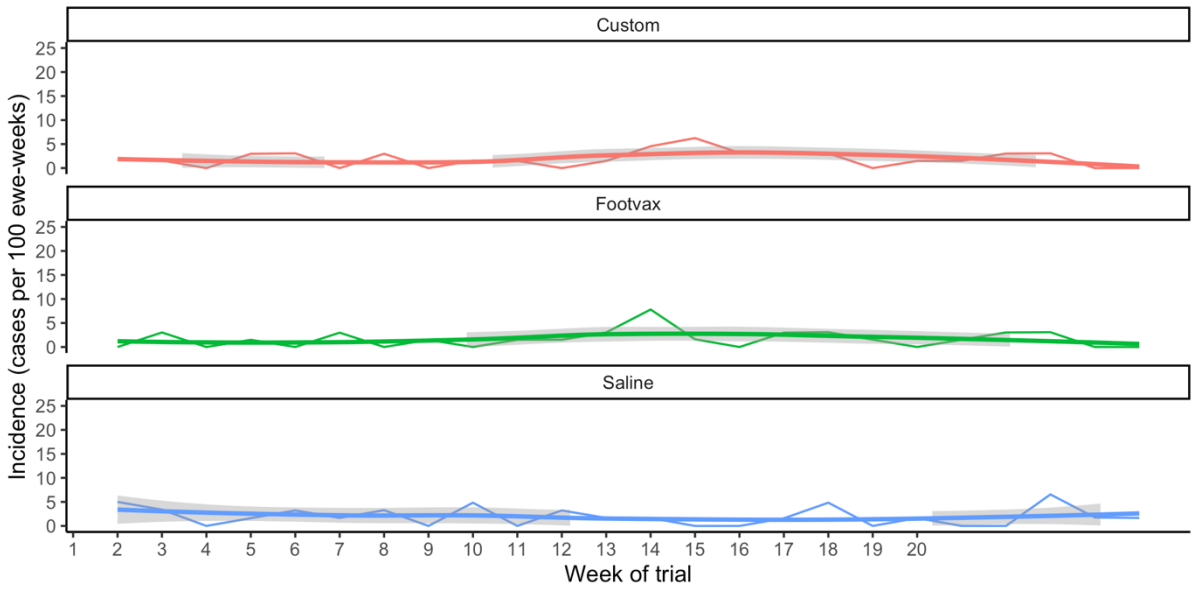

Figure A- 5 Weekly incidence of lameness by treatment group in flock 2. The thin line shows the true incidence, the thick line is fitted using Loess regression for which the 95% CI is shaded in grey.

