The role of lambs, time and space in persistence of *Dichelobacter nodosus*, the causative agent of footrot

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Training

As part of the students, modules in statistics, programming and data analysis were completed in the first year, as well as a mini project at the University of Leicester. I. also attended a workshop at the Max Planck Institute on analysis of social network data.

Declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by myself, under the supervision of my supervisors Professor Laura Green and Professor Matt Keeling and has not been submitted in any previous application for any degree.

The work presented (including data generated and data analysis) was carried out by the author except in cases outlined below:

- Dr Joanne Winter provided the dataset of farm management practices and prevalence of lameness in ewes and lambs, collected in 2013
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Summary

Lameness in sheep is a significant health and welfare problem in UK sheep flocks, with economic impacts that cost sheep farmers in both lost productivity and costs of treatment. Most lameness is caused by footrot, an infectious bacterial disease. The causative agent of footrot is *Dichelobacter nodosus*, which persists on the feet of infected sheep and is found transiently in soil, suggesting the most likely route of transmission between sheep is via the environment.

Both ewes and lambs are affected by footrot, although the disease can present differently. While there is a large evidence base for management of lameness in ewes, less is known about how management practices are associated with prevalence of lameness in lambs. Two cross-sectional, questionnaire-based studies were used to identify relationships between management of lambs and prevalence of lameness in ewes and lambs and found managements linked with high prevalence of lameness in ewes are also associated with prevalence of lameness in lambs.

Network-based diffusion analysis was used to investigate possible transmission pathways of *D. nodosus* using data from a longitudinal observational study of a flock of ewes and their lambs. Over the two-week study period, ewes were more likely to become lame without social contact with other sheep – presumably as some were already infected prior to lambing. These infectious ewes most likely acted as a reservoir of infection that led to infection in their lambs. Twin lambs were less likely to become lame over the study period, which may be because they spend less time with their mother than single lambs. These insights into the spread of *D. nodosus* may suggest that it would be beneficial for farmers to avoid turning out lame ewes and their lambs with the rest of the flock after lambing.

List of Abbreviations

Abbreviation	Definition
AHDB	Agricultural and Horticultural Development Board
AI	Association Index
AIC	Akaike's Information Criterion
AICc	Akaike's Information Criterion - corrected
BH	Benjamini-Hochberg
BIC	Bayesian Information Criterion
CI	Confidence interval
CODD	Contagious Ovine Digital Dermatitis
cTADA	Continuous time of acquisition diffusion analysis
D. nodosus	*Dichelobacter nodosus*
dTADA	Discrete time of acquisition diffusion analysis
EID	Electronic Identification
F. necrophorum	*Fusiobacterium necrophorum*
FAWC	Farm Animal Welfare Council
GEN-BS	Gaussian elastic net model run on boot-strap data
GLM	Generalised linear model
GMPL	Geometric mean prevalence of lameness
GPS	Global Positioning System
HR	Hazard ratio
ID	Interdigital dermatitis
L	Lower
LASSO	Least absolute shrinkage and selection operator
LC	Latent class
LCA	Latent class analysis
LiE	Average annual prevalence of lameness in ewes
LiL	Average annual prevalence of lameness in lambs
N	Number
NB-GLM	Negative binomial generalised linear model
NBDA	Network-based diffusion analysis
NSA	National Sheep Association
OADA	Order of acquisition diffusion analysis
OR	Odds ratio

PEN-BS	Poisson elastic net model run on boot-strap data
Pi	Participation coefficient
PST	Proportion of cases solved by social transmission
Q	Newman’s modularity coefficient
QP-GLM	Quasi-Poisson generalised linear model
Ref	Reference
RFID	Radio Frequency Identification
SD	Standard deviation
SE	Standard error
SFR	Severe footrot
TADA	Time of acquisition diffusion analysis
U	Upper
Zi	Normalised measure of an individual’s interactions within its module

Chapter 1 General Introduction to footrot and methods for establishing associations between lameness, foot lesions and management practices in sheep

1.1 Lameness and implications for welfare of sheep

Lame sheep are found in almost every flock in England, with the mean period prevalence of lameness estimated at 3.5% (Winter et al., 2015). Lameness in sheep is a serious health and welfare issue which costs the UK sheep industry up to £80 million a year (Wassink et al., 2010b), with costs incurred through lost productivity, time, and treatment of lame sheep (Winter and Green, 2017). Lost productivity occurs because lame sheep are in pain (Fitzpatrick et al., 2006, Ley et al., 1994), which leads to weight loss (Marshall et al., 1991), reduced wool growth (Marshall et al., 1991) and reduced lambing percentage (Wassink et al., 2010b). Lambs that are lame have reduced growth rate (Nieuwhof et al., 2008b, Wassink et al., 2010b) and so are older at slaughter (Wassink et al., 2010b).

1.2 Summary of common endemic causes of foot lameness in sheep

Lameness has both infectious and non-infectious causes. In the United Kingdom, the predominant causes of lameness are the infectious bacterial diseases, footrot and contagious ovine digital dermatitis (CODD) (Kaler and Green, 2009, Winter et al., 2015). Footrot has two stages – interdigital dermatitis (ID), where the interdigital skin becomes inflamed and severe footrot (SFR), where the hoof horn starts to separate from the underlying tissue (Figure 1.1). In CODD, the primary lesion initiates at the coronary band, and progresses to horn separating from the laminae from the coronary band, often leading to complete detachment of the horn capsule (Winter, 2008).

Figure 1.1 Clinical presentation of interdigital dermatitis, showing inflammation of the digital skin (left) and severe footrot, where the hoof horn has started to separate from the underlying tissue (right)

Non-infectious causes of lameness in ewes are less prevalent than infectious causes in the UK (Winter et al., 2015) and include granulomas - proliferations of highly vascularised tissue that protrude through hoof horn, white line lesions - where the hoof wall separates from the underlying laminae (Winter, 2008) and shelly hoof – which presents as detachment of the hoof horn wall, leading to lameness if debris impacted in the cavity penetrates into living tissue and an abscess forms (Winter, 2008).

1.3 Bacteria associated with persistence and spread of footrot and the role of the environment

1.3.1 *Dichelobacter nodosus*

The causal agent of footrot is *Dichelobacter nodosus* (Beveridge, 1941). Load of *D. nodosus* plays the primary role in disease initiation and progression, with the load of *D. nodosus* on the interdigital skin increasing the week prior to development of ID (Witcomb et al., 2014). Prior damage to skin, for example from long grass or wet conditions, is a prerequisite for development of footrot because it enables *D. nodosus* to invade the epidermis (Beveridge, 1941). Consequently, *D. nodosus* causes disease in damp environments (Graham and Egerton, 1968, Smith et al., 2014) when it survives for short periods on damp pasture or bedding, and when skin is susceptible to invasion. Footrot does not occur in, hot or cold, dry climates when the environment and skin are not conducive to survival and invasion (Stewart, 1989, Clifton et al., 2019).

D. nodosus is able to persist on footrot affected feet for two to at least six weeks but not on healthy feet of sheep with footrot (Clifton et al., 2019), and is found only transiently in the environment (Clifton et al., 2019). This indicates cross-contamination from diseased feet to healthy within a sheep because of spatial co-location (Clifton et al., 2019) – raising the question of whether spatial co-location of feet from other sheep might also be is a risk factor for development of footrot.

Conditions in England are usually suitable for year-round transmission of *D. nodosus* and development of footrot (Green and George, 2008) although there is some seasonality to occurrence of ID (Wassink et al., 2004). Different soils and temperatures may affect survival of *D. nodosus,* with laboratory studies indicating that *D. nodosus* survives for longer in clay soils, lower temperatures and higher moisture content (Cederlöf et al., 2013, Muzafar et al., 2016, Clifton et al., 2019).

1.3.2 *Fusobacterium necrophorum*

Fusobacterium necrophorum is a secondary invader that multiplies after footrot has developed (Witcomb et al., 2015; Clifton et al., 2019) and is associated with increased disease severity (Beveridge, 1941, Witcomb et al., 2014). *F. necrophorum* is host-dependent and shed into the environment in faeces by only a few animals - while it can be detected in soil, this is only in wet conditions, on the soil surface and where sheep spend the majority of their time e.g. around a feed trough, which indicates transient contamination of the environment, as with *D. nodosus* (Clifton et al., 2019).

1.3.3 Other bacteria

Other bacterial species that have been proposed to exacerbate foot lesions include *Spirochaeta pernortha* (Beveridge, 1936), *Treponema podovis* (Egerton et al., 1969) and *Cornybacterium pyogenes* (Roberts et al., 1972). There is a dysbiosis of the interdigital skin in footrot, with distinct bacterial communities seen in healthy and footrot affected feet (Maboni et al., 2016, McPherson et al., 2019, Blanchard et al., 2021). Disease with *D. nodosus* reduces diversity of bacteria on the foot, increasing abundance of not only *D. nodosus* but also of species such as *Mycoplasma fermentans* and *Porphyromonas asaccharolytica* (Blanchard et al., 2021) and triggers a shift from Gram positive aerobic taxa to Gram negative anaerobic taxa (McPherson et al., 2019).

1.4 Relationships between lameness and footrot lesions in ewes and lambs

Both ewes and lambs are susceptible to footrot (Wassink et al., 2010b), but lambs are more likely to develop ID than SFR (Grogono-Thomas and Johnston, 1997, Wassink et al., 2004). Sheep are born naïve to *D. nodosus*, but it can be detected on their feet within hours of birth (Muzafar et al., 2015), and clinical signs of disease have been seen on lambs at fourteen days after exposure to *D. nodosus* positive ewes in a trial (Kuhnert et al., 2019).

When prevalence of lameness in ewes is high, prevalence of lameness in lambs is also high (Kaler et al., 2010b, Winter et al., 2015) and similarly, prevalence of ID lesions is higher in lambs when prevalence is higher in ewes (Wassink et al., 2004). Flock level observations suggest that the dynamics of spread of footrot in lambs may be different from ewes since prevalence of ID lesions in lambs peaks dramatically in late spring and early summer (Figure 1.2), while prevalence of ID in ewes remains more consistent over the year (Wassink et al., 2004). Possible explanations for this include large numbers lambs susceptible to disease in spring without any immunity or that lamb feet are more prone to damage and subsequent entry of bacteria, than those of ewes (Wassink et al., 2004), or that there are increased density of sheep when lambs are present, providing more opportunity for transmission (Russell et al., 2013).

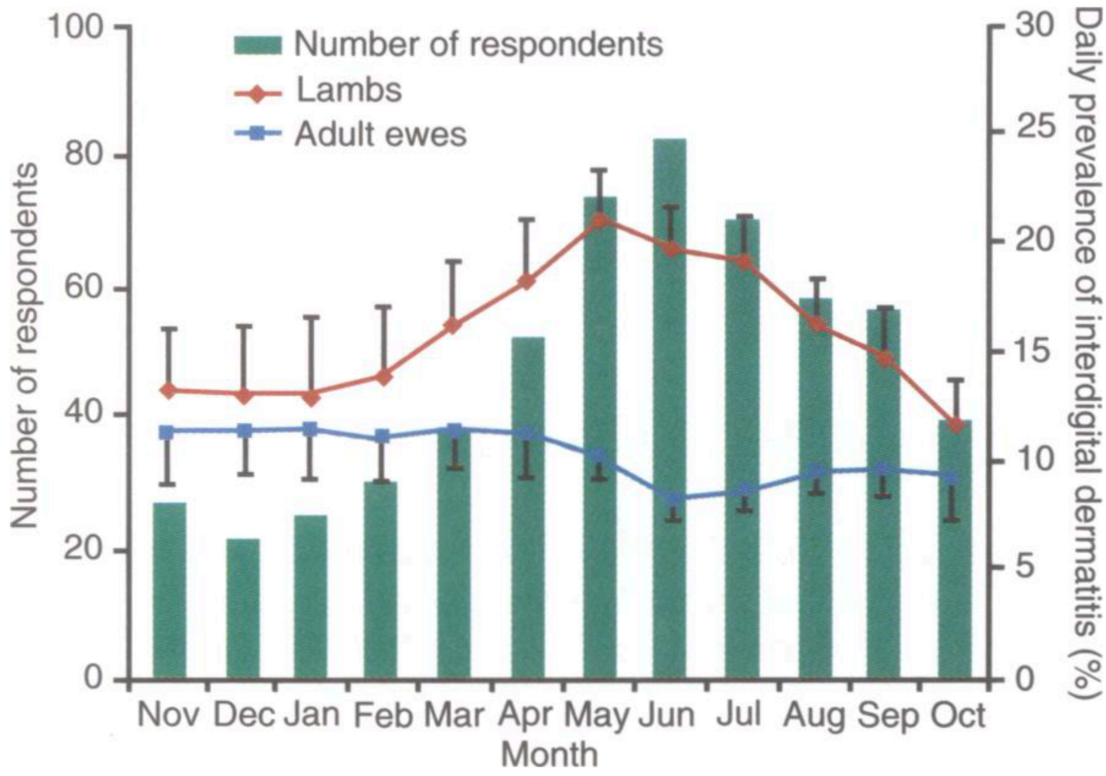


Figure 1.2 Wassink et al., (2004) Months, indicated by the respondents, with the highest prevalence of interdigital dermatitis in the flock, and the mean (se) daily prevalence reported during those months for ewes and lambs

1.5 Recognition of lameness by farmers and researchers, and lameness as a proxy for detection of footrot

The results of many studies use farmers (Best et al., 2020, Kaler and Green, 2009, Prosser et al., 2019, Winter et al., 2015) or researchers (Kaler et al., 2010a, Kaler et al., 2011, Wassink et al., 2010b, Witt and Green, 2018) to estimate the prevalence of lameness in sheep. Locomotion scoring systems are used to measure lameness according to factors such as stride length, duration of weight bearing and body posture. The first for sheep was designed in 1989 (Ley et al., 1989) and a validated scoring system (Kaler et al., 2009) exists, which has both high inter and intra observer agreement (ICC = 0.93 and 0.90, respectively, weighted kappa = 0.93 and 0.91, respectively). Researcher estimates of prevalence of lameness in a flock using the Kaler et al. (2009) locomotion scoring system, were highly correlated with farmer estimates of the prevalence of lameness where farmers did not use the scoring system (Spearman's rank correlation coefficient = 0.73) (King, 2013).

Farmers recognise lame sheep and differentiate severity of gait change intrinsically (Kaler and Green, 2008b).

Since the majority of lameness in England is caused by footrot (Winter et al., 2015), lameness has been used as a proxy for footrot disease, in both questionnaire-based studies (Kaler and Green, 2009, Prosser et al., 2019, Winter et al., 2015) and observational studies (Kaler et al., 2010a, Wassink et al., 2010b). An observational study (Kaler et al., 2011) with five, weekly observations of 60 sheep indicated that as the severity of lesions increased, the locomotion scores increased. If lameness is considered as a diagnostic test for footrot, it likely has a high sensitivity and can correctly identify sheep that are likely to have disease – in the study by Kaler et al., (2011) footrot lesions were present on at least one foot on 83% of observations of lame sheep, suggesting lame sheep are likely to have footrot. However, it is possible to miss cases of footrot if lameness is used as a proxy – in the same study footrot lesions were seen on 27% of observations when sheep were not lame, although the majority of these lesions were mild (Kaler et al., 2011).

1.6 The evidence base for management practices associated with prevalence of lameness in sheep flocks in the United Kingdom

1.6.1 Treatment of footrot

The current recommended treatment for SFR in ewes is administration of parenteral and topical antibiotics within three days of a sheep becoming lame (Kaler et al., 2010a, Wassink et al., 2010b). When farmers adhere to this protocol the prevalence of lameness can be <2% (Wassink et al., 2010b). No observational studies have been carried out for lambs, but the recommended treatment for ID is to spray all four feet with topical antibiotic, and to use parenteral antibiotics where there are signs of SFR (Sheep Veterinary Society, 2013).

1.6.2 Management factors associated with lameness in ewes

There are many factors that are associated with prevalence of lameness in ewes. Factors that contribute to a low prevalence of lameness in ewes include recognition of mildly lame sheep (Kaler and Green, 2009, Winter et al., 2015), treatment of individual sheep within three days of recognition of lameness (Kaler et al., 2010a), treatment of individual sheep when the first in the group is lame compared to waiting until >1 are lame (Winter et al., 2015) and separation of lame sheep from the rest of the flock (Kaler and Green, 2009, Winter et al., 2015). Assuming infectiousness is correlated with presence of lesions, rapid, effective, treatment of sheep with footrot would act to reduce the overall flock prevalence by reducing the force of infection if fewer animals are transmitting bacteria.

The efficacy of some flock management practices can depend on factors such as facilities or equipment available (Wassink et al., 2010b, Witt and Green, 2018) if the farmer has other time commitments (Witt and Green, 2018) or how well the management is performed – for example, some farmers under-dose sheep with antibiotics (Green et al., 2020). Examples of management practices with mixed evidence for efficacy include vaccination of sheep with FootVax™ and footbathing. Vaccination of sheep with FootVax™ is associated with lower flock prevalence of lameness but only when used for >5 years (Prosser et al., 2019, Best et al., 2020). Footbathing ewes to prevent ID is associated with lower prevalence of lameness (Witt and Green, 2018, Winter et al., 2015) while footbathing to treat SFR is associated with higher prevalence of lameness (Winter et al., 2015). Specific products may be more detrimental to the health of sheep feet than others – use of formalin in footbaths has been associated with high prevalence of shelly hoof (Reeves et al., 2019). Formalin is carcinogenic and causes skin inflammation in sheep (Ross, 1983) and hard, keratinous material in the interdigital skin (Pryor, 1959).

Other management practices used by farmers to control lameness have been shown to be of no benefit at best, or detrimental at worst. One of these is foot trimming, both as a routine management to control lameness or as a treatment for footrot. Causing bleeding during a routine foot trim is associated with high prevalence of lameness in ewes (Prosser et al., 2019, Winter et al., 2015) and with granulomas (Reeves et al., 2019), possibly due to the damage to the foot structure and resultant proliferation of connective tissues. Foot trimming to treat footrot increases recovery time (Kaler et al., 2010a), presumably due to the damage caused to the foot.

1.6.3 Environmental factors associated with lameness in ewes

There are also environmental factors which are associated with prevalence of lameness. Hill flocks have lower prevalence of lameness compared to lowland flocks (Winter et al., 2015) and sheep are also more likely to have footrot when grazing heavily poached ground compared to where there is good coverage of grass (Angell et al., 2018, Vittis and Kaler, 2020). Housing of sheep during the previous lambing season is also associated with higher prevalence of lameness (Witt and Green, 2018).

1.6.4 Management factors associated with lameness in lambs

Currently, there is little evidence for management practices associated with lameness in lambs. Factors associated with an annual average prevalence of >5% of ID in lambs are sometimes or never catching lame ewes to treat them compared to always, sometimes or never treating lame ewes with parenteral antibiotics compared to always, showing sheep at agricultural events compared to not and turning sheep onto a field free from livestock for >2 weeks after footbathing compared to not doing so (Wassink et al., 2004).

1.6.5 Environmental factors associated with lameness in lambs

Altitude has been associated with prevalence of ID in lambs, with increased risk for lambs kept on farm land of 100m or less above sea level, compared to >100m (Wassink et al., 2004), although other factors that are associated with lameness are also associated with altitude – these include weather, stocking rate and breed of sheep.

1.7 Practical considerations for study design and analysis to identify associations between management, lameness and foot lesions

1.7.1 Study types used to identify associations between lame sheep, foot lesions and management

Lameness in sheep has been studied extensively using both longitudinal observational studies and cross-sectional studies. Summaries of the advantages and disadvantages of using the two designs are in Table 1.1 (Mann, 2012, Merrill, 2019).

Table 1.1 Summary of typical strengths and weaknesses of cross-sectional and longitudinal observational study designs (Mann,2012, Merrill, 2019)

Study type	Strengths	Weaknesses
Cross-sectional	• Allow many farms to be studied at once • Allow study of many risk factors at once • Unlikely to be ethical difficulties about measuring exposure • Often relatively cheap	• No data on time relationship between exposure and development of disease • Not feasible with rare exposures • Potential for retrospective observation/recall bias
Longitudinal	• Allow determination of cause and effect • Can collect time relationship between exposure and development of disease • Can assess several outcomes	• Potential for issues with follow up of individuals • Potential for difficulty for controlling of confounders (e.g. between farms)

Cross-sectional studies on lameness in sheep typically involve a paper-based or online questionnaire, which request a farmer estimate of the average number of lame sheep in the flock, flock size and management practices over a specified time period (Angell et al., 2014, Best et al., 2020, Dickins et al., 2016, Kaler and Green, 2009, Prosser et al., 2019, Reeves et al., 2019, Wassink et al., 2004, Winter et al., 2015). These are distributed to farmers using third-party organisations, although some are from random selection of Defra holdings (Winter et al., 2015).

Longitudinal observational studies are designed to elucidate the biology between lameness, foot lesions and management using temporal relationships. They focus on a small number of flocks or sheep. To date they have been used to identify that foot lesions develop before lameness (Kaler et al., 2011), the temporal associations between changing climate and hoof horn growth rate and development of foot lesions (Smith et al., 2014), the effect of different treatment types (Green et al., 2007, Kaler et al., 2010a, Wassink et al., 2010b) and the effect of flock-specific lameness control plans (Witt and Green, 2018). Longitudinal studies have also been used to identify associations between *D. nodosus* and foot characteristics, such as hoof conformation (Best et al., 2021, Kaler et al., 2010b), lesion development and severity (Witcomb et al., 2014) and to identify sites of bacterial persistence (Clifton et al., 2019). The evidence for temporal associations from longitudinal studies, and that sheep farmers rarely change their management practices (Wassink et al., 2010b) strengthens the likelihood that management strategies identified by cross-sectional studies influence the flock prevalence of lameness.

1.7.2 Considerations for inferential modelling to identify risk factors for lameness in sheep at the flock level

In multivariable model selection, different model structures, analytic workflows, and variable selection techniques give rise to different covariate selection because the methods have different selection criteria for the covariates and different limitations in ability to manage confounding (Botvinik-Nezer et al., 2020, Lima et al., 2020b, Lima et al., 2020a, Lima et al., 2021, Tercerio, 2003). Consequently, the covariates selected are not consistent between modelling approaches, which is a weakness of such modelling. The following section outlines factors influencing the identification of risk factors for lameness in sheep flocks.

1.7.2.1 Model choice

There are three main aims to creating statistical models. Models can be predictive, with the aim of accurately predicting an outcome variable; explanatory, where the model explains differences in the outcomes variable value by differences in values or categories of explanatory variables; or descriptive, where they capture associations between the outcome and explanatory variables (Shmueli, 2010). Models in lameness literature tend to be descriptive and explanatory (Best et al., 2020, Kaler and Green, 2009, Prosser et al., 2019, Winter et al., 2015) – with difficulty in exact prediction of proportion of lame sheep noted by Witt and Green (2018) as there is some under-prediction of the extreme values, as even models such as the quasi-Poisson or negative binomial, which are designed to deal with over-dispersion (Ver Hoef and Boveng, 2007), do not always adequately capture the over-dispersion in lameness between farms.

General, or generalised linear statistical models (GLM) are a standard choice to analyse data from cross-sectional epidemiological studies. These models allow a specification of the relationship between the mean and the variance (McCullagh and Nelder, 1989), and measures such as risk ratios can be calculated to compare risk in exposed and unexposed groups (Dohoo et al., 2003). In studies of lameness in sheep the outcome variable 'average annual number of lame sheep in the flock' tends to be over-dispersed (Prosser et al., 2019, Best et al., 2020) and structures such as the negative binomial or quasi-Poisson GLM models are appropriate model types since they incorporate extra parameters to model the over-dispersion. The choice of which of these models is more appropriate (in terms of fit, or coefficient estimation) is situation dependent (Gardner et al., 1995, Tercerio, 2003, Ver Hoef and Boveng, 2007).

1.7.2.2 Variable selection

Methods to accurately identify the variables most likely to have a true association with the outcome are essential – particularly where results will be used to make real-life changes to management of sheep on farm. Variable selection can be particularly challenging with "wide" data, where the number of explanatory variables is large relative to the number of responses.

Traditional methods of variable selection to identify risk factors for lameness in sheep are test-based (Desboulets, 2018) and often combined with a step-wise selection process (Dohoo et al., 2003). Variable selection approaches based on p-values are common in the lameness literature, e.g. O'Kane et al., (2017), Winter et al., (2015) and Wassink et al., (2003), with some use of other likelihood-based measures such as AIC e.g. Prosser et al., (2019). The advantage of stepwise selection methods is that the user can view and assess various combinations of models (Shtatland et al., 2008) but there is a risk that models are over-fitted to the data, resulting in non-reproducible results in a wider population and misleading information on the importance of management practices on control of lameness. Complex correlation structures between variables increase the risk of overfitting models (Kuhn and Johnson, 2013, Hastie et al., 2015) and have been identified between variables such as recognition of lameness and decisions over when to treat sheep (Winter et al., 2015). Methods that have been used in studies involving sheep to control for correlation between variables include selection of the most biologically relevant variable (Witt and Green, 2018), use of a statistically determined cut-off value to remove highly correlated variables (Best et al., 2020, Witt and Green, 2018) or including both variables in a model (Winter et al., 2015) but in practice, these decisions are arbitrary.

1.7.2.3 Other methods to control over-fitting of models and increase confidence in variable selection

There are other methods to control over-fitting and improve confidence in variable selection that have not yet been employed in analysis of data from questionnaires about lame sheep but have been used in other studies of animal health (Lima et al., 2020a) and in proof of concept studies using simulated data that would relate to animal health data (Lima et al., 2020b).

1.7.2.3.1 Penalised regression models

Penalised regression models provide a trade-off between bias and variance by adding a penalty to the sum of squared errors (Kuhn and Johnson, 2013). Use of penalised regression models, particularly when combined with additional bootstrap methods, can help answer the question of whether many predictor variables have small effects on the prevalence of lameness, or if a small number of variables that have the biggest effects on prevalence of lameness be identified?

1.7.2.3.2 Boot-strap methods – selection stability

Selection stability aims to address an often ignored problem of variable selection – how robust the model is to small perturbations of the dataset (Sauerbrei and Schumacher, 1992). Boot-strap methods involve repeated sub-sampling of the data, and re-running models on these samples. Covariate stability (Austin and Tu, 2004, Hastie et al., 2015, Heinze et al., 2018, Meinshausen and Bühlmann, 2010), calculated as the proportion of times a covariate is selected by a model repeatedly run on sub-samples of the dataset, can be used to discriminate true positive explanatory variables from “noise” variables (Austin and Tu, 2004, Lima et al., 2021). “Noise” variables would be those that are only associated with the outcome on certain farms, that are not selected when these farms are not included in the bootstrap sample.

1.7.2.3.3 Triangulation of multiple methods

Triangulation of multiple models has been proposed as a method to explore “between-method” variability, with the idea that variables identified as important by several methods are less likely to be artefacts (Munafò and Davey Smith, 2018). The idea is to integrate results from several model types, each making different assumptions and with different sources of bias to derive a reliable answer (Lawlor et al., 2016). When variables are selected by different approaches, confidence in the association between variable and the outcome is increased (Lima et al., 2020b).

1.8 Summary

The focus of the PhD was to investigate the role of lambs, time and space in persistence of *D. nodosus* using lameness as a proxy for footrot disease and presence of *D. nodosus*. There were two parts to the thesis. The first used cross-sectional studies with farmer-reported estimates of lameness in the flock and management practices used in the same year to investigate relationships between management of lambs and prevalence of lameness in both ewes and lambs, and the second used a longitudinal observational study of lambs and ewes to investigate associations between spatial co-location of sheep and new cases of lameness.

The aims of this thesis were to:

- Use robust modelling methods to investigate whether there are specific risk factors for lameness in lambs that are different from those in ewes and how management of lambs impacts flock-level prevalence of lameness in both ewes and lambs
- To investigate whether spatial-temporal co-location of sheep is a risk factor for developing lameness and whether lameness changes social behaviour

Chapter 2 Management practices associated with prevalence of lameness in lambs in 2012 – 2013 in 1271 English sheep flocks

The contents of this chapter have been published in Lewis and Green, 2020, Management Practices Associated with Prevalence of Lameness in Lambs in 2012-2013 in 1271 English Sheep Flocks, Frontiers in Veterinary Science, 7:519601. The full published paper is available at https://doi.org/10.3389/fvets.2020.519601 with the exceptions of Figures 2.3, 2.4 and 2.5 which have been added for further illustration. The Materials and Methods, Results, Discussion and Conclusion are from the pre-print and Table 2.2 and Table 2.9 have been added from the Supplementary Material contents.

2.1 Introduction

The aim of this chapter was to investigate two hypothesises – 1) that there are specific flock management practices associated with prevalence of lameness in lambs, and 2) some management practices associated with prevalence of lameness in ewes are also associated with prevalence of lameness in lambs. These hypotheses were investigated using an existing dataset of farmer responses to a questionnaire about prevalence of lameness and management practices used in ewes and lambs, supplied by Dr Joanne Winter.

2.2 Materials and Methods

2.2.1 Ethical approval details

Ethical approval was obtained from the University of Warwick's Biomedical and Scientific Research Ethics Committee (BSREC), BSREC 159-01-12; approved December 7, 2011. The farmers were all informed of the purpose of the study and their right to withdraw at any point, responding to the questionnaire was indication that they consented to participate. All participants owned or rented a farm, indicating they were over sixteen years old and could consent to participation in the study.

2.2.2 Questionnaire design and administration

Data came from a postal questionnaire (available online in the Supplementary Material for the published paper) developed at the Universities of Warwick and Nottingham (Winter et al., 2015), requesting information on prevalence of lameness in lambs and ewes and management practices to control lameness for the period May 2012 to April 2013. The questionnaire was sent to a random sample (stratified by county and size) of 4000 lowland sheep farmers in England reported to have >200 ewes. Up to two reminder letters were sent to non-respondents with a second copy of the questionnaire with the second reminder. Double data entry was carried out by an outside agency (Wyman Dillon Ltd, Bristol) and then data were cleaned and stored in Microsoft Excel as described by Winter et al. (2015). A total of 1348 questionnaires were returned after two reminders; 1271 (31.8%) responses were usable.

2.2.3 Descriptive statistics

Data analysis was carried out in RStudio v3.4.1 (R Core Team, 2017). Responses were excluded from analysis if data on annual mean period prevalence of lameness in lambs (LiL) or lameness in ewes (LiE) or ewe flock size was missing. The number and percentage of farmers using each management strategy are available online in the Supplementary Material from Winter et al., (2015). Flocks were categorised by prevalence of LiL and LiE into ≤2%, >2–5%, >5-10% and >10%. These categories were based on the FAWC targets of <2% of the national flock lame by 2021 and ≤5% of the national flock lame by 2016 (FAWC 2011); >5-10% from the global mean prevalence of lameness in 2013 and 2004 respectively (Kaler and Green, 2009, Winter et al., 2015) and >10%, the flock prevalence of LIE deemed unacceptable by farmers (Liu et al., 2018).

The number and percentage of flocks managed using each practice was calculated for each category of LiL – these are available online in the Supplementary Material for the published paper. The relationship between the geometric mean LiE and LiL was investigated using paired Wilcoxon tests and Spearman's correlation coefficient tests. The questionnaire included a photograph and descriptions of ID, SFR, CODD and shelly hoof. Farmers were asked to name each lesion and estimate its prevalence in ewes in their flock. For the analysis the prevalence of each lesion, including correct and incorrect naming of the lesion was used as in Winter et al., (2015) and Reeves et al., (2019) because farmers recognise lesions, but do not always name them correctly (Kaler and Green, 2008a). The prevalence of lesions in lambs was not available.

2.2.4 Latent class analysis of methods of treatment used by farmers to treat interdigital dermatitis and severe footrot in ewes and lambs

Two separate latent class analyses (LCA) for lambs and ewes were used to determine typologies of farmers by treatment of ID and SFR using the '*poLCA'* R package (Linzer and Lewis, 2011), which identifies latent classes using the expectation-maximisation algorithm (Linzer and Lewis, 2011). Models ranging from two to seven classes were obtained by running 500 repetitions of each model using 20,000 iterations of the expectation-maximisation algorithm to increase confidence the final solution for each model had converged on a global maximum solution. Goodness of fit statistics (Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC) and G-Goodness of fit test) were calculated, with the BIC used as the primary selection criterion (Nylund et al., 2007), to determine the optimum number of classes. The posterior probability for each farmer being in a class and the conditional probability that farmers in a class were practising a management were calculated.

The geometric mean LiE and LiL and associated 95% confidence intervals were calculated within latent classes. Wilcoxon tests with the Benjamini-Hochberg correction (Benjamini and Hochberg, 1995) were used to investigate differences in prevalence of lameness by latent class for lambs and ewes.

2.2.5 Structure of data and associations between variables

The questions on management of lameness were grouped into ten categories: recognising and catching lame sheep, treatment of ID and SFR in lambs, treatment of ID and SFR in ewes, routine trimming of sheep, footbathing, vaccination, whole flock antibiotic treatment, farm biosecurity and farm and farmer characteristics. When a question asked about sheep and did not specify lambs or ewes, it was assumed to relate to management of both groups. Associations between explanatory variables were investigated using Pearson's chi-square test for associations between categorical variables, with Cramer's V statistic to indicate the strength of the association calculated using the *lsr* R package (Navarro, 2015), Kruskal-Wallis tests for categorical and continuous variables (Hollander and Wolfe, 1973), and Pearson's correlation coefficient tests for two continuous variables (Schober et al., 2018).

2.2.6 Multinomial modelling of associations with management practices and prevalence of lameness in lambs and ewes

Three models were created, these were 1) LiL with flock management practices and options for treatment of footrot in lambs as explanatory variables (Model 1), 2) LiL with flock management practices and options for treatment of footrot in ewes as explanatory variables (Model 2) and 3) LiE with flock management practices and treatment of ewes as explanatory variables (Model 3).

The models took the form:

$$\mathrm{logit}(\pi_{\mathrm{1k/pi0k}}) = \beta_{\mathrm{0k}} + \sum \beta_0 x + e_k$$

$$\mathrm{logit}(\pi_{\mathrm{2k/pi0k}}) = \beta_{\mathrm{1k}} + \sum \beta_1 x + e_k$$

$$\mathrm{logit}(\pi_{\mathrm{3k/pi0k}}) = \beta_{\mathrm{2k}} + \sum \beta_2 x + e_k$$

where the baseline is ≤2% lameness and $\mathrm{logit}(\pi_{\mathrm{1k/pi0k}})$ = the probability of having >2-5% lameness, $\mathrm{logit}(\pi_{\mathrm{2k/pi0k}})$ = the probability of >5-10% lameness, $\mathrm{logit}(\pi_{\mathrm{3k/pi0k}})$ = the probability of having >10% lameness. $\beta_0 x$, $\beta_1 x$ and $\beta_2 x$ are a series of coefficients for explanatory variables for each category of prevalence of lameness, and e_k is the residual variance. The '*multinom'* function from the '*nnet'* package (Venables and Ripley, 2002) of R was used.

Initially, for each model ten sub-models were built. In each sub-model univariable associations between each explanatory variable and the prevalence of lameness were assessed and the variable with the lowest AIC was selected. Multivariable sub-models were then built using a manual forward stepwise process (Dohoo et al., 2003), with the variable with p≤0.05 and the lowest AIC score the next variable added to the multivariable model. Interactions between variables were tested by fitting the same model with an interaction term, these would have been included if they improved the AIC score and were biologically plausible. The sub-models were merged using a manual forward stepwise approach as above. All variables not in the final model were re-tested to check for residual confounding (Cox and Wermuth, 1996). Model fit was assessed using the Hosmer-Lemeshow test for multinomial models using the '*generalhoslem'* package (Jay, 2017).

2.3 Results

2.3.1 Prevalence of lameness in ewes and lambs

The geometric mean prevalence of LiL and LiE were 2.4% (95% CI = 2.1-2.6), and 3.4% (95% CI = 3.2-3.7) respectively; the LiL and LiE within a flock were significantly positively correlated (r = 0.62, p<0.01) and LiL was significantly lower than LiE. The geometric mean LiL was also lower than LiE in of the ≤2% lameness category but higher in the >10% lameness category (Table 2.1), indicating that the distribution of prevalence of LiL was more dispersed than LiE.

Table 2.1 Geometric mean period prevalence of lameness and 95% confidence interval in lambs and ewes by category of prevalence of lameness from 1271 flocks in England.

Prevalence of lameness (%)	Lambs		Ewes	
	N (%)	GM % prevalence (95% CI)	N (%)	GM % prevalence (95% CI)
≤2	553 (43.5)	0.7 (0.6-0.9)	413 (32.5)	1.1 (1.0-1.3)
>2-5	456 (35.9)	4.1 (4.0-4.2)	544 (42.8)	4.1 (4.0-4.2)
>5-10	165 (13.0)	8.7 (8.4-8.9)	222 (17.5)	8.6 (8.4-8.8)
>10	97 (7.6)	19.4 (18.0-20.9)	92 (7.2)	18.3 (17.2-19.4)

1. GM = Geometric mean, N = number of flocks, CI = Confidence Interval

2.3.2 Associations between variables

Strong associations were identified for treatment of ewes and lambs (Table 2.2), with farmers likely to treat their ewes and lambs similarly, with the strongest associations between using foot spray to treat both ID and SFR in both ewes and lambs and using antibiotic injection to treat SFR in both ewes and lambs.

Table 2.2 Strength of association between a treatment variable used in ewes and lambs for 732 flocks with complete responses to these questions in 2012-2013

Treatment of ewes	Treatment of lambs					
	Foot trimming		Antibiotic injection		Foot spray	
	SFR	ID	SFR	ID	SFR	ID
Foot trimming (SFR)	0.35*	0.20*	0.09	0.09	0.15*	0.10*
Foot trimming (ID)	0.21*	X	0.08	X	0.10*	0.10*
Antibiotic injection (SFR)	0.12*	0.10*	0.50*	0.21*	0.14*	0.10*
Antibiotic injection (ID)	0.08	X	X	X	0.08	0.10*
Foot spray (SFR)	0.13*	0.11*	0.11*	0.08*	0.63*	0.53*
Foot spray (ID)	0.12*	0.12*	0.11*	0.11*	0.48*	0.65*

1. * indicates where p<0.05 from the chi squared test, indicating a significant association between the practices. X is where expected values were less than 5, so a p value from the chi squared test could not be calculated. Categories for the treatments include never, sometimes, usually and always.