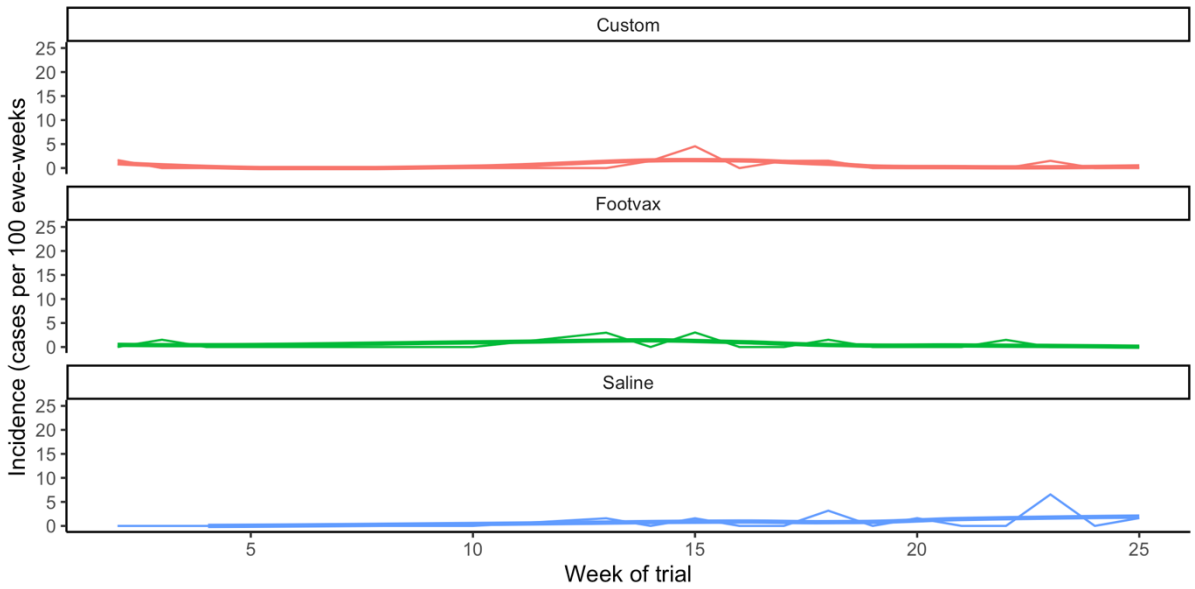

Figure A- 6 Weekly incidence of footrot by treatment group in flock 2. The thin line shows the true incidence, the thick line is fitted using Loess regression.

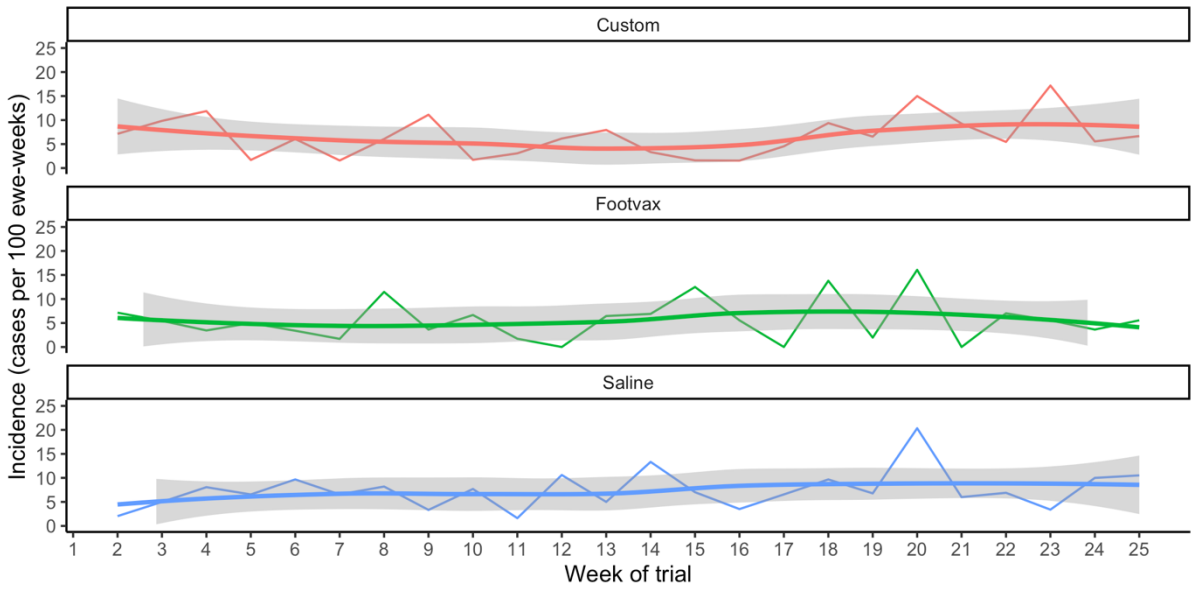

Figure A- 7 Weekly incidence of lameness by treatment group in flock 3. The thin line shows the true incidence, the thick line is fitted using Loess regression for which the 95% CI is shaded in grey.

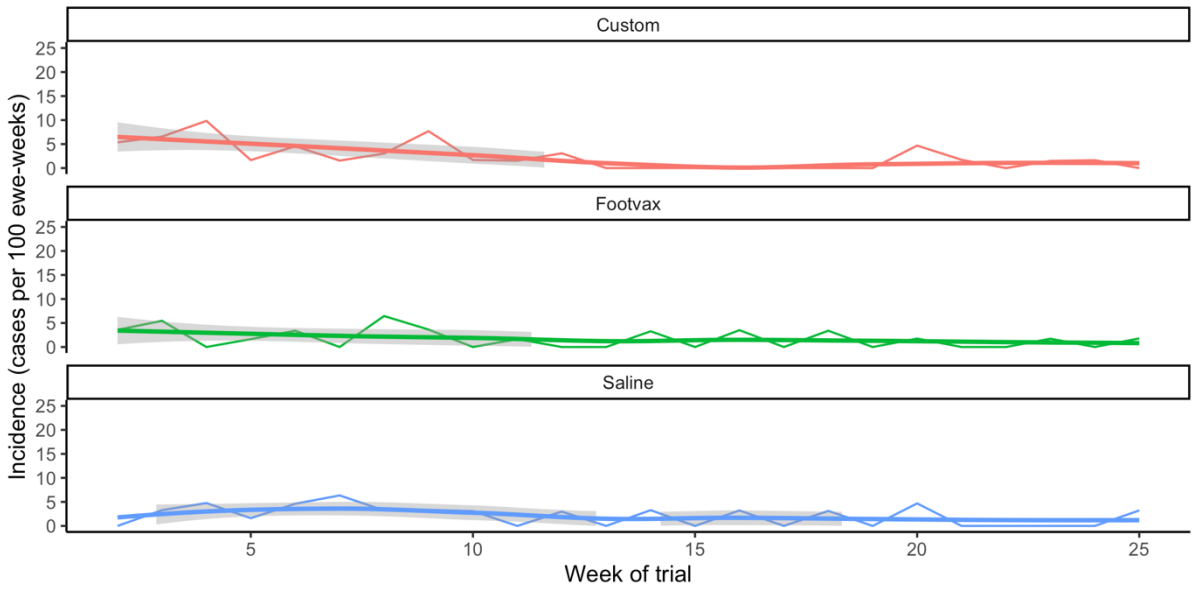

Figure A- 8 Weekly incidence of footrot by treatment group in flock 1. The thin line shows the true incidence, the thick line is fitted using Loess regression for which the 95% CI is shaded in grey.

Appendix 14 Number of lameness and footrot cases per ewe

Table A- 23 The number of lameness cases recorded per ewe between week 7 and 25 of the trial (percentage of all ewes in treatment group)

	Number of lameness cases						Mean
	0	1	2	3	4	5	(SD)
Flock 1							
Custom	28	11	9	4	3	0	1.0
R-Pilus	(50.9%)	(20.0%)	(16.4%)	(7.3%)	(5.5%)		(1.2)
Footvax®	27	14	9	6	0	2	1.0
	(46.6%)	(24.1%)	(15.5%)	(10.3%)		(3.4%)	(1.3)
Saline	14	26	15	4	2	1	1.3
	(22.6%)	(41.9%)	(24.2%)	(6.5%)	(3.2%)	(1.6%)	(1.1)
Flock 2							
Custom	49	13	4	1	0	0	0.4
R-Pilus	(73.1%)	(19.4%)	(6.0%)	(1.5%)			(0.7)
Footvax®	46	19	2	0	0	0	0.3
	(68.7%)	(28.4%)	(3.0%)				(0.5)
Saline	46	14	2	1	0	0	0.3
	(73.0%)	(22.2%)	(3.2%)	(1.6%)			(0.6)
Flock 3							
Custom	21	22	19	4	1	0	1.1
R-Pilus	(31.3%)	(32.8%)	(28.4%)	(6.0%)	(1.6%)		(1.0)
Footvax®	22	22	14	3	1	0	1.0
	(35.5%)	(35.5%)	(22.6%)	(4.8%)	(1.6%)		(1.0)
Saline	19	23	14	7	4	0	1.3
	(28.4%)	(34.3%)	(20.9%)	(10.4%)	(6.0%)		(1.2)

Table A- 24 The number of footrot cases recorded per ewe between week 7 and 25 of the trial (percentage of all ewes in treatment group)

	Number of footrot cases					Mean
	0	1	2	3	4	(SD)
Flock 1						
Custom R-Pilus	37 (67.3%)	10 (18.2%)	3 (5.5%)	3 (5.5%)	2 (3.6%)	0.6 (1.1)
Footvax®	39 (67.2%)	10 (17.2%)	3 (5.2%)	5 (8.6%)	1 (1.7%)	0.6 (1.0)
Saline	27 (44.3%)	21 (34.4%)	10 (16.4%)	2 (3.3%)	1 (1.6%)	0.8 (0.9)
Flock 2						
Custom R-Pilus	60 (89.6%)	7 (10.4%)	0	0	0	0.1 (0.3)
Footvax®	61 (91.0%)	6 (9.0%)	0	0	0	0.1 (0.3)
Saline	53 (84.1%)	10 (15.9%)	0	0	0	0.2 (0.4)
Flock 3						
Custom R-Pilus	50 (74.6%)	16 (23.9%)	1 (1.5%)	0	0	0.3 (0.5)
Footvax®	47 (75.8%)	14 (22.6%)	1 (1.6%)	0	0	0.3 (0.5)
Saline	46 (68.7%)	20 (29.9%)	1 (1.5%)	0	0	0.3 (0.5)