2.3.3 Latent class analyses of treatments for footrot in ewes and lambs

The LCAs for lambs and ewes were both optimal with four typologies of treatment for footrot, although the class attributes were different for lambs and ewes. Fit statistics for all tested models (2-7 classes) are in Appendix 1 and 2 along with the standard errors for class conditional probabilities (Appendix 3 and 4).

For typologies of treatment of lambs, the geometric mean LiL ranged from 1.0-3.5% (Table 2.3). Flocks in LC1 had significantly ($p<0.05$) lower LiL than flocks in LC2, LC3 and LC4. The prevalence of LiE did not differ significantly across typologies for treatment of lambs.

For typologies of treatment for ewes, the geometric mean LiE ranged from 1.8-4.2% (Table 2.3). In contrast to treatment of lambs, LC1 and LC2 had significantly ($p<0.05$) lower LiE than LC3 and LC4, with no significant difference in LiE between LC1 and LC2,and LC3 and LC4 (Table 2.3).

Table 2.3 Latent class models for treatment of lambs and ewes. Number of flocks, geometric mean period prevalence of lameness and 95% confidence intervals for treatment of lambs (823 flocks) and treatment of ewes (908 flocks) with footrot in England, 2012-2013

Latent Class	Number of flocks	GM prevalence of lameness (95% CI) LiL	LiE
Treatment of lambs			
LC1	117	1.0 (0.6-1.7)[a]	2.8 (2.0-3.9)[a]
LC2	214	2.8 (2.2-3.5)[b]	3.7 (3.2-4.3)[a]
LC3	257	3.1 (2.3-3.7)[b]	4.0 (3.6-4.4)[a]
LC4	235	3.5 (3.1-4.1)[b]	3.9 (3.5-4.3)[a]
Total	823		
Treatment of ewes			
LC1	86	1.1 (0.6-2.1)[a]	1.8 (1.0-3.1)[a]
LC2	134	2.4 (1.8-3.2)[ab]	3.2 (2.9-3.7)[a]
LC3	198	2.5 (1.9-3.3)[b]	3.9 (3.5-4.4)[b]
LC4	490	3.0 (2.6-3.4)[b]	4.2 (3.9-4.5)[b]
Total	908		

1. GM = Geometric mean, 95 % CI = 95% confidence interval, LiL: period prevalence of lameness in lambs. LiE: period prevalence of lameness in ewes. Where superscripts differ across columns, prevalence of lesion or lameness differs ($p\leq0.05$) between latent classes, as indicated by the Benjamini-Hochberg adjusted p-value from paired Wilcoxon tests.

LC analyses identified one typology (LC1) both in lambs (Figure 2.1) and ewes (Figure 2.2), where farmers had little lameness and used treatment infrequently, suggesting a low prevalence of lameness in some flocks that was stable with only using treatments for ID and SFR 'sometimes'. In these flocks, some farmers reported zero lameness (Table 2.3) and ewes in flocks in LC1 were significantly ($p<0.05$) less likely to have ID (geometric mean prevalence (GMP) = 1.06, 95% CI (0.48-2.34) than LC2 (GMP = 4.01%, 95% CI 3.02-5.32 (Table 2.4). For treatment of ewes but not lambs, there was a typology where farmers followed 'best practice' (LC2, Figure 2.2). Given that there was no significant difference in prevalence of LiE in LC1 and LC2, these farmers were actively controlling lameness successfully. Ewe flocks in LC3 and LC4 had significantly higher prevalence of LiE (LC3: GMP = 3.9%, 95% CI = 3.5-4.4), LC4: GMP = 4.2%, 95 % CI = 3.9-4.5)) and did not follow 'best practice' guidelines. Farmers in LC3 'usually' treated SFR with foot spray and antibiotic injection but were less likely to treat within 3 days (Figure 2.2), while farmers in LC4 treated SFR with detrimental managements including 'always' using foot spray with foot trimming (Figure 2.2). These flocks also had significantly ($p<0.05$) higher prevalence of CODD lesions than flocks in LC2 (Tables 2.4 and 2.5). Typologies for treatment of lambs in LC 2-4 were less distinct and the prevalence of LiL was not significantly different. Farmers mostly used antibiotic injection 'sometimes' (Figure 2.1), and 'usually' or always used topical treatment to treat ID and SFR in lambs.

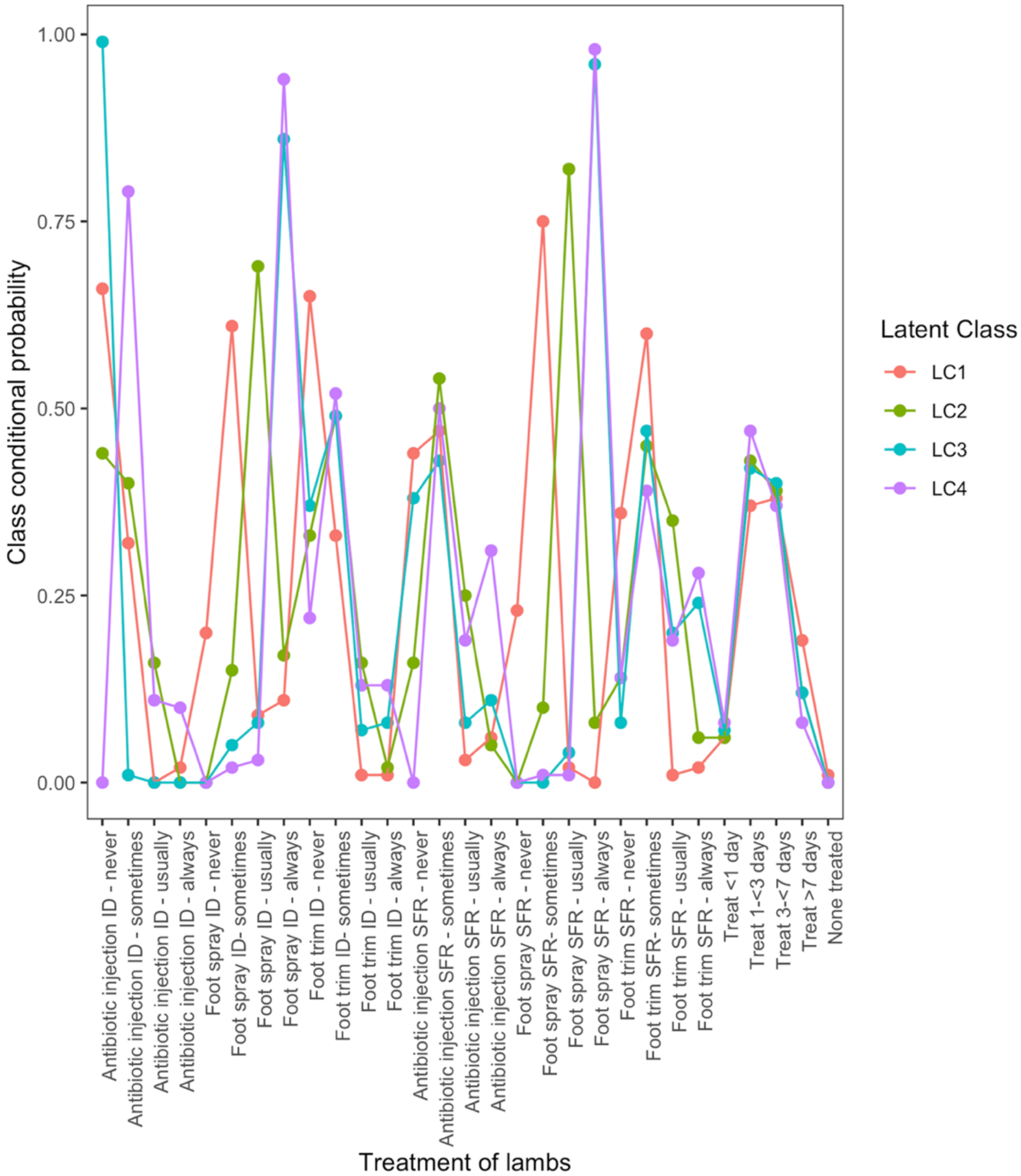


Figure 2.1 Class conditional probabilities that a farmer used a type and frequency of treatment on lambs with interdigital dermatitis or severe footrot from a four-class latent class model, for 823 flocks of sheep in England, 2012-2013.

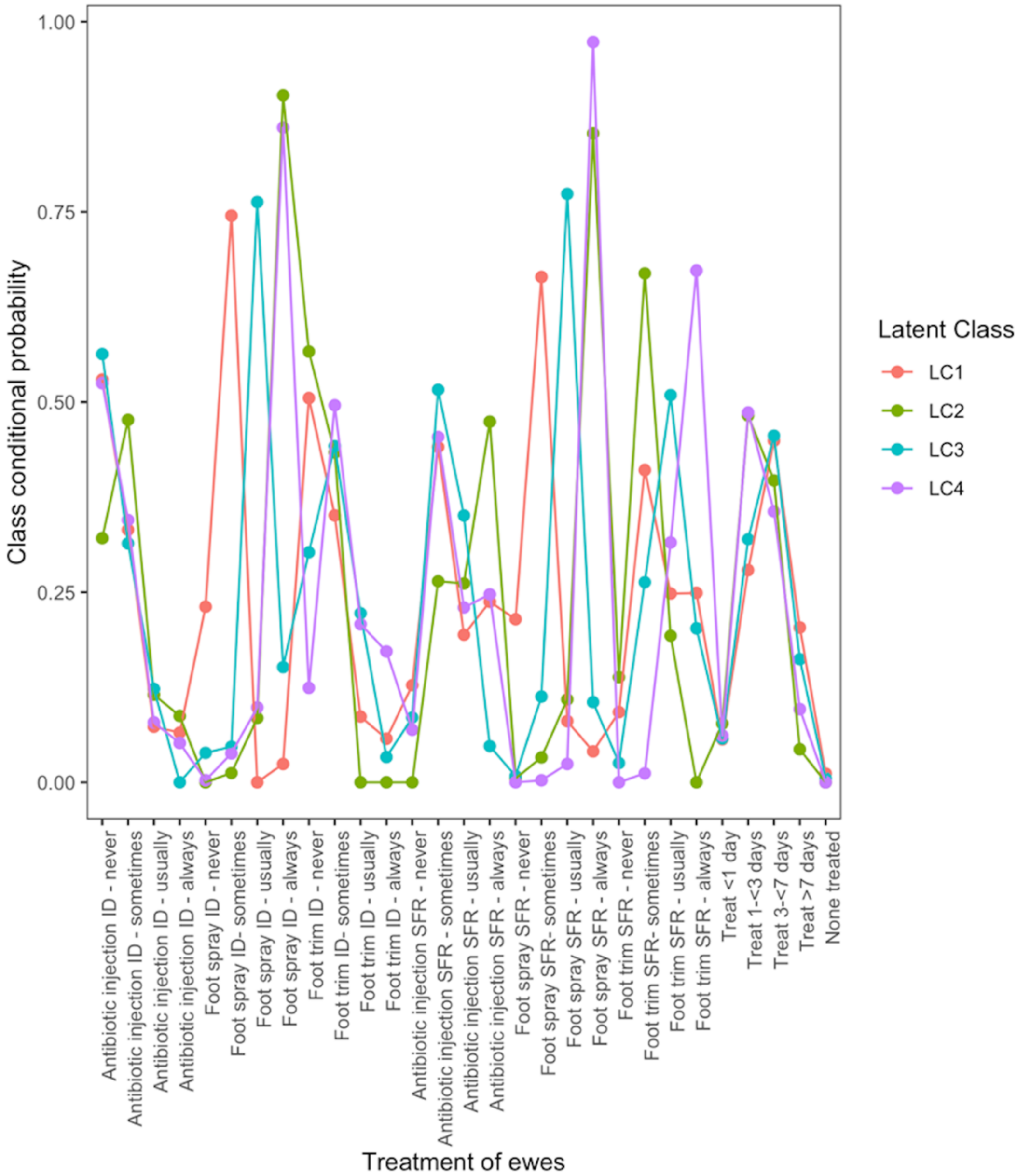


Figure 2.2 Class conditional probabilities that a farmer used a type and frequency of treatment on ewes with interdigital dermatitis or severe footrot from a four-class latent class model for 908 flocks of sheep in England, 2012-2013.

Table 2.4 Prevalence of farmers reporting lameness and lesions in their flock by latent class for treatment of lambs (842 flocks) and ewes (902 flocks), and percentage of missing observations by latent class.

Latent class	Farmer reported presence	Percentage of flocks reporting absent / present					
		Lame ewes	Lame lambs	Interdigital dermatitis	Severe footrot	CODD	Shelly hoof
Treatment of lambs							
1	Absent	2.6	8.5	12.0	17.1	47.9	27.4
	Present	97.4	91.5	81.2	75.2	45.3	36.8
	Not reported	0.0	0.0	6.8	7.7	6.8	35.9
2	Absent	0.5	1.9	5.1	9.8	35.0	20.1
	Present	99.5	98.1	85.5	82.2	55.6	47.7
	Not reported	0.0	0.0	9.3	7.9	9.3	32.2
3	Absent	0.0	1.2	2.3	8.9	34.6	19.8
	Present	100.0	98.8	90.3	84.8	58.0	49.0
	Not reported	0.0	0.0	7.4	6.2	7.4	31.1
4	Absent	0.0	0.4	2.1	11.5	41.3	19.6
	Present	100.0	99.6	91.9	83.4	51.1	43.0
	Not reported	0.0	0.0	6.0	5.1	7.7	37.4
Treatment of ewes							
1	Absent	5.8	8.1	11.6	15.1	40.7	25.6
	Present	94.2	91.9	83.7	76.7	52.3	47.7
	Not reported	0.0	0.0	4.7	8.1	7.0	26.7
2	Absent	0.0	2.2	1.5	9.7	50.7	23.1
	Present	100.0	97.8	94.0	86.6	42.5	36.6
	Not reported	0.0	0.0	4.5	3.7	6.7	40.3
3	Absent	0.0	2.5	7.1	10.1	36.9	17.7
	Present	100.0	97.5	82.8	82.3	52.0	42.9
	Not reported	0.0	0.0	10.1	7.6	11.1	39.4
4	Absent	0.0	1.4	2.9	11.0	38.0	22.7
	Present	100.0	98.6	90.6	82.4	55.5	45.5
	Not reported	0.0	0.0	6.5	6.5	6.5	31.8

Table 2.5 Geometric mean and 95% confidence intervals for prevalence of foot lesions in ewes by latent class for treatment of lambs (842 flocks) and treatment of ewes (908 flocks).

Latent class	GM % prevalence and 95% CI (%)			
	Interdigital dermatitis	Severe footrot	Contagious ovine digital dermatitis	Shelly hoof
Lambs				
LC1	1.09 (0.54-2.18) [a]	0.47 (0.21-1.03) [a]	0.01 (0.00-0.03) [a]	0.03 (0.01-0.10) [a]
LC2	2.65 (1.82-3.85) [ab]	1.09 (0.68-1.73) [a]	0.05 (0.02-0.09) [ab]	0.11 (0.05-0.22) [a]
LC3	3.92 (3.06-5.02) [b]	1.19 (0.80-1.79) [a]	0.06 (0.03-0.11) [b]	0.13 (0.07-0.27) [a]
LC4	3.52 (2.77-4.47) [ab]	0.89 (0.56-1.42) [a]	0.03 (0.01-0.05) [ab]	0.10 (0.05-0.21) [a]
Ewes				
LC1	1.06 (0.48-2.34) [a]	0.51 (0.21-1.24) [a]	0.02 (0.01-0.07) [ab]	0.06 (0.02-0.18) [a]
LC2	4.01 (3.02-5.32) [bc]	0.98 (0.56-1.72) [a]	0.01 (0.00-0.02) [b]	0.05 (0.02-0.14) [a]
LC3	1.84 (1.18-2.87) [ab]	1.15 (0.71-1.89) [a]	0.04 (0.02-0.08) [a]	0.11 (0.05-0.27) [a]
LC4	3.54 (2.93-4.27) [c]	0.95 (0.69-1.30) [a]	0.04 (0.03-0.06) [a]	0.08 (0.05-0.14) [a]

1. GM: Geometric mean, 95 % CI: 95% confidence interval (lower, upper), ID: interdigital dermatitis, SFR: severe footrot, CODD: contagious ovine digital dermatitis, SH = shelly hoof. Where superscripts differ across columns, prevalence of lesion or lameness differs ($p \leq 0.05$) between latent classes.

2.3.4 Multivariable models of associations between management of lameness and prevalence of lameness in lambs and ewes

Summaries of variables in the sub-model for each group of explanatory variables are found in Appendix 5 and 6. Since flock managements and treatment choices were similar for ewes and lambs within flocks (Table 2.2), separate models were developed to investigate flock managements and treatments of lambs (Model 1 (Table 2.6) and flock management and treatment of ewes (Model 2, Table 2.7) associated with LiL and associations between management and treatment of ewes and LiE (Model 3, Table 2.8). A comparison of covariates in each of the three models is shown in Figures 2.3, 2.4 and 2.5.

Similarly to the LC analyses, farmers with ≤2% LiL were less likely to use treatments whilst farmers with LiL >2% were more likely to use antibiotic injection and foot trimming to treat SFR in lambs and ewes (Table 2.6, Table 2.7, Table 2.8). Farmers with >10% prevalence of LiE were more likely to delay treatment of lame ewes until >10 sheep in a group were lame compared with one, and treat all sheep >3 days after onset of lameness compared with the first day they were seen lame (Model 3, Table 2.8). LiL >10% was associated with farmers recognising lameness at locomotion score (Kaler et al., 2009) >1 compared with 1 (Model 3). Routine managements that are detrimental to control of lameness in ewes (Winter et al., 2015) were also more frequently used in flocks with higher prevalence of lameness than in flocks with ≤2% lameness: farmers were more likely to footbath to treat SFR when LiL was >5-10% and >10%; farmers were more likely to vaccinate ewes with Footvax™ to treat footrot when LiL was >2-5% (Table 2.6). Farmers were also more likely to footbath to treat SFR when LiE >2% and less likely to footbath to prevent ID when LiE >10%. Reduced implementation of biosecurity practices was associated with >2% LiL and LiE (Table 2.6, Table 2.7, Table 2.8).

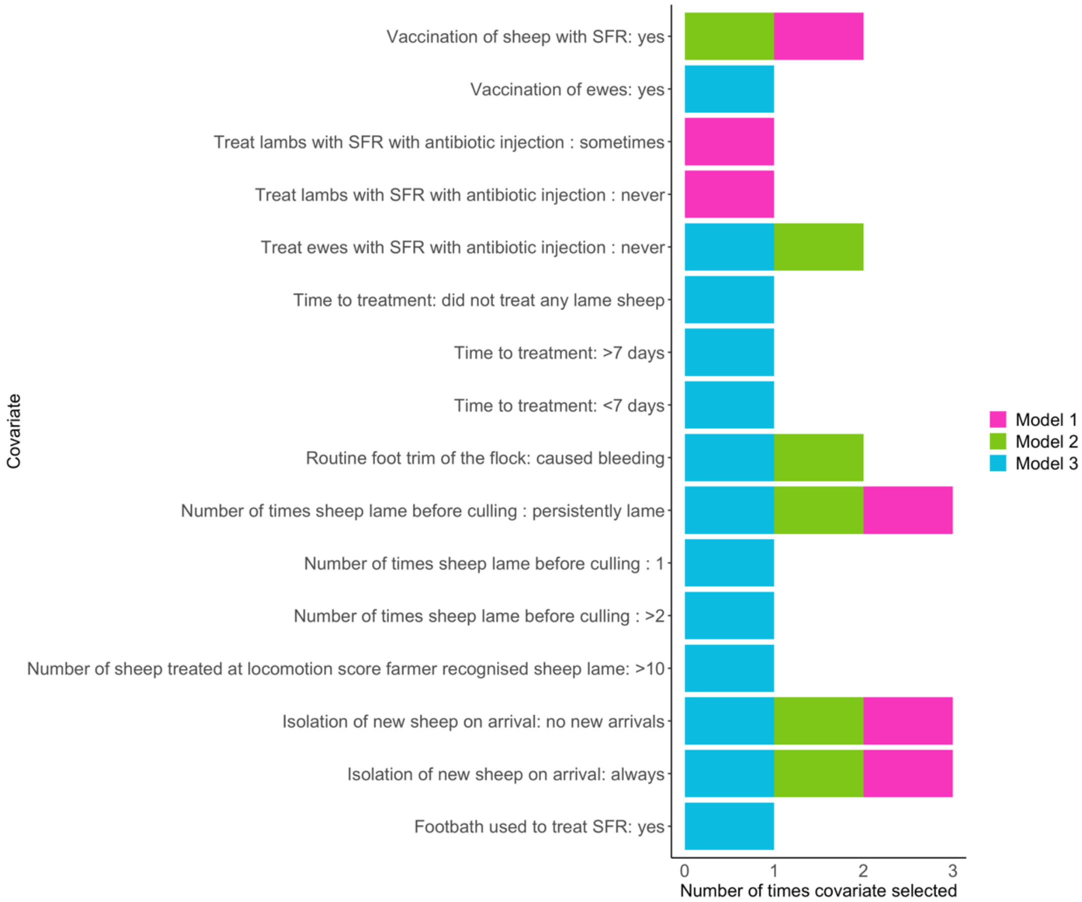


Figure 2.3 Comparison of covariates associated with having >2-5% lame sheep in the three models: Model 1, (pink - treatment used for lambs and flock management practices) Model 2 (green - treatment used for ewes and flock management practices) and Model 3 (blue - treatments for ewes and flock management practices)

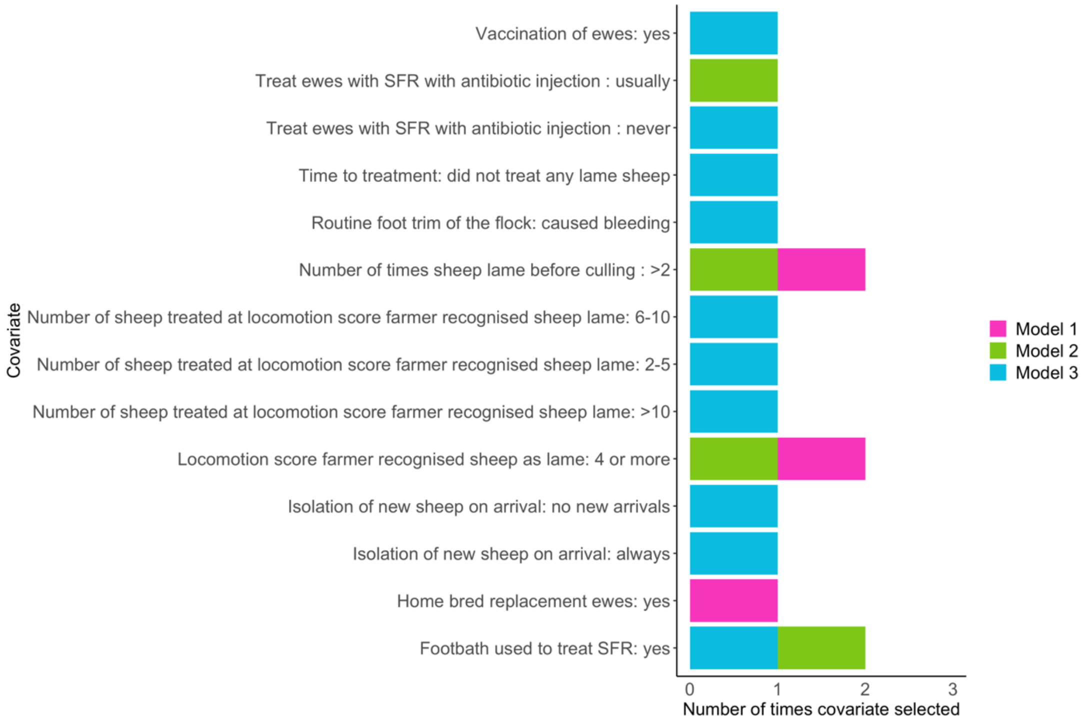


Figure 2.4 Comparison of covariates associated with having >5-10% lame sheep in the three models: Model 1, (pink - treatment used for lambs and flock management practices) Model 2 (green - treatment used for ewes and flock management practices) and Model 3 (blue - treatments for ewes and flock management practices)

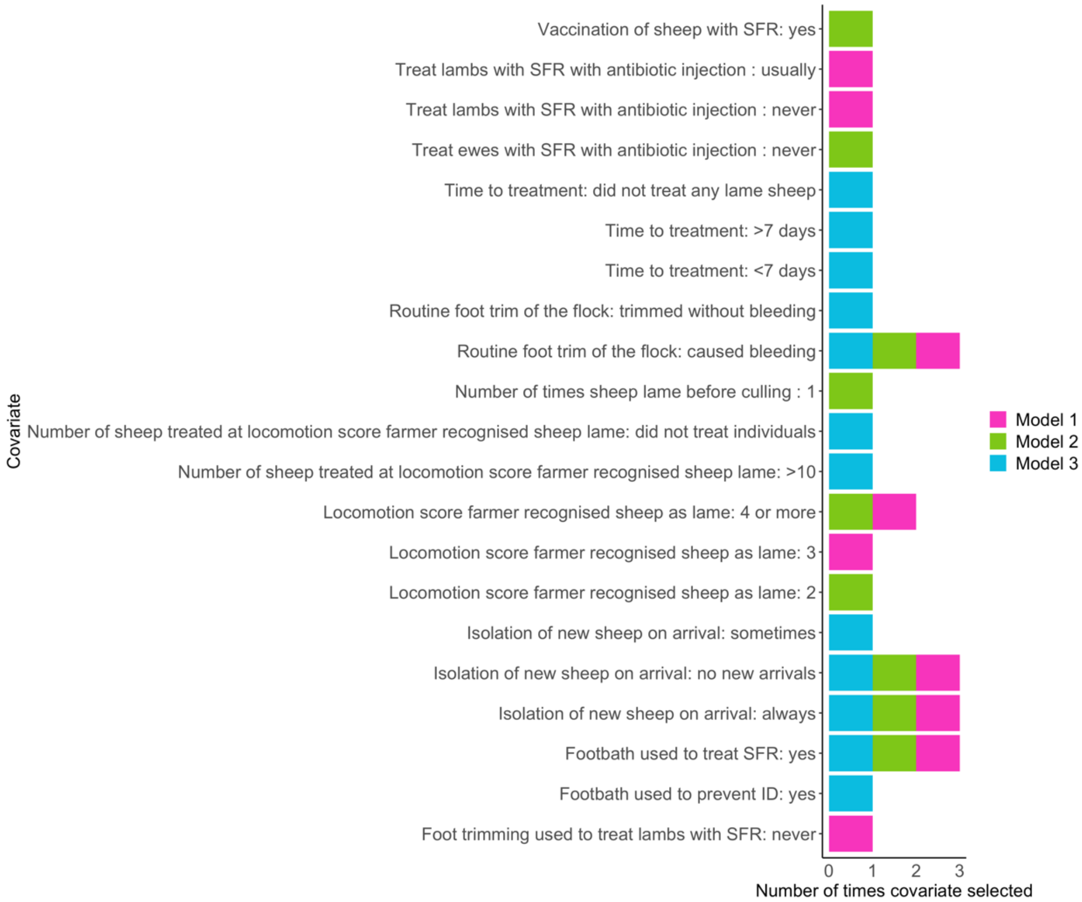


Figure 2.5 Comparison of covariates associated with having >2-5% lame sheep in the three models: Model 1, (pink - treatment used for lambs and flock management practices) Model 2 (green - treatment used for ewes and flock management practices) and Model 3 (blue - treatments for ewes and flock management practices)

Table 2.6 Multivariable multinomial model of factors associated with prevalence of lameness in lambs in 842 flocks of sheep in England, 2012-2013.

Prevalence of lameness and variable		N	%	OR	LCI	UCI	P-value
Antibiotic injection used to treat lambs with SFR							
≤2%	Always	38	11.0	Ref			
	Usually	48	13.9	-			
	Sometimes	160	46.4	-			
	Never	99	28.7	-			
>2-5%	Always	58	18.0	-			
	Usually	55	17.1	0.64	0.36	1.16	0.14
	Sometimes	**141**	**43.8**	**0.48**	**0.29**	**0.80**	**<0.01**
	Never	**68**	**21.1**	**0.42**	**0.24**	**0.73**	**<0.01**
>5-10%	Always	15	13.5	-			
	Usually	15	13.5	0.82	0.34	1.96	0.65
	Sometimes	63	56.8	1.01	0.49	2.07	0.98
	Never	18	16.2	0.56	0.24	1.30	0.18
>10%	Always	11	17.2	-			
	Usually	**7**	**10.9**	**0.32**	**0.10**	**1.00**	**0.05**
	Sometimes	37	57.8	0.54	0.23	1.25	0.15
	Never	**9**	**14.1**	**0.27**	**0.09**	**0.76**	**0.01**
Foot trimming used to treat lambs with SFR							
≤2%	Always	54	15.7	Ref			
	Usually	64	18.6	-			
	Sometimes	145	42.0	-			
	Never	82	23.8	-			
>2-5%	Always	58	18.0	-			
	Usually	74	23.0	1.07	0.64	1.80	0.80
	Sometimes	145	45.0	1.10	0.69	1.75	0.68
	Never	45	14.0	0.69	0.40	1.19	0.18
>5-10%	Always	28	25.2	-			
	Usually	22	19.8	0.82	0.34	1.96	0.65
	Sometimes	55	49.5	1.01	0.49	2.07	0.98
	Never	6	5.4	0.56	0.24	1.30	0.18
	Always	12	18.8	-			
>10%	Usually	16	25.0	1.16	0.47	2.83	0.75
	Sometimes	35	54.7	1.30	0.59	2.89	0.52
	Never	**1**	**1.6**	**0.11**	**0.01**	**0.87**	**0.04**
Vaccinate sheep with SFR using FootVax™							
≤2%	No	341	98.8	Ref			

	Yes	4	1.2	-			
>2-5%	No	309	96.0	-			
	Yes	**13**	**4.0**	**3.32**	**1.03**	**10.67**	**0.04**
>5-10%	No	108	97.3	-			
	Yes	3	2.7	1.89	0.39	9.18	0.43
>10%	No	62	96.9	-			
	Yes	2	3.1	3.65	0.59	22.71	0.16
Footbath to treat SFR							
≤2%	No	242	70.1		Ref		
	Yes	103	29.9	-			
>2-5%	No	202	62.7	-			
	Yes	120	37.3	1.19	0.85	1.68	0.31
>5-10%	No	65	58.6	-			
	Yes	46	41.4	1.30	0.81	2.08	0.28
>10%	No	27	42.2	-			
	Yes	**37**	**57.8**	**2.63**	**1.45**	**4.75**	**<0.01**
Locomotion score farmer recognised sheep as lame							
≤2%	1	200	58.0		Ref		
	2	112	32.5	-			
	3	31	9.0	-			
	4 or more	2	0.6	-			
>2-5%	1	167	51.9	-			
	2	121	37.6	1.30	0.92	1.83	0.14
	3	32	9.9	1.27	0.72	2.22	0.41
	4 or more	2	0.6	1.12	0.15	8.20	0.91
>5-10%	1	52	46.8	-			
	2	46	41.4	1.58	0.98	2.57	0.06
	3	9	8.1	1.18	0.51	2.75	0.70
	4 or more	**4**	**3.6**	**7.37**	**1.23**	**44.30**	**0.03**
>10%	1	26	40.6	-			
	2	24	37.5	1.78	0.94	3.37	0.08
	3	**11**	**17.2**	**2.83**	**1.17**	**6.82**	**0.02**
	4 or more	**3**	**4.7**	**10.61**	**1.47**	**76.28**	**0.02**
Routine foot trim the flock							
≤2%	Did not trim	163	47.2		Ref		
	Trimmed without bleeding	26	7.5	-			
	Caused bleeding	156	45.2	-			
>2-5%	Did not trim	132	41.0	-			
	Trimmed without bleeding	19	5.9	1.04	0.53	2.02	0.92
	Caused bleeding	171	53.1	1.39	0.99	1.94	0.06

>5-10%	Did not trim	59	53.2	-			
	Trimmed without bleeding	2	1.8	0.24	0.05	1.08	0.06
	Caused bleeding	50	45.0	0.79	0.50	1.27	0.33
>10%	Did not trim	12	18.8	-			
	Trimmed without bleeding	4	6.3	2.91	0.80	10.57	0.10
	Caused bleeding	**48**	**75.0**	**4.16**	**2.03**	**8.53**	**<0.01**
Number of times sheep lame before culling							
≤2%	Did not cull when lame	183	53.0				
	Lame once	18	5.2	Ref			
	Lame twice	50	14.5	-			
	Lame >2 times	79	22.9	-			
	Persistently lame	15	4.3	-			
>2-5%	Did not cull when lame	154	47.8	-			
	Lame once	9	2.8	0.60	0.25	1.42	0.25
	Lame twice	26	8.1	0.87	0.53	1.43	0.58
	Lame >2 times	96	29.8	1.39	0.94	2.05	0.09
	Persistently lame	**27**	**8.4**	**2.10**	**1.04**	**4.21**	**0.04**
>5-10%	Did not cull when lame	47	42.3	-			
	Lame once	2	1.8	0.48	0.10	2.25	0.35
	Lame twice	15	13.5	1.41	0.70	2.82	0.34
	Lame >2 times	**42**	**37.8**	**2.12**	**1.25**	**3.58**	**0.01**
	Persistently lame	5	4.5	1.24	0.41	3.74	0.70
>10%	Did not cull when lame	33	51.6	-			
	Lame once	0	0.0	0.00	0.00	4.18e109	0.94
	Lame twice	7	10.9	0.87	0.34	2.21	0.77
	Lame >2 times	20	31.3	1.54	0.78	3.04	0.21
	Persistently lame	4	6.3	1.40	0.40	4.84	0.60
Isolation of new sheep on arrival							
≤2%	Never	33	9.6	Ref			
	Sometimes	28	8.1	-			
	Usually	46	13.3	-			
	Always	164	47.5	-			
	No new sheep	74	21.4	-			
>2-5%	Never	46	14.3	-			
	Sometimes	29	9.0	0.59	0.29	1.20	0.15
	Usually	55	17.1	0.72	0.38	1.35	0.30
	Always	**138**	**42.9**	**0.51**	**0.30**	**0.87**	**0.01**

	No new sheep	**54**	**16.8**	**0.53**	**0.29**	**0.97**	**0.04**
>5-10%	Never	17	15.3	-			
	Sometimes	7	6.3	0.46	0.16	1.33	0.15
	Usually	18	16.2	0.66	0.28	1.55	0.34
	Always	49	44.1	0.52	0.25	1.06	0.07
	No new sheep	20	18.0	0.71	0.31	1.64	0.43
>10%	Never	11	17.2	-			
	Sometimes	7	10.9	0.50	0.16	1.60	0.25
	Usually	9	14.1	0.35	0.12	1.04	0.06
	Always	30	46.9	0.45	0.19	1.06	0.07
	No new sheep	**7**	**10.9**	**0.29**	**0.09**	**0.88**	**0.03**
Home breeding replacement ewes							
≤2%	No	98	28.4		Ref		
	Yes	247	71.6	-			
>2-5%	No	112	34.8	-			
	Yes	210	65.2	0.78	0.55	1.12	0.18
>5-10%	No	45	40.5	-			
	Yes	**66**	**59.5**	**0.55**	**0.33**	**0.89**	**0.02**
>10%	No	25	39.1	-			
	Yes	39	60.9	0.82	0.44	1.52	0.53

1. N = number of flocks, %: percent, OR = odds ratio, LCI = lower confidence interval, UCI = upper confidence interval, Ref = reference category. Where p≤0.05, OR marked in bold, indicating a significant difference from the baseline (according to Wald's test of significance).

Table 2.7 Multivariable multinomial model of management practices and treatments used on ewes associated with prevalence of lameness in lambs in 973 flocks of sheep in England, 2012-2013.

Prevalence of lameness and variable		N	%	OR	LCI	UCI	P-value
Treat ewes with SFR with antibiotic injection							
≤2%	Always	99	23.6		Ref		
	Usually	101	24.0	-			
	Sometimes	176	41.9	-			
	Never	44	10.5	-			
>2-5%	Always	100	27.9	-			
	Usually	85	23.7	0.71	0.47	1.08	0.11
	Sometimes	152	42.5	0.75	0.51	1.09	0.13
	Never	**21**	**5.9**	**0.47**	**0.25**	**0.88**	**0.02**
>5-10%	Always	21	17.2	-			
	Usually	**49**	**40.2**	**2.09**	**1.15**	**3.81**	**0.02**
	Sometimes	46	37.7	1.23	0.68	2.23	0.49
	Never	6	4.9	0.72	0.26	1.98	0.53
>10%	Always	19	26.0	-			
	Usually	20	27.4	0.81	0.39	1.67	0.56
	Sometimes	33	45.2	0.80	0.41	1.55	0.51
	Never	**1**	**1.4**	**0.11**	**0.01**	**0.92**	**0.04**
Footbath to treat SFR							
≤2%	No	300	71.4		Ref		
	Yes	120	28.6	-			
2-5%	No	229	64.0	-			
	Yes	129	36.0	1.28	0.94	1.75	0.12
5-10%	No	72	59.0	-			
	Yes	**50**	**41**	**1.61**	**1.04**	**2.49**	**0.03**
>10%	No	32	43.8	-			
	Yes	**41**	**56.2**	**2.86**	**1.68**	**4.85**	**<0.01**
Vaccinate sheep with SFR using FootVax™							
≤2%	No	416	99.0		Ref		
	Yes	4	1.0	-			
>2-5%	No	345	96.4	-			
	Yes	**13**	**3.6**	**4.46**	**1.40**	**14.20**	**0.01**
>5-10%	No	118	96.7	-			
	Yes	4	3.3	3.40	0.80	14.48	0.10
>10%	No	70	95.9	-			
	Yes	**3**	**4.1**	**7.00**	**1.40**	**35.07**	**0.02**
Locomotion score farmer recognised sheep as lame							

≤2%	1	240	57.1	Ref			
	2	132	31.4	-			
	3	45	10.7	-			
	4 or more	3	0.7	-			
>2-5%	1	185	51.7	-			
	2	134	37.4	1.33	0.97	1.83	0.08
	3	36	10.1	1.08	0.66	1.78	0.75
	4 or more	3	0.8	1.31	0.26	6.68	0.75
>5-10%	1	58	47.5	-			
	2	49	40.2	1.38	0.88	2.17	0.16
	3	10	8.2	0.93	0.43	2.01	0.86
	4 or more	**5**	**4.1**	**6.14**	**1.36**	**27.69**	**0.02**
>10%	1	30	41.1	-			
	2	**29**	**39.7**	**1.81**	**1.02**	**3.22**	**0.04**
	3	11	15.1	1.98	0.87	4.49	0.10
	4 or more	**3**	**4.1**	**7.14**	**1.28**	**39.85**	**0.03**
Routine foot trimming the flock							
≤2%	Did not trim	199	47.4	Ref			
	No bleeding caused	34	8.1	-			
	Caused bleeding	187	44.5	-			
>2-5%	Did not trim	151	42.2	-			
	No bleeding caused	21	5.9	0.90	0.49	1.65	0.74
	Bleeding caused	**186**	**52.0**	**1.38**	**1.01**	**1.87**	**0.04**
>5-10%	Did not trim	63	51.6	-			
	Trimmed without bleeding	4	3.3	0.44	0.15	1.32	0.14
	Caused bleeding	55	45.1	0.95	0.62	1.47	0.83
>10%	Did not trim	18	24.7	-			
	No bleeding caused	5	6.8	2.04	0.66	6.27	0.21
	Bleeding caused	**50**	**68.5**	**3.25**	**1.77**	**5.95**	**<0.01**
Culling sheep when lame							
≤2%	Did not cull	224	53.3	Ref			
	1	19	4.5	-			
	1-<2	63	15.0	-			
	>2	98	23.3	-			
	Persistently lame	16	3.8	-			
>2-5%	Did not cull	177	49.4	-			
	1	9	2.5	0.62	0.27	1.42	0.25
	1-<2	41	11.5	0.75	0.47	1.18	0.22
	>2	103	28.8	1.21	0.85	1.73	0.29
	Persistently lame	**28**	**7.8**	**2.22**	**1.14**	**4.33**	**0.02**

>5-10%	Did not cull	54	44.3	-			
	1	3	2.5	0.59	0.16	2.14	0.42
	1-<2	16	13.1	1.10	0.57	2.10	0.78
	>2	**44**	**36.1**	**1.63**	**1.00**	**2.66**	**0.05**
	Persistently lame	5	4.1	1.31	0.45	3.84	0.62
>10%	Did not cull	36	49.3	-			
	1	**0**	**0.0**	**0.00**	**0.00**	**0.00**	**<0.01**
	1-<2	8	11.0	0.81	0.34	1.89	0.62
	>2	24	32.9	1.39	0.76	2.56	0.29
	Persistently lame	5	6.8	1.80	0.58	5.58	0.31
Isolation of new sheep on arrival							
≤2%	Never	41	9.8		Ref		
	Usually	33	7.9	-			
	Sometimes	55	13.1	-			
	Always	196	46.7	-			
	No new arrivals	95	22.6	-			
>2-5%	Never	49	13.7	-			
	Usually	37	10.3	0.83	0.43	1.58	0.57
	Sometimes	60	16.8	0.79	0.44	1.40	0.41
	Always	**154**	**43.0**	**0.58**	**0.36**	**0.95**	**0.03**
	No new arrivals	**58**	**16.2**	**0.49**	**0.28**	**0.84**	**0.01**
>5-10%	Never	18	14.8	-			
	Usually	9	7.4	0.52	0.20	1.35	0.18
	Sometimes	21	17.2	0.70	0.32	1.53	0.37
	Always	51	41.8	0.53	0.27	1.03	0.06
	No new arrivals	23	18.9	0.62	0.30	1.31	0.21
>10%	Never	13	17.8	-			
	Usually	6	8.2	0.38	0.12	1.17	0.09
	Sometimes	11	15.1	0.44	0.17	1.16	0.10
	Always	**35**	**47.9**	**0.46**	**0.21**	**0.99**	**0.05**
	No new arrivals	**8**	**11.0**	**0.25**	**0.09**	**0.69**	**0.01**

1. N = number of flocks, % = percent, OR = odds ratio, LCI = lower confidence interval, UCI = upper confidence interval, Ref = reference category. Where p≤0.05, OR marked in bold, indicating a significant difference from the baseline (according to Wald's test of significance).

Table 2.8 Multivariable multinomial model of management practices and ewe treatments for severe footrot (SFR) and interdigital dermatitis (ID) associated with prevalence of lameness in ewes in 964 flocks of sheep in England, 2012-2013.

Prevalence of lameness and variable		N	%	OR	LCI	UCI	P-value
Treat ewes with SFR with antibiotic injection							
≤2%	Always	85	27.0	Ref			
	Usually	69	21.9	-			
	Sometimes	116	36.8	-			
	Never	45	14.3	-			
>2-5%	Always	101	24.2	-			
	Usually	116	27.8	1.29	0.83	2.01	0.25
	Sometimes	184	44.1	1.08	0.72	1.63	0.70
	Never	**16**	**3.8**	**0.28**	**0.14**	**0.56**	**<0.01**
>5-10%	Always	37	22.3	-			
	Usually	50	30.1	1.22	0.68	2.19	0.50
	Sometimes	70	42.2	0.88	0.51	1.50	0.63
	Never	**9**	**5.4**	**0.32**	**0.13**	**0.80**	**0.01**
>10%	Always	15	22.7	-			
	Usually	17	25.8	1.18	0.51	2.73	0.70
	Sometimes	32	48.5	0.94	0.44	2.00	0.87
	Never	2	3.0	0.21	0.04	1.07	0.06
Footbath to treat SFR							
≤2%	No	241	76.5	Ref			
	Yes	74	23.5	-			
>2-5%	No	263	63.1	-			
	Yes	**154**	**36.9**	**1.51**	**1.05**	**2.18**	**0.03**
>5-10%	No	87	52.4	-			
	Yes	**79**	**47.6**	**2.40**	**1.52**	**3.79**	**<0.01**
>10%	No	34	51.5	-			
	Yes	**32**	**48.5**	**2.81**	**1.51**	**5.22**	**<0.01**
Footbath to prevent ID							
≤2%	No	219	69.5	Ref			
	Yes	96	30.5	-			
>2-5%	No	246	59.0	-			
	Yes	171	41.0	1.22	0.86	1.74	0.26
>5-10%	No	99	59.6	-			
	Yes	67	40.4	1.01	0.64	1.59	0.97
>10%	No	50	75.8	-			
	Yes	**16**	**24.2**	**0.44**	**0.22**	**0.87**	**0.02**
Vaccinate ewes using FootVax™							

≤2%	No	248	78.7	Ref			
	Yes	67	21.3	-			
>2-5%	No	347	83.2	-			
	Yes	**70**	**16.8**	**0.62**	**0.41**	**0.94**	**0.02**
>5-10%	No	148	89.2	-			
	Yes	**18**	**10.8**	**0.39**	**0.21**	**0.71**	**<0.01**
>10%	No	56	84.8	-			
	Yes	10	15.2	0.65	0.29	1.45	0.30
Time to treatment of sheep with SFR							
≤2%	<1 day	30	9.5	Ref			
	<3 days	159	50.5	-			
	<7 days	98	31.1	-			
	>7 days	26	8.3	-			
	None treated	2	0.6	-			
>2-5%	<1 day	17	4.1	-			
	<3 days	190	45.6	1.88	0.95	3.73	0.07
	<7 days	**165**	**39.6**	**2.48**	**1.22**	**5.03**	**0.01**
	>7 days	**45**	**10.8**	**2.81**	**1.19**	**6.59**	**0.02**
	Individuals not treated	**0**	**0.0**	**0.00**	**0.00**	**0.00**	**<0.01**
>5-10%	<1 day	8	4.8				
	<3 days	56	33.7	0.94	0.38	2.35	0.89
	<7 days	85	51.2	2.13	0.85	5.33	0.11
	>7 days	17	10.2	1.63	0.54	4.95	0.39
	Individuals not treated	**0**	**0.0**	**0.00**	**0.00**	**0.00**	**<0.01**
>10%	<1 day	1	1.5				
	<3 days	22	33.3	3.68	0.45	30.04	0.22
	<7 days	**33**	**50.0**	**9.44**	**1.15**	**77.58**	**0.04**
	>7 days	**10**	**15.2**	**11.10**	**1.20**	**102.86**	**0.03**
	Individuals not treated	**0**	**0.0**	**0.74**	**0.74**	**0.74**	**<0.01**
Number of sheep treated							
≤2%	1	67	21.3	Ref			
	2-5	177	56.2	-			
	6-10	41	13.0	-			
	>10	27	8.6	-			
	Individuals not treated	3	1.0	-			
>2-5%	1	54	12.9	-			
	2-5	223	53.5	1.19	0.76	1.86	0.45
	6-10	78	18.7	1.52	0.86	2.67	0.15

	>10	**59**	**14.1**	**1.88**	**1.00**	**3.51**	**0.05**
	Individuals not treated	3	0.7	2.42	0.20	28.77	0.49
>5-10%	1	9	5.4	-			
	2-5	**89**	**53.6**	**2.78**	**1.26**	**6.10**	**0.01**
	6-10	**36**	**21.7**	**3.85**	**1.59**	**9.29**	**<0.01**
	>10	**30**	**18.1**	**5.99**	**2.36**	**15.22**	**<0.01**
	Individuals not treated	2	1.2	8.69	0.56	134.73	0.12
>10%	1	8	12.1	-			
	2-5	16	24.2	0.46	0.18	1.18	0.11
	6-10	24	36.4	2.34	0.89	6.15	0.08
	>10	**18**	**27.3**	**2.96**	**1.05**	**8.38**	**0.04**
	Individuals not treated	**0**	**0.0**	**0.00**	**0.00**	**0.00**	**<0.01**
Routine foot trim the flock							
≤2%	Did not trim	174	55.2	Ref			
	No bleeding caused	26	8.3	-			
	Bleeding caused	115	36.5	-			
>2-5%	Did not trim	183	43.9	-			
	No bleeding caused	25	6.0	1.28	0.68	2.42	0.44
	Bleeding caused	**209**	**50.1**	**1.71**	**1.22**	**2.40**	**<0.01**
	Did not trim	58	34.9	-			
>5-10%	No bleeding caused	8	4.8	1.46	0.59	3.63	0.41
	Bleeding caused	**100**	**60.2**	**2.42**	**1.56**	**3.76**	**<0.01**
	Did not trim			-			
>10%	**No bleeding caused**	**4**	**6.1**	**4.11**	**1.15**	**14.65**	**0.03**
	Bleeding caused	**47**	**71.2**	**5.53**	**2.80**	**10.93**	**<0.01**
Isolation of new sheep on arrival							
≤2%	Never	29	9.2	Ref			
	Sometimes	24	7.6	-			
	Usually	41	13.0	-			
	Always	150	47.6	-			
	No new arrivals	71	22.5	-			
>2-5%	Never	56	13.4	-			
	Sometimes	34	8.2	0.49	0.23	1.03	0.06
	Usually	59	14.1	0.54	0.28	1.03	0.06
	Always	**190**	**45.6**	**0.47**	**0.27**	**0.82**	**0.01**

	No new arrivals	**78**	**18.7**	**0.55**	**0.30**	**1.00**	**0.05**
>5-10%	Never	24	14.5	-			
	Sometimes	22	13.3	0.68	0.29	1.63	0.39
	Usually	37	22.3	0.85	0.39	1.85	0.69
	Always	**56**	**33.7**	**0.34**	**0.17**	**0.67**	**<0.01**
	No new arrivals	**27**	**16.3**	**0.46**	**0.21**	**0.98**	**0.04**
>10%	Never	12	18.2	-			
	Sometimes	**4**	**6.1**	**0.18**	**0.05**	**0.72**	**0.01**
	Usually	9	13.6	0.35	0.12	1.06	0.06
	Always	**32**	**48.5**	**0.38**	**0.16**	**0.90**	**0.03**
	No new arrivals	**9**	**13.6**	**0.26**	**0.09**	**0.75**	**0.01**
Number of times sheep lame before culling							
≤2%	Did not cull lame sheep	174	55.2	Ref			
	1	20	6.3	-			
	1 to <2	47	14.9	-			
	>2	64	20.3	-			
	Persistently lame	10	3.2	-			
>2-5%	Did not cull lame sheep	188	45.1	-			
	1	**8**	**1.9**	**0.37**	**0.15**	**0.91**	**0.03**
	1 to <2	55	13.2	1.03	0.64	1.64	0.92
	>2	**136**	**32.6**	**1.83**	**1.24**	**2.72**	**<0.01**
	Persistently lame	**30**	**7.2**	**2.29**	**1.06**	**4.95**	**0.04**
>5-10%	Did not cull lame sheep	88	53.0	-			
	1	3	1.8	0.30	0.08	1.11	0.07
	1 to <2	19	11.4	0.71	0.37	1.36	0.30
	>2	46	27.7	1.25	0.75	2.09	0.39
	Persistently lame	10	6.0	1.45	0.55	3.82	0.45
>10%	Did not cull lame sheep	33	50.0	-			
	1	1	1.5	0.38	0.05	3.19	0.38
	1 to <2	7	10.6	0.68	0.26	1.75	0.42
	>2	21	31.8	1.65	0.83	3.30	0.16
	Persistently lame	4	6.1	1.70	0.46	6.36	0.43

1. N = number, % = percent, OR = odds ratio, LCI = lower confidence interval, UCI = upper confidence interval, Ref = reference category. Where p≤0.05, OR marked in bold, indicating a significant difference from the baseline (according to Wald's test of significance).

2.3.5 Multivariable model fit

The Hoslem test did not indicate lack of fit of any model (Model 1: $p = 0.85$, Model 2: $p = 0.74$, Model 3: $p = 0.66$). The numbers of flocks observed and predicted to be in each category of the multinomial models are shown in Table 2.9.

Table 2.9 Numbers of flocks observed and predicted in each category of the three multinomial models (Model 1: lameness in lambs, flock managements and treatment used for lambs, Model 2: lameness in lambs, flock managements and treatment used for ewes, Model 3: lameness in ewes, flock managements and treatment used for ewes)

Model	Number of flocks observed in category	Number of flocks predicted in category			
		≤2%	>2-5%	>5-10%	>10%
Model 1	≤2%	240	100	0	5
	>2-5%	142	176	2	2
	>5-10%	49	52	8	2
	>10%	16	42	3	3
Model 2	≤2%	307	110	1	2
	>2-5%	189	167	2	0
	>5-10%	67	50	4	1
	>10%	28	41	3	1
Model 3	≤2%	157	149	7	2
	>2-5%	89	310	11	7
	>5-10%	25	124	14	3
	>10%	6	52	4	4

2.4 Discussion

This is the first investigation of individual treatment practices and flock managements associated with the prevalence of lameness in lambs in the United Kingdom, and globally. One highly novel finding was a group of flocks with low prevalence of lameness in lambs and ewes that were only sometimes treating sheep lame with footrot (LC1 for lambs and ewes). The most logical explanation for this association is that the cause of lameness was primarily non-infectious. This is supported by evidence that more farmers within this class reported having no ID or SFR lesions in ewes in the flock than in other latent classes (Table 2.4). If infectious causes of lameness were present and farmers delayed treatment, the prevalence of lameness would increase because the duration of each case and spread of disease would occur without prompt treatment (Wassink et al., 2010). An alternative hypothesis for why lack of treatment was associated with low prevalence of lameness in a flock is that there is a non-linear dynamic infection process in footrot and that at ≤2% prevalence of lameness a low force of infection occurs and so footrot spreads slowly. Vaccination could contribute to slow spread of disease and flocks with >2-5% or >5-10% LiE were less likely to vaccinate ewes with FootVax™, the commercially available vaccine in the UK, than those with ≤2% LiE (Supplementary Table 8). Finally, environmental conditions (Depiazzi et al., 1998, Graham and Egerton, 1968) or host genetics (Niggeler et al., 2017, Nieuwhof et al., 2008a) might have protected flocks sufficiently to reduce the force of infection.

A second novel finding was that no farmers with LiL >2% are currently using best practice (Model 1). Farmers in LC 2 – 4 delayed treatment, waited until lambs had more severe locomotion scores or waited until several lambs in a group were lame. These variables are all correlated (Winter et al., 2015) and associated with high prevalence of lameness in ewes. From the current study, and the infectious nature of footrot, we can conclude that as with ewes (Winter et al., 2015), prompt treatment of the first mildly lame lamb in a group would reduce the flock prevalence of LiL.

The current British Veterinary Association (BVA) guidelines on appropriate use of antimicrobial products recommend that while use of antimicrobials in farm animals should be minimized, they should be used when appropriate, to treat clinically diseased animals (British Veterinary Association, 2019). Our results provide evidence that some farmers are using antimicrobial products appropriately to manage lameness. These were farmers with lamb and ewe flocks in LC1 with a low prevalence of infectious causes of lameness where antibiotics were rarely used, and ewes in LC2 where 'best practice' controlled infectious causes of lameness (Wassink et al., 2010b, Kaler et al., 2010a). However, for ewes in flocks in LC3 and 4 and lambs in flocks in LC 2 – 4 potentially more antibiotic treatments are being used, because they are administered too late to prevent onward spread of infectious causes of lameness.

A third novel finding was that farmers were more likely to practice therapeutic foot trimming of lame lambs in flocks with LiL >10% (Model 1). There was no association between therapeutic foot trimming of ewes and LiL (Model 2) indicating that the effect if trimming feet on lameness is direct, that is, the prevalence of lameness in lambs was only high when farmers trimmed the feet of lame lambs. There is strong evidence that therapeutic foot trimming of lame ewes delays recovery from footrot (Kaler et al., 2010) and that foot trimming is associated with development of granulomas (Reeves et al., 2019) which cause chronic lameness, and so the high flock prevalence of lameness in lambs from foot trimming is possibly not unexpected but this is useful evidence to present to farmers to influence their behaviour (Green et al., 2020).

There were no management practices associated with prevalence of LiL that have not previously been associated with LiE. However, those identified can be used to improve control of lameness in lambs and so contribute to the FAWC 2021 target of <2% prevalence of lameness in sheep flocks in the UK. These are discussed briefly below.

Farmers with >2% LiL were less likely to take measures to prevent introduction of disease onto the farm, including introducing new sheep and not quarantining new sheep (Table 5) both previously associated with prevalence of lameness in ewes (Winter et al., 2015; Wassink et al., 2004). Bringing in new sheep is particularly associated with introduction of CODD (Angell et al., 2014, Dickins et al., 2016). Whole flock management practices previously associated with LiE, and now associated with LiL, included feet bleeding when routine foot trimming the flock compared with not routinely trimming the flock, culling sheep when persistently lame and footbathing to treat SFR. Lambs are not typically vaccinated against footrot and it is not known whether vaccinating ewes with FootVax™ protects lambs . There was no association between vaccination of ewes and prevalence of LiL. Flocks with >2-5% LiL were more likely to vaccinate ewes to treat SFR than those with <2% LiL, demonstrating a reactive approach to managing lameness rather than a preventive approach.

The data for this study came from a questionnaire where the lamb flock size was not known, preventing analysis of data on lambs using an over-dispersed Poisson model as used by Winter et al., (2015) for ewes. The percentage of lame lambs per flock was known and so consequently a multinomial model was used. A model for LiE was built to investigate risk factors for ewes using the same family of models and compared with the Winter et al. (2015) results. Not as many risk factors for ewes were significant in the multinomial model as in the over-dispersed Poisson model, which might be explained by the categorization of the outcome variable which can result in data loss potentially reducing the power to detect significant associations (Altman et al., 1994).

2.5 Conclusion

In conclusion, low prevalence of lameness in lambs is associated with low prevalence of infectious foot diseases and that no farmers are using best practice to treat lame lambs. In addition, foot trimming lame lambs is as detrimental as foot trimming lame ewes. We identified three distinct flock types and farmer behaviours: low prevalence of lameness with little treatment of lameness and where foot trimming and footbathing were not practised, low prevalence of lameness where 'best practice' treatment and vaccination were used, again without foot trimming and footbathing and higher prevalence of lameness associated with foot trimming, footbathing, poor biosecurity and poor treatment options. We conclude that adopting best practice and avoiding foot trimming and footbathing and practicing good biosecurity would reduce the prevalence of lameness in lambs and contribute to the FAWC target of <2% flock prevalence of lameness by 2021.

Chapter 3 Multiple model triangulation to identify factors associated with lameness in British sheep flocks

The contents of this chapter have been published in Lewis et al., 2021, Multiple model triangulation to identify factors associated with lameness in British sheep flocks, Preventive Veterinary Medicine. The full published paper and Supplementary Material can be found at: https://doi.org/10.1016/j.prevetmed.2021.105395. The Methods, Results and Discussion and Conclusion have been taken from the pre-print.

3.1 Introduction

The aim of this chapter was to further investigate how management practices are associated with prevalence of lameness in lambs, and how management of lambs impacts prevalence of lameness in ewes, using the results of a more recent questionnaire sent to farmers in 2018 that collected further information on where managements for lambs differs from those for ewes. Since identification of causal factors can be challenging when the number of explanatory variables is large in relation to the number of observations, multiple model triangulation was used to identify the covariates most likely to be truly associated with the prevalence of lameness in sheep flocks in Great Britain.

3.2 Materials and Methods

3.2.1 Questionnaire design and administration

Ethical approval (reference number BSREC 67/18-19) was granted by the University of Warwick. The aim of the questionnaire (designed by Jessica Witt (JW) and Laura Green (LG)) was to collect updated figures for flock-level prevalence of lameness in ewes and lambs, and their association with management practices and to widen the target population of sheep flocks from England only to include Welsh and Scottish flocks. The questionnaire (available online in the Supplementary Material for the published paper) had six sections – causes of lameness, patterns of lameness, management of the flock, culling and replacement of ewes and farm, and flock, characteristics. Questions were mostly closed, with some options for free text answers.

In 2018, 2000 questionnaires were sent to a random sample of farmers in England selected by the Agricultural and Horticultural Development Board (AHDB), and a further 600 to farmers in Scotland, and 600 in Wales selected by the National Sheep Association (NSA). Two reminder letters were sent.

3.2.2 Data cleaning and re-structuring of explanatory variables

Data were double entered by Wyman Dillon Ltd, Bristol, returned and stored as an Excel file, and cleaned manually by Kate Lewis (KL) and JW, checking each response for errors and inconsistencies against the original questionnaire.

Questionnaires were useable when farmers reported the annual period prevalence of lameness in ewes and lambs, and the number of ewes and lambs in the flock (450 responses), and questions were useable if they were answered by >85% of farmers. Where >85% but not all farmers answered a question a "missing" category was created, for continuous variables the data were categorised into quintiles with a sixth "missing" category and for categorical variables one category was "missing" data. Use of this "missing" category resulted in dataset of 310 completely answered responses used for modelling work.

Data management and analyses were conducted using RStudio v3.6.0 (R statistical software, R Core Team, 2019). Descriptive statistics, measures of central tendency and dispersion and frequency distributions, were used to explore each variable and to inform recoding of variables for analysis. There were 57 categorical variables which were coded as 105 dummy variables for the elastic net models (Kassambara, 2018) using *fastDummies* (Kaplan, 2020). Associations between variables were explored using contingency tables and chi-square tests of association.

3.2.3 Models of associations between management practices and the prevalence of lameness in ewes

3.2.3.1 Model types 1 and 2: Generalised linear models

Two model structures appropriate for over-dispersed count data, the quasi-Poisson (QP-GLM) and negative binomial (NB-GLM), were used.

The models took the form:

$$\text{Number of lame ewes}_i \sim \alpha + \text{offset}(\log(\text{number of ewes in flock}_i)) + \sum \beta_i X_i + e$$

where ~ is the log link function, α the intercept, i the ith flock offset by the natural log of the number of ewes in the flock i and β_i the coefficients for a series of predictor variables, X_i, and e the residual error. Confidence intervals were obtained by profiling the likelihood using *MASS* (Venables and Ripley, 2002).

Initially, four models were built using subsets of the variables (treatment of ewes and lambs, management of the flock, replacement of the flock, and the flock environment). Country and flock size were forced into each model. For the NB-GLM, a manual forwards stepwise selection process (Dohoo et al., 2009) was used to select variables for inclusion in the model using the *MASS* package (Venables and Ripley). For the quasi-Poisson model manual selection and the *stats* base package was used (R Core Team, 2019). Variables remained in the sub-models when the p-value from a Wald's test of significance was <0.10.

Two final multivariable models were built from the sub-models using a forwards stepwise approach with variables retained in the model when p<0.05 (Wald's test). All variables were re-tested in the final multivariable model to check for residual confounding (Cox and Wermuth, 1996) and interactions between variables in the final model were checked, to be included if biologically relevant and significant (p<0.05). Model fit was checked by ranking predicted and observed numbers of lame sheep per flock and summing them in deciles and comparing the distributions of the deciles (Cameron and Trivedi, 2013). Since model fit indicated that that the adjusted Poisson models did not correct sufficiently for over dispersion of the outcome variable, an additional dummy variable was created that identified flocks in the tenth decile as "problem flocks" – with a prevalence of lameness in ewes ≥7.1% and in lambs ≥8.5%. The "problem flock" variable was forced into the final models to evaluate model fit and retained where model fit was improved and it did not impact on the coefficients of other variables in the model.

3.2.3.2 Model types 3 and 4: Elastic net models with covariate selection stability

Because the specification of the response variable can influence model results (Tercerio, 2003) two distributions were used for model triangulation. These were:

1) A Poisson distribution with the outcome number of lame ewes in the flock, offset by the natural logarithm of the number of ewes in the flock ("PEN-BS")
2) A Gaussian log10(x+1) with the outcome log10((1+the number of lame ewes)/number of ewes in the flock), giving a rate ("GEN-BS")

Models were fitted using the *glmnet* (Friedman et al., 2009) and *caret* R packages (Kuhn, 2020). The elastic net is designed to implement a balance between ridge regression and the least absolute shrinkage and selection operator (LASSO) penalties (Friedman et al., 2010). Full details of the model algorithms is in Friedman et al., (2010), but essentially the elastic net solves the problem:

$$SSE_{enet} = \frac{1}{2n}\sum_{i}^{n}(y_i - \hat{y}_i)^2 + \lambda[\sum_{j}^{P}\left(\frac{1}{2}(1-\alpha)\beta_j^2\right) + \alpha\beta_j]$$

Where, for the Gaussian family, SSE_{enet} is the elastic net loss function to be minimised, i represents each farm, n the number of farms, y_i the observed outcome for the ith farm and $\hat{y}_i$ the predicted outcome for the ith farm. The penalisation parameter is λ, with j, a predictor variable, p the total number of predictor variables, and α the mixing parameter that defined the relative proportion of penalisation on either the sum of the square of the coefficients (β^2) or the unsquared coefficients (β).

For the Poisson regression model, *glmnet* uses an outer Newton loop, and an inner weighted least-squares loop to optimise the penalised log likelihood, using the equation:

$$\min_{\beta o,\beta} -\frac{1}{N}\mathrm{l}(\beta|\mathrm{X},\mathrm{Y}) + \lambda\left((1-\alpha)\sum_{i=1}^{N}\beta_i^2/2) + \alpha\sum_{i=1}^{N}|\,\beta_i|\right)$$

Three further parameters were calculated for these models using a bootstrap procedure of 100 resamples (Hastie et al., 2015):

- Covariate stability: the percentage of times a covariate was selected in the elastic net model over the 100 bootstrap samples
- Coefficient 95% confidence intervals (Steyerberg, 2019): the 2.5 and 97.5 percentile values from the distribution of covariate coefficients from the bootstrap samples when the variable was selected
- Bootstrap p-values: the smaller proportion of a coefficient's values on one side of zero across the 100 bootstrap samples. For example, if a covariate was selected in the model in 80 of 100 bootstrap samples (i.e. a stability of 80%) and 10 of these were all greater (or all less) than zero, then the bootstrap p-value would be 10/80=0.125.

For each elastic net model, from each of the 100 bootstrap samples, ten-fold cross validation, repeated 10 times was used to find the values λ and α (from a wide grid of parameter values) that minimised model mean absolute error (MAE). Values for α for both models ranged from 0.1 to 1.0 at 0.1 increments, and values for λ ranged from 0-30 for the PEN-BS model and 0-2 for the GEN-BS model, with distributions of the optimal value from each sample stored after each run to ensure a sufficient range had been used. The distribution of parameter values used are provided in Appendix 7.

A cut-point selection stability of >80% and a bootstrap p-value of <0.05 were chosen to identify predictor variables retained in the final model (Lima et al., 2020a).

A similar methodological approach was taken to identify predictor variables most consistently associated with prevalence of lameness in lambs. The four model types used were the same as those used to model the ewe data but using the number of lame lambs per flock as the numerator and number of lambs born as the denominator for the outcome variable.

3.3 Results

3.3.1 Response rate and flock characteristics of ewes and lambs in flocks in Great Britain, 2018

A total of 523 (16.3%) questionnaires were returned, with 450 containing the average prevalence of lameness in ewes and lambs and the flock size, a useable response rate of 14.1%. The useable response rate was reasonably similar by country – England – 15.2%, Scotland 11.7%, Wales – 12.7%. There were 310 responses useable for modelling purposes (9.7%). The geographical distribution of respondents is in Appendix 8.

Flocks in Scotland were larger than flocks in England and Wales (Table 3.1) and some factors differed significantly between the three countries; including altitude, exposure to clay soil, an open flock and proportion of flocks vaccinating ewes with FootVax™ (MSD Animal Health). The numbers and percentages of farmers using each management practice are available online in the Supplementary Material for the published paper.

The geometric mean prevalence of lameness was 1.4% (95% CI 1.2-1.7) of ewes and 0.6% (95% CI 0.5-0.9) of lambs (Table 3.1), with a moderate within flock correlation between the prevalence of lameness in ewes and lambs (Spearman's rank correlation rho = 0.60, p<0.001). Infectious bacterial diseases were the predominant cause of lameness in both ewes and lambs, 87.3% of farmers reported ID, 75.3% reported SFR and 36.9% reported CODD (Appendix 9).

Table 3.1 Flock size and prevalence of lameness in ewes and lambs in 450 flocks of sheep in Great Britain (October 2017-September 2018)

	Overall	England	Scotland	Wales
Flock characteristics (number)				
Responses	450	304	70	76
Number of ewes				
Median (95% CI)	250 (220-300)	200 (165-235)[a]	545 (375-650)[b]	325 (230-500)[a]
Range	4-5000	4-5000	4-2400	5-1800
Number of lambs born				
Median (95% CI)	420 (350-490)	319 (270-400)[a]	775 (600-900)[b]	500 (350-700)[a]
Range	5-7500	6-7500	5-3546	10-2200
Prevalence of lameness - ewes				
GM % (95% CI)	1.4 (1.2-1.7)	1.5 (1.2-1.9)	1.2 (0.8-1.9)	1.1 (0.6-1.7)
Median % (95% CI)	2.0 (2.0-2.5)	2.4 (2.0-2.9)	2.0 (1.3-2.0)	2.0 (1.5-2.9)
Range %	0-39	0-39	0-30	0-15
Prevalence of lameness - lambs				
GM % (95% CI)	0.6 (0.5-0.9)	0.6 (0.4-0.9)	0.5 (0.2-1.2)	1.0 (0.5-2.0)
Median % (95% CI)	2.0 (2.0-2.1)	2.0 (2.0-2.7)	1.6 (1.0-2.0)	2.0 (1.9-3.0)
Range (%)	0-80	0-80	0-50	0-13

1. Superscripts (a, b, c) indicate significant (Benjamini-Hochberg adjusted p-value ≤0.05) difference between countries, by post-hoc Wilcoxon tests.

2. GM = geometric mean, CI = confidence interval

3.3.2 Triangulation of associations between management practices and prevalence of lameness in ewes

The NB-GLM selected the fewest predictor variables (8), followed by the QP-GLM (13), the PEN-BS (17), with most selected by the GEN-BS (24), although the number selected by the latter two is determined by the threshold bootstrap value selected. The final model for each method is in Appendices 11,13,15 and 16 and a visual assessment of fit of the generalised linear models in Appendix 14.

Triangulation across model types identified ten variables associated with the prevalence of lameness. Only four variables were selected in all four model types and six in three of four models of ewes (Figure 3.1, Table 3.2). It was noticeable that the estimates and confidence intervals for each variable were similar across statistical methods (Table 3.2).

The extra parameter to adjust for high prevalence of lameness was selected in all four models. In addition, in all four models there was a higher prevalence of lameness associated with 5-100% feet bleeding during routine foot trimming compared with not foot trimming at all. There was a lower prevalence of lameness when farmers reported no lame ewes to treat compared with treating lame ewes in 0-3 days. Flocks where sheep were kept on peat soil compared with no peat soil also had a lower prevalence of lameness.

Variables associated with a higher prevalence of lameness in three of the four models (Figure 3.1, Table 3.2) were footbathing the flock to treat SFR and always using formalin in footbaths both compared with not footbathing at all, vaccination of sheep with FootVax™ for <1 year compared with not using Footvax™ at all, and never quarantining new or returning sheep for >3 weeks, compared with always doing so. A lower prevalence of lameness was associated with flocks vaccinated with FootVax™ for >5 years, compared with not using FootVax™ at all.

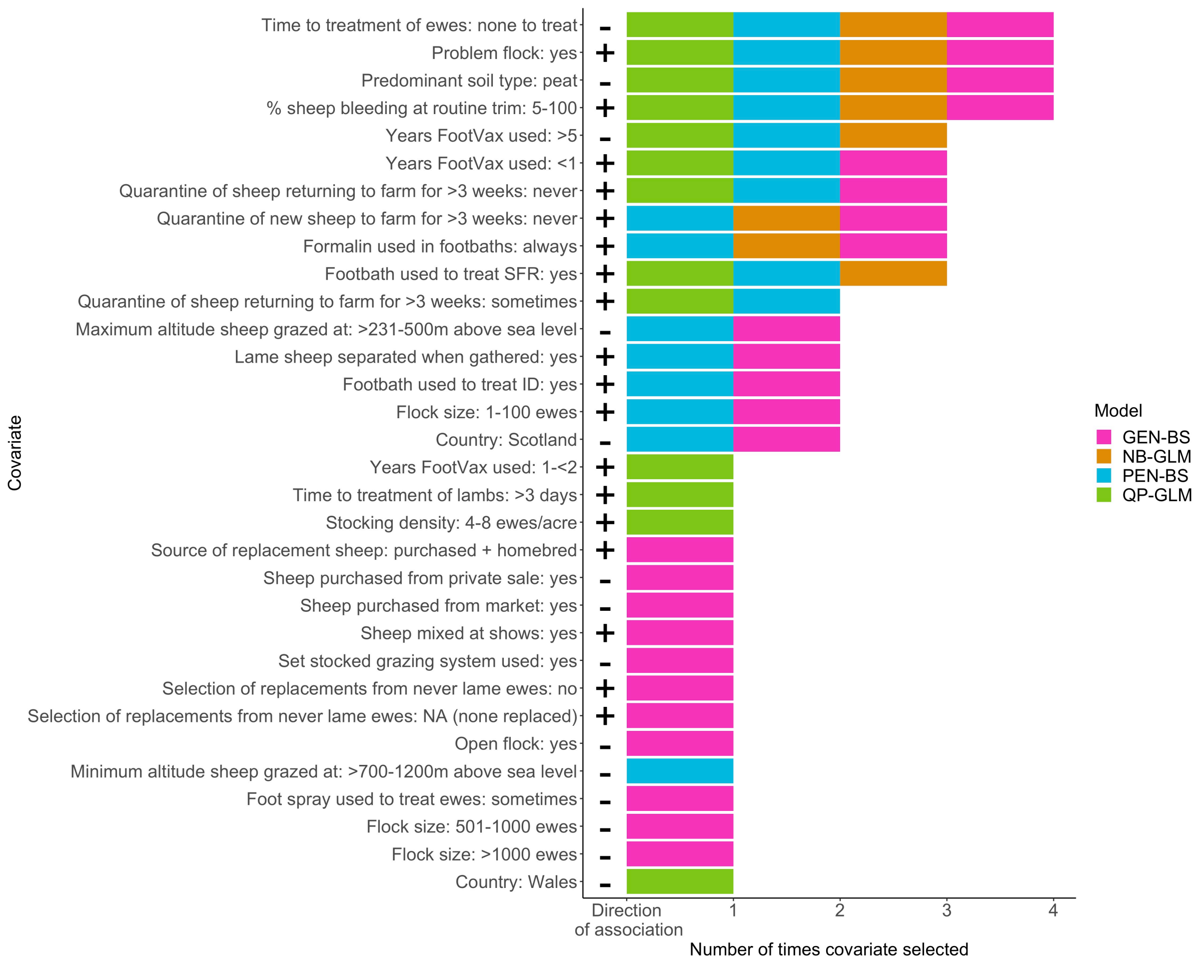


Figure 3.1 The number of times covariates were selected in final models for association with prevalence of lameness in ewes for the four model types (Quasi-Poisson GLM (QP-GLM), Negative Binomial GLM (NB-GLM) boot-strapped Poisson models (PEN-BS) and Gaussian log(x+1) model (GEN-BS). Predictors that were not selected at all are not shown.