Appendix 15 Frailty models for lameness events for all flock data with Custom R-Pilus as the reference treatment

Table A- 25 Frailty model for time to first lameness event after week 6 of the trial in all flocks, reference treatment = Custom R-Pilus

Variable	No.	%	HR	SE	p value
Treatment					
Custom R-Pilus	91	45	Ref		
Footvax®	92	44	1.10	0.15	0.52
Saline	113	55	**1.42**	**0.14**	**0.01**
Flock					
3	134	68	Ref		
1	106	61	0.98	0.13	0.87
2	56	23	**0.29**	**0.16**	**<0.01**
Previously lame					
No	136	43	Ref		
Yes	160	54	**1.38**	**0.12**	**<0.01**
Random effect					
Flock	Variance = 1.55×10^{-5}				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p < 0.05$ and are indicated in bold.

Table A- 26 Frailty model for all lameness events after week 6 of the trial in all flocks, stratified by flock, reference treatment = Custom R-Pilus

Variable	No.	%	HR	SE	p value
Treatment					
Custom R-Pilus	153	46	Ref		
Footvax®	146	48	1.01	0.12	0.94
Saline	190	54	**0.26**	**0.11**	**0.04**
Previously lame					
No	214	45	Ref		
Yes	275	53	**1.42**	**0.10**	**<0.01**
Random effect					
Flock	Variance < 0.01				
Ewe	Variance = 0.09				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p < 0.05$ and are indicated in bold.

Appendix 16 Survival model results for lameness in Purchased and Existing ewes in flock 1

Table A- 27 Cox PH model for time to first lameness event after week 6 of the trial for Purchased ewes in flock 1

Variable	No.	%	HR	Lower 95% CI	Upper 95% CI
Treatment					
Saline	21	95	Ref		
Footvax®	13	65	**0.47**	**0.23**	**0.96**
Custom R-Pilus	18	95	0.98	0.52	1.69
Previously lame					
No	33	83	Ref		
Yes	19	90	0.94	0.52	1.69
Concordance = 0.57 (se = 0.052)					

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when the CI does not include unity and are indicated in bold.

Table A- 28 Cox PH model for time to first lameness event after week 6 of the trial for Existing ewes in flock 1

Variable	No.	%	HR	Lower 95% CI	Upper 95% CI
Treatment					
Saline	27	69	Ref		
Footvax®	18	46	0.66	0.36	1.18
Custom R-Pilus	9	25	**0.25**	**0.12**	**0.54**
Previously lame					
No	32	43	Ref		
Yes	22	55	1.67	0.96	2.89
Concordance = 0.63 (se = 0.035)					

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when the CI does not include unity and are indicated in bold.

Table A- 29 Frailty model for all lameness events after week 6 of the trial for Purchased ewes in flock 1

Variable	No.	%	HR	SE	p value
Treatment					
Saline	40	65	Ref		
Footvax®	30	60	0.84	0.27	0.51
Custom R-Pilus	40	75	1.19	0.25	0.50
Previously lame					
No	66	65	Ref		
Yes	44	70	1.37	0.23	0.17
Random effect					
Ewe	Variance = 0.13				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p \leq 0.05$ and are indicated in bold.

Table A- 30 Frailty model for all lameness events after week 6 of the trial for Existing ewes in flock 1

Variable	No.	%	HR	SE	p value
Treatment					
Saline	41	58	Ref		
Footvax®	30	60	0.68	0.31	0.20
Custom R-Pilus	13	30	**0.31**	**0.37**	**<0.01**
Previously lame					
No	44	42	Ref		
Yes	40	54	**1.92**	**0.28**	**0.02**
Random effect					
Ewe	Variance = 0.61				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p \leq 0.05$ and are indicated in bold.

Appendix 17 Frailty model for lameness events in flock 2

Table A- 31 Frailty model for all lameness events after week 6 of the trial in flock 2

Variable	No.	%	HR	SE
Treatment				
Saline	21	31	Ref	
Footvax®	24	35	1.03	0.32
Custom R-Pilus	23	34	1.07	0.32
Random effect				
Ewe	Variance = 0.42			

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p \leq 0.05$ and are indicated in bold.

Appendix 18 Frailty models for all flock data with Custom R-Pilus as the reference treatment

Table A- 32 Frailty model for time to first footrot event after week 6 of the trial in all flocks, reference treatment = Custom R-Pilus

Variable	No.	%	HR	SE	p value
Treatment					
Custom R-Pilus	42	14	Ref		
Footvax®	40	15	0.98	0.22	0.91
Saline	65	23	**1.70**	**0.20**	**0.01**
Flock					
3	53	21	Ref		
1	71	37	**1.84**	**0.19**	**<0.01**
2	23	6	0.70	0.26	0.17
Previous FR					
No	91	18	Ref		
Yes	56	27	**1.76**	**0.17**	**<0.01**
Random effect					
Flock	Variance = 1.62×10^{-5}				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p \leq 0.05$ and are indicated in bold.

Table A- 33 Frailty model for all footrot events after week 6 of the trial in all flocks, reference treatment = Custom R-Pilus

Variable	No.	%	HR	SE	p value
Treatment					
Custom R-Pilus	58	17	Ref		
Footvax®	57	17	0.99	0.19	0.98
Saline	83	23	1.43	**0.18**	**0.04**
Flock					
3	56	18	Ref		
1	119	39	**2.81**	**0.17**	**<0.01**
2	23	5	0.68	0.26	0.14
Previous footrot					
No	117	17	Ref		
Yes	81	24%	**1.92**	**0.16**	**<0.01**
Random effect					
Flock	Variance < 0.01				
Ewe	Variance = 0.14				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p < 0.05$ and are indicated in bold.

Appendix 19 Survival model results for footrot in Purchased and Existing ewes in flock 1

Table A- 34 Cox PH model for time to first footrot event after week 6 of the trial for Purchased ewes in flock 1

Variable	No.	%	HR	Lower 95% CI	Upper 95% CI
Treatment					
Saline	19	86	Ref		
Footvax®	12	55	0.63	0.29	1.36
Custom R-Pilus	17	85	1.07	0.54	2.12
FR on week 1					
No	44	73	Ref		
Yes	4	100	2.07	0.69	6.22
Lame on week 1					
No	46	78	Ref		
Yes	2	40	0.46	0.10	2.06
Previous footrot event					
No	31	70	Ref		
Yes	17	85	1.21	0.65	2.26
Concordance = 0.58 (se = 0.05)					

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when the CI does not include unity and are indicated in bold.

Table A- 35 Cox PH model for time to first footrot event after week 6 of the trial for Existing ewes in flock 1

Variable	No.	%	HR	Lower 95% CI	Upper 95% CI
Treatment					
Saline	15	31	Ref		
Footvax®	7	21	**0.36**	**0.14**	**0.90**
Custom R-Pilus	1	3	**0.06**	**<0.01**	**0.42**
FR on week 1					
No	14	13	Ref		
Yes	9	36	**2.94**	**1.13**	**7.61**
Lame on week 1					
No	16	14	Ref		
Yes	7	37	**3.00**	**1.03**	**8.69**
Previous footrot event					
No	15	15	Ref		
Yes	8	30	1.45	0.48	4.39
Concordance = 0.80 (se = 0.04)					

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. CI = confidence interval. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when the CI does not include unity and are indicated in bold.

Table A- 36 Frailty model for all footrot events after week 6 of the trial for Purchased ewes in flock 1

Variable	No.	%	HR	SE	p value
Treatment					
Saline	35	60	Ref		
Footvax®	26	54	0.94	0.29	0.84
Custom R-Pilus	32	68	1.10	0.27	0.74
FR on week 1					
No	87	61	Ref		
Yes	6	55	1.09	0.47	0.86
Lame on week 1					
No	89	62	Ref		
Yes	4	44	0.53	0.58	0.26
Previously footrot event					
No	54	57	Ref		
Yes	39	67	**1.76**	**0.24**	**0.02**
Random effect					
Ewe	Variance = 0.10				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p \leq 0.05$ and are indicated in bold.

Table A- 37 Frailty model for all footrot events after week 6 of the trial for Existing ewes in flock 1

Variable	No.	%	HR	SE	p value
Treatment					
Saline	16	28	Ref		
Footvax®	9	18	0.45	0.43	0.06
Custom R-Pilus	1	2	**0.07**	**1.03**	**0.01**
FR on week 1					
No	15	13	Ref		
Yes	11	31	2.10	0.48	0.12
Lame on week 1					
No	17	14	Ref		
Yes	9	33	2.12	0.53	0.16
Previously footrot event					
No	16	14	Ref		
Yes	10	28	1.45	0.56	0.51
Random effect					
Ewe	Variance = 0.10				

No. represents the number of events in each category and % represents the events as percentage of all observations in the category. Ref is the reference (baseline) category for comparison. Hazard ratios are statistically significant when $p \leq 0.05$ and are indicated in bold.