Table 3.2 Covariates associated with prevalence of lameness in ewes selected by triangulation in three or four of four model types (Quasi-Poisson generalised linear model, Negative binomial generalised linear model, bootstrap Poisson Elastic net and bootstrap Gaussian elastic net) in 310 flocks of sheep in Great Britain from October 2017-September 2018

Covariate	N	%	QP-GLM	NB-GLM	PEN-BS	GEN-BS
			RR (95% CI)	RR (95% CI)	RR (95% CI)	Coefficient (95% CI)
Problem Flock (Decile 10 - ≥7.14% lameness)						
No	279	90.0	Ref	Ref		
Yes	31	10.0	3.12 (2.67-3.62)	3.72 (2.99-4.65)	2.89 (2.25-4.06)	0.42 (0.33-0.49)
Predominant soil type - peat						
No	265	85.5	Ref	Ref		
Yes	45	14.5	0.77 (0.65-0.90)	0.79 (0.65-0.95)	0.82 (0.66-0.98)	-0.08 (-0.16--0.00)
Time to treatment of ewes with SFR						
0-3 days	165	53.2	Ref	Ref		
>3 days	141	45.5				
None to treat	4	1.3	0.07 (0.00-0.41)	0.08 (0.01-0.29)	0.43 (0.15-0.83)	-0.49 (-0.86--0.27)
% sheep feet bleeding at routine trim						
Did not trim	115	37.1	Ref	Ref		
0	50	16.1				
>0-<5	104	33.5				
5-100	41	13.2	1.31 (1.13-1.51)	1.32 (1.07-1.62)	1.36 (1.17-1.60)	0.11 (0.04-0.19)
Footbath to treat SFR						
No	230	74.2	Ref	Ref		
Yes	80	25.8	1.27 (1.12-1.42)	1.17 (1.01-1.36)	1.13 (1.00-1.38)	
Formalin used in footbaths						
Did not footbath	66	21.3	Ref	Ref		
Always	85	27.4		1.36 (1.07-1.73)	1.12 (1.01-1.23)	0.04 (0.00-0.19)

Covariate	N	%	QP-GLM	NB-GLM	PEN-BS	GEN-BS
			RR (95% CI)	RR (95% CI)	RR (95% CI)	Coefficient (95% CI)
Sometimes	79	25.5				
Never	80	25.8				
Quarantine sheep returning to farm for >3 weeks						
Always	60	19.4	Ref	Ref		
Sometimes	49	15.8				
Never	94	30.3	1.27 (1.07-1.50)		1.17 (1.03-1.38)	0.05 (0.01-0.12)
Missing	107	34.5				
Quarantine new sheep to farm for >3 weeks						
Always	162	52.3		Ref		
Sometimes	56	11.0				
Never	58	18.7		1.28 (1.06-1.55)	1.17 (1.02-1.42)	0.07 (0.00-0.14)
Did not purchase	34	18.1				
Years FootVax™ used						
Did not vaccinate	219	70.6	Ref	Ref		
<1	10	3.2	1.56 (1.27-1.89)		1.42 (1.09-1.84)	0.17 (0.07-0.37)
1-<2	19	6.1				
2-5	32	10.3				
>5	30	9.7	0.75 (0.60-0.92)	0.72 (0.57-0.90)	0.84 (0.69-0.99)	

1. N = number of flocks, RR = risk ratio, CI = confidence interval, QP-GLM = quasi-Poisson generalised linear model, NB-GLM = negative binomial generalised linear model, PEN-BS = Poisson elastic net model run on bootstrap data, GEN-BS = Gaussian elastic net model run on bootstrap data, SFR = severe footrot, ID = interdigital dermatitis, Ref = reference category

3.3.3 Triangulation of associations between management practices and prevalence of lameness in lambs

The QP-GLM selected the fewest predictor variables (16), followed by the NB-GLM (19), the PEN-BS (23) with most selected by the GEN-BS (25). The full model for each method is in Appendices 18,20,22 and 23, with visual assessment of fit of the generalised linear models in Appendix 21.

Triangulation identified 12 variables - five were selected in all four model types and a further seven in three of four models (Figure 3.2, Table 3.3), fewer than in each model type. As for ewes, estimates and confidence intervals for each predictor variable were similar across statistical methods (Table 3.3).

Three of the variables associated with lower prevalence of lameness in lambs were environmental - flocks kept on peat soil compared with no peat, flocks in Scotland compared with England and flocks grazed at >230-500m above sea level compared with ≤230m. Two of the variables associated with a lower prevalence of lameness in lambs were managemental - never using antibiotic injection to treat lambs with SFR compared with always and having no lame lambs to treat compared with treating lame lambs in 0-3 days. However, treating lambs >3 days after recognition of lameness compared to within 0-3 days was associated with a higher prevalence of lameness.

Ewe management practices associated with a higher prevalence of lameness in lambs were: 5-100% of ewes bleeding during routine foot trimming compared with not foot trimming at all; always foot trimming ewes with SFR compared with never doing so; not knowingly selecting replacement ewes from ewes that were never lame compared to always doing so; and replacement sheep both purchased and homebred compared with only homebred. One flock variable was associated with a higher prevalence of lameness in lambs, this was footbathing the flock to treat ID compared with not footbathing at all.

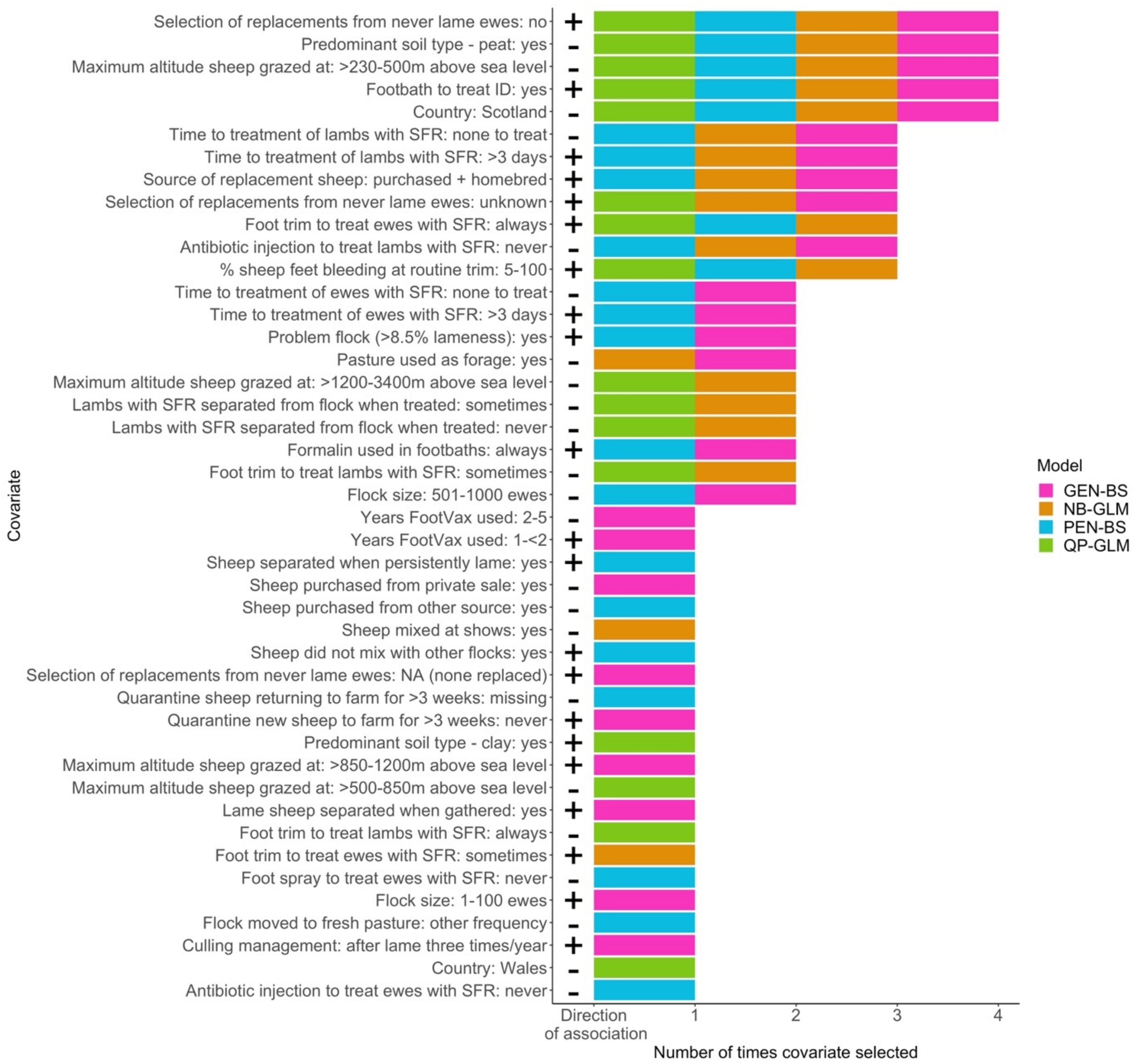


Figure 3.2 The number of times covariates were selected in final models for association with prevalence of lameness in lambs for the four model types ((Quasi-Poisson GLM (QP-GLM), Negative Binomial GLM (NB-GLM) boot-strapped Poisson models (PEN-BS) and Gaussian log(x+1) model (GEN-BS). Predictors that were not selected at all are not shown.

Table 3.3 Covariates associated with prevalence of lameness in lambs selected by triangulation in three or four of four model types (Quasi-Poisson generalised linear model, Negative binomial generalised linear model, bootstrap Poisson Elastic net and bootstrap Gaussian elastic net) in 310 flocks of sheep in Great Britain from October 2017-September 2018

Covariate	N	%	QP-GLM	NB-GLM	PEN-BS	GEN-BS
			RR (95% CI)	RR (95% CI)	RR (95% CI)	Coefficient (95% CI)
Country						
England	219	70.6	Ref	Ref		
Scotland	43	13.9	0.52 (0.35-0.75)	0.71 (0.52-0.96)	0.84 (0.66-0.97)	-0.07 (-0.19--0.01)
Wales	48	15.5				
Footbath to treat ID						
No	170	54.8	Ref	Ref		
Yes	140	45.2	1.64 (1.25-2.17)	1.35 (1.09-1.68)	1.22 (1.07-1.57)	0.09 (0.03-0.18)
Maximum altitude flock was grazed at (m above sea level)						
0-230	52	16.8	Ref	Ref		
>230-500	52	16.8	0.49 (0.31-0.78)	0.69 (0.48-0.99)	0.86 (0.59-0.98)	-0.07 (-0.21--0.00)
>500-850	61	19.7				
>850-1200	56	18.1				
>1200-3400	42	13.5				
Missing	47	15.2				
Selection of replacements from ewes that were never lame						
Yes	86	27.7	Ref	Ref		
No	87	28.1	2.07 (1.47-2.92)	1.77 (1.34-2.34)	1.25 (1.06-1.60)	0.08 (0.01-0.22)
Unknown	99	31.9	1.61 (1.15-2.27)	1.38 (1.04-1.84)		0.05 (0.00-0.15)
Not applicable	38	12.3				
Predominant soil type - peat						
No	265	85.5	Ref	Ref		

Covariate	N	%	QP-GLM	NB-GLM	PEN-BS	GEN-BS
			RR (95% CI)	RR (95% CI)	RR (95% CI)	Coefficient (95% CI)
Yes	45	14.5	0.53 (0.35-0.78)	0.64 (0.48-0.87)	0.84 (0.68-0.98)	-0.08 (-0.19--0.01)
% sheep feet bleeding at routine foot trim						
Did not foot trim	115	37.1	Ref	Ref		
0	50	16.1				
>0-<5	104	33.5				
5-100	41	13.2	1.91 (1.34-2.72)	1.48 (1.07-2.07)	1.19 (1.01-1.48)	
Foot trim to treat ewes with SFR						
Never	51	16.5	Ref	Ref		
Sometimes	97	31.3				
Always	162	52.3	2.13 (1.24-3.68)	1.95 (1.26-3.01)	1.12 (0.98-1.25)	
Antibiotic injection to treat lambs with SFR						
Always	109	35.2		Ref		
Sometimes	136	43.9				
Never	65	21.0		0.71 (0.53-0.95)	0.92 (0.71-1.00)	-0.05 (-0.15-0.00)
Source of replacement sheep						
Homebred	164	52.9		Ref		
Purchased	42	13.5				
Homebred + purchased	94	30.3		1.55 (1.21-1.97)	1.12 (0.98-1.26)	0.07 (0.01-0.13)
Not applicable	10	3.2				
Time to treatment of lambs with SFR						
0-3 days	161	51.9		Ref		
>3 days	131	42.3		1.51 (1.22-1.87)	1.15 (1.02-1.35)	0.06 (0.01-0.15)

Covariate	N	%	QP-GLM	NB-GLM	PEN-BS	GEN-BS
			RR (95% CI)	RR (95% CI)	RR (95% CI)	Coefficient (95% CI)
None to treat	18	5.8		0.04 (0.01-0.12)	0.66 (0.12-0.95)	-0.37 (-0.61--0.15)

1. N = number of flocks, RR = risk ratio, CI = confidence interval, QP-GLM = quasi-Poisson generalised linear model, NB-GLM = negative binomial generalised linear model, PEN-BS = Poisson elastic net model run on bootstrap data, GEN-BS = Gaussian elastic net model run on bootstrap data, SFR = severe footrot, ID = interdigital dermatitis, Ref = reference category

3.3.4 Variable stability

In the elastic net bootstrapped models for both ewes and lambs, predictor variables with high stability tended to have lower p-values (Figure 3.3) and so there was a clear demarcation of 'important' variables that comprised the 'final model'.

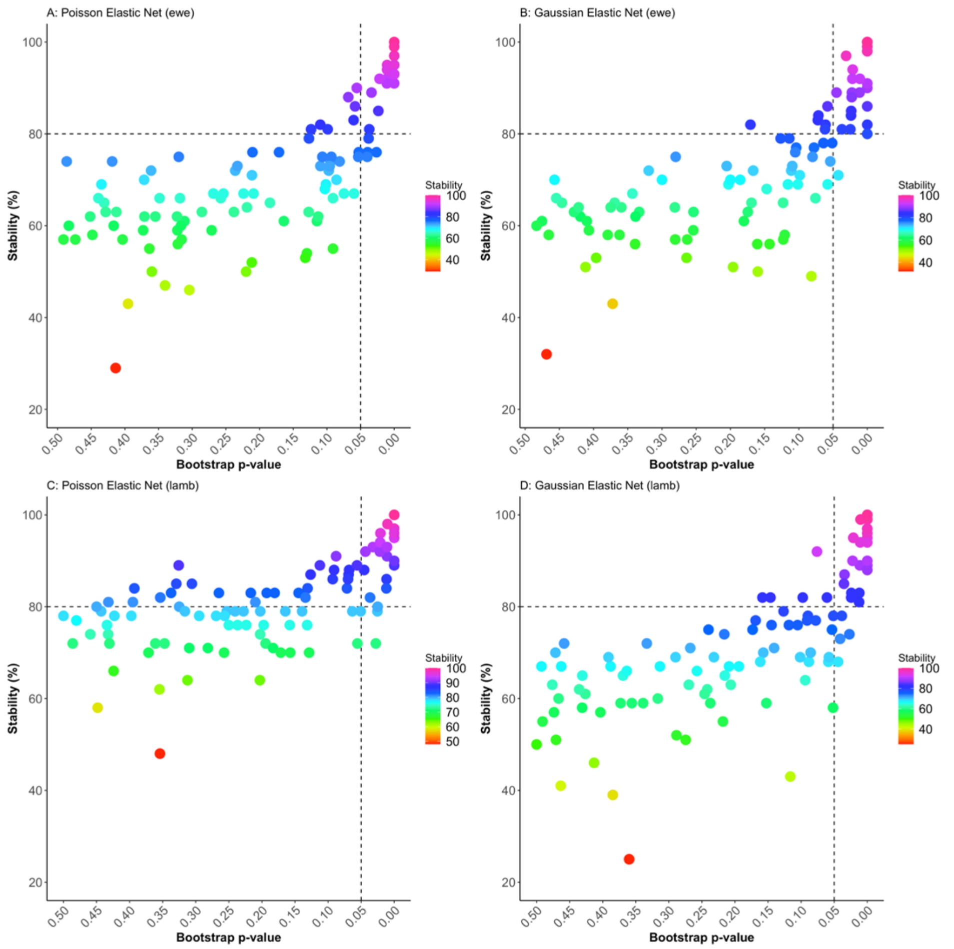


Figure 3.3 Stability (the proportion of times the predictor was selected by the elastic net model in the 100 boot-strapped samples) vs boot-strap p-value (the proportion of times the coefficient for the predictor was > or < than 0 (depending on the median coefficient) in the Poisson (A) and Gaussian (B) elastic net models for management practices associated with prevalence of lameness in ewes, and in Poisson (C) and Gaussian (D) elastic net models for management practices associated with prevalence of lameness in lambs in 310 sheep flocks in Great Britain from October 2017-September 2018

3.4 Discussion

Our study is the first to implement multiple model triangulation to identify robust associations between farm management practices and the prevalence of lameness in sheep flocks. Previous triangulation of models in animal health research used continuous outcome data (Lima et al., 2020a), our results indicate that triangulation is equally useful with Poisson models: three of the four models were for count data (Ver Hoef and Boveng, 2007), whilst one assumed a loglinear function. Triangulation highlighted a small set of variables in three or four model types (Figures 3.1 and 3.2). These variables are therefore likely to be the most reliable management practices associated with prevalence of lameness in this sample and more likely to be informative for the population of sheep flocks in GB because triangulation reduces the impact of bias from each modelling method, strengthening confidence that selected covariates have a true association with the outcome and would be reproduceable (Lawlor et al., 2016).

Some of the triangulated variables in our study have been reported in previous studies whilst others are new. In addition, analysing ewe and lamb data in separate models has highlighted that some management practices for ewes and the whole flock influence the prevalence of lameness in lambs. We can also learn from management of footrot by disease severity, because lambs are less likely to develop SFR than ewes (Appendix 9) and so risks for lambs with ID equate to risks for ewes with SFR. These are discussed below.

There was an increased risk of lameness in both ewes and lambs when 5-100% of sheep feet bled after routine foot trimming and when foot trimming was part of treatment of ewes with SFR (Tables 3.2, 3.3, Figure 3.1 and 3.2). Feet bleeding during routine foot trimming has been associated with higher prevalence of lameness in ewes (Winter et al., 2015, Prosser et al., 2019) and foot trimming ewes with SFR delays healing (Kaler et al., 2010), consequently, it is consistent that these practices were associated with higher prevalence of lameness in ewes. However, it is less clear why foot trimming ewes was associated with a higher prevalence of lameness in lambs. Foot trimming lambs as a direct risk for increased prevalence of lameness was reported in Lewis and Green (2020) and in the current study farmers who foot trimmed ewes to treat SFR were more likely to also foot trim lambs as part of treatment for footrot ($p<0.01$, Appendix 26), and so it is possible that only the ewe variable was selected in the models. Alternatively, the risk to lambs might be indirect, because foot trimming may increase the prevalence of ewes with footrot (Kaler et al., 2010), which would increase the prevalence of footrot in lambs.

Another ewe variable that was associated with lower prevalence of lameness in lambs was conscious selection of replacement ewes from dams that were never lame (Table 3.3). Such selection increases resistance or resilience to footrot which is mildly heritable (Nieuwhof et al., 2008a, Raadsma et al., 1994, Skerman et al., 1988). The results indicate that closed flocks could derive benefits in control of lameness from planned selection programmes.

There was one environmental factor associated with lameness in both ewes and lambs. The prevalence of lameness was lower in ewes and lambs in flocks on predominately peat compared with no peat soil (Tables 3.2 and 3.3). Peat has a lower pH than other soil types (Wheeler et al., 2010), which could affect survival of *D. nodosus* or other bacteria in the foot and so change the interdigital skin microbial community. A laboratory study (Muzafar et al., 2015) reported longer survival of *D. nodosus* in clay rich soils, indicating some difference in survival by soil type, but peat soils were not included in that study. However, there are other plausible explanations for this association. For example, flocks on peat are also likely to be at low stocking density because it is marginal land, and low stocking density is associated with lower prevalence of footrot (Wassink et al., 2003, Kaler and Green, 2009). Flock management might also explain the lower prevalence of lameness in lambs in flocks in Scotland compared with England and Wales as Scottish flocks were larger and on higher ground (Table 3.1, Online Supplementary Material Table 2). One other environmental factor was associated with a lower prevalence of lameness in lambs. This was when flocks were kept at a maximum altitude of >230m-500m above sea level compared with ≤230m. A similar association between altitude and prevalence of ID was reported by (Wassink et al., 2004). However, as with peat soils, higher altitudes are associated with marginal land, lower air temperature, low stocking density, which are all associated with prevalence of footrot (Wassink et al., 2003, Wassink et al., 2004)

Analysing data for lambs and ewes separately has increased insight into good management practices to control lameness in lambs and ewes. In the current study, footbathing to treat ID and SFR was associated with a higher prevalence of lameness in lambs and ewes respectively, compared with not using footbaths at all. Lambs rarely develop SFR (Appendix 9) and so ID is the common presenting sign of footrot, whereas ewes do develop SFR. These results highlight that treating any stage of footrot with footbaths is less effective than individual rapid treatment of lame sheep, or indeed not having any lame sheep to treat. This is probably both because farmers delay treatment until sufficient sheep are lame to use a footbath (Kaler and Green, 2009) but also because footbaths are not an effective treatment of SFR (Wassink et al., 2010a). Overall, our paper has highlighted that footbathing is not an effective management to minimise footrot in lambs or ewes.

Our study provides the first evidence that formalin footbaths are associated with a higher prevalence of lameness in ewes than other footbath products. Footbathing with formalin has been associated with flock-presence of shelly hoof and foot granulomas (Reeves et al., 2019). Granulomas are very painful and affected ewes are lame (Winter et al., 2015), given that the geometric mean prevalence of granuloma lesions in ewes in affected flocks in the current study was 0.8% and the mean prevalence of lameness overall was 1.4%, these lesions could account for much of higher prevalence of lameness in those flocks using formalin. Of the 152 farmers that reported sheep with granulomas, 86.2% used a footbath, with 31.6% always using formalin, while of the 147 who reported no granulomas, 71.4% used a footbath, and 23.1% always used formalin, which was significantly lower (Fisher's exact test, p = 0.02, Appendix 24).

The complex risk pattern associated with time since starting to vaccinate with FootVax™ (a lower risk when >5 years and an increased risk when <1 year and no difference where vaccination used 2-5 years) was identified via the triangulation in ewe models but not lamb models. This association with ewes was first reported in 2019 (Prosser et al., 2019) and then by Best et al., (2020). Only 20.9% of farmers vaccinated ewes, with only 2.9% for <1 year and 8.9% for >5 years but the variable was robust in our triangulated approach, suggesting a real effect. Vaccinating ewes was not, however, associated with a lower prevalence of lameness in lambs. This suggests that lambs were not protected indirectly from vaccination of ewes.

Never quarantining new or returning sheep for >3 weeks, compared with always doing so were associated with a higher prevalence of lameness in ewes, as in Winter et al. (2015); 20.0% of farmers always quarantined returning stock for > 3 weeks. Footrot is highly endemic (Prosser et al., 2020) but the robustness of this risk indicates that there is still a benefit from quarantine for > 3 weeks. This might be because quarantine prevents the introduction of new strains of *D. nodosus* to a flock and also reduces the risk of introducing contagious ovine digital dermatitis (Dickins et al., 2016).

There were a small number of flocks with no lame ewes (4) or lambs (18). Not surprisingly, but very encouragingly, these flocks had a lower prevalence of lameness than flocks with lame ewes, even if treated within 3 days of becoming lame. Despite this, our study highlights, for the first time, that treatment of lame lambs within 3 days of onset of lameness was associated with a lower prevalence of lameness than treatment after 3 days. This has been reported previously in ewes, where rapid treatment is the highest attributable risk to maintain a low prevalence of lameness (Grant et al., 2018, Prosser et al., 2019). Our study supports this management practice in lambs.

Whilst treating lambs in >3 days after recognising lameness compared with 0-3 days was selected by the triangulation process in lamb models it was not in the ewe triangulated variables (Figure 3.1), suggesting that time to treatment was not as reliable a variable in the ewe models as in the lamb models. One explanation for this is that time to treatment of lambs was more consistent than for ewes. This might be because lambs remain on farm for 4-6 months and are handled regularly and so regular treatment is given, whilst ewes management varies throughout the production year and e.g. some farmers do not treat lame ewes during pregnancy (O'Kane et al., 2017) or when harvesting (Witt and Green, 2018). This might indicate that the question needs refining for future studies by including aspects of the production cycle.

Flocks where lambs with SFR were never treated with antibiotic injection to treat lamb lambs had a lower prevalence of lameness than flocks where lambs were always treated with antibiotic injection – this was also reported in Lewis and Green (2020). Current Sheep Veterinary Society guidelines only recommend treating lambs with antibiotic injection if clinical signs of SFR are present, and to use antibiotic foot spray alone for signs of ID (Sheep Veterinary Society, 2013). Our questionnaire did not ask about recognition of lameness – recognition of lameness only at high locomotion scores was identified as a risk factor for higher prevalence of lameness in lambs (Lewis and Green, 2020) and it is possible that the farmers who never used antibiotic injection were treating lambs promptly with foot spray, recognising lame lambs at low locomotion scores and that this was sufficient to prevent progression to SFR and the need to use antibiotic injection in lambs.

The geometric mean prevalence of all lameness was low in the current study conducted in 2018, ewes - 1.4% (95% CI 1.2-1.7) and lambs - 0.6% (95% CI 0.5-0.9) compared with previous estimates in English sheep flocks of 4.2% in 2015 (Prosser et al., 2019), and 3.5% in 2013 (Winter et al., 2015). The summer of 2018 was unusually dry (Met Office., 2018) which would have reduced the prevalence of footrot (Clifton et al., 2019, Graham and Egerton, 1968, Smith et al., 2014). It would be interesting to see estimates from a wet summer to see how well footrot is controlled in conditions conducive to spread of disease. However, if the flocks in the current study are representative of flocks in Great Britain, then the FAWC (Farm Animal Welfare Council, 2011) target of a national flock prevalence of lameness of <2% by 2021 is getting closer to being achieved. However, even in 2018 approximately 60% of flocks had >2% lameness (Appendix 27).

Standard limitations of questionnaire studies apply to this research. One limitation of cross-sectional studies is determining causality. Sheep farmers rarely change their management practices (Wassink et al., 2010a) and therefore management practices in 2018 are likely to be those used in 2017, strengthening the likelihood that associations between management practices and prevalence of lameness are temporally likely to be causal. In addition, other study types have identified similar associations (Kaler et al., 2010a, Wassink et al., 2010b, Witt and Green, 2018). The response proportion was lower than other paper-based studies (Kaler and Green, 2009, Winter et al., 2015). This is an increasing trend in the livestock industry and might be because of the number of questionnaires and forms farmers are now asked to complete.

3.5 Conclusion

In conclusion, our study illustrates that triangulation of results from different model types identifies a robust set of variables associated with prevalence of lameness in ewes and lambs. Some of these associations have been associated with prevalence of lameness previously, while others are reported for the first time. These risks are likely to be the most reliable for reduction of prevalence of lameness on sheep farms since multiple model triangulation reduces the likelihood of false positive associations.

Chapter 4 Introduction to social relationships in sheep flocks and methodologies to study animal behaviour and the impact of disease

4.1 Sociality in sheep, a literature review

4.1.1 Interactions between adult sheep

Ewes spend most of their day grazing (Morgan and Arnold, 1974) and other activities include ruminating and idling (Pokorna et al., 2013). Most ewe-ewe contacts are very short, lasting less than a minute (Ozella et al., 2020) and rarely involve actual touch (Manlove et al., 2017). When ewes do touch, the most common forms of contact are rubbing and head-butting (Norton et al., 2012).

When a flock consists of only ewes, there is no evidence that the sheep sub-divide into social communities (Ozella et al., 2020), but ewes do have individual preferences for other ewes (Ozella et al., 2020). Some pairs of ewes will actively seek each other out, while others avoid each other (Van der Waal, 2016). Associations are stronger between ewes of a similar age in domestic sheep (Ozella et al., 2020, Doyle et al., 2016) but wild sheep of a similar age may avoid each other, although this effect is inconsistent across years (Van der Waal, 2016). Individual personality traits can also affect how ewes associate, with "bold" ewes more likely to split into subgroups to explore novel environments than "shy" ewes (Michelena et al., 2009).

Younger ewes are found more centrally in social networks compared with older ewes (measured by eigenvector centrality, defined in Table 4.1) (Van der Waal, 2016). There is a weak effect of similarity in reproductive status (ewes with or without a lamb at heel), where ewes were more likely to associate with those of the same reproductive status (Van der Waal, 2016). Relatedness between ewes did not affect the strength of social bonds (Van der Waal, 2016).

4.1.2 Interactions between sheep when lambs are present

4.1.2.1 Ewe-lamb interactions

Ewes can recognise their lambs and locate them from at least 10m within a day of parturition (Morgan, 1970) but lambs cannot reliably find their mothers until 8 days old (Morgan, 1970). Lambs show strong maternal preferences for their mother compared to other adult ewes (Norton et al., 2012, Manlove et al., 2017, Hinch et al., 1987) and they are usually found in close spatial proximity to their mother. Average distances for young lambs from their mother (up to 10 weeks old) are 1.0m to 5.2m, depending on lamb and ewe activity (Hinch et al., 1987, Galeana et al., 2007, Morgan and Arnold, 1974). Both male and single lambs are found further away from their mothers compared to female or twin-born lambs (Morgan and Arnold, 1974, Shillito Walser and Williams, 1986). Bonds between ewes and lambs only appear to decrease once lambs are of 7 months old (Arnold and Pahl, 1974, Grubb and Jewell, 1966).

Ewes and their lambs are often found engaging at the same activity at the same time – for example if ewes are lying or walking, so are their lambs (Morgan and Arnold, 1974). The exception to this is grazing, as when ewes are grazing, their lambs are likely to be playing with other lambs (Morgan and Arnold, 1974). As lambs age, their dams spend more time grazing (Morgan and Arnold, 1974).

Lamb-dam contacts are frequent and of long duration (Manlove et al., 2017) and include feeding. Lambs tend to suck throughout the day and night (Ewbank, 1964, Ewbank, 1964). Most ewes will only allow their own lambs to feed (Ewbank, 1967) but very young lambs will attempt to suck from non-related ewes (Norton et al., 2012). For the first one to two weeks of a lamb's life, their dam will allow them to suck at any time but as the lambs age, the ewe will discourage some attempts (Ewbank, 1967) and as lambs age, the number of suckling activities declines (Morgan and Arnold, 1974, Ewbank, 1967).

4.1.2.2 Lamb-lamb interactions

The time that lambs spend playing increases from birth up to when they are 10 days old and this is gradually replaced by grazing (Morgan and Arnold, 1974).

Contact between lambs and other members of the flock decreases as they age (Norton et al., 2012). Lamb contacts with other sheep tend to involve sniffing and rubbing (Norton et al., 2012).

Twin-born lambs have a strong preference for their sibling and spend more time in contact with each other in the 24-hour period after birth (14.7 hours/day) than with their mother (9.25 hours/day) (Broster et al., 2010). After weaning, the association between pairs of twin lambs will temporarily increase (Shillito Walser and Williams, 1986).

Twin lambs suckle more frequently compared to single lambs for the first four weeks of life but the same amount after four weeks (Ewbank, 1964, Ewbank, 1967) and some ewes will only allow twins to feed if both are present (Ewbank, 1967).

4.1.2.3 Ewe-ewe interactions in the presence of lambs

Overall levels of contact between sheep in a flock are higher when lambs are in the flock than when there are only adult ewes (Norton et al., 2012, Manlove et al., 2017). However, ewes reduce their contact with each other, particularly immediately after lambing (Broster et al., 2010, Manlove et al., 2017) and when suckling very young lambs (Norton et al., 2012).

4.2 Considerations for modelling disease and social relationships within animal systems

There are three stages involved in direct transmission of disease: susceptible and infectious individuals must come into contact, secondly the pathogen must be transferred from the infectious individual to the susceptible individual and finally that individual must become infected. Contact networks are able to give insight into the first step of the process since the variation in contact patterns can determine the likelihood that an individual becomes infected.

The following factors (Craft, 2015, White et al., 2017) need to be carefully considered for the specific disease system in question:

- Are there factors that mediate individual variability in susceptibility and exposure?
- Are there ‘trait-based’ features that are predictive of infection status?
- How does the community structure or group-living affect the spread of pathogens?
- Are there feedbacks between network position and infection status?
- Are there other factors that affect social behaviour?

These considerations are outlined below in context of spread of footrot. Relatively few studies have considered contact in sheep and disease spread, therefore examples and considerations from other animal systems are drawn on.

4.2.1 Factors that mediate individual variability in susceptibility and exposure

Factors that mediate individual variability in susceptibility and exposure include the type of contact made between two individuals, because not all types of contact have equal chance of transmitting disease, the relationships between animals and genetic susceptibility to disease. These are discussed below.

4.2.1.1 Type of contact necessary to transmit *D. nodosus* between sheep and consideration of knowledge of transmission dynamics of *D. nodosus*

Defining how a contact relates to pathogen transmission is one of the most challenging considerations. Transmission can be either direct – where a susceptible and infectious host are within a specified distance for a specified time, or indirect, where there is some form of environmental persistence or vector of the pathogen (Craft, 2015). Examples of direct social interactions that are correlated with disease risk in animal systems include transmission of bovine tuberculosis between meerkats, where specific social interactions e.g. grooming infected individuals have more influence on risk of transmission than the total time spent with an infectious individual (Drewe, 2010) and rabies in racoons, which is spread primarily through bites (Rupprecht et al., 2002), which are more frequent in winter because racoons co-den in the winter, and consequently there are seasonal epidemics (Hirsch et al., 2016).

Strictly speaking transmission of *D. nodosus* between sheep is indirect because *D. nodosus* is spread when diseased and infectious feet contaminate the environment (pasture or bedding) with *D. nodosus* (Clifton et al., 2019) and sheep become infected from stepping on the contaminated environment. However, the pathogen survives for a very short time, probably less than 2 days even in optimal damp conditions (Beveridge, 1941, Clifton et al., 2019, Whittington, 1995) and so it is possible that sheep that spend time in close proximity to infectious sheep are more likely to become infected themselves.

The transmission dynamics of footrot are that susceptible sheep can become infected, then diseased (Green and George, 2008). The estimated time to develop symptoms of disease post infection is one week (Egerton et al., 1969, Roberts and Egerton, 1969) and sheep can recover from mild disease in about two weeks (Beveridge, 1941), although recovery rates without treatment are generally thought to be low (Kaler et al., 2010).

The presence of footrot lesions is not a necessary condition for sheep to potentially transmit disease – sheep can test positive for *D. nodosus* without showing clinical signs of infection, although in many sheep these will develop within days (Kuhnert et al., 2019) and strains of *D. nodosus* only persist on diseased feet (Clifton et al., 2019). The load of *D. nodosus* is highest at the start of disease expression (Witcomb et al., 2014) but it is unknown for how long sheep remain infectious following expression of disease symptoms – poor hoof conformation follows footrot infection (Smith et al., 2017) and misshaped feet have been found to have high loads of *D. nodosus* (Best et al., 2021), which could increase potential for transmission from sheep that have previously shown symptoms of disease.

Lambs are born naïve to *D. nodosus* but it can be detected on feet with 5-13 hours of birth (Muzafar et al., 2015) and clinical signs can be seen as early as two weeks after contact with a diseased sheep (Kuhnert et al., 2019). Since lambs in this study system were less than four weeks old, it is likely that any clinical signs seen were their first footrot disease event.

4.2.1.2 Relationships between individuals that influence the likelihood of disease transmission

Individuals do not interact with all other individuals in the same manner. Kinship relationships in particular may influence disease dynamics since animals that are related may interact differently with each other compared with animals that are not related. In wild big-horn sheep, contact with a dam infected with *Mycoplasma ovipneumoniae* had approximately 5 times the odds of producing a lamb-mortality event compared to an identical contact (as measured by proximity) with an infected non-pregnant ewe (Manlove et al., 2017), suggesting that there may be something different about the dam-lamb contact that increases the chance of the lamb becoming infected.

There are links between mother-offspring relationships and lameness in domestic sheep, with ewes more likely to become lame if their offspring are lame, and lambs are more likely to become lame if their dam or sibling is lame (Kaler et al., 2010b).

4.2.1.3 Individual susceptibility to lameness and the influence of previous disease, immunity and heritability

There is no long-term immunity to footrot (Egerton and Roberts, 1971), leading to re-infection, and once disease has occurred, it is likely to occur again (Kaler et al., 2010a). There is thought to be some mild heritability for resistance to footrot (Skerman et al., 1988, Raadsma et al., 1994, Nieuwhof et al., 2008a) but these studies have short time scales and/or limited observations. Selective breeding trials have resulted in some success in breeding for footrot resistance – for example in Broomfield Corriedale sheep (Skerman and Moorhouse, 1987). Additionally, theoretical modelling studies suggest infection parameters (infection rate, bacterial death rate) make it difficult to truly determine the effect of genetic traits (Russell et al., 2013).

4.2.2 Trait-based features that are predictive of infection status

For some diseases, trait-based features such as age or morphology can indicate high risk for disease– for example in honey bees, age dictates the spatial organisation of the colony and older bees leave the hive to forage and so are the most likely to become infected and introduce disease into a colony (Naug, 2008).

There are several traits that are associated with lameness in sheep. Ewes that have poor foot conformation and are >4 years of age are more likely to become lame (Kaler et al., 2010b), while male lambs are more likely to become lame than female lambs, as are single vs twin lambs (Kaler et al., 2010b).

4.2.3 The effect of community structure or group living on the spread of pathogens

The modularity of a network is the extent to which it is compartmentalised into distinct communities. Networks with high modularity, or greater community structure, tend to be more resistant to disease invasion (Salathé and Jones, 2010) – for example in ants, social networks of colonies infected with spores of *Metarhizium brunneu* become more modular, which limits the spread of infection (Stroeymeyt et al., 2018). However, modularity creates the potential for individuals to act as “super-spreaders” by moving through compartments of a network, and individuals that visit other groups can be more likely to be infected with disease themselves, which is the case for male meerkats that move between groups and bovine tuberculosis infection (Drewe, 2010).

Domestic sheep networks are very dense, with sheep contacting almost every other member of the flock (Norton et al., 2012) and show little modularity when only ewes are present (Ozella et al., 2020). However, wild sheep are known to form nursery groups, where groups of ewes are associated with particular lambs in addition to their own (Manlove et al., 2014, Manlove et al., 2017). This can result in disease impacting some portions of populations much more intensely than others – during epidemics of pneumonia in big-horn flocks, lamb mortality varies between ewe sub-populations, suggesting localised pathogen transmission and that group stability acts as a buffer to spread of disease in a population (Manlove et al., 2014).

4.2.4 Feedbacks between network position and infection status

The effect of disease on social behaviour has important implications for transmission dynamics across networks, by either accelerating or reducing disease spread. Some diseases act to improve the likelihood of pathogen transmission, for example infection with *Toxoplasma gondii* in rats affects innate fear responses, such as causing a preference in infected rats for cat-scented areas to increase the chance of predation (Berdoy et al., 2000), but does not affect other social behaviours such as dominance hierarchy or mating success (Berdoy et al., 1995). Other diseases have been associated with reductions in sociality across many species, including influenza in humans (Van Kerckhove et al., 2013), liposaccharide induced sickness in wild vampire bats (Ripperger et al., 2020), pathogenic fungal infections in ants (Bos et al., 2012) and experimentally induced infection in mice (Lopes et al., 2016).

The extent of the behaviour change depends on the relationship between animals – for example, mother-offspring interactions are much less affected by disease events than other relationships (Stockmaier et al., 2020) and also the type of interaction measured, with social behaviours that have greater benefit to inclusive fitness, such as food sharing, less affected than interactions such as allo-grooming (Stockmaier et al., 2020). Social animals can also make judgements about care provided for others when sick – ants perform less grooming and more antimicrobial disinfection for nestmates contaminated with heterologous pathogens compared to homologous ones - allowing ants to acquire less infectious particles of heterologous pathogens, reducing potential for super infection of the colony (Konrad et al., 2018).

Lameness in any animal comes from pain and can influence behaviour, particularly the “time-budget” of their day. Lame cows, sows and chickens (Galindo and Broom, 2002, Blackie et al., 2011, Weeks et al., 2000, Gregoire et al., 2013) spend more time lying compared to sound animals. Lame cows also spend less time feeding compared to sound cows (Galindo and Broom, 2002, Blackie et al., 2011), and are also less likely to start aggressive interactions (Galindo and Broom, 2002). Anecdotal evidence suggests that farmers report that lame sheep cluster together, especially at the back of the flock when gathered, which corresponds to results from Doughty et al., (2018), where lame sheep were likely to be further back in the flock compared to non-lame sheep when sheep were moved along a track, following a researcher with food (Doughty et al., 2018).

4.2.5 Other factors that affect social behaviour in sheep

Environmental conditions influence ewe behaviour, with an increased tendency for ewes to cluster together when the wind chill index increases (Ozella et al., 2020), and contacts become longer as both temperature and precipitation increase (Doyle et al., 2016), which can be due to shared use of resources, such as hedges for shelter (Doyle et al., 2016, Ozella et al., 2020).

Sheep in “resource-poor” environments make more contacts than sheep in “good-resource” plots (Freire et al., 2012) suggesting that food availability affects sheep social patterns. Sheep may be attempting to obtain information about food sources by aggregating with others (Freire et al., 2012).

4.3 Methodological approaches for inferring relationships between social structure and disease transmission

4.3.1 Observation of social networks with biologging devices

Biologging refers to the tracking of individual animals by attaching equipment that collects information about location, behaviour or physiology, and allows the study of behaviour to timescales that would not be possible by human eye alone. Co-location of animals can be recorded by several methods – Radio Frequency Identification (RFID) radio signals, ultrasonic telemetry, Global Positioning System (GPS) co-ordinates etc. The equipment used for this study were spatial proximity loggers (Cattuto et al., 2010), which record tag-to-tag communication via RFID signals when in a specified range and have previously been used in animal systems (Ozella et al., 2020, Wilson-Aggarwal et al., 2019).

Considerations around use of proximity tag tracking devices in animal systems include the weight of the tag relative to the animal - which should be <5-10% of the individual's body weight (Sikes et al., 2016, Wilson et al., 1996), the ability to attach a tag to the animal safely i.e. without causing harm to the animal, including considering whether the animal will grow, battery life and data storage. Several studies have attached proximity sensors to ewes using collars or harnesses (Broster et al., 2012a, Hobbs-Chell et al., 2012, Paganoni et al., 2020, Ozella et al., 2020) and their use is not thought to affect locomotion (Hobbs-Chell et al., 2012). However, there is less research using proximity tags on lambs.

4.3.2 Measures of centrality

Measures of centrality describe how connected each individual node (animal) in the network is by assessing the connections of an individual node and that node's links in various different ways – for example degree investigates only the contacts of that node, whereas eigenvector centrality, flow betweenness and transitivity consider the links of the contacts of that node – fully described in Table 4.1. In combination with statistical techniques such as generalised linear models, measures of centrality can be used to test hypotheses about the effect of individual-level characteristics (such as age, sex or disease status) on the individual's position in the network. Some examples include that flow-betweenness differs for badgers with and without bovine tuberculosis (Weber et al., 2013), transitivity is negatively correlated with prevalence of *Cryptosporium* spp. in Belding's ground squirrels (VanderWaal et al., 2013) and artificially immunologically challenged bats have lower strength, degree and eigenvector centrality compared to healthy bats (Ripperger et al., 2020).

Table 4.1 Definition of various individual-level measures of network centrality and example animal disease systems that they have been applied in.

Individual-level measure of centrality	Definition	Example animal disease system applied in
Degree	The number of links of a node	Immunologically challenged bats (Ripperger et al., 2020)
Weighted degree/strength	The sum of a links weights in a weighted network	Immunologically challenged bats (Ripperger et al., 2020)
Eigenvector centrality	The first non-negative eigenvector value obtained by transforming an adjacency matrix linearly	Immunologically challenged bats (Ripperger et al., 2020)
Betweenness	The number of times a node is included in the shortest paths generated by every combination of two nodes	Gut microbial transmission – rhesus macaques (Balasubramaniam et al., 2019)
Flow betweenness	A second measure of betweenness centrality that measures the centrality of an individual as a function of the "flow" through it than purely with respect to the shortest paths	*M. bovis* infection – bushtail possums (Corner et al., 2003) *M. bovis* infection – badgers (Weber et al., 2013) *M. bovis* infection – meerkats (Drewe et al., 2010)

Closeness	A measure related to the normalised mean path length from that node to all other individuals in the network	*M. bovis* infection – bushtail possums (Corner et al., 2003)
Transitivity	The number of triangles in the network, compared to the number of triplets	*Cryptosporium* spp. - Belding's ground squirrels (VanderWaal et al., 2013)

4.3.3 Community detection and individual-level metrics related to the role of disease spread

Social networks are often complex structures. There are several methods to identify sub-groups within a network, such as cliques, k-cores and modularity. Chapter 7 will focus on modularity, the extent to which nodes exhibit clustering as greater density within the clusters and less density between them (Newman, 2006). This has been used previously for sheep (Ozella et al., 2020) to study social structure at a group level and how environmental influences (space available, weather patterns) influence the group level social behaviour.

Modularity approaches can be extended to create individual-level metrics (Guimerà and Nunes Amaral, 2005) that describe an individual's potential role in disease spread (Silk et al., 2017). This has been used in badgers, where social networks correlate with tuberculosis infection (Weber et al., 2013) - infected badgers make both more contacts and more contacts that are out of their own community compared with uninfected badgers (Silk et al., 2017).

4.3.4 Network-based diffusion analysis

Network-based diffusion analysis (NBDA) infers social transmission of a behaviour or disease if the pattern of spread of a behaviour or disease follows the connections of a social network by assuming that a trait will spread faster between individuals that spend more time together than those that spend less time together (Hoppitt and Laland, 2013). NBDA was primarily developed to assess transmission of behaviour across a network, for example revealing that a naturally occurring foraging invention (lobtail feeding, where a whale slaps its tail on the water before blowing bubbles to engulf its prey) was transmitted via social learning through populations of humpback whales in the Gulf of Main (Allen et al., 2013). In a behavioural context, NBDA estimates the strength of social learning relative to asocial learning, where social learning is learning that is facilitated by observation or interaction with another individual (Hoppitt and Laland, 2013), and asocial learning is where a behaviour is learned independently of others (Hasenjager et al., 2021) – for example through trial and error or direct interaction with the environment (Hoppitt et al., 2010b).

The theory of NBDA can also be applied to transmission of disease and in this context, social transmission of disease would occur when animals become infected through connection to other infected animals, and asocial transmission would be those that became infected with no connection to infected animals, which could be taken transmission attributable to either the environment or possibly genetic aspects – although evidence for heritability of susceptibility to footrot infection is weak (discussed above). In the context of sheep lameness social connection refers to spatial proximity as indicated by the proximity sensors, with the assumption that this is sufficient for transmission of *D. nodosus* between sheep and subsequent development of lameness. If there is no effect of social transmission across the social network, this would indicate that there is no influence of spatial proximity on risk of developing lameness.

4.4 Aims

The following chapters investigated the relationships between disease, lameness, and spatial co-location of sheep by considering the points above using social network-based methodologies above to consider the questions posed by Craft et al., 2015 and White et al. 2017 to explore how, or if, spatial co-location of sheep is related to lameness. There are two main hypotheses to explore – 1) Sheep that are lame have different contact patterns compared to those that are not lame, and 2) Spatial co-location of sheep with lame sheep is a risk factor for developing lameness. Table 4.2 summaries how the analyses in the following chapters apply to the considerations outlined above.

Table 4.2 Summary of the analytical methods used to address the considerations outlined by Craft et al., 2015 and White et al., 2017 to explore the disease dynamics of lameness in a flock of ewes and their lambs.

Consideration	**Analysis**
Factors that mediate individual variability in susceptibility and exposure	• Kinship relationships – general linear mixed effects models for in and out of family degree centrality – Chapter 7 • Type of contacts made by sheep – considered pre-trial • Prior immunity/genetics – considered pre-trial
Trait-based features and lameness/foot lesion status	• Binomial mixed effect models to identify associations between individual-level trials and lameness in the trial – Chapter 7 • General linear mixed effects models to investigate associations between degree centrality and lameness – Chapter 7
Community structure and group living	• Identification of sub-groups and community structure using Newman's modularity approach – Chapter 7 • Use of individual-level metrics (Guimerà and Nunes Amaral, 2005) to describe how an individual's position in the network is related to potential for disease spread – Chapter 7
Network position and infection status	• General linear mixed models to investigate associations between network centrality and lameness – Chapter 7 • Network-based diffusion analysis – Chapter 8
Other factors affecting social behaviour	• Generalised linear models for association between number of communities and weather data – Chapter 7

Chapter 5 Methods used to determine contact rates and lameness in a flock of Poll Dorset Sheep

This chapter provides the methods used for the farm trial to collect data on individual sheep characteristics, including lameness and foot lesions and contact rates based on spatial co-location using proximity sensors.

5.1 Study population

The study population consisted of 63 Poll Dorset ewes located in Cullompton, Devon, United Kingdom. Sheep are typically seasonally polyestrous (Hafez, 1952). with reducing daylight hours stimulating sexual activity. However, Poll Dorset ewes are sexually active all year round and can produce lambs in August-November in the Northern Hemisphere as well as December – May, the more typical lambing season. The breeding cycle on the trial farm (described fully in Ozella et al., 2020) was typical for Poll Dorset breeders, with the flock producing lambs from September to mid-October.

5.2 Farm management - lambing process and location of sheep

The location of sheep during the trial is shown in Figure 5.1. Ewes lambed outside throughout September (Field 1) and were brought into individual 'family' pens within 24 hours of parturition to bond, and then turned out onto a new field (Field 2). By the 30th September, 50/63 ewes had lambed and this was deemed sufficient to deploy the sensors. After the sensors were deployed (described below) on 1st October, ewes and lambs were turned out onto a new field (Field 3). The fields were strip grazed (Figure 5.1). Ewes and lambs were initially turned out into Section A of Field 3 (1.7 acres), the temporary fence between Section A and B was removed on the 4th October, to provide a total area of 3.3 acres (Section A+ Section B), then the fence to Section C removed on the 8th October, to provide a total area of 4.9 acres (Section A + Section B + Section C).

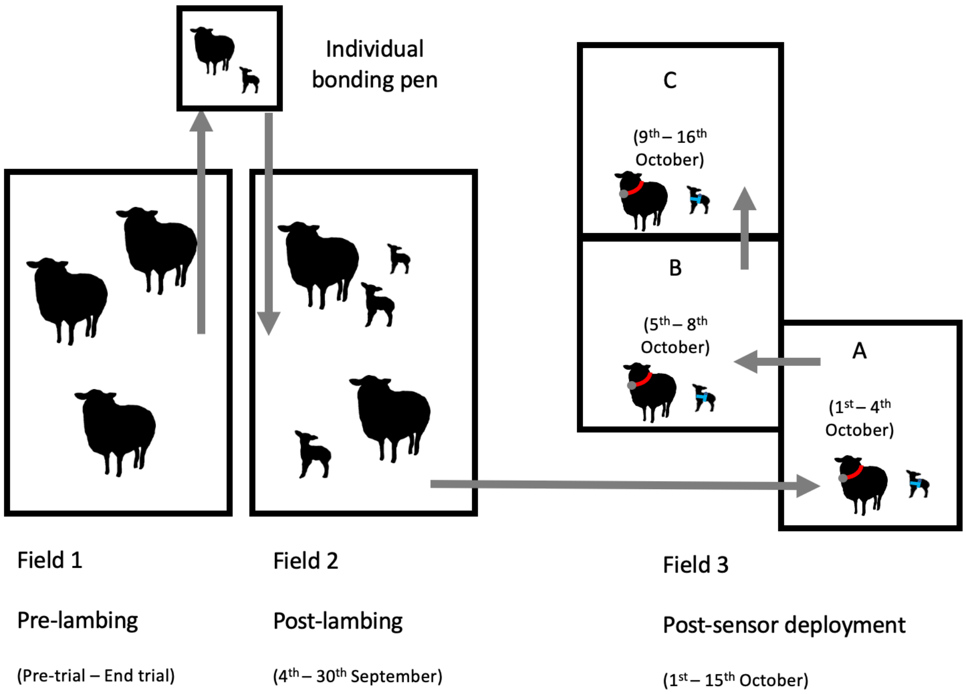


Figure 5.1 Location of sheep during the trial. Ewes moved from the pre-lambing to post-lambing field after spending at least 24 hours in a bonding pen. Sensors were deployed on 1st October 2019 and the flock was moved to Field 3. Dates correspond to the field the flock was in when locomotion was scored.

5.3 Exclusions from sensor deployment and characteristics of selected sheep

Graham and Joss Langford (Garland Hayes Farm) provided records of lambing details, pedigree and electronic identification (EID) numbers. Characteristics of the flock in Field 3 are presented in Table 5.1. There were 120 sheep that entered Field 3 on the 1st October. Of these, five lambs were untagged because they were too small for the sensor harness and two of these left the field during deployment of sensors (death and removal for bottle feeding) leaving 118 sheep (50 ewes, 68 lambs) at the end of the study period. The 68 lambs ranged from 5-27 days old on the 1st October.

Table 5.1 Flock characteristics for 118 sheep that were in Field 3 post sensor deployment from the 1st-15th October

Characteristic		**Ewes (N = 50)**			**Lambs (N= 68)**	
Categorical		**N**	**%**		**N**	**%**
Lambs raised	1	28	56.0	1	27	39.7
	2	22	44.0	2	41	60.3
Sex	Ewe	50	100.0	Ewe	37	54.4
	Ram	-	-	Ram	31	45.6
Continuous		**Mean**	**Range**		**Mean**	**Range**
Age	Years	4	2-9	Days	15	5-27
Birth weight	kg	-	-	kg	5.0	2-7

1. N = number of sheep, kg = kilogram, lambs raised refers to the number of lambs a ewe mothered during the study period (some lambs died between birth and the beginning of the study, and darm protocol was to foster triplets onto singles)

5.4 The proximity sensing platform, choice of biologging equipment, attachment to sheep and date of deployment

The proximity sensing platform was designed by the SocioPatterns collaboration consortium (http://www.sociopatterns.org/), with open-source hardware based on the OpenBeacon project (http://www.openbeacon.org/). The sensors (Cattuto et al., 2010) have previously been deployed on ewes (Ozella et al., 2020) and dogs (Wilson-Aggarwal et al., 2019).

Proximity sensors provide insight into the relative location of sheep to other sheep and do not provide absolute information on the location of the sheep. Since transmission of *D. nodosus* occurs via the environment, other sensing systems, such as GPS, which give information on location could have also provided insight into transmission pathways. However, since *D. nodosus* is found in similar quantities in both high traffics area of the field (e.g. gateways) and low traffic areas (Clifton et al., 2019), spatial proximity to other sheep was likely most relevant for the current study. The study also took place at a small spatial resolution (sheep in one field), and GPS tags are still thought to be most appropriate for studies where animals interact over at least tens of metres (Hughey et al., 2018). Additionally, the current study was part of a larger project and sheep were also fitted with accelerometers, and therefore it would not have been practical to fit sheep with a third sensor due to the balance of the two on a harness/collar and the combined weight may have been more than the recommended <5-10% of the individual's body weight (Sikes et al., 2016, Wilson et al., 1996).

Poll Dorsets have strong maternal behaviours, which helps to minimise the risk of lamb rejection as a result of attaching sensors. Neck collars were used to attach sensors to ewes and an adjustable harness was used for lambs, in order to accommodate growth (Figure 5.2). Lambs were checked daily and if the harnesses needed adjustment, individual lambs were caught in the field. The sensors had a total weight of ~6g (sensor ~ 2.7g, lithium coin battery ~ 3g), memory of ~1000 hours of contact events and battery life of ~ 25 days. Sensors have to be face-to-face with each other to record a contact. The sensors were deployed on the 1st October 2019 and removed on 15th October 2019, giving 13 complete midnight-midnight periods of data.

Figure 5.2 Sensor placement on ewes using a neck collar, and lambs using a harness

5.5 Processing of data from the sensors

The wearable proximity sensors use RFID technology to assess proximity. The sensors feature a bi-directional radio interface and transmit packets carrying a unique identifier as a data payload, which can be received by other tags located nearby. The exchange of these low power radio packets is used as a proxy for spatial proximity (Cattuto et al., 2010) using the attenuation, which is the difference between the received and transmitted power. An attenuation threshold of -75 dBm was used to allow detection of sheep co-located within 1.0-1.5m, and a contact was identified when the devices exchange at least one radio packet in a 20 second time interval. Contacts were considered broken if no radio packets were exchanged in a 20 second interval. Contact data was stored locally in the memory of each sensor, and the data were processed at the Institute for Scientific Interchange, Turin, Italy and returned as a CSV file with the date, time and length of contact recorded between $sensor_i$ and $sensor_j$. Each proximity sensor had a unique identification number and Emily Price (University of Exeter) provided the document which linked the identity of the proximity sensors to the sheep EID numbers.

5.6 Locomotion scoring of sheep

Sheep locomotion was scored using the validated scale below (Table 5.2) from Kaler et al. (2009). Locomotion scores were recorded on paper and entered manually into Microsoft Excel. Data collection forms are in Appendix 28.

Table 5.2 Criteria from the Kaler et al., 2009 locomotion scoring system for sheep

Criteria - all required for score	Locomotion score 0	1	2	3	4	5	6
Bears weight evenly on all four feet	■						
Uneven posture, but no clear shortening of stride		■	■	■	■	■	
Short stride on one leg compared to others		■	■	■	■	■	
Visible nodding of head in time with short stride			■				
Excessive flicking of head, more than nodding, 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							■

1. Shaded squares indicate where the criteria was required

5.6.1 Locomotion scoring prior to sensor deployment and identification of sheep post lambing

Ewes were acclimatised to locomotion scoring by a researcher (KL) walking among them in the field for four weeks to minimise disruption to their natural behaviour. Acclimatisation took place for four weeks in September, with all ewes' locomotion scored on at least five occasions, whether ewes were pregnant in Field 1 or with lambs in Field 2. The flock was scored twice in the first and second weeks of

September, and once in the third and fourth week. Ewes were not scored if they were lambing or in bonding pens after parturition.

Before lambing, ewes did not have numbered flanks and so locomotion scores were not linked to individual ewes. Therefore, locomotion scores from Field One were collected as the frequency of sheep with each locomotion score, this was repeated three times on each visit due to the difficulty of accurately collecting scores at a flock level when sheep are unmarked. The mean weekly prevalence of lameness (lame was defined as locomotion score ≥2) was calculated from percentage of ewes at each score from the three repeated scorings.

Once ewes had lambed, their flank was given a unique number using livestock marker paint to ensure they could be identified. Lambs were marked with the same number as their dam, with a paint spot put on the head of the larger twin. Ewes and lambs locomotion was scored in Field 2.

5.6.2 Scoring locomotion in Field 3 after sensors had been deployed

Once sensors were deployed and sheep had been placed in Field 3, ewe and lamb locomotion was scored each day from the 1st-15th October.

5.7 Treatment of lame sheep

Sheep were treated at the farmer’s discretion according to their normal protocol and gathered for treatment when ‘enough’ sheep were lame. Sheep were gathered for treatment on 3rd, 5th and 9th October. The standard treatment for ID was to spray all four feet of lame ewes and lambs with topical antibiotic. The farmer thought that two severely lame lambs may have joint ill and these were treated with a course of antibiotic injections (Betamox™) starting on the 5th October and finishing on the 9th October. At the lesion check on the 15th, all lambs with ID were treated with topical antibiotic spray on all four feet.

Table 5.3 The number of sheep treated with antibiotic foot spray on each occasion sheep were gathered

	Number treated with antibiotic foot spray (%)		
Date	All	Ewes	Lambs
03-10-2019	5 (4.2)	0 (0.0)	5 (7.4)
05-10-2019	2 (1.7)	2 (4.0)	0 (0.0)
07-10-2019	5 (4.2)	3 (6.0)	2 (2.9)
09-10-2019	9 (7.6)	6 (12.0)	0 (0.0)
15-10-2019	26 (22.0)	0 (0.0)	26 (38.2)

5.8 Identification of foot lesions

The farmer stated that ID and non-infectious lesions such as granuloma had been observed in the flock, he had not seen evidence of CODD or SFR.

Foot lesion data was collected only once at the end of the study (15/10/2019) to minimise disruption in the trial. The experienced researcher who scored the foot lesions (Liz Nabb - LN) was blind to the locomotion scores. Ewes and lambs were gathered into a pen. Lambs were lifted and removed from the pen to inspect their feet before any ewes were scored. Ewes were run through a race and then caught as they were released from the race and held against the side of a pen by a handler and feet inspected by lifting each foot in turn. The scoring system from Moore et al., 2005 was used for ewes, and the criteria for ID lesions adapted for lambs by LN since the size of their feet made it difficult to determine when <10% of the interdigital space was affected by a lesion. Criteria for the scoring systems are shown in Table 5.4 and Table 5.5.

Other foot lesions were identified using classical definitions (white line disease, fibroma and granuloma).

Table 5.4 The scoring system from Moore et al., 2005 for interdigital dermatitis and severe footrot lesions in ewes

Foot lesion	Criteria
Interdigital dermatitis	
0	Clean interdigital foot with no lesions
1	Slight interdigital dermatitis, partial loss of hair, slight redness but dry
2	Slight interdigital dermatitis, partial/complete loss of hair, redness, pasty scum (<10% of interdigital area affected)
3	Moderate interdigital dermatitis, partial/complete loss of hair, redness, pasty scum (10-50% of interdigital area affected)
4	Severe interdigital dermatitis, partial/complete loss of hair, redness, pasty scum (>50% of interdigital area affected)
Severe footrot	
0	No under-running of the wall or sole of the digit
1	Under-running of the horn on the wall and/or sole of the digit
2	Extensive under-running and detachment of the horn involving the sole and wall of the digit

Table 5.5 The adapted scoring system (Moore et al., 2005) used for identification of interdigital dermatitis lesions in lambs

Foot lesion	Criteria
Interdigital dermatitis	
0	Clean interdigital foot with no lesions
1	Slight interdigital dermatitis, partial loss of hair, slight redness but dry
2	<10% of the skin was hairless, grey and rough in texture (pitted or eroded)
3	10-50% of the skin was hairless, grey and rough in texture (pitted or eroded)
4	>50% of the skin was hairless, grey and rough in texture (pitted or eroded)
4+	The interdigital space was hairless, reddish, swollen, cracked or ulcerated – evidence there was an inflammatory process. No under-running of the hoof wall.

5.9 Weather over the study

Daily meteorological data were collected using a Davis Vantage Pro2 Plus weather station. A descriptive summary of the weather in the study is in Table 5.6. The weather in October was generally unsettled with frequent low pressure systems and rain belts crossing the country (Met Office, 2019). Daily mean 24-hour temperatures remained relatively consistent over the study, but some days were wetter and windier than others - the 11th October had over twice as much rainfall as any other day in the study and the highest mean windspeeds (Table 5.6).

Table 5.6 Daily mean and standard deviation for weather-related variables (temperature, humidity, windspeed, windchill, THW index, and rainfall) over the entire study period)

	Temperature (°C)		Humidity (%)		Windspeed (mph)		Windchill (°C)		THW Index (°C)		Rainfall (inches)
Date	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Total**
01-10-2019	13.8	1.8	95.8	3.6	5.5	2.6	13.1	2.6	13.5	2.6	0.30
02-10-2019	9.2	2.6	84.5	10.8	3.5	2.6	8.5	2.7	8.5	2.5	0.00
03-10-2019	9.8	3.5	93.3	5.7	4.2	3.2	9.2	3.0	9.3	3.0	0.17
04-10-2019	12.1	1.0	93.2	5.0	4.4	2.1	11.6	1.0	11.9	1.0	0.09
05-10-2019	13.0	1.1	96.7	3.4	2.4	2.2	12.9	1.1	13.1	1.1	0.37
06-10-2019	11.7	1.9	91.0	7.8	4.6	2.1	11.1	1.8	11.3	1.8	0.06
07-10-2019	11.6	2.2	97.7	1.1	4.1	3.1	11.0	2.2	11.2	2.2	0.12
08-10-2019	11.4	1.5	91.3	5.9	4.2	1.8	11.0	1.6	11.1	1.6	0.03
09-10-2019	9.4	1.3	93.5	3.8	3.9	1.3	8.8	1.3	9.0	1.3	0.19
10-10-2019	11.4	2.5	94.1	4.7	5.6	3.3	10.6	1.9	10.7	1.9	0.04
11-10-2019	12.6	1.4	98.2	0.7	7.4	4.2	11.3	0.9	11.6	0.9	1.01
12-10-2019	10.1	1.1	97.8	1.6	1.7	0.8	10.1	1.1	10.3	1.0	0.38
13-10-2019	10.4	1.2	95.9	5.0	3.1	2.2	10.0	1.0	10.2	1.0	0.50
14-10-2019	10.4	0.5	98.5	0.6	3.5	2.1	9.9	0.6	10.2	0.5	0.40
15-10-2019	10.9	1.6	95.3	5.2	2.3	2.4	10.7	1.7	10.9	1.6	0.07

1. SD = standard deviation, THW = temperature, humidity and wind index, mph = miles per hour

Chapter 6 Flock prevalence of lameness and individual attributes associated with lameness in ewes and lambs

6.1 Introduction

While establishing if contact patterns between sheep are important for influencing disease risk is the predominant focus of subsequent chapters, it is equally important to consider if there are trait-based features that are associated with disease development (White et al., 2017), particularly since there is evidence that certain sheep-level attributes pre-dispose sheep to lameness. Ewes are more likely to become lame if they are more than four years old compared with not (Kaler et al., 2010b), while lambs are more likely to become lame if they are male compared to female, or single-born compared to twin-born (Kaler et al., 2010b, Wassink et al., 2010b). This chapter summarises the prevalence of lameness recorded in the Devon flock pre and post- lambing and relationships between individual sheep attributes and lameness to determine whether any trait-based features were predictive of either lameness during the trial or presence of a foot lesion at the end of the trial.

6.2 Methods

Methods to collect locomotion scores and foot lesions are described in chapter 5. Sheep were defined as lame at locomotion score ≥2, the score at which it is recommended farmers catch and treat lame sheep, as these sheep are likely to have foot lesions (Kaler et al., 2011) . Infectious foot lesions were defined as an ID score of ≥1, or a SFR score of ≥1 (Table 5.4, Table 5.5).

6.2.1 Agreement between locomotion scoring of unmarked sheep in September

Two methods for assessment of intra-observer reliability were used to assess the reliability of the scores collected from Field One. These were Cohen's weighted Kappa with squared weights (using the *irr* package - (Gamer et al., 2019)) and the average Kendall's rank correlation coefficient (using *stats* - (R Core Team, 2019)), which was calculated for pairwise set of scores, and a mean taken from these.

6.2.2 Sheep-level characteristics associated with lameness

Two-level binomial generalised linear mixed effects models were used to determine relationships between individual characteristics of sheep and lameness. Individual sheep characteristics were divided into three sections – those related to both birth characteristics (age, sex and single/twin for lambs, age and number of lambs raised for ewes), those related to foot lesion characteristics (infectious, non-infectious, multiple feet affected and whether or not the sheep was treated during the trial), and those related to the location of the sheep (space available and whether or not the sheep were gathered on the day). The association between lameness and time was tested by using day as a fixed effect in the model, and day as a first and second-degree polynomial term.

Data were analysed separately for ewes and lambs, with the outcome variable whether or not a sheep was lame on a day, with random variables for individual and day. Models were constructed using the *glmer* function from *lme4* (Bates et al., 2015) in RStudio version 4.3 using default options. Confidence intervals were constructed using Wald's test and p-values came from Wald's test.

Univariable models were constructed for each sheep-level attribute. Multivariable models were built only from birth characteristics, using a forward stepwise process where if the p-value from a likelihood ratio test ≤0.05, this was considered a significant improvement in the model. Interactions between birth characteristics were tested for where biologically plausible and added if the likelihood ratio test indicated a significant improvement in the model.

6.2.3 Sheep-level characteristics associated with presence of infectious foot lesions

Univariable binomial generalised linear models were used to test associations between sheep-level birth characteristics and presence of infectious foot lesions on

15.10.2019. Whether or not the sheep had a family member with an infectious foot lesion at the end of the trial was also tested in these models. Lameness was not used as an independent variable in these models.

6.2.4 Relationships between lameness and presence of infectious foot lesions

Relationships between lameness and presence of foot lesions at the end of the trial were explored using four metrics (sensitivity, specificity, accuracy and balanced accuracy). Metrics were computed when lameness was defined at different cut-off points (scores of ≥1, at ≥2 and ≥3). These were also computed for lameness over different time scales (sheep lame on the 15.10.19 and lame in three, seven or fourteen days prior to 15.10.2019)

6.3 Results

6.3.1 Flock prevalence of lameness

6.3.1.1 Prevalence of lameness in ewes in September (prior to sensor deployment)

Overall prevalence of lameness in ewes ranged from 22.4% in Week 1 to 7.9% in Week 4 when the majority of ewes had lambed (Table 6.1). The intra-observer agreement between the three repeated flock locomotion scoring of the unmarked ewes was good (Table 6.2).

Table 6.1 Prevalence of lameness in ewes throughout September. Scores are combined from both unmarked sheep that had not yet lambed, and sheep that had lambed and could be individually identified.

September	Number of days scored	Locomotion score	Mean number of sheep (%)
Week 1	2	0-1	48 (76.8)
		≥2	14 (22.4)
		Not scored	1 (1.6)
Week 2	2	0-1	49 (81.0)
		≥2	12 (19.0)
		Not scored	0 (0.0
Week 3	1	0-1	45 (72.0)
		≥2	18 (28.0)
		Not scored	0 (0.0)
Week 4	1	0-1	52 (82.5)
		≥2	5 (7.9)
		Not scored	6 (9.5)

Table 6.2 Intra-observer agreement for the three runs in September for the locomotion scores in the field in September

September	Date	Mean Kendall's rank correlation coefficient	Mean weighted Kappa statistic
Week 1	04-09-2019	0.92	0.73
Week 1	05-09-2019	0.98	0.98
Week 2	11-09-2019	0.97	0.91
Week 2	12-09-2019	1.00	0.96
Week 3	18-09-2019	0.98	0.79
Week 4	26-09-2019	1.00	1.00

6.3.1.2 Prevalence of lameness in lambs in September (prior to sensor deployment)

There were 11 lambs in Field 2 on the 11th September, and 65 by the 26th. Lambs were first lame at around two weeks old. The first lamb was lame in Week 3 (Lamb 3A, 12 days old), and was the only lame lamb that week out of the 40 present (2.5%). By week 4, this had risen to 3/65 lambs (4.6% - Lambs 6B, 13B and 15A, mean age = 14 days, range = 13-18).

6.3.1.3 Prevalence of lameness in ewes and lambs in October (post sensor deployment)

The daily prevalence of lameness ranged from 13.6% to 20.3% (Figure 6.3) from the 1st to the 15th October 2019. Of the 118 sheep, 48 (40.7%) sheep, 56.0% of ewes and 29.4% of lambs were lame on at least one day of the 13 days sensors were deployed.

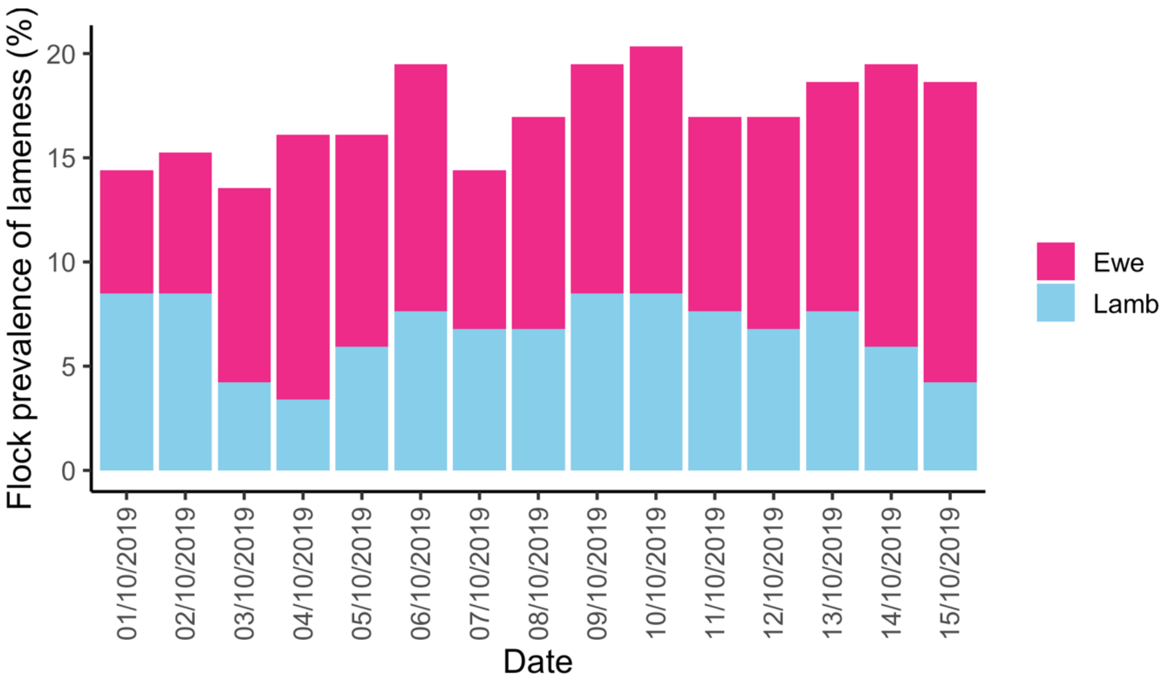


Figure 6.3 Flock prevalence of lameness (%) recorded on the day sensors were deployed, 13 days of the trial, and the day sensors were removed.

6.3.1.4 Prevalence of lameness in October and locomotion scores for individual sheep

More ewes (24.5%) were lame over the study than single lambs (14.4%) or twin lambs (9.3%) (Table 6.3). Individual locomotion scores for each ewe and lamb are shown in Figure 6.2 (ewes) and Figure 6.3 (lambs). Of the sheep that were lame, sheep were lame for a median of 4 days, with a range from 1-15 days. Combined over the study period, there were 89 changes from a sound to lame state from day to day, and 84 from sound to lame, with 1280 instances of sheep remaining sound, and 197 where sheep remained lame.

Table 6.3 Total number of observations of lame (locomotion score ≥2) and sound (locomotion score 0-1) ewes, single, and twin lambs from 1st-15th October.

Sheep	Locomotion score	N	%
Ewe	0-1	566	75.5
	≥2	184	24.5
Single lamb	0-1	409	85.6
	≥2	69	14.4
Twin lamb	0-1	490	90.7
	≥2	50	9.3

1. N = number of observations

Figure 6.2 Daily locomotion scores for 50 ewes from 1st-15th October. Sound sheep (locomotion score 0 or 1) are shown in white or grey, respectively, then lame sheep (locomotion score of ≥2) are shown in pink-red.

Figure 6.3 Daily locomotion scores for 68 lambs from 1st-15th October. Sound sheep (locomotion score 0 or 1) are shown in white or grey, respectively, then lame sheep (locomotion score of ≥2) are shown in pink to red.

6.3.2 Causes of lameness

On the final day of the trial, 38.2% of lambs and 32.0% of ewes had an ID lesion (Table 6.4) and only one ewe showed any evidence of SFR with the ID. White line abscesses and fibroma were seen in ewes but not lambs. Some (5 ewes) had both an infectious and non-infectious cause of lameness. Non-infectious causes of lameness for lambs included granuloma (2 lambs) and a possible shoulder injury (1 lamb) (Table 6.4).

Table 6.4 Number and percentage of ewes and lambs with each potential cause of lameness identified at the end of the study

Potential cause of lameness		Ewes		Lambs	
		N	%	N	%
Infectious foot lesions					
Interdigital dermatitis score	0	34	68.0	42	61.8
	1	0	0.0	0	0.0
	2	3	6.0	0	0.0
	3	7	14.0	15	22.1
	≥4	6	12.0	11	16.2
Severe footrot score	0	49	98.0	68	100.0
	1	1	2.0	0	0.0
	≥2	0	0.0	0	0.0
Non-infectious foot lesions					
White line disease	No	42	84.0	68	100.0
	Yes	8	16.0	0	0.0
Fibroma	No	42	84.0	68	100.0
	Yes	8	16.0	0	0.0
Granuloma	No	50	100.0	66	97.1
	Yes	0	0.0	2	2.9
Heel ulcer	No	49	98.0	68	100.0
	Yes	1	2.0	0	0.0

Potential cause of lameness		Ewes		Lambs	
		N	%	N	%
Broken claw at toe	No	49	98.0	68	100.0
	Yes	1	2.0	0	0.0
Uneven claw size	No	49	98.0	68	100.0
	Yes	1	2.0	0	0.0
Suspected shoulder injury	No	50	100.0	67	98.5
	Yes	0	0.0	1	1.5

1. N = number of sheep

6.3.3 Relationships between lameness at different locomotion scores and presence of foot lesions on 15.10.2019

Classifying sheep as lame at locomotion scores of ≥3 gave the highest sensitivity for detection of foot lesions (0.86 for ewes, 0.93 for lambs), but specificity was low (0.07 for ewes, 0.08), whereas classifying sheep as lame at locomotion scores of ≥2 had the highest balanced accuracy for detecting foot lesions (0.55 for ewes, 0.59 for lambs). These are found in Appendix 29.