Appendix 20 Distribution of litter size and number of living lambs per ewe at exit examination

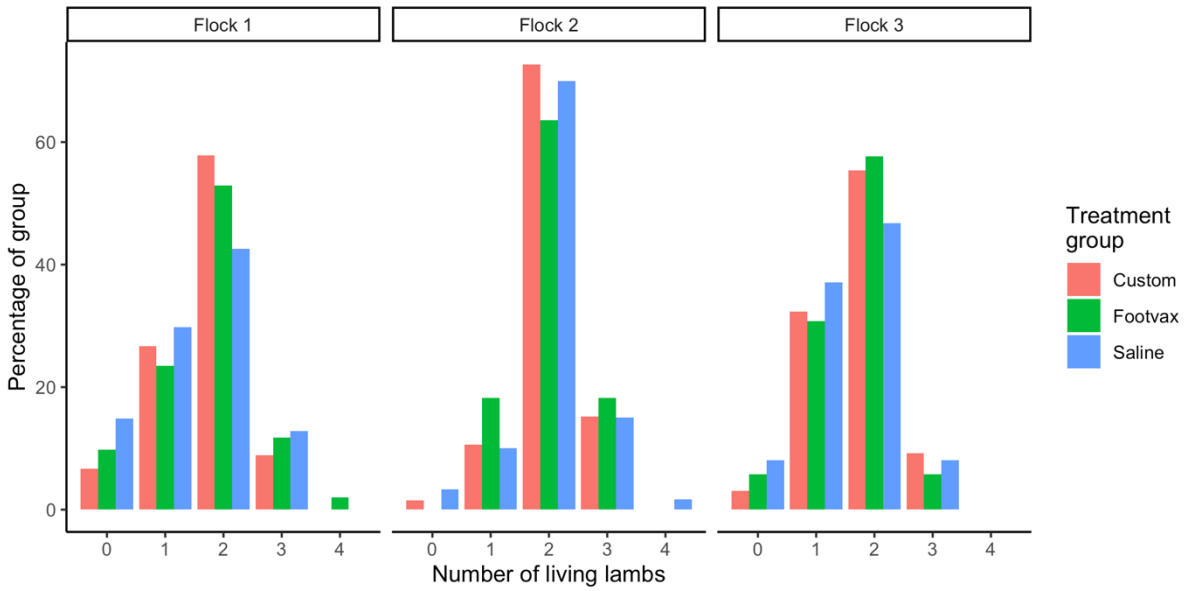

Figure A- 9 Distribution of litter size (all lambs born) per ewe for each flock by treatment group

Table A- 38 The distribution of living lambs per ewe at time of exit examination by treatment group and flock (percentage of all ewes in the treatment group)

	0	1	2	3+	Total
Flock 1					
Custom R-Pilus	3 (6.7%)	12 (26.7%)	26 (57.8%)	4 (8.9%)	45
Footvax®	5 (9.8%)	12 (23.5%)	27 (52.9%)	7 (13.8 %)	51
Saline	7 (14.9%)	14 (29.8%)	20 (42.6%)	6 (12.8%)	47
Total	15 (10.5%)	38 (26.6%)	73 (51.0%)	17 (11.9%)	143
Flock 2					
Custom R-Pilus	1 (1.5%)	7 (10.6%)	48 (72.7%)	10 (15.2%)	66
Footvax®	0	12 (18.2%)	42 (63.6%)	12 (18.2%)	66
Saline	2 (3.3%)	6 (10.0%)	42 (70.0%)	10 (16.7%)	60
Total	3 (1.6%)	25 (13.0%)	132 (68.8%)	32 (16.7%)	192
Flock 3					
Custom R-Pilus	2 (3.1%)	21 (32.3%)	36 (55.4%)	6 (9.2%)	65
Footvax®	3 (5.8%)	16 (30.8%)	30 (57.7%)	3 (5.8%)	52
Saline	5 (8.1%)	23 (37.1%)	29 (46.8%)	5 (8.1%)	62
Total	10 (5.6%)	60 (33.5%)	95 (53.1%)	14 (7.8%)	179