6.3.4 Associations between sheep-level attributes and lameness

6.3.4.1 Univariable binomial mixed effects models for association between ewe characteristics and lameness

Only having received treatment for lameness, compared with not was associated with lameness in ewes (OR = 86.73, 95% = 3.72-2020.77, Table 6.5). Other sheep-level attributes (age, number of lambs raised and presence of foot lesions) were not associated with lameness. Neither space available or time were associated with lameness (Table 6.5).

Table 6.5 Univariable associations from generalised binomial mixed effects models between lameness (score ≥2) in 50 ewes and sheep-level attributes for the 13 days sensors were attached

	N (%)	OR	LCI	UCI	P-value	Random effect variance	
						Sheep	Day
Ewe characteristics							
Ewe age (years)							
+1 year	160 (24.6)	1.22	0.83	1.80	0.310	3.75	0.00
Ewe age (years)							
0-4	90 (21.6)	Ref					
>4	70 (29.9)	2.02	0.57	7.18	0.279	3.72	0.00
Lambs raised							
1	109 (26.2)	Ref					
2	51 (21.8)	0.62	0.17	2.29	0.447	3.82	0.00
Foot lesions							
Infectious foot lesion at end of study							
No	107 (24.2)	Ref					
Yes	53 (25.5)	1.07	0.29	4.02	0.919	3.82	0.00
Non-infectious cause at end of study							
No	88 (21.8)	Ref					
Yes	72 (29.2)	1.82	0.52	6.39	0.347	3.73	0.00
Multiple affected feet at end of study							
No	124 (22.7)	Ref					
Yes	36 (34.6)	2.52	0.50	12.74	0.265	3.94	0.00
Sheep treated during study							
No	150 (23.5)	Ref					
Yes	**10 (90.9)**	**86.73**	**3.72**	**2020.77**	**0.005**	**3.90**	**0.00**
Space							
Area available							
1.7 acres	34 (22.7)	Ref					

	N (%)	OR	LCI	UCI	P-value	Random effect variance	
						Sheep	Day
3.3 acres	47 (23.5)	1.07	0.50	1.98	0.824	3.29	0.00
4.9 acres	79 (26.3)	1.35	0.77	2.36	0.301		
Sheep gathered							
No	115 (25.6)	Ref					
Yes	45 (22.5)	0.78	0.48	1.26	0.308	3.84	0.00
Time							
Day	-	1.04	0.98	1.11	0.163	3.86	-
Day 1st polynomial	-	53.77	0.20	-14505.12	0.163	3.86	-
Day 2nd polynomial	-	54.06	0.20	14673.06	0.163	3.86	-
		0.80	0.00	228.67	0.958		-

1. N = number of observations of sheep that were lame within the category, % = percentage of observations of sheep that were lame within the category, OR = odds ratio, LCI = lower confidence interval, UCI = upper confidence interval, P-value from Wald's test of significance, Ref = reference category

6.3.4.2 Univariable binomial mixed effects models for association between lamb characteristics and lameness

Univariable associations (Table 6.6) showed lambs that were lame during in the trial were heavier at birth (OR = 2.19, 95% CI = 1.04-4.60) and were more likely to have an infectious foot lesion on the final day of the trial (OR = 4.28, 95% CI = 1.24-14.73) compared with not, be diagnosed with a non-infectious potential cause of lameness (OR = 63.32, 95% CI = 4.98-804.98) compared with not, have multiple feet affected by foot lesions (OR = 5.68 (95% CI = 1.56-20.66) compared with not, and to have received treatment over the trial (OR = 73.63, 95% CI = 7.15-758.32) compared with not.

Table 6.6 Univariable associations from generalised binomial mixed effects models between lameness (score ≥2) in 68 lambs and birth characteristics, foot lesion characteristics and management variables for the 13 days sensors were attached

Predictor	N (%)	OR	LCI	UCI	P-value	Random effect variance	
						Sheep	Day
Birth characteristic							
Lamb age (days)							
+ 1 unit	104 (11.8)	0.90	0.80	1.01	0.062	4.60	0.00
Age (days)							
5-9	27 (15.3)	Ref					
10-11	21 (11.9)	1.27	0.24	6.61	0.780	4.79	0.00
12-16	20 (11.3)	1.05	0.17	6.59	0.956		
17-19	18 (10.2)	0.38	0.05	2.75	0.340		
20-27	18 (10.2)	0.35	0.05	2.51	0.295		
Lambs raised							
1	60 (14.5)	Ref					
2	44 (9.5)	0.27	0.07	1.03	0.055	4.67	0.00
Lambs born							
1	42 (12.4)	Ref					
2 or 3	62 (11,4)	0.51	0.13	1.90	0.312	0.474	0.00
Sex							
Female	47 (9.8)	Ref					
Male	57 (14.1)	1.79	0.50	6.40	0.373	4.61	0.00
Birth weight (kg)							
+ 1 unit	**104 (11.8)**	**2.19**	**1.04**	**4.60**	**0.038**	**4.35**	**0.00**
Foot lesion characteristics							
Infectious foot lesion at end of study							
No	41 (7.5)	Ref					

Predictor	N (%)	OR	LCI	UCI	P-value	Random effect variance	
						Sheep	Day
Yes	**63 (18.6)**	**4.28**	**1.23**	**14.75**	**0.021**	**4.07**	**0.00**
Non-infectious cause of lameness at end of study							
No	82 (9.7)	Ref					
Yes	**22 (56.4)**	**63.32**	**4.98**	**804.99**	**0.001**	**3.60**	**0.00**
Multiple feet affected by foot lesions at end of study							
No	50 (7.9)	Ref					
Yes	**54 (21.9)**	**5.68**	**1.56**	**20.66**	**<0.001**	**3.94**	**0.00**
Sheep treated for lameness during study							
No	90 (10.4)	Ref					
Yes	**14 (187.5)**	**73.63**	**7.15**	**758.32**	**<0.001**	**4.43**	**0.00**
Management and time							
Area available							
1.7 acres	19 (9.4)	Ref					
3.3 acres	32 (11.8)	1.47	0.71	3.45	0.303	4.75	0.00
4.9 acres	53 (13.0)	1.75	0.88	3.45	0.108		
Sheep gathered							
No	74 (12.1)	Ref					
Yes	30 (11.0)	0.85	0.49	1.14	0.576	4.69	0.00
Day	-	1.03	0.97	1.10	0.346	4.70	-
Day 1st polynomial	-	36.42	0.02	63915.56	0.346	4.70	-
Day 2nd polynomial	-	41.96	0.02	87605.13	0.338	4.72	-
	-	0.07	0.00	145.51	0.502		

1. N = number of observations of sheep that were lame within the category, % = percentage of observations of sheep that were lame within the category, OR = odds ratio, LCI = lower confidence interval, UCI = upper confidence interval, P-value from Wald’s test of significance

6.3.4.3 Multivariable binomial mixed effects models for association between lamb birth characteristics and lameness

A multivariable model (Table 6.7) for the lamb birth characteristics suggested an interaction effect between the number of lambs born (i.e. single vs twin/triplet) and their birth weights, and once this was accounted for, twin lambs were less likely to be lame during the trial compared to single lambs. Younger lambs were also less likely to be lame during the trial (OR = 0.89, 95% CI = 0.80-0.99, Table 6.7).

Table 6.7 Multivariable associations from generalised binomial mixed effects models between lameness (score ≥2) in 68 lambs and sheep-level attributes

	N (%)	OR	LCI	UCI	P-value
Birth weight					
+ 1 unit	104 (11.8)	0.89	0.30	2.58	0826
Lambs born					
1	47 (13.4)	Ref			
2 or 3	**57 (10.7)**	**0.00**	**0.00**	**0.68**	**0.040**
Lamb age (days)					
+ 1 unit	104 (11.8)	0.89	0.80	0.99	0.038
Interaction					
Birth weight * Lambs born		**4.94**	**1.07**	**22.95**	**0.041**
Random effects		*Variance*			
Sheep	68	3.66			
Day	13	0.00			
Residual	-	3.29			

1. N = number of observations that were lame within the category, OR = odds ratio, LCI = lower confidence interval, UCI = upper confidence interval, P-value from Wald's test of significance, Ref = reference category
2. Variables added to multivariable model when p<0.05 from likelihood ratio test and highlighted when Wald's P-value <0.05.

6.3.5 Associations between sheep-level attributes and presence of a foot lesion at the end of the trial

6.3.5.1 Univariable binomial models for ewe-level attributes associated with presence of an infectious foot lesion on 15.10.2019

No ewe-level attributes were associated with having an infectious foot lesion on 15.10.2019 (Table 6.8).

Table 6.8 Univariable binomial models for association between ewe-level attributes and presence of an infectious foot lesion on 15.10.2019 for 50 ewes in the flock

Predictor	N (%)	OR	LCI	UCI	P-value
Lambs raised					
1	10 (31.3)				
2	6 (33.3)	1.10	0.31	3.75	0.880
Lamb with infectious foot lesion at end of study					
No	9 (34.6)	Ref			
Yes	7 (29.3)	0.78	0.23	2.57	0.680
Ewe age (years)					
+ 1 unit	16 (32.0)	0.78	0.51	1.14	0.181
Ewe age (years)					
0-4	6 (42.9)	Ref			
>4	36 (27.8)	0.51	0.14	1.90	0.309

1. N = number of observations with a foot lesion seen at the end of the study, % = percentage of sheep with foot lesion in that category, OR = odds ratio, CI = confidence interval, P-value from Wald's test of significance, Ref = reference category

6.3.5.2 Univariable binomial models for lamb-level attributes associated with presence of an infectious foot lesion on 15.10.2019

Univariable models (Table 6.9) suggested that male lambs were more likely to have an infectious foot lesion compared to females (OR = 2.88, 95% CI = 1.06-8.15, p = 0.004). No other characteristics were associated with lameness in a multivariable model.

Table 6.9 Univariable associations from binomial models for association between lamb-level attributes and presence of an infectious foot lesion on 15.10.2019 for 68 lambs in the flock

Predictor	N (%)	OR	LCI	UCI	P-value
Lambs raised					
1	10 (27.0)	Ref			
2	16 (39.0)	1.09	0.40	3.02	0.869
Sex					
Female	10 (27.0)	Ref			
Male	**31 (51.6)**	**2.88**	**1.06**	**8.15**	**0.004**
Birth weight (kg)					
1 unit +	26 (38.2)	1.25	0.74	2.22	0.414
Age (days)					
1 unit +	26 (38.2)	0.97	0.89	1.06	0.530
Age (days)					
5-9	5 (35.7)	Ref			
10-11	4 (28.6)	0.72	0.14	3.56	0.686
12-16	8 (57.1)	2.40	0.54	11.70	0.259
17-19	6 (46.2)	1.54	0.33	7.54	0.582
20-27	3 (23.1)	0.54	0.09	2.86	0.475
Dam with infectious foot lesion at end of study					
No	19 (58.7)	Ref			
Yes	7 (41.3)	0.66	0.22	1.90	0.453
Dam or sibling with infectious foot lesion at end of study					
No	15 (35.7)	Ref			
Yes	11 (42.3)	1.32	0.48	3.61	0.587

1. N = number of observations with a foot lesion seen at the end of the study, % = percentage of sheep with foot lesion in that category, OR = odds ratio, CI = confidence interval, P-value from Wald's test of significance, Ref = reference category

6.4 Discussion

The aim of this chapter was to establish which, if any, trait-based features were predictive of lameness in the flock of Poll-Dorset sheep and determine potential causes of lameness in the flock. As suggested by the farmer, ID lesions were prevalent in the flock – with 38.2% of lambs and 32.0% of ewes diagnosed with an ID score of ≥1 at the end of the trial.

In this study, older ewes (>4 years) were not more likely to be lame compared to younger ewes, which is in contrast to Kaler et al., 2010. However, the majority (76.0%) of ewes in this flock were >4 years of age which could explain this difference. In the multivariable model for lambs, younger lambs were less likely to be lame than older lambs. The youngest lambs were only 5 days old on the day sensors were deployed and since it takes around two weeks for clinical signs of foot lesions to be seen in lambs post exposure to *D. nodosus* positive ewes (Kuhnert et al., 2019), it is possible that the very youngest lambs had not yet had sufficient time to develop clinical signs by the end of the trial.

For lambs, both male and single lambs are more likely to become lame than female or twin lambs (Kaler et al., 2010b), most likely because they are heavier and therefore more susceptible to lameness (Egerton et al., 1989). In this study, male lambs were more likely to have ID lesions compared to females (Table 6.9). However, due to the difficulty in lesion scoring lambs (the size of their feet combined with the mud), if male lambs tend to be larger it may have been easier to see the lesions on their feet. Having an ID or SFR lesion was not associated with ewes being lame – all sheep in the study had ID rather than SFR and since lesion severity is correlated with severity of lesions (Kaler et al., 2011), this could have resulted in ewes with lesions not being identified as lame. The mud may have also obscured foot lesions in ewes – no ewes were scored with an ID score of 1 (Table 6.4), where less than 10% of the interdigital space is affected (Table 5.4). Given the high prevalence of ID lesions in the flock, it seems unlikely that there would not be some ewes with small lesions.

Additionally, some lame sheep were treated with antibiotic foot spray during the trial, which may have resulted in the lesions healing, and therefore not identified at the end of the trial. Feet could not be checked at the start of the trial due to farmer concerns about small lambs being crushed when the sheep were gathered. Sheep had to be lame in order to be treated by the farmer, and as a result, that sheep that were treated during the trial were more likely to be lame compared to not is most likely a function of the farm management, as sheep were treated when "enough" sheep in the group were lame for it to be worth gathering them.

There was no difference in whether or not sheep were lame based on the area available to them. Higher stocking densities are associated with increased prevalence of footrot/lameness at the flock level (Angell et al., 2018, Kaler and Green, 2009, Winter et al., 2015) and the stocking density of this flock was high (~10 ewes/acre when the full field was available). However, sheep were not less likely to be lame as the field size increased, which may be a reflection of the strip grazing management as sheep only spent a few days in each area before it was increased. *D. nodosus* does not persist in the environment, only on diseased sheep feet (Clifton et al., 2019). Since the new areas had not been grazed by sheep at least since September when the trial started, it was likely that it was "clean" pasture when the flock was allowed access.

6.5 Conclusion

In conclusion, this chapter established that ewes were lame prior to lambing and that ID lesions, and therefore *D. nodosus* strains, were present in the flock. Lame lambs were more likely to have an ID lesion compared to not. However, conditions during the lesion scoring may have obscured some lesions, and some would have healed due to treatment, and therefore, combined with evidence from other studies, it is likely that lameness is a suitable proxy for infection with *D. nodosus* in this flock. Twins were less likely to be lame than singles, which may be because they tend to be lighter than singles. Age, or number of lambs raised were not associated with either lameness or presence of ID lesions at the end of the trial in ewes.

Chapter 7 Social contact patterns in a flock of ewes with lambs at foot and the impact of lameness on social patterns

7.1 Introduction

Current knowledge of social contact patterns in sheep is summarised in chapter 4, and trait-based features associated with lameness in this flock are summarised in chapter 6. Less is known about the impact of lameness on social contact patterns in sheep. Lameness is painful and determining how lameness affects social behaviour is a key part of determining the role of lambs in persistence of *D. nodosus* within a flock – for example, if sheep that have footrot are not often found in close spatial proximity to other sheep, this reduces the chance that other sheep will be picking up bacteria transiently shed into the environment from the infected sheep.

Community detection provides a way to quantify the social organisation of group-living animals, by identifying if there are sub-groups of animals that interact more with each other than with other animals. Previous studies on domestic sheep (Ozella et al., 2020) found that when flocks consist of only ewes, sheep do not divide into sub-groups, although ewes do show individual-level preferences for each other (Ozella et al., 2020, Michelena et al., 2009). In wild sheep, lambs interact mainly with lambs of a similar age (Hass and Jenni, 1993), but no work has currently looked at community structure in domestic flocks when lambs are present.

Community-based approaches can be extended to create individual-level metrics (Guimerà and Nunes Amaral, 2005) that describe an individual's potential role in disease spread (Silk et al., 2017). The relationship between disease burden and modularity depends on the trade-off between global disease spread across the whole network and local spread within subgroups (Sah et al., 2017), with individuals that make high proportions of contacts outside of their own module having the most potential to spread disease to different sub-groups within the network, while individuals that make high proportions of contacts within their own module are most likely to spread disease within their sub-group. . One example of where disease can become "trapped " within sub-groups of a network is pneumonia outbreaks in wild big-horn lambs – as the network fragmentation increases, the outbreak size decreases (Sah et al., 2017).

The aim of this chapter was to use the methods outlined in chapter 4 to answer the following questions:

- How does lameness impact on community structure, if a community structure is identified?
- Does community structure impact on the spread of lameness through the flock?
- Are there feedbacks between network position and whether or not sheep are lame?
- Are there other factors that affect social behaviour?

7.2 Methods

Details about the proximity sensing platform, deployment, farm and sheep are fully described in chapter 5.

7.2.1 Network creation

Both social structure, and health status can change on a daily basis, therefore weighted, daily time-aggregated networks were constructed using *igraph* (Csardi and Nepusz, 2006) in RStudio v4.0.3. The weight of an $edge_{ij}$ correspond to the total time spent in contact between sheep *i* and sheep *j* on each day of the trial, where time refers to the exchange of a least one radio packet in 20 second interval. A day was considered as midnight-midnight, since sheep only sleep transiently in short bouts of up to 40 minutes (Munro, 1957). Data were used only from sensors where each sheep in the family group made at least one contact per day (94 sheep).

7.2.2 Network descriptive statistics

Basic network statistics were calculated using *igraph* (Csardi and Nepusz, 2006) for each daily network to determine how sheep interacted on a daily basis, and to assess how the network structure could impact on disease spread. Measures were chosen that describe how connected the flock was. These included:

- Density: the proportion of observed ties to the maximum possible number of ties
- Diameter: the length of the longest shortest path across the network, where a path is the shortest number of steps required to go from sheep A to sheep B
- Mean distance: the average path length in the graph, calculated from all the shortest paths between all nodes

7.2.3 Community detection and the role of the individual in disease spread through network communities

Many social networks are made up of densely connected subgroups that are only connected to other groups by weak ties. The aim of community detection is to identify internally cohesive subgroups thar are somewhat separated from other groups or individuals – and this structure can then be linked to the potential of individuals to spread disease.

7.2.3.1 Community detection with Newman's modularity clustering algorithm

Modularity quantifies the extent to which a network can be divided into smaller groups. Newman's modularity clustering algorithm (Newman, 2006) was used to find densely connected sub-graphs within the network by calculating the leading non-negative eigenvector of the modularity matrix of the graph. Sub-graphs are where networks are made up of densely connected sub-groups, that are themselves connected only by weak ties.

The total number of communities formed each day was calculated, and the modularity coefficient (Q) was computed for each aggregated network (per day).

Modularity measures how good the division of a graph is with respect to a division – in this case the divisions (referred to as components) are the assigned community memberships from the detection algorithm. Q was calculated using *igraph* (Csardi and Nepusz, 2006) as:

$$Q = \frac{1}{2(m)} \sum_{i,j} \left(A_{ij} - \frac{k_i k_j}{2m} \right) \delta(c_i, c_j),$$

where *m* is the number of edges in the network, A_{ij} is the element (here, the weight of the edge) of the adjacency matrix A, in row *i* (sheep i) and column *j* (sheep j*)*, $k_i$is the degree of sheep i *i*, k_j is the degree of sheep *j*, c_i is the type, or component of sheep *i*, c_j that of sheep *j*, the sum goes over all *i* and *j* pairs of vertices, and δ *(x,y)* is 1 if *x*=*y* and 0 otherwise.

7.2.3.2 Metrics related to the role of the individual in disease spread through network communities

Two individual-level metrics that rely on module assignment that relate to the role of the individual in disease spread were calculated (Guimerà and Nunes Amaral, 2005, Silk et al., 2017). These were:

P_i: the proportion of an individual's interactions with individuals from the same vs different modules

$$P_i = \sum_{S=1}^{N_m} \left(\frac{D_{is}}{K_i} \right)^2$$

where D_{is} is the strength of within-module connections and K_i is the individual's overall strength. Individuals with lower Pi may be linked to disease transmission as inter-module interactions are likely to allow epidemics to spread through a structured social network (Silk et al., 2017).

Z_i: a normalised measure of the strength of an individual's interactions within its module

$$z_i = \frac{D_i - \overline{D_{Si}}}{\sigma_{D_{si}}}$$

where D_i is the total strength of within module connections, $\overline{D_{si}}$ is the mean number of the strength within-module connections from that module and $\sigma_{D_{si}}$ the standard deviation around this mean. Individuals with high Zi are likely to play a role in spreading infection through local regions of the network (Silk et al., 2017). Daily Zi values could not be calculated for sheep where only two sheep were assigned to a community and the in-going strength was equal – where this occurred, the Zi was set to 0. If only one sheep was assigned to the community, and all in-going connections were 0, the Zi was also set to 0.

These measures were calculated on a daily basis for both sound sheep, and sheep that were classified as lame – at scores of ≥1, ≥2 and ≥3 to assess the impact of lameness severity on the results, and for sheep with and without infectious foot lesions at the end of the trial.

7.2.3.3 Identification of the connections that are affected by lameness

Linear mixed effects models were used to assess the relationship between the Pi and Zi and lameness/presence of foot lesions at the end of the trial in ewes, single lambs and twin lambs using *lme4* (Bates et al., 2015). Day of the trial and each individual sheep were included as random effects. Other sheep-level attributes (age, and sex of lambs) were tested in univariable and multivariable models.

7.2.3.4 Other factors that influence community formation – weather and space available

Generalised linear models with a Poisson error function were used to assess the relationship between the daily number of communities formed by the sheep as detected by Newman's modularity algorithm and daily mean weather-related variables. The weather-related variables assessed were mean daily temperature, humidity, THW index, wind speed, wind chill and total rainfall. Model fit was determined by the deviance test, where the residual deviance of the model is compared to a chi-square distribution.

7.2.4 Node centrality of individual sheep and relationship to lameness

Centrality describes how well-connected a node is. The measure of centrality used was strength, which corresponded to the weight of the edges - the total time spent in contact between sheep *i* and sheep *j* on each day of the trial, as measured by the proximity sensors in 20 second intervals. Three models were used in order to assess how lameness impacts on different relationships between sheep, using node strength as the outcome variable in each model.

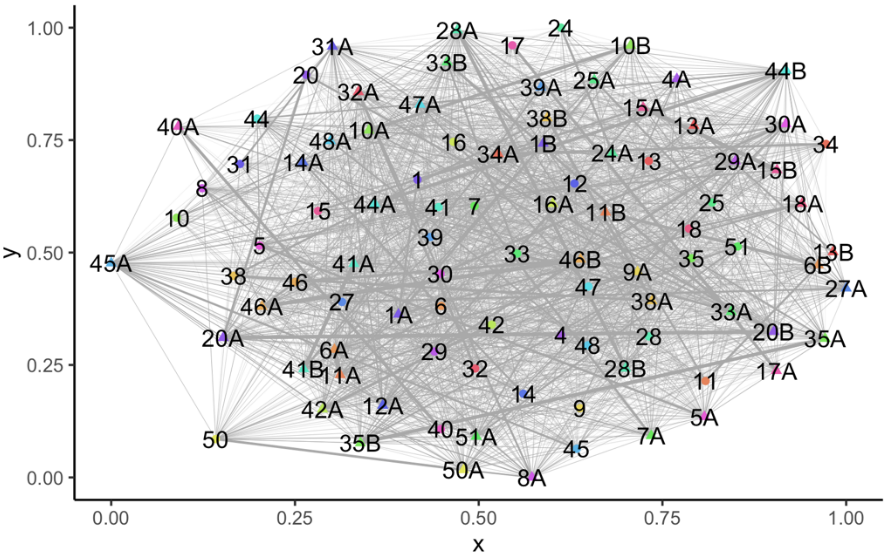
- Model 1 - all connections: used all contacts made by each sheep each day. Fixed effects were included for lameness, whether the sheep was a ewe, single or twin lamb, the space available and whether or not the sheep were gathered on the day. Random effects were used for each individual sheep, the family group and day of the trial.
- Model 2 - family connections: used only contacts that were made between sheep in the same first-generation family group as the outcome variable each day. Fixed effects were included for lameness, whether the sheep was a ewe, single or twin lamb, the space available and whether or not the sheep were gathered on the day. Random effects were used for each individual sheep and day of the trial.
- Model 3 - out of family connections: used only contacts that were made between sheep not in the same first-generation family group as the outcome variable each day. Fixed effects were included for lameness, whether the sheep was a ewe, single or twin lamb, the space available and whether or not the sheep were gathered on the day. Random effects were used for each individual sheep and day of the trial.

7.3 Results

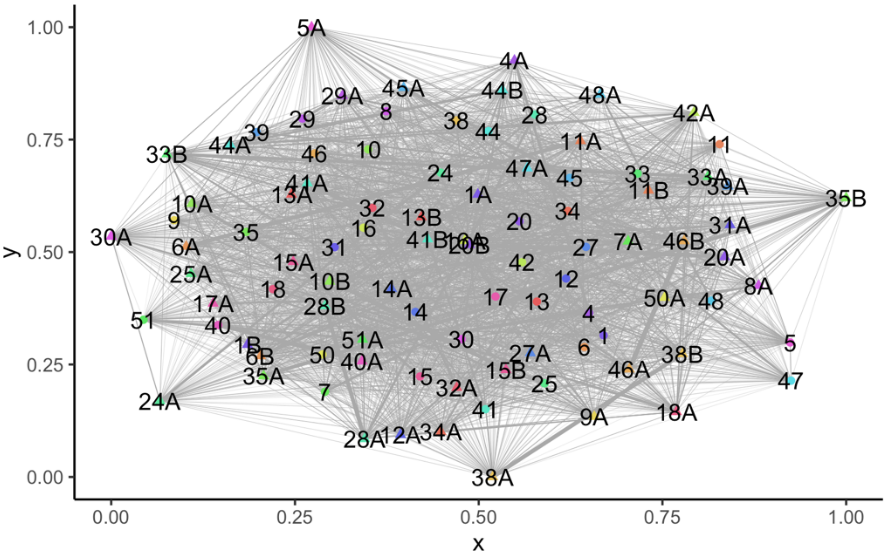
7.3.1 Visualisation of daily networks

Networks are visualised in Figure 7.1. The number of nodes in each daily network was 94, and the number of edges ranged from 1338 (October 14) to 3754 (October 3). Networks were dense, with edge density (the proportion of observed ties to the maximum possible ties), ranging from 0.31 (October 14) - 0.86 (October 3) and sheep contacting most other members of the flock each day (Figure 7.1). The diameter of the networks (the longest of the shortest paths across the network) ranged from 60 (October 2-10) – 100 (October 11-14), with the mean distance between nodes ranging from 1.14 (October 3) - 1.69 (October 14).

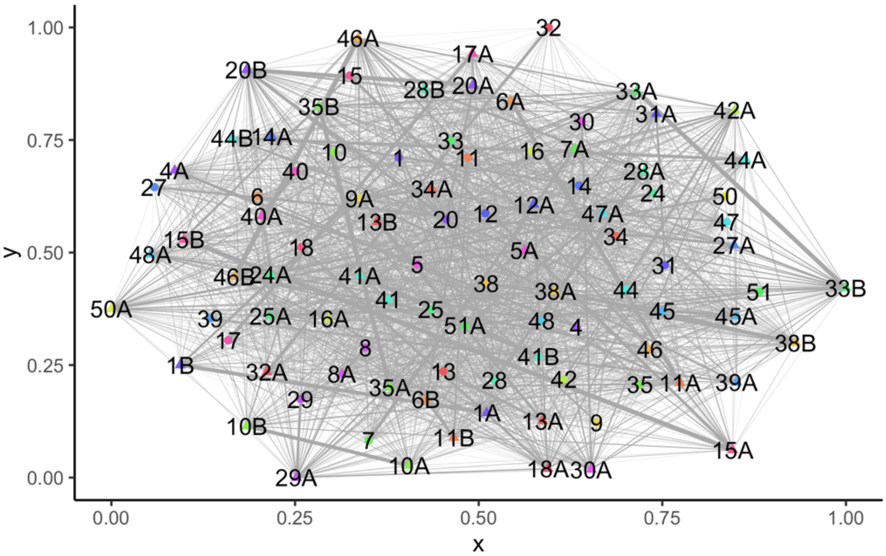
October 2



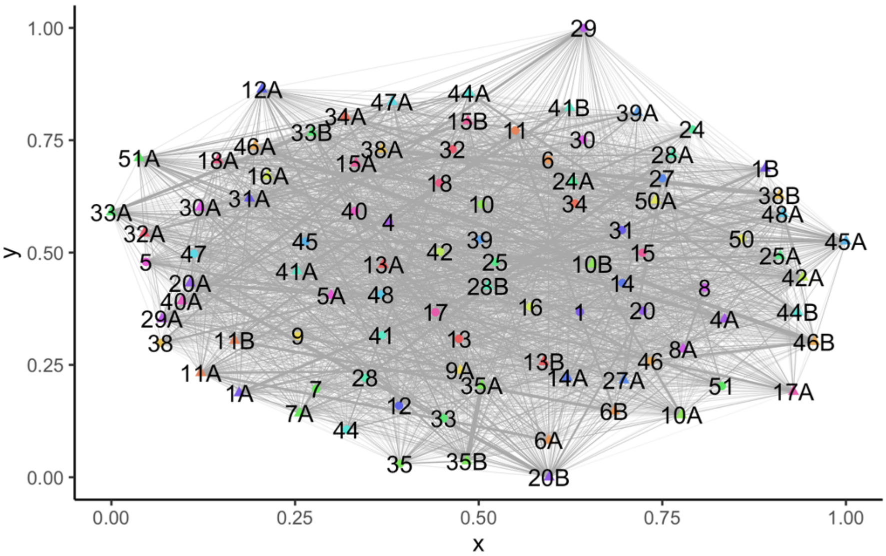
October 3



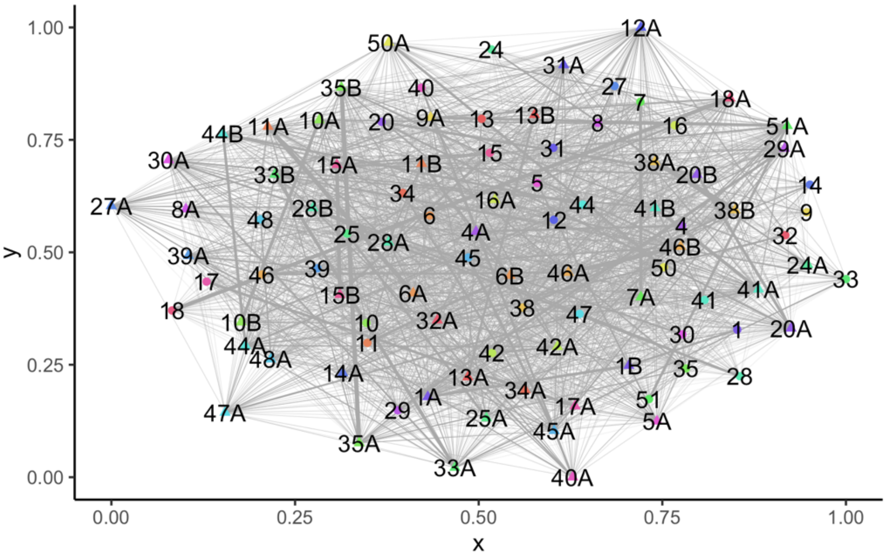
October 4



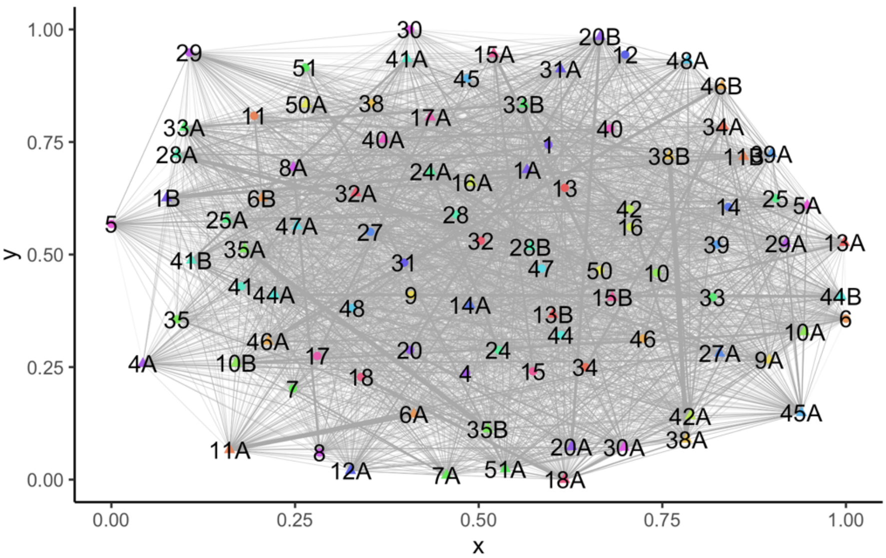
October 5



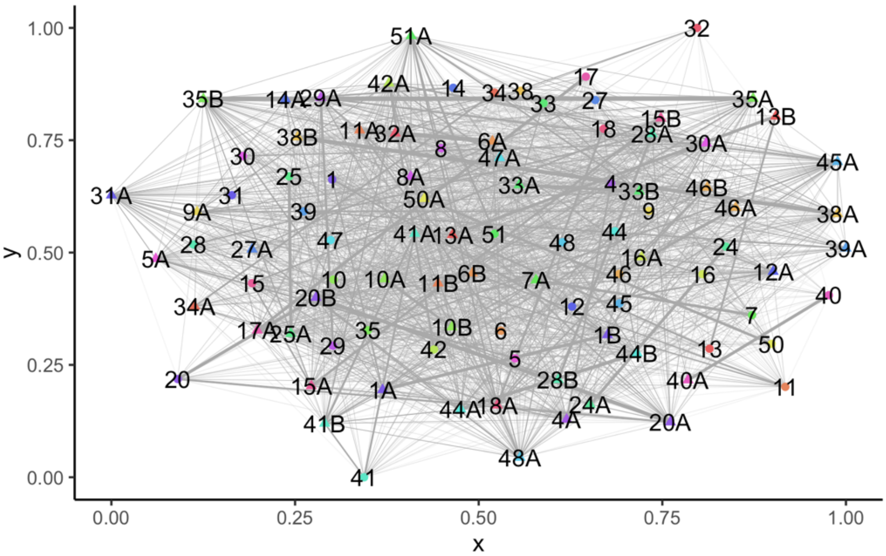
October 6



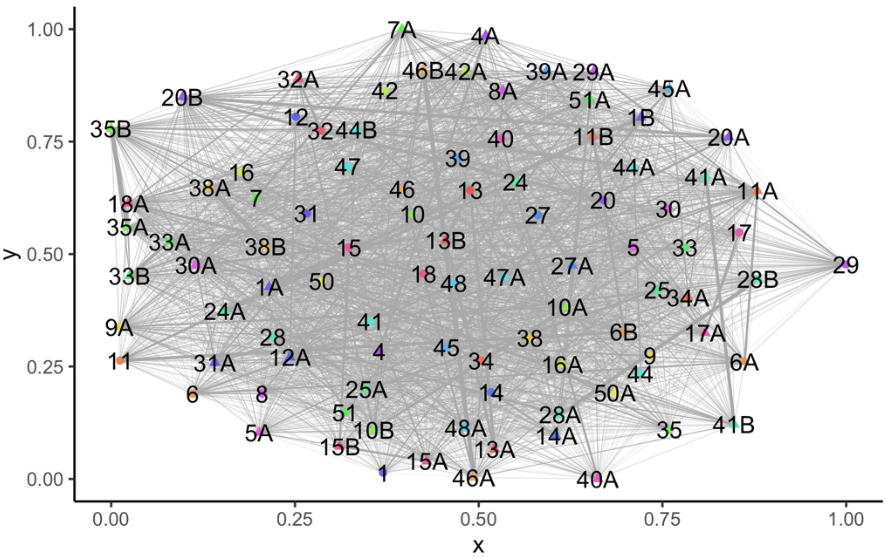
October 7



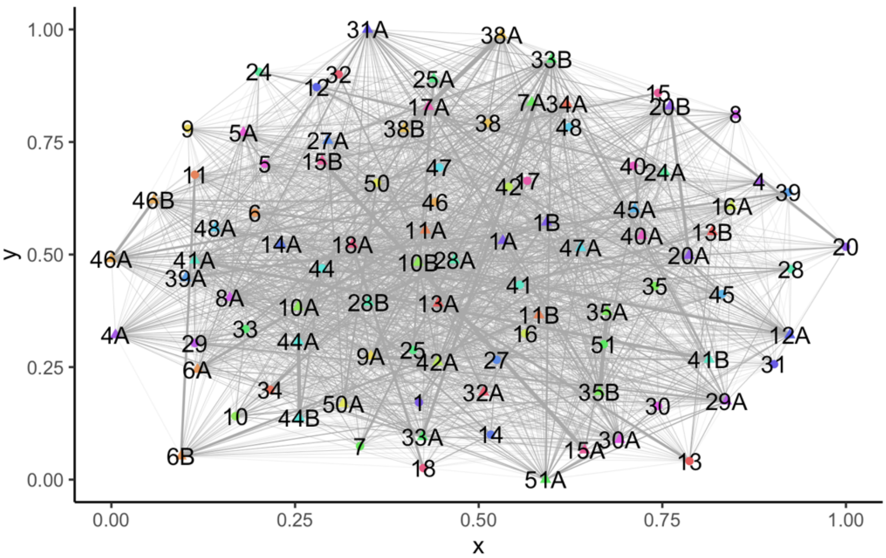
October 8



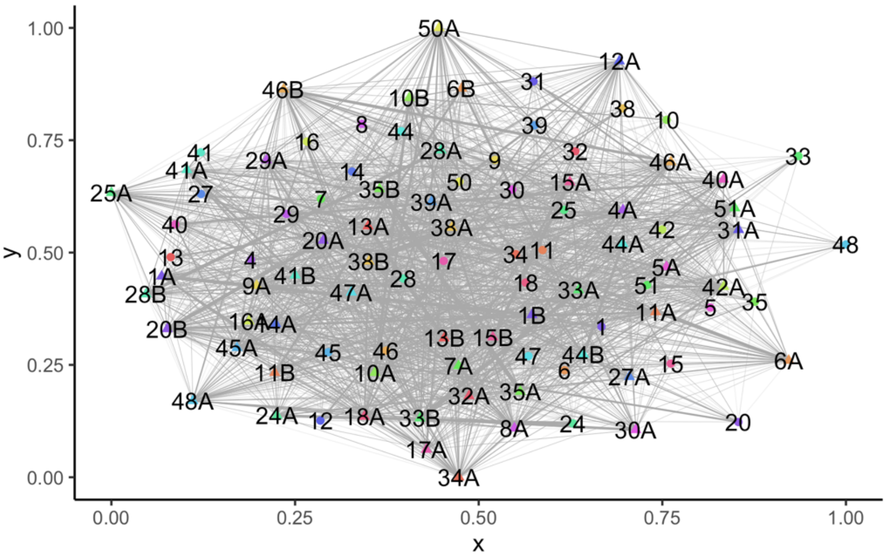
October 9



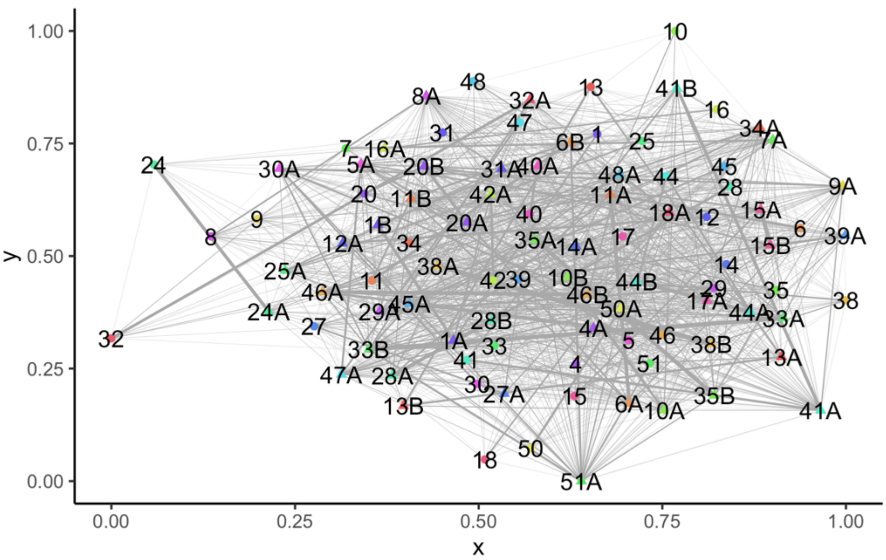
October 10



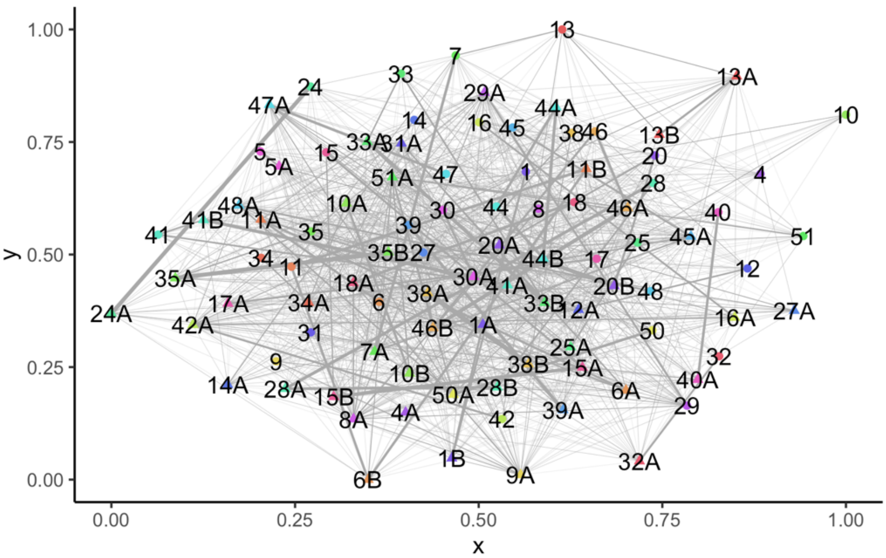
October 11



October 12



October 13



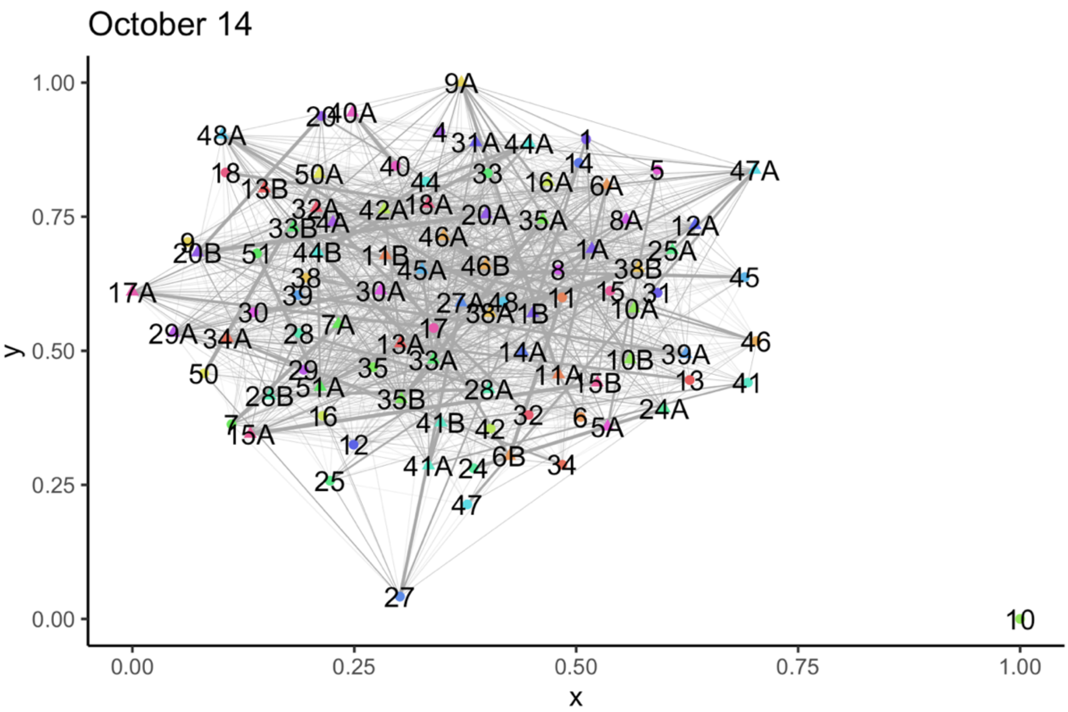
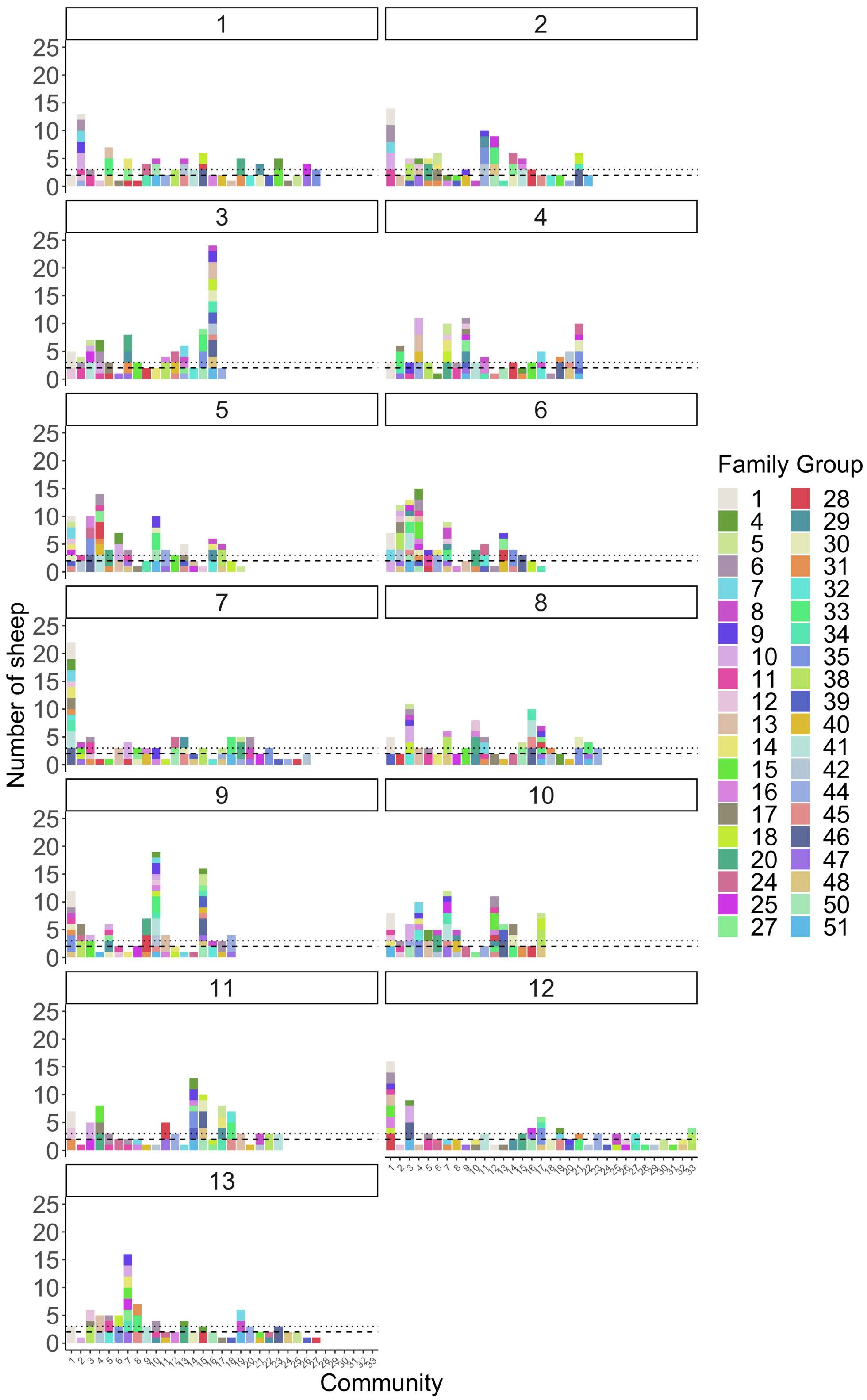


Figure 7.1 Network visualisation for the 13 time-aggregated weighted networks for 94 sheep using the Kamada-Kawai layout. Ewes are labelled by numbers, with their lambs indicated by the same number, with A indicating the first lamb and B the second if the ewe had twins, with nodes for ewes as circles and lambs triangles.

7.3.2 Community detection – modular structure of daily networks

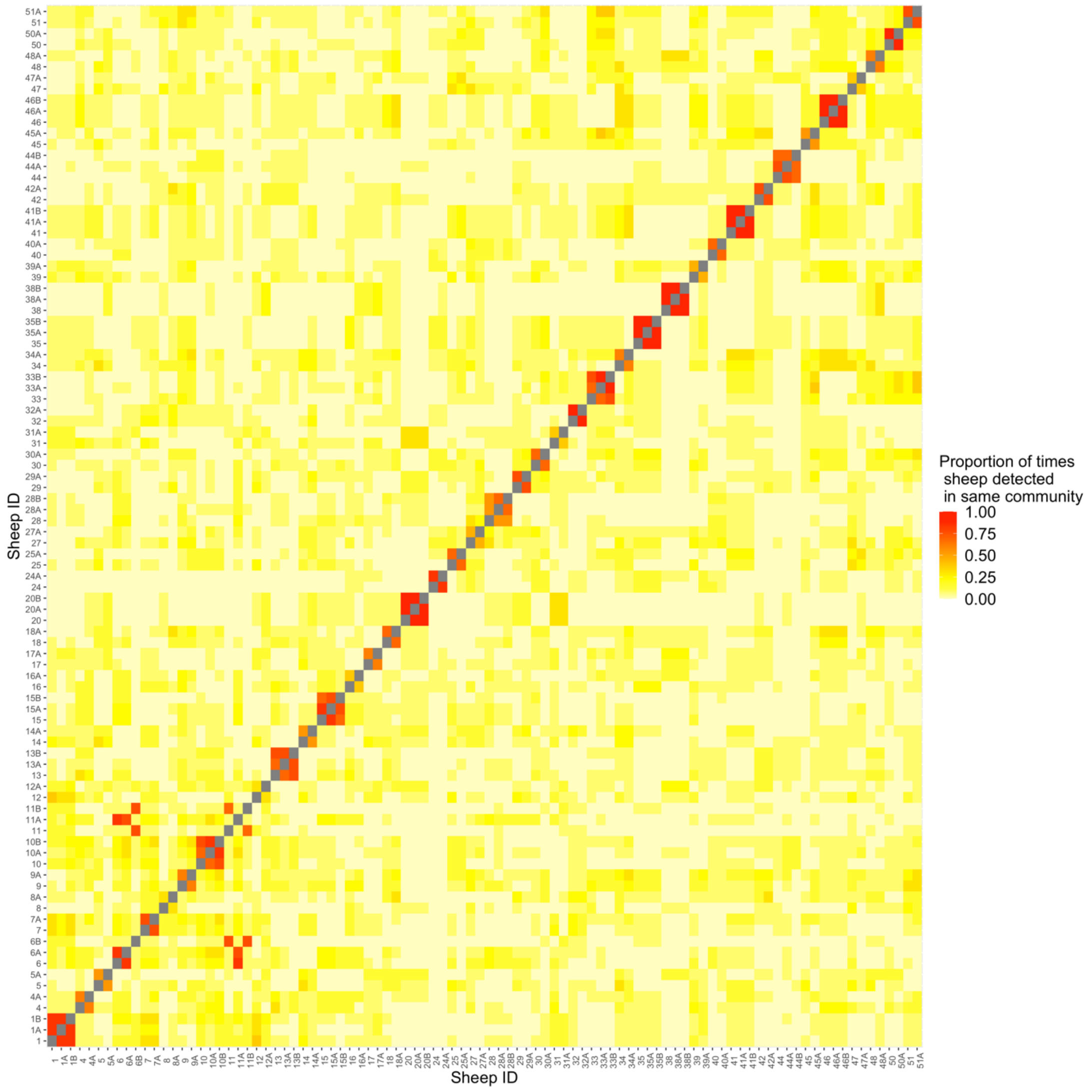
Community detection with Newman's modularity algorithm suggested that sheep clustered into communities - Newman's Q ranged from 0.31-0.58. The number of communities formed on a daily basis ranged from 17-33 (Figure 7.2), with the number of sheep in a community ranging from 1 to 24. Only family members tended to be found consistently in the same grouping (Figure 7.2, Figure 7.3).

Figure 7.2 Clustering of sheep into communities on a daily basis (2nd-14th October), as determined by Newman's modularity.



Colours correspond to the 40 first-generation family groups and box labels to the day of the study. The dashed line is placed at two sheep, and the dotted line at three sheep.

Figure 7.3 The proportion of times pairs of sheep were found in the same community over the 13 days. Sheep are ordered by family group, ewes are indicated by a number and their lambs by a letter, with the ordering symmetrical on the x and y axis.



7.3.3 Individual metrics related to disease spread between and within communities

7.3.3.1 Participation coefficients and normalised measure of an individual's interactions within modules for ewes, single and twin lambs

Single lambs had the lowest mean participation coefficients (Table 7.1), suggesting single lambs make more contacts outside of their own module than ewes or twin lambs. Twins had much higher Zi (measure of interactions within their own module) than singles or ewes, which is most likely due to the interaction with their twin since the communities formed on a daily basis were fairly transient (Figure 7.3), with only sheep in the same family likely to be found in the same community on a daily basis (Figure 7.3).

Table 7.1 Summary of the mean participation coefficient (Pi) and normalised measure of an individual's interactions (ZI) within its module for ewes, single and twin lambs.

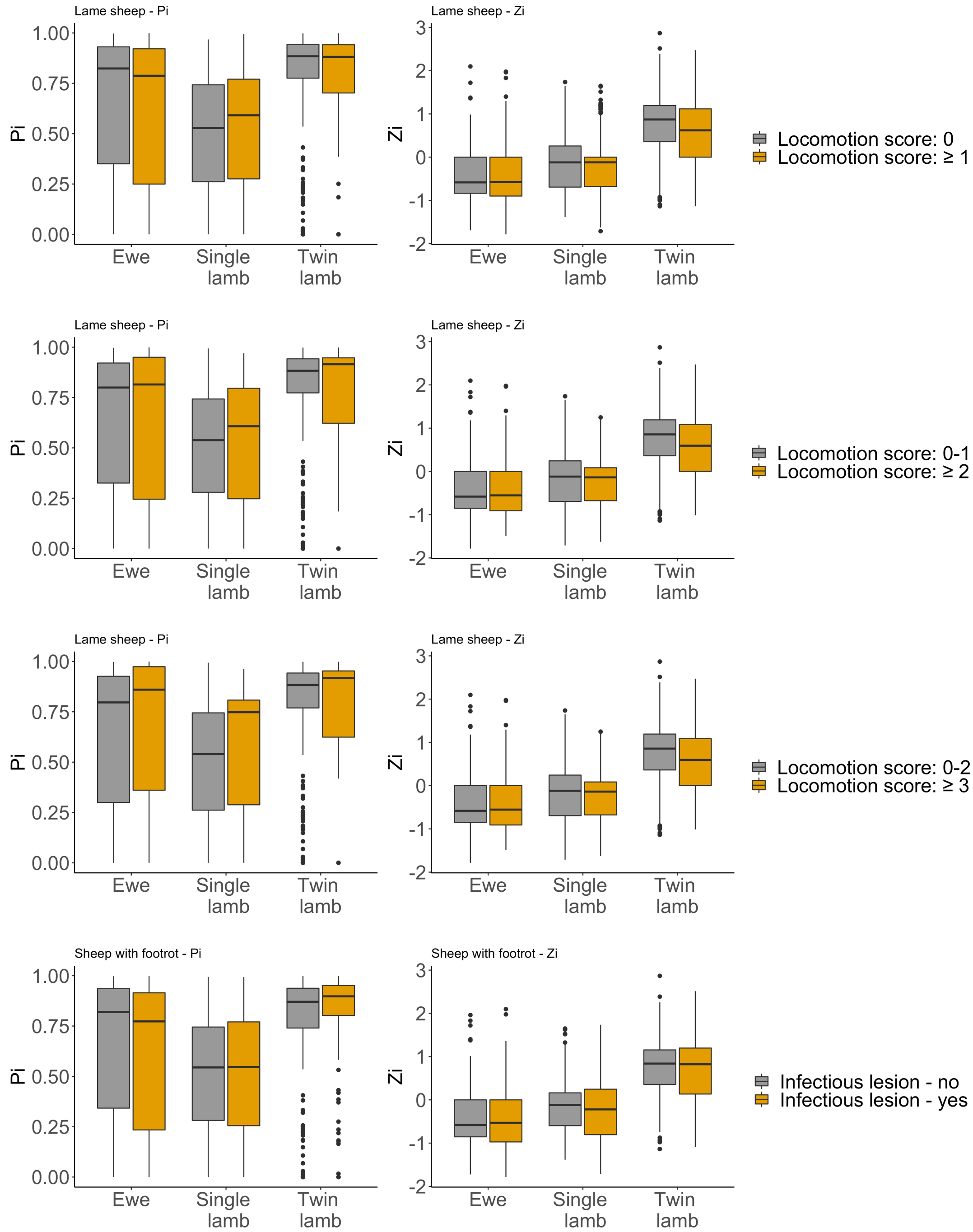
Sheep	N	Pi		Zi	
		Mean	SD	Mean	SD
Ewe	520	0.64	0.34	-0.44	0.62
Single	338	0.51	0.29	-0.14	0.71
Twin	364	0.79	0.25	0.75	0.72

1. N = number of observations of sheep, SD = standard deviation

7.3.3.2 The effect of lameness and presence of infectious foot lesions on participation coefficients and normalised measure of an individual's interactions within modules for ewes, single and twin lambs

Distributions of participation coefficients and the normalised measure of an individual's interactions within its module when sheep were classified as lame at scores of ≥1, ≥2 and ≥3 and when sheep had an infectious lesion at the end of the study are shown in Figure 7.4. Regardless of lameness severity classification Pi and Zi in ewes were similar (Figure 7.4). There appeared to be some differences for lambs (Figure 7.4), with lame single lambs making higher proportions of contacts within their module, and lame twins making fewer contacts within their module, suggesting that there could be some effect of lameness on their social contact patterns.

Figure 7.4 Distribution of participation coefficients and the normalised measure of an individual's interactions within its module when sheep were classified as lame at scores of ≥1, ≥2 and ≥3 and when sheep had a infectious lesion at the end of the study



However, univariable models of the associations between lameness and Pi/Zi suggested there was no significant difference ($p<0.05$, Wald's test of significance) between sheep with a locomotion score of ≥2 compared to 0-1 for ewes, single or twin lambs.

Table 7.2 Linear mixed effects models for association between Pi (participation coefficient) and Zi (normalised measure of an individual's interactions within its own module) and lameness for ewes, single and twin lambs

Sheep	N (%)	Pi		Zi	
		β (95% CI)	P-value	β (95% CI)	P-value
Ewes					
Intercept		0.64 (0.57-0.71)	<0.001	-0.45 (-0.55 - -0.36)	<0.001
Fixed effects					
Locomotion score					
0-1	393 (75.6)	Ref		Ref	
≥2	127 (24.4)	0.01 (-0.06-0.08)	0.848	0.06 (-0.08-0.19)	0.403
Random effects		Variance			
Individual	40	0.02		0.06	
Day	13	0.01		0.00	
Residual	-	0.10		0.33	
Single lambs					
Intercept		0.51 (0.46-0.56)	<0.001	-0.13 (-0.26-0.11)	0.064
Fixed effects					
Locomotion score					
0-1	284 (84.0)	Ref		Ref	
≥2	54 (16.0)	0.01 (-0.07-0.10)	0.751	-0.05 (-0.26-0.16)	0.648
Random effects		*Variance*		*Variance*	
Individual	26	0.01		0.08	
Day	13	0.00		0.00	
Residual	-	0.07		0.42	
Twin lambs					
Intercept		0.80 (0.75-0.84)	<0.001	0.64 (0.60-0.93)	<0.001
Locomotion score					

Sheep	N (%)	Pi		Zi	
		β (95% CI)	P-value	β (95% CI)	P-value
0-1	332 (91.2)	Ref		Ref	
≥2	32 (8.8)	-0.07 (-0.17-0.03)	0.145	-0.17(-0.46- 0.12)	0.251
Random effects		*Variance*		*Variance*	
Individual	32	0.01		0.14	
Day	13	0.00		0.01	
Residual	-	0.06		0.37	

1. N = number of observations of sheep, β = coefficient, CI = Wald's confidence interval, P-value = P-value from Wald's test of significance, Ref = reference category

The presence of an infectious foot lesion at the end of the study was not associated with any effect on the social patterns of sheep (Table 7.3).

Table 7.3 Linear mixed effects models for association between Pi (participation coefficient) and Zi (normalised measure of an individual's interactions within its own module) and presence of an infectious foot lesion at the end of the study for ewes, single and twin lambs

Sheep	N (%)	Pi		Zi	
		β (95% CI)	P-value	β (95% CI)	P-value
Ewes					
Intercept		0.65 (0.58-0.73)	<0.001	-0.45 (-0.56- -0.34)	<0.001
Fixed effects					
Infectious foot lesion at end of study					
No	363 (70.0)	Ref		Ref	
Yes	156 (30.0)	-0.04 (-0.14-0.07)	0.460	0.03 (-0.16-0.23)	0.738
Random effects		Variance			
Individual	40	0.02		0.06	
Day	13	0.01		0.00	
Residual	-	0.10		0.33	
Single lambs					
Intercept		0.51 (0.45-0.57)	<0.001	-0.11 (-0.27-0.06)	0.193

Sheep	N (%)	Pi		Zi	
		β (95% CI)	P-value	β (95% CI)	P-value
Fixed effects					
Infectious foot lesion at end of study					
No	221 (65.4)	Ref		Ref	
Yes	117 (34.6)	0.00 (-0.08-0.09)	0.942	-0.08 (-0.36-0.20)	0.584
Random effects		Variance			
Individual	26	0.01		0.09	
Day	13	0.00		0.00	
Residual	-	0.07		0.42	
Twin lambs					
Intercept		0.78 (0.73-0.83)	<0.001	0.76 (0.56-0.97)	<0.001
Fixed effects					
Infectious foot lesion at end of study					
No	221 (60.7)	Ref		Ref	
Yes	143 (39.3)	0.03 (-0.05-0.10)	0.452	-0.03 (-0.35—0.29)	0.848
Random effects		*Variance*		*Variance*	
Individual	28	0.01		0.15	
Day	13	0.00		0.01	
Residual	-	0.06		0.37	

1. N = number of observations of sheep, β = coefficient, CI = Wald's confidence interval, P-value = P-value from Wald's test of significance, Ref = reference category

7.3.3.3 The effect of other sheep-level attributes (age and sex) on participation coefficients and the normalised measure of an individual's interactions within modules for ewes, single and twin lambs

Univariable models suggested that there was no significant difference in either the proportion of contacts made out of the module, or within the module as the age of either ewes, or lambs increased (Table 7.4) or for male lambs compared to female lambs (Table 7.5). Neither age or sex of lambs was significantly associated with Pi or Zi in a multivariable model also including lameness as predictor variable.

Table 7.4 Linear mixed effects models for association between Pi (participation coefficient) and Zi (normalised measure of an individual's interactions within its own module) and age for ewes, single and twin lambs

Sheep	N	Pi		Zi	
		β (95% CI)	P-value	β (95% CI)	P-value
Ewes					
Intercept		0.66 (0.52-0.79)	<0.001	-0.24 (-0.48—0.00)	0.046
Age (years)					
+ 1 unit	520 (100.0)	-0.00 (-0.03-0.03)	0.848	-0.04 (-0.10 – 0.01)	0.09
Random effects		*Variance*		*Variance*	
Individual	40	0.02		0.05	
Day	13	0.01		0.00	
Residual	-	0.10		0.33	
Single lambs					
Intercept		0.57 (0.45-0.68)	<0.001	-0.01 (-0.35-0.34)	0.976
Age (days)					
+ 1 unit	338 (100.0)	-0.00 (-0.01-0.00)	0.298	-0.01 (-0.03- 0.01)	0.422
Random effects		*Variance*		*Variance*	
Individual	26	0.01		0.08	
Day	13	0.00		0.00	
Residual	-	0.07		0.42	
Twin lambs					
Intercept		0.87 (0.77-0.97)	<0.001	1.09 (0.68-1.50)	<0.001
Age (days)					
+ 1 unit	364 (100.0)	0.01 (-0.01-0.00)	0.07	-0.02 (-0.05-0.00)	0.082
Random effects		*Variance*		*Variance*	
Individual		0.01		0.13	
Day	13	0.00		0.01	
Residual	-	0.06		0.37	

1. N = number of observations of sheep, β = coefficient, CI = Wald's confidence interval, P-value = P-value from Wald's test of significance

Table 7.5 Linear mixed effects models for association between Pi (participation coefficient) and Zi (normalised measure of an individual's interactions within its own module) and sex in single and twin lambs

Sheep	N (%)	Pi		Zi	
		β (95% CI)	P-value	β (95% CI)	P-value
Single lambs					
Intercept		0.50 (0.44-0.57)	<0.001	-0.11 (-0.29-0.07)	0.218
Fixed effects					
Sex					
Female	182 (53.8)	Ref		Ref	
Male	156 (42.6)	0.03 (-0.06-0.11)	0.547	-0.05 (-0.31- 0.22)	0.729
Random effects		*Variance*		*Variance*	
Individual	26	0.01		0.09	
Day	13	0.00		0.00	
Residual	-	0.07		0.42	
Twin lambs					
Intercept		0.82 (0.76-0.87)	<0.001	0.86 (0.63-0.1.09)	<0.001
Fixed effects					
Sex					
Female	169 (46.4)	Ref		Ref	
Male	195 (53.6)	-0.05 (-0.13 -0.02)	0.148	-0.20 (-0.50-0.10)	0.197
Random effects		*Variance*		*Variance*	
Individual	28	0.01		0.14	
Day	13	0.00		0.01	
Residual	-	0.06		0.37	

1. N = number of observations, β = coefficient, CI = Wald's confidence interval, P-value = P-value from Wald's test of significance, Ref = reference category

7.3.4 Environmental influences on sheep behaviour

7.3.4.1 Univariable Poisson models for the effect of environmental variables and the number of communities formed on a daily basis

Higher mean wind-speeds, increases in the mean daily THI index and increases in the mean daily WCI were associated with a decrease in the number of communities formed per day (RR = 0.92, 95% CI = 0.84-1.00, RR = 0.92, 95% CI = 0.83-1.01, and RR = 0.91, 95% CI = 0.83-1.01, respectively) suggesting sheep were gathering together in larger numbers, with more sheep found in each community. The deviance goodness of fit test indicated no lack of fit of the model of the either mean daily windspeed, mean daily THI or WCI (p =0.619, 0.355, 0.580, respectively). No space-use variables (whether or not the sheep were gathered, or the space available to them) were associated with the number of communities formed per day.

Table 7.6 Univariable models for associations between daily weather-related variables and the number of communities formed by the sheep each day, as detected by Newman's modularity algorithm.

Environmental predictor	N (%)	RR	LCI	UCI	P-value
Weather					
Intercept		54.23	19.67	148.10	
Mean THI (°C)	**13 (100.0)**	**0.92**	**0.83**	**1.01**	**0.077**
Intercept		37.10	17.62	78.06	
Mean WCI (°C)	**13 (100.0)**	**0.91**	**0.83**	**1.01**	**0.079**
Intercept		30.98	21.59	44.28	
Mean windspeed (mph)	**13 (100.0)**	**0.92**	**0.84**	**1.00**	**0.064**
Intercept		22.21	18.88	25.99	
Total rainfall (cm)	13 (100.0)	1.02	0.55	1.54	0.940
Space use					
Intercept		23.33	20.32	26.66	
Sheep gathered - no	9 (69.2)	Ref			
Sheep gathered – yes	4 (30.8)	0.90	0.70	1.16	0.428

Environmental predictor	N (%)	RR	LCI	UCI	P-value
Intercept		20.00	15.36	25.50	
Area available– 1.7 acres	4 (30.8)	Ref			
Area available – 3.3 acres	4 (30.8)	0.98	0.72	1.32	0.879
Area available– 4.9 acres	5 (38.5)	1.09	0.82	1.43	0.563

1. N = number of observations, RR = risk ratio, LCI = lower 95% Wald's confidence interval, UCI = upper 95% Wald's confidence interval, THI = temperature humidity index, WCI = wind chill index. Ref = Reference category, P-value from Wald's test of significance.

2. Variables with $p<0.05$ are highlighted in bold.

7.3.5 Measures of centrality

7.3.5.1 Descriptive statistics – node strength

Twin lambs spent the most amount of time with other sheep and had higher mean strength in the network compared to ewes and single lambs (Table 7.7). There was little difference in mean strength for lame and sound ewes, but mean strength appeared lower for lame lambs compared to sound lambs (Table 7.7).

Table 7.7 Summary of the daily node strength values for sound vs lame ewes, single and twin lambs over the 13 days

Sheep	Loco-motion score	N (%)	Node strength		
			Mean	SD	Range
Ewe	0-1	393 (75.6)	10,215.47	5,279.73	0-28760
	≥2	127 (24.4)	10,631.34	4,376.59	3100-21940
Single lamb	0-1	284 (84.0)	20,171.83	8,306.36	400-46500
	≥2	54 (16.0)	16,280.00	6,428.22	3540-34140
Twin lamb	0-1	332 (91.2)	31,418.19	10,462.20	8920-62240
	≥2	32 (8.8)	24,289.38	12,832.52	6260-53500

1. N = number of observations of sheep, SD = standard deviation

7.3.5.2 Linear mixed effects model of the effect of age of sheep, lameness and space use variables on daily node strength

Both single and twin lambs had higher node strength compared to ewes, indicating that they spent more time in close proximity to other sheep than ewes (Table 7.8). Sheep that were lame had significantly reduced node strength compared to sheep that were not lame (Table 7.8) and node strength was also reduced when sheep had the full field available to them, compared to only the first section (Table 7.8). Gathering the sheep compared to not had a small positive coefficient (β = 1963.22) but was not associated with a significant increase in node strength (Table 7.8). P-values obtained from node permutation of the networks were similar to those from Wald's test (Figure 7.5).

Table 7.8 Linear mixed effects model for association between node strength and lameness, with fixed effects for sheep age, space available and whether or not the sheep were gathered on a day and random effects for individual sheep, family group and day.

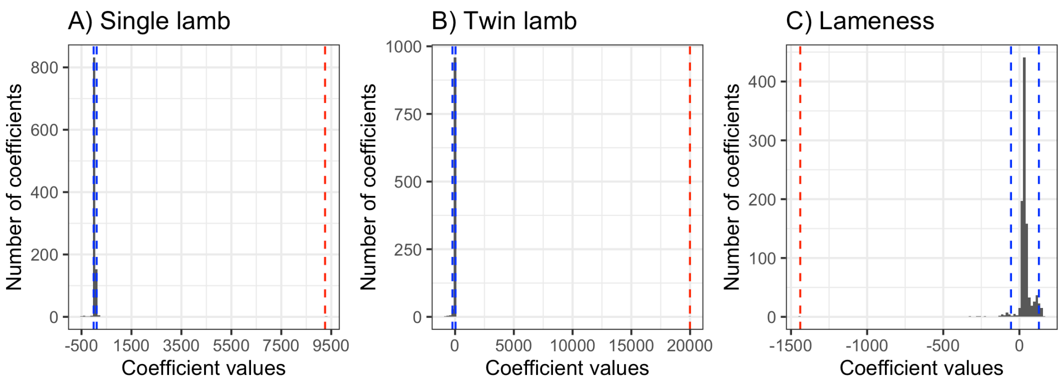
Predictor	**N**	**β**	**SE**	**LCI**	**UCI**	**P-value** Node Permutation	**P-value** Wald's test
Intercept		12894.54	1299.94	10346.70	15442.37	-	<0.001
Fixed effects							
Ewe	520	Ref					
Single	**338**	9252.37	1170.93	6957.40	11547.34	**<0.001**	**<0.001**
Twin	**364**	19987.75	1233.36	17570.41	22405.08	**<0.001**	**<0.001**
Locomotion score							
0-1	1009	Ref					
≥2	**213**	-1439.30	475.57	-2371.40	-507.21	**<0.001**	**0.002**
Area available							
1.7 acres	470	Ref					
3.3 acres	376	-1705.33	1138.95	-3937.62	526.96	-	0.134
4.9 acres	**376**	**-5988.22**	**1221.39**	**-8382.10**	**-3594.35**	**-**	**<0.001**
Sheep gathered							

Predictor	N	β	SE	LCI	UCI	P-value Node Permutation	P-value Wald's test
No	846	Ref					
Yes	376	1956.18	1138.95	-276.11	4188.47	-	0.086
Random effects		*Variance*		*SD*			
Individual	94	18293321.40		4277.07			
Family group	40	8730735.98		2954.78			
Day	13	2318576.68		1522.69			
Residual	-	25916253.64		5090.80			

1. β = coefficient from the model, SE = standard error, T = t-statistic, LCI = lower 95% Wald's confidence interval, UCI = upper 95% Wald's confidence interval, P-value$_{\text{Node Permutation}}$ = p-value derived from 1000 node permutations, P-value$_{\text{Wald's test}}$ = Wald's test of significance, Ref = reference category

2. P-values were not calculated for space use and gathering days using node permutation since these were the same for each sheep on each day of the trial.

Figure 7.5 Distribution of the coefficient values from node permutation, of 1000 random networks.



The red line indicates the observed coefficient from the model, and the blue lines are the 2.5% and 97.5% percentiles of the values obtained from the models using the permuted data.

7.3.5.3 Linear mixed effects models for the effect of lameness on different types of connections made between sheep

Both single and twin lambs make more out of family connections compared to ewes (Table 7.9) whereas only twin lambs have stronger in-family connections compared to ewes (Table 7.10). Lameness does not impact on the strength of family connections (Table 7.9) but does reduce the strength of out of family connections when sheep are considered as lame at scores of ≥2 (Table 7.9). The strength of both in-family and out-of family connections were reduced when sheep had access to the full 4.9 acres compared with 1.7 acres.