Appendix 21 Distribution of BCS change by treatment groups over the trial

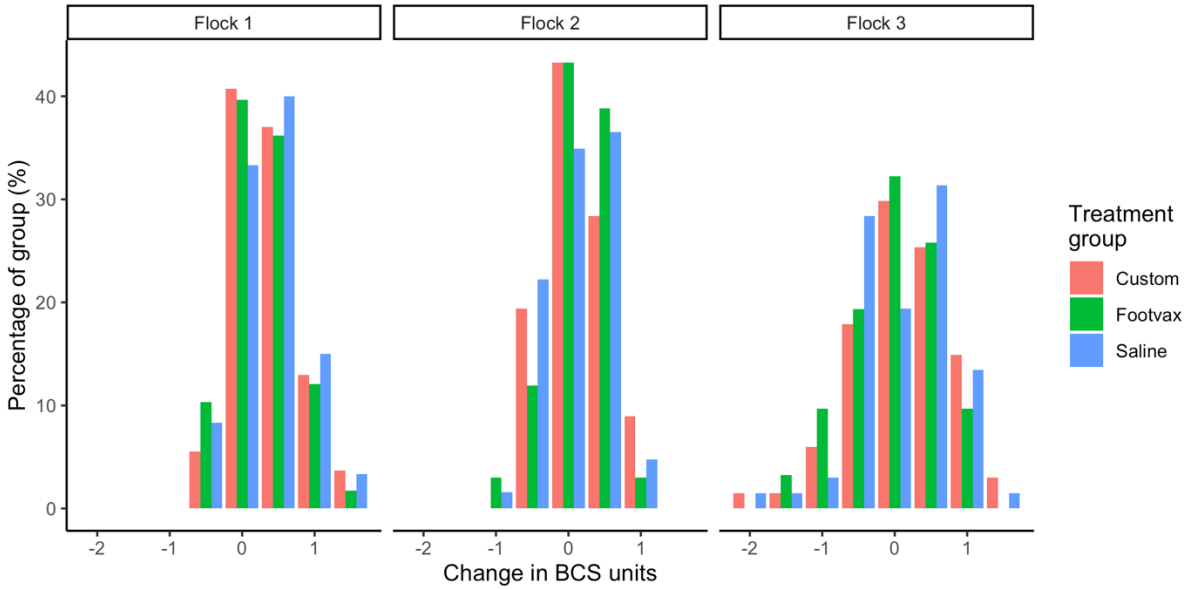

Figure A- 10 Distribution of change in BCS units in ewes between week 1 and 17 of the trial by treatment group for each flock

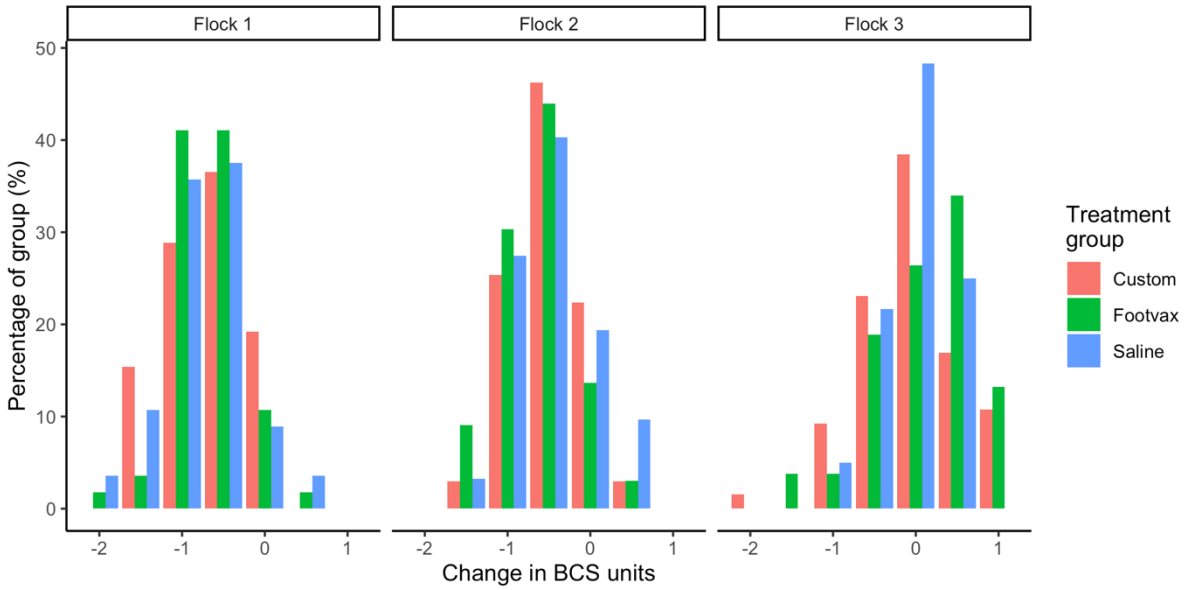

Figure A- 11 Distribution of change in BCS units in ewes between week 17 of the trial and lambing by treatment group for each flock