Table 7.9 Linear mixed effects model for the effect of age and lameness diagnosed at varying severity on the out-going strength (i.e the contacts made with sheep that were not in the same family)

Predictor	**N**	**β**	**SE**	**LCI**	**UCI**	**P-value**
Intercept		7171.67	1335.76	4553.62	9,789.72	<0.001
Fixed effects						
Age						
Ewe	520					
Single	338	**9573.63**	**1270.31**	**7083.87**	**12063.39**	**<0.001**
Twin	**364**	**9519.42**	**1243.41**	**7082.39**	**11956.46**	**<0.001**
Locomotion score						
0-1	1009	Ref				
≥2	**213**	-1125.41	355.92	-1823.00	-427.82	**0.002**
Area available						
1.7 acres	470	Ref				
3.3 acres	376	353.37	1386.96	-2365.01	3,071.76	0.799
4.9 acres	**376**	**-5523.31**	**1487.36**	**-8438.49**	**-2608.13**	**<0.001**
Sheep gathered						
No	846	Ref				
Yes	376	-25628.84	1386.78	-5346.87	89.20	0.058
Random effects		*Variance*		*SD*		
Individual		24312579.43		4,930.78		

Predictor	N	β	SE	LCI	UCI	P-value
Day		4078543.85		2,019.54		
Residual		14312288.26		3,783.16		

1. N = number of observations, β = coefficient from the model, SE = standard error, SD = standard deviation, LCI = lower 95% Wald's confidence interval, UCI = upper 95% Wald's confidence interval, p-value from Wald's test of significance, ref = reference category

2. Variables significant at p <0.05 are highlighted in bold.

Table 7.10 Linear mixed effects model for the effect of age and lameness diagnosed at varying severity on the in-going strength (i.e. the contacts made with sheep in the same family)

Predictor	N	β	SE	LCI	UCI	P-value
Intercept		7,267.26	1,052.16	5,205.06	9,329.47	<0.001
Fixed effects						
Age						
Ewe	520	Ref				
Single	338	-460.43	1221.73	-2854.98	1934.12	0.706
Twin	**364**	**10733.27**	**1195.84**	**8389.48**	**13077.07**	**<0.001**
Locomotion score						
0-1	1009	Ref				
≥2	213	-293.14	338.51	-956.61	370.32	0.386
Area available						
1.7 acres	470	Ref				
3.3 acres	376	-1578.81	934.36	-3410.13	252.51	0.091
4.9 acres	**376**	**-2510.53**	**1002.01**	**-4474.44**	**-546.62**	**0.012**
Sheep gathered						
No	873	Ref				
Yes	388	-382.58	934.39	-2213.95	1,448.80	0.682
Random effects		*Variance*		*SD*		
Individual	94	22511704.97		4744.65		

Predictor	N	β	SE	LCI	UCI	P-value
Day	13	1782997.83		1335.29		
Residual	-	12941879.27		3597.48		

1. N = number of observations, β = coefficient from the model, SE = standard error, SD = standard deviation, LCI = lower 95% Wald's confidence interval, UCI = upper 95% Wald's confidence interval, p-value from Wald's test of significance, ref = reference category

2. Variables significant at p <0.05 are highlighted in bold.

7.4 Discussion

Results from this chapter suggest domestic sheep with lambs at foot cluster into social communities which are driven by environmental variables that lead sheep to seek shelter. Lame sheep spend less time in contact with other sheep compared to non-lame sheep – but the connections that are affected are with sheep outside of the family group sheep, rather than family connections, and single and twin lambs have different potential to spread disease through the flocks.

The extent of the impact of sickness behaviours varies depending on the relationship between animals (Stockmaier et al., 2020). For example, mother-offspring relationships in bats are less affected when bats are artificially immune challenged compared to other relationships (Stockmaier et al., 2020). Poll Dorset ewes have strong maternal instincts, which may be why family connections are not affected by lameness in this group of sheep and lameness was only associated with a reduction in the strength of out-of-family connections (Table 7.9). Sick animals can prioritise actions that have greater benefit to fitness (Stockmaier et al., 2020) and it may be that lame ewes with lambs at foot prioritise looking after their lambs over other social interactions and similarly, lame lambs may prioritise sucking from their mother over playing with other lambs. Further work would look at social networks created from interaction data or other behavioural data to determine if the daily time-budget is altered for lame sheep. There is some evidence for this already - lambs with footrot lie down more frequently and for shorter duration than non-affected lambs (Härdi-Landerer et al., 2017). If behaviours such as grazing are reduced, this could provide a reason why lambs with footrot are slower to reach finishing weights than lambs without (Wassink et al., 2010b).

This is the first study to find that lameness impacts the social contact patterns of twin lambs in a different way to single lambs (Table 7.2), presumably due to the lack of contact with their twin. Twin lambs have different contact patterns to single lambs – in the same study, their contact patterns are more stable over time than singles or ewes (Ozella et al., submitted) and they associate more with their litter-mate more than other lambs of the same age (Walser et al., 1981) and than their dam (Broster et al., 2010, Broster et al., 2012b). This study also found that twin lambs had higher total amounts of time spent with family members compared to ewes, while single lambs did not, as twins spend so much time together.

The individual-level metrics relating to an individual's potential to spread disease through regions of the network indicated that single lambs tend to have lower Pi values than twin lambs. Although single lambs spend more time with their mother than twins, both in this study (Table 8.4) and others (Morgan and Arnold, 1974, Broster et al., 2010), the current study suggests that they are also more likely to make contacts outside of their family group, which may be due contacting other lambs to play. As a result, single lambs may be the most likely to become infected with *D. nodosus* via association with other sheep and spread disease through different regions of the network. Conversely, twin lambs may be more likely to spread disease through their own region of the network. That lambs and ewes are more likely to become lame if a family member is lame was reported by Kaler et al., (2010a).

The community detection algorithm suggested that the communities formed in this flock were transient and altered daily (Figure 7.2 and Figure 7.3). It is possible that communities would become more stable over a longer time period as sheep do have preferential attractions for specific sheep, both as ewes (Ozella et al., 2020), and lambs, with lambs most likely to play with other lambs of a similar age (Hass and Jenni, 1993) or size (Berger, 1980). Increasing the space available to the sheep, and the associated increase in grass availability may have affected sheep behaviour, as ewes may be more motivated to graze where the new grass was situated, which may result in reductions in social contact - poor resource availability has been associated with increases in contact between sheep (Freire et al., 2012). Opening up the field also reduced stocking density and so would lower contact - increased stocking density is also associated with more contacts between ewes and lambs (Broster et al., 2012b). In the current study, sheep also made fewer contacts when the full field was available to them compared to only the first section (Table 7.8, Table 7.9, Table 7.10).

Weather conditions affected the social contact patterns in the flock - increased wind-speed was associated with a decrease in the number of communities formed per day. This is likely to be due to sheep gathering around the hedge-rows at the edge of the field – clustering of ewes in response to the wind chill index has been previously observed (Ozella et al., 2020) and lambs may be even more likely to seek protection from the elements as they do not have the fleece of adult sheep. The type of shelter available influences the amount of contact between ewes and their lambs (Broster et al., 2010), lambs may feel more protected by certain types of shelter and less likely to seek out their mother, or conversely, if lambs are easily visible, ewes may be less likely to stop grazing to seek out their lamb (Broster et al., 2010). Other weather patterns that affect social contact patterns include temperature, as sheep cluster under trees for shade (Broster and Doyle, 2013, Kawai, 1989) – however, this study was conducted in Autumn in the UK, with average daily temperatures between 9 and 14°C (Table 5.6) so sheep were unlikely to be seeking shade.

7.5 Conclusion

Domestic ewes with young lambs at foot divide into social communities and the environment (particularly wind-speed) influences the number of communities formed because sheep group together differently depending on the weather conditions. Lameness reduces connections between sheep, predominately out-of-family connections are reduced in lame sheep. Twin lambs and single lambs have different contact patterns, which could equate to different risks of disease transmission/acquisition.

Chapter 8 Network-based diffusion analysis to determine the role of different social networks in transmission of lameness in a sheep flock

8.1 Introduction

8.1.1 Definition of a diffusion

A "diffusion" refers to the spread of a trait within a group, where individuals move from a naïve to an informed state (Hoppitt and Laland, 2013). Network-based diffusion analysis (NBDA) models the acquisition of a trait as a stochastic process in which, in any given time, each naïve individual has a transmission rate which determines the likelihood of acquiring the trait, and individuals can acquire the trait of interest through either social, or asocial transmission. Asocial transmission is where a trait is acquired independently from others, while social transmission comes from a lasting causal influence from one individual to a second, which increases the likelihood that the second individual acquires the trait (Hoppitt and Laland, 2013).

In terms of disease, the standard NBDA model describes a simple contagion - if a disease is socially transmitted, then the spread should follow the connections in a social network that represent opportunities for social transmission. In this analysis, sheep move from a sound (locomotion score of 0 or 1) to a lame state (locomotion score of ≥2), as a result of either social or asocial acquisition of lameness or both. Social transmission is naïve sheep becoming lame through close spatial connection with lame sheep, and asocial transmission through non-spatial environmental contamination or other factors that cause lameness.

8.1.2 Use of social networks to test hypotheses about transmission

NBDA enables detection and quantification of the impact of social transmission of disease because the social network is the key predictor in the analysis. Comparing different social networks enables different hypotheses about transmission in the

NBDA to be tested. Examples of different social networks that can be used to consider different hypotheses include whether the acquisition of a trait is better predicted by proximity, kinship, or other types of social interaction. For example, in ravens social networks based on affiliative interactions predict the order in which ravens are able to solve a task better than social networks based on either proximity or aggressive interactions (Kulahci et al., 2016). The networks need to represent a potential for social learning opportunity e.g. in whales, only proximity networks where whales are within two body lengths of each other predict their ability to acquire a novel foraging behaviour (Allen et al., 2013). This can be difficult to quantify accurately in some study systems – for example, if dyads were recorded only based on nearest neighbours, an animal could perform the trait of interest, and be observed by multiple animals, but only the closest of these would be recorded as the "nearest neighbour".

The social networks used can be either static or dynamic. A static network a_{ij} gives the total number of times i observed j until the time at which i learned the behaviour, whereas in a dynamic network, a_{ij} is the number of times i has observed j perform the target behaviour prior to time t. In some circumstances, dynamic networks can provided a more complete record of "who transmitted to whom" (Hobaiter et al., 2014), but if networks are broken down into time periods that are too small for the subject of interest, estimates of connection strength can become less precise (Hoppitt and Farine, 2018).

8.1.3 Type of NBDA – OADA and TADA

There are different types of NBDA – these are order of acquisition diffusion analysis (OADA) and time of acquisition diffusion analysis (TADA), which can use either continuous time (cTADA) or discrete time periods (dTADA). The key differences (Hasenjager et al., 2021) between the NBDA variants are summarised in Table 8.1.

Table 8.1 Summary of the key differences (Hasenjager et al., 2021) between order of acquisition diffusion analysis (OADA), continuous time of diffusion analysis (cTADA) and discrete time of acquisition diffusion analysis (dTADA).

	OADA	cTADA	dTADA
Data required for the order of acquisition of behaviour	Only the order that individuals acquired the behaviour required	Precise times of acquisition needed	The time period within which the individual first acquired the behaviour
Shape of the baseline rate function $\lambda_o(t)$	No assumptions – other than it is the same for every individual	Assumes shape of the baseline rate (Constant, Weibull or Gamma)	Assumes shape of the baseline rate (Constant, Weibull or Gamma)

8.1.4 Aims

The aim of this chapter was to use NBDA to estimate the impact of social transmission on acquisition of lameness throughout the 13-day study period using different types of social networks in order to elucidate possible transmission pathways of *D. nodosus*, using lameness as a proxy for infection. The social networks were a network based on first-generation family connections, which would represent either transmission from mother to offspring, or offspring to mother, one based on association indexes calculated from the proximity data obtained from the sensors and one where each sheep had equal connection to other sheep, to determine if transmission occurred homogenously through the group. Multi-network NBDA was used to quantify the relative importance of transmission along these different networks over the 13-day study period.

8.2 Methods

8.2.1 Choice of NBDA variant

Since locomotion scores were collected once for each 24-hour period from midnight to midnight, dTADA models where lameness occurred within a time period were most appropriate as the order and exact time within a day that individuals became lame was not known. The finest time granularity that for the networks that could be used was 24-hour periods and the largest was the whole 13-day study period.

The basic NBDA model (Hoppitt et al., 2020) is fitted by maximum likelihood and can be expressed as:

$$\lambda_i(t) = \lambda_o(t)(1 - z_i(t)\,)\,(s\sum_{j=1}^{N} a_{ij}z_j(t) + 1)$$

where $\lambda_i(t)$ is the rate at which individual *i* acquires the target behaviour as a function of time, $\lambda_o(t)$ is a baseline rate function, $z_i(t)$ is the 'status' of individual *i* at time *t* (1 = informed; 0 = naïve), *N* is the number of individuals in the population and a_{ij} is a non-negative value indicating the connection strength from *j* to *i* in a social network. S is the key output parameter, which is the relative strength of social transmission to the rate of asocial learning of the target behaviour.

Individual-level predictor variables were included in the model by expanding the formula to:

$$\lambda_i(t) = \lambda_o(t)(1 - z_i(t)\,)\,(e^{\Gamma_i}s\sum_{j=1}^{N} a_{ij}z_j(t) + e^{B_i})$$

$$\mathrm{B}_i = \sum_{k=1}^{V} \beta_i X_{k,i}\,,$$

$$\Gamma_i = \sum_{k=1}^{V} \gamma_k X_{k,i},$$

where $x_{k,i}$ is the value of the kth variable for individual i, β_k is the coefficient of the effect of variable k on asocial learning, and γ_k is the coefficient of the effect of variable k on social transmission.

8.2.2 Outcome variable - the order of acquisition of lameness

Sheep were considered to have a case of lameness if they had a locomotion score of ≥2 on at least two days of the trial (1^{st}-15^{th} October) and the first day the locomotion score was ≥2 was taken to be the day that they acquired the lameness. Sheep that were first seen lame on the 1^{st} October (Day 0 of the study, when sensors were deployed) were included in the diffusion as seeded demonstrators. All lame sheep in the study were assumed to have the possibility of being infectious.

8.2.3 Explanatory variables and model selection

8.2.3.1 Social networks used to test hypotheses about transmission

Three static social networks and one dynamic network were tested as predictors in the NBDA. These were:

- Static association network over the whole study period:

  $$AI = \frac{x_{ab}}{x_{ab} + x_a + x_b}$$

  Where the association index (AI) equals the number of sampling periods (x) with individual a and b observed associated divided by the number of sampling periods individual a and b were observed associated and the number of sampling periods individual a was observed without b and vice versa, where the sampling period is the 20 second temporal window detected by the proximity sensors.
- Dynamic association network – where the association index was calculated as above for the static network, but for daily 24-hour (midnight-midnight) time periods.

- Kinship network: where 1 indicates a first-generation family relationship between sheep and 0 where sheep were not first-generation family members. Fostered lambs were considered related to the ewe that they had been fostered onto.
- Homogenous networks – all network connections were set to 1, indicating all network connections are of equal strength. If the homogenous network is favoured over the measured social network, it would imply that either transmission occurs homogenously within the group, or that the measured network differs substantially from the real transmission pathways.

2.3.2 Model selection – individual-level variables and choice of baseline rate function in dTADA using static networks

The fit of the three static model combinations of individual-level predictor variables affecting social/asocial learning with different baseline functions were compared using AICc, relative support and AICc weights, where appropriate. These were defined as in the NBDA package (Hoppitt et al., 2020), which was used to create all models:

AICc: a sample size corrected version of the AIC, recommended to be used when N/k < 40 (Burnham and Anderson, 2002), where

$$AIC_c = 2 * k * (N/(N - k - 1) - 1 + 2 * loglik$$

Where k = the number of parameters in the model, N is the number of acquisition events, summed across the diffusion events (Hoppitt and Laland, 2011) and loglik is the negative log likelihood for the model.

Relative support: quantifies the ratio of the probabilities that each model is the one with the best Kullback-Leibler information – calculated as the difference in AICc between two models - $e^{\Delta AIC/2}$

Akaike weight: the probability that model i is the best K-L model in the set, accounting for sampling error. First the AIC difference between each model (i) and the best model is taken using $\Delta_i = AIC_{c_i} - AIC_{c_{best}}$, then the Akaike weight for model i is the $w_i = e^{(-\frac{1}{2}\Delta i)} / \sum_j e^{(-\frac{1}{2}\Delta j)}$

8.2.3.3 Effect of individual-level variables

Unconstrained NBDA models (Hoppitt and Laland, 2013) allow individual-level predictor variables to be estimated independently and therefore have different effects on social transmission and asocial transmission (i.e. βk and γk are estimated independently).

Models were tested where individual level dummy-coded predictor variables (lamb vs ewe, and twin vs not) could affect:

- Both social and asocial transmission
- Social transmission only
- Asocial transmission only
- A null model without the individual-level predictor variables included

8.2.3.4 Effect of assumptions about the baseline rate of acquisition of the trait ($\lambda_O(t)$)

If the baseline acquisition of lameness ($\lambda_O(t)$) changes over time, spurious effects of social transmission can be detected because NBDA estimates the strength of social transmission compared to asocial transmission. Therefore, three different assumptions about the baseline rate of acquisition of lameness were tested. These were:

- Constant baseline: allows the rate of acquisition in the absence of social transmission to remain constant over time
- Gamma distribution: allows for a systematic increase or decrease in the asocial rate of acquisition over time
- Weibull: allows for an increasing baseline rate when an additional shape parameter κ >1, a constant baseline if κ =1 and a decreasing baseline rate where κ <1.

8.2.3.5 Testing whether inclusion of an effect for social learning (s parameter) improves the model

Models were also tested where lameness was never acquired through social transmission by constraining the s parameter in the model to be 0 – meaning that the social network is essentially not used as a predictor.

8.2.3.6 Summary of the different combinations of tested models

Table 8.2 shows the different combinations of individual-level predictors, baseline and social network predictors tested.

Table 8.2 Model number reference table for each combination of individual-level variables and baseline rates of acquisition. Cell entries are labelled with the model number.

		Model number			
Static social network predictor		**Social and asocial learning**	**Social learning only**	**Asocial learning only**	**Not included**
First-generation family network	Constant	1	2	3	4
	Weibull	14	15	16	17
	Gamma	27	28	29	30
Association network	Constant	5	6	7	8
	Weibull	18	19	20	21
	Gamma	31	32	33	34
Homogenous network	Constant	9	10	11	12
	Weibull	22	23	24	25
	Gamma	35	36	37	38
No social network effect estimated	Constant	-	-	13	-
	Weibull	-	-	26	-
	Gamma	-	-	39	-

8.2.4 Model selection – choice of baseline rate function in dTADA using a dynamic network and time changing management variables

For the dynamic models, all predictors were assumed to only affect asocial learning. The time-changing predictor variables were dummy coded as follows:

- Whether or not the sheep were gathered on a day (1 = yes, 0 = no)
- Whether or not the sheep had access to both Field A and Field B compared with only Field A (1 = yes, 0 = no)
- Whether or not sheep had access to Field C, Field B and Field A (4.9 acres) compared with only Field A (1.7 acres) or access to Field A and Field B (3.3 acres) compared to only Field A (1 = yes, 0 = no)

As for the static models, the dummy coded lamb/twin variables were included, and models with a social transmission component were compared to models without a social transmission component, using the three different baseline rates for acquisition of lameness (Constant, Gamma and Weibull).

8.2.5 Parameter estimation

8.2.5.1 Static networks

Multi-model inferencing (Burnham and Anderson, 2002, Hoppitt et al., 2020) was used to calculate median estimates of the parameters across the model sets to allow more robust inference about the strength of transmission though the different networks. A lower limit calculation for the s parameter was performed for each model that contained the s parameter, by using profile likelihood function to search between 0 and the maximum likelihood estimate for s in each model, since NBDA can have a lot of certainty about the lower limit for s but not the upper limit due to high asymmetry in the profile likelihood. The proportion of cases solved by social transmission (PST) corresponding to the calculated lower limit for s were calculated by:

$$p_{social,e} = \frac{e^{\Gamma_i(t_e) s \sum_{j=1}^{N} a_{ij}(t_e) z_j(t_e)}}{e^{\Gamma_i(t_e) s \sum_{j=1}^{N} a_{ij}(t_e) z_j(t_e)} + e^{\mathrm{B}_i(t_e)}}$$

Where *i* is the individual that learned during event *e* and t_e is the time at which event *e* occurred. This is the predicted relative social transmission rate divided by the predicted total relative learning rate for *i* at the time of learning. The mean of $p_{social,\, e}$ across all events is the estimated proportion events that occurred by social transmission.

Confidence intervals for the overall best fitting model (judged by AICc) were obtained using the profile likelihood function for each included parameter and the interactive procedure suggested by Hasenjager et al., (2021).

8.2.5.2 Dynamic network

The best fitting model from the combinations of predictor variables tested was evaluated using AICc and AICc weights. Profile likelihood confidence intervals for the best fitting model as for the best fitting static model.

8.3 Results

8.3.1 The diffusion curve

Of the 94 sheep with working sensors, 14 sheep were lame on the 1st October, 5 ewes, 5 single lambs and 4 twin lambs (Table 8.3), 2 of the twins were related. Of the 32 sheep that became lame from the 2nd onwards, 19 were ewes, 9 were single lambs and 4 were twin lambs, from 23 different family units, 8 of which had more than one sheep in the family lame. By the end of the study, a total of 46 sheep had been seen lame (Table 8.3).

The cumulative percentage of the flock that became lame is shown in Figure 8.1. The curve had a very slight s-shape suggesting a possibility of social transmission (due to the acceleration in transmission as more sheep become lame) although this is not always a reliable indicator for social transmission (Hoppitt et al., 2010a).

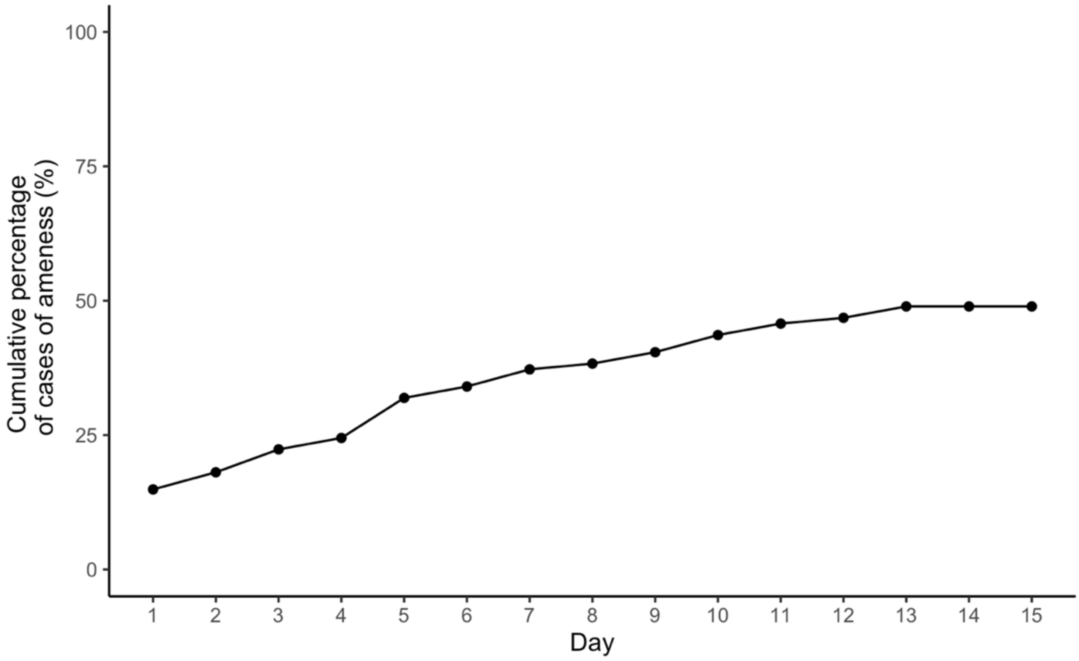


Figure 8.1 The cumulative percentage of the flock that were cases of lameness during the trial from the 94 sheep with working sensors.

Table 8.3 The number and percentage of ewes, single and twin lambs with a case of lameness during the trial

Sheep	Lame from 1st-14th October (Day 0-Day 13)	Lame 1st October (Day 0)	Lame from 2st-14th October (Day 1-13)
	N (%)	N (%)	N (%)
Ewe	24 (60.0)	5 (12.5)	19 (47.5)
Single lamb	14 (53.8)	5 (19.2)	9 (34.6)
Twin lamb	8 (28.6)	4 (14.3)	4 (14.3)

1. N = number of sheep

8.3.2 Association indexes between sheep

Descriptive statistics for the association indexes in different dyad types (where a dyad refers to a pair of sheep) from the static association network are in Table 8.4. Association indexes were much higher between related sheep than non-related sheep. Twin lambs had higher association indexes with each other than their mother, and single lambs had higher association indexes with their mother than twin lambs with their mother (Table 8.4)

Table 8.4 Association indexes for dyadic associations between related and non-related sheep from the static association network calculated over the 13-day study period.

Dyad type	Mean	Median	Min	Max	SD
Ewe–offspring	0.183	0.169	0.123	0.000	0.494
Ewe–non-kin sheep	0.002	0.001	0.005	0.000	0.212
Single lamb–mother	0.233	0.210	0.100	0.062	0.494
Single lamb–non-kin sheep	0.003	0.002	0.004	0.000	0.032
Twin lamb– mother	0.088	0.078	0.049	0.000	0.180
Twin– non-kin sheep	0.003	0.001	0.010	0.000	0.334
Twin– other twin	0.282	0.297	0.133	0.002	0.472

1. Min = minimum, max = maximum, sd = standard deviation

8.3.3 Static network models – identification of the best fitting model

Models using the first-generation family connections as the social network predictor tended to have higher Akaike Weight than either the association or homogenous networks (Table 8.5). Models including the lamb and twin predictor variables affecting only asocial transmission performed better than models either without the individual-level predictor variables included at all or affecting social learning only – model details are found in Table 8.2. The best fitting model (Model 3) used the network of first-generation family connections with the lamb and twin predictor variables affecting only the rate of asocial transmission and a constant baseline rate of acquisition (Table 8.5).

Table 8.5 AICc, Delta AICc, Akaike Weight and relative support for each model in the tested model set using social networks of first-generation family connections, association indexes, a homogenous network where all connections are set to one and asocial models where the social network was not used as predictor.

Model	Social network predictor	Baseline	AICc	Delta AICc	Akaike Weight	Relative Support
3	Family	Constant	259.58	0.00	0.44	1.00
16	Family	Weibull	261.60	2.02	0.16	0.36
29	Family	Gamma	261.84	2.26	0.14	0.32
7	Association	Constant	263.06	3.48	0.08	0.18
1	Family	Constant	264.85	5.27	0.03	0.07
20	Association	Weibull	264.98	5.40	0.03	0.07
13	Asocial	Constant	265.10	5.52	0.03	0.06
33	Association	Gamma	265.24	5.66	0.03	0.06
5	Association	Constant	266.86	7.28	0.01	0.03
14	Family	Weibull	267.45	7.87	0.01	0.02
26	Asocial	Weibull	267.49	7.91	0.01	0.02
39	Asocial	Gamma	267.60	8.03	0.01	0.02
27	Family	Gamma	267.67	8.09	0.01	0.02
11	Homogenous	Constant	267.72	8.14	0.01	0.02
18	Association	Weibull	269.73	10.15	0.00	0.01
31	Association	Gamma	269.91	10.33	0.00	0.01
24	Homogenous	Weibull	270.13	10.55	0.00	0.01
37	Homogenous	Gamma	270.41	10.83	0.00	0.00
4	Family	Constant	271.37	11.79	0.00	0.00
8	Association	Constant	272.67	13.09	0.00	0.00
17	Family	Weibull	272.81	13.23	0.00	0.00
30	Family	Gamma	273.00	13.42	0.00	0.00
12	Homogenous	Constant	273.29	13.71	0.00	0.00

Model	Social network predictor	Baseline	AICc	Delta AICc	Akaike Weight	Relative Support
21	Association	Weibull	274.10	14.52	0.00	0.00
34	Association	Gamma	274.31	14.73	0.00	0.00
2	Family	Constant	274.81	15.23	0.00	0.00
25	Homogenous	Weibull	275.26	15.68	0.00	0.00
38	Homogenous	Gamma	275.39	15.81	0.00	0.00
6	Association	Constant	276.54	16.96	0.00	0.00
15	Family	Weibull	276.80	17.22	0.00	0.00
28	Family	Gamma	276.98	17.40	0.00	0.00
19	Association	Weibull	278.01	18.43	0.00	0.00
32	Association	Gamma	278.24	18.66	0.00	0.00
10	Homogenous	Constant	278.36	18.78	0.00	0.00
23	Homogenous	Weibull	280.71	21.13	0.00	0.00
36	Homogenous	Gamma	280.84	21.26	0.00	0.00
9	Homogenous	Constant	NA	-	-	-
22	Homogenous	Weibull	NA	-	-	-
35	Homogenous	Gamma	NA	-	-	-

8.3.4 The best fitting static network

Model 3 (Table 8.6) estimated 24.8% of cases were solved by social transmission across family groups, with the lower confidence interval for this estimated at 15.0% (Table 8.7). There was no significant difference in the rate of asocial transmission of lameness between lambs and ewes but being a twin lamb compared to single lamb was associated with a reduction in the rate of asocial transmission of lameness (HR = 2.70x10-09, 95% CI = ∞-0.39), meaning twin lambs were less likely to become lame during the study without connections to other lame sheep.

Table 8.6 Full model with 95% profile likelihood confidence intervals for Model 3

Predictor	Baseline	PST	MLE	SE	HR	LCI	UCI
Scale (1/rate)	-		17.11	4.40			
S	-	0.25	0.45	0.24	-	0.13	1.20
Lamb- yes	Lamb - no	-	-0.53	0.47	0.20	0.19	1.41
Twin - yes	Twin - no	-	-19.73	8324.93	2.70×10^{-09}	∞	0.39

1. PST = proportion of cases solved by social transmission, MLE = maximum likelihood coefficient estimate for the parameter, SE = standard error, HR = hazard ratio, LCI = lower profile 95% confidence interval for the hazard ratio, UCI = upper profile 95% confidence interval for the hazard ratio

8.3.5 Calculation of lower limits for the s parameter in models that estimate social transmission

Within the model set where an s parameter was estimated, the lower confidence interval for the s parameter was >0 in models that used the first-generation family connections as the social network predictor (Model 3, 16 and 29, Table 8.7) and included the lamb and twin-lamb dummy-coded predictors with an effect on asocial learning only. In these models, the lower confidence interval for the proportion of cases solved by social transmission was low (~1% of cases solved by social transmission, Table 8.7).

Table 8.7 Lower limit of the profile confidence interval for the s parameter, estimated proportion of cases solved by social transmission and overall Akaike Weight, and Akaike Weights calculated across each social network predictor.

Model	LCI s	PST	Delta AICc	Akaike Weight Overall	Akaike Weight Adjusted	Akaike Weight Cumulative
Family						
3	0.13	0.15	0.00	0.44	0.55	0.55
16	0.13	0.15	2.02	0.16	0.20	0.76

Model	LCI s	PST	Delta AICc	Akaike Weight Overall	Akaike Weight Adjusted	Akaike Weight Cumulative
29	0.13	0.15	2.26	0.14	0.18	0.94
1	0.00	0.14	5.27	0.03	0.04	0.98
14	0.00	0.14	7.87	0.01	0.01	0.99
27	0.00	0.14	8.09	0.01	0.01	1.00
4	0.00	0.00	11.79	0.00	0.00	1.00
17	0.00	0.00	13.23	0.00	0.00	1.00
30	0.00	0.00	13.42	0.00	0.00	1.00
2	0.00	0.00	15.23	0.00	0.00	1.00
15	0.00	0.00	17.22	0.00	0.00	1.00
28	0.00	0.00	17.40	0.00	0.00	1.00
Association						
7	0.12	0.04	3.48	0.08	0.51	0.51
20	0.12	0.04	5.40	0.03	0.20	0.71
33	0.12	0.04	5.66	0.03	0.17	0.88
5	0.00	0.13	7.28	0.01	0.08	0.96
18	0.00	0.13	10.15	0.00	0.02	0.97
31	0.00	0.13	10.33	0.00	0.02	0.99
8	0.00	0.00	13.09	0.00	0.00	0.99
21	0.00	0.00	14.52	0.00	0.00	1.00
34	0.00	0.00	14.73	0.00	0.00	1.00
6	0.00	0.00	16.96	0.00	0.00	1.00
19	0.00	0.00	18.43	0.00	0.00	1.00
32	0.00	0.00	18.66	0.00	0.00	1.00
Homogenous						
11	0.00	0.00	8.14	0.01	0.60	0.60
24	0.00	0.00	10.55	0.00	0.18	0.78

Model	LCI s	PST	Delta AICc	Akaike Weight Overall	Akaike Weight Adjusted	Akaike Weight Cumulative
37	0.00	0.00	10.83	0.00	0.16	0.93
12	0.00	0.00	13.71	0.00	0.04	0.97
25	0.00	0.00	15.68	0.00	0.01	0.98
38	0.00	0.00	15.81	0.00	0.01	1.00
10	0.00	0.00	18.78	0.00	0.00	1.00
23	0.00	0.00	21.13	0.00	0.00	1.00
36	0.00	0.00	21.26	0.00	0.00	1.00
9	-	-	-	-	-	-
22	-	-	-	-	-	-
35	-	-	-	-	-	-

1. LCI = lower confidence interval for the model s parameter, PST = proportion of cases solved by social transmission, corresponding to the lower limit for s
2. Overall Akaike Weights refer to the full model set, while the adjusted and cumulative Akaike weights are calculated within each social network predictor model set.

8.3.6 Model averaged parameter estimates – incorporating model selection uncertainty

Model averaged terms identified a role of social transmission across the family network, but not the association or homogenous networks (Table 8.8). The model averaged terms from all model sets suggested that both lambs compared to ewes, and twins compared to singles, were becoming lame at a slower rate than ewes (Table 8.8) and these variables were associated with the rate of asocial transmission (i.e. becoming lame without connections to lame sheep), rather than the rate of social transmission (becoming lame with connections to lame sheep).

Table 8.8 Median model averaged terms for the s parameters (family, association and homogenous) and other parameters across all network models (family, association over the whole 13-day study period and homogenous)

Median model averaged terms	**Baseline**	**Median model averaged term**
S1 – family network	-	0.45
S2 – association network	-	0.00
S3 – homogenous network	-	0.00
Asocial – lamb	Ewe	-0.53
Asocial – twin	Single	-19.73
Social – lamb	Ewe	0.00
Social – twin	Single	0.00

8.3.7 Baseline support

The constant baseline models received 3.16 times as much support as the Gamma baseline models, and 2.81 times as much support as the Weibull baseline models (Table 8.9), indicating that assuming that the rate of acquisition of lameness in the absence of social transmission remains constant over time fitted the data better than allowing for increases or decreases in the asocial rate of acquisition over time according to either a Gamma or Weibull distribution.

Table 8.9 support for the models across the different baseline rate of acquisition.

Baseline	**Support**	**Number of models**
Constant	0.60	13
Gamma	0.19	13
Weibull	0.20	13

8.3.8 Dynamic models with time-varying management-related variables

Models with the s parameter estimated tended to perform better than models with the s parameter estimated (Table 8.10). The best fitting dynamic model included the predictor variables for lambs and twins and whether or not the sheep were gathered (Table 8.10).

In the best fitting model (Table 8.11), gathering sheep compared to not was associated with an increase in the rate of asocial transmission of lameness (HR = 2.50, 95% CI = 1.02-7.45). Being a lamb compared to ewe was not significantly associated with the rate of asocial transmission of lameness, but being a twin lamb compared to single was associated with a decrease in the rate of asocial transmission of lameness (HR = 1.03x10-8, 95% CI =∞-0.71), again suggesting twin lambs were less likely to become lame without connections to other lame sheep. A lower limit for this could not be estimated because of the asymmetry in the profile likelihood – this is because, as far as the model is concerned, it is possible that only ewes, when compared to twins, are able to become lame asocially, i.e. without social connection with other sheep.

Table 8.10 AICc and AICc weight for models with different combination of predictor variables, including time changing managements, affecting the rate of asocial learning and including an s parameter

Predictor (baseline)						
Lamb: yes	**Twin: yes**	**Sheep gathered: yes**	**Area available: 3.3 acres**	**Area available: 4.9 acres**	**AICc**	**AICc Weight**
(No)	**(No)**	**(No)**	**(1.7 acres)**	**(1.7 acres)**		
Models with s estimated						
Constant baseline						
+	+	+	+	+	266.37	0.03
+	+		+	+	265.53	0.05
+	+	+			262.64	0.22
+	+				263.83	0.12

Predictor (baseline)						
Lamb: yes	Twin: yes	Sheep gathered: yes	Area available: 3.3 acres	Area available: 4.9 acres	AICc	AICc Weight
(No)	(No)	(No)	(1.7 acres)	(1.7 acres)		
Gamma baseline						
+	+	+	+	+	269.79	0.01
+	+		+	+	267.75	0.02
+	+	+			264.94	0.07
+	+				266.06	0.04
Weibull baseline						
+	+	+	+	+	269.82	0.01
+	+		+	+	267.87	0.02
+	+	+			264.86	0.07
+	+				265.81	0.05
Models without s estimated						
Constant baseline						
+	+	+	+	+	268.70	0.01
+	+		+	+	268.90	0.01
+	+	+			263.98	0.11
+	+				265.10	0.06
Gamma baseline						
+	+	+	+	+	271.95	0.00
+	+		+	+	270.88	0.00
+	+	+			266.71	0.03
+	+				267.60	0.02
Weibull baseline						
+	+	+	+	+	271.98	0.00
+	+		+	+	271.01	0.00

Predictor (baseline)						
Lamb: yes	Twin: yes	Sheep gathered: yes	Area available: 3.3 acres	Area available: 4.9 acres	AICc	AICc Weight
(No)	(No)	(No)	(1.7 acres)	(1.7 acres)		
+	+	+			266.68	0.03
+	+				267.49	0.02

Table 8.11 The best fitting dynamic NBDA model comparing ewes, single and twin lambs and whether sheep were gathered as predictors

Predictor	Baseline	MLE	SE	HR	LCI	UCI
Scale (1/rate)	-	26.26	10.39			
Lamb - yes	Ewe	2.23	1.53	-	0.06	9.59
Twin - yes	Single	-0.65	0.54	0.52	0.13	1.35
Sheep gathered - yes	No	-18.39	8337.43	$1.03x^{10-8}$	∞	0.71

1. MLE = maximum likelihood coefficient estimate, SE = standard error, HR = hazard ratio, LCI = lower profile 95% confidence interval, UCI = upper profile 95% confidence interval

8.4 Discussion

The NBDA analysis indicated social transmission of lameness occurs within family groups (Table 8.5, Table 8.6) in this flock of ewes and young lambs. Model averaged terms suggested that lambs, particularly twins, were more likely to become lame following contact with lame sheep compared to ewes (Table 8.8).

Ewes were lame prior to lambing (Table 6.1), and overall flock prevalence of lameness was high (8-28% in the weeks throughout September), indicating that *D. nodosus* was present in the flock (along with conformation from the farmer that ID lesions were common in his sheep). Some ewes would have become lame without contact with lame sheep in the study period because as they were already infected with *D. nodosus*, and it can take around 2 weeks for clinical signs to show, which is why ewes in the study had the highest rate