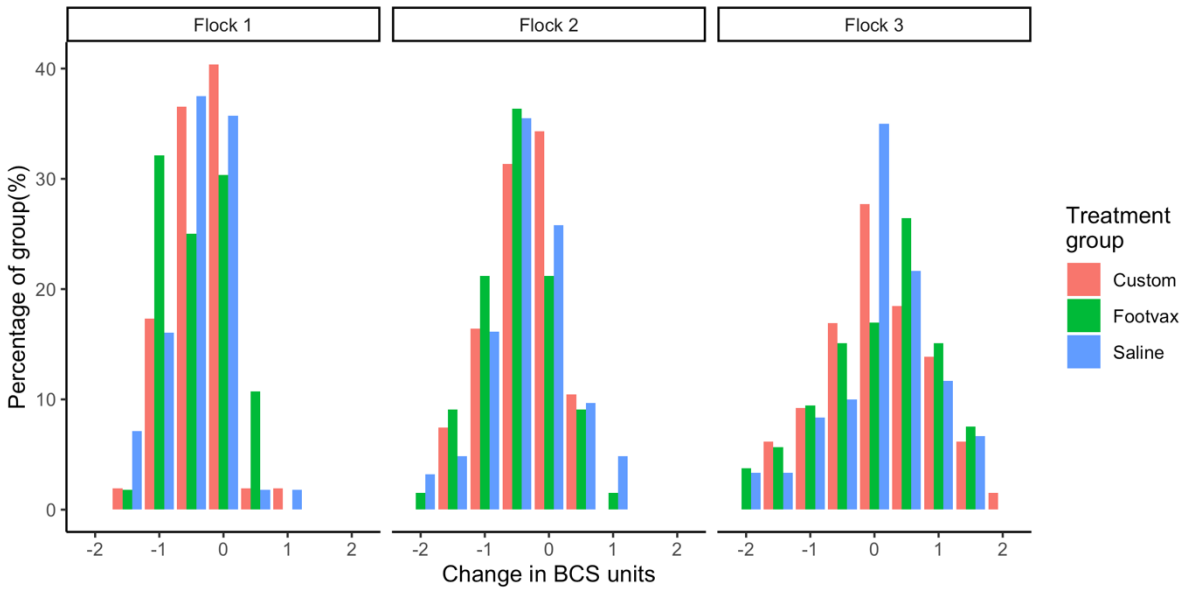

Figure A- 12 Distribution of change in BCS units in ewes between week 1 of the trial and lambing by treatment group for each flock

Appendix 22

Table A- 39 The minimum number of D. nodosus copies at which each serogroup was detected in any of the samples from the trial that underwent serogroup PCR testing

Serogroup	*AprV2/AprB2* copies
A	1020
B	333
C	331
F	113
H	80
I	435

Appendix 23

Table A- 40 Serogroup combinations on the interdigital swabs of ewes with footrot lesions at whole flock examinations in flock 1 by origin. ND = No serogroup detected on D. nodosus positive sample

	Entry			Mid			Exit		
	Custom	Footvax	Saline	Custom	Footvax	Saline	Custom	Footvax	Saline
Existing									
H	3	2	3		1	1	2	2	3
HI	1		4						1
I		1						1	
AH							1		
AHI		1							
BH		1					2		
BCH								2	
BHI								1	
CH		1					1		
CHI						1		1	2
C							1		
B		1						1	
BC								1	
ND		1	2			2	3		
Total	4	8	9	0	1	4	10	9	6
Purchased									
H			1			2		1	
HI					1	2		2	
I								1	
BH				1		1		2	2
BHI					1		1		
ABH				1		1	1	1	1
ACH						1			
BCHI									1
B							1		
AB			1						1
BC				1					
ND	1	1						2	
Total	1	1	2	3	2	7	3	9	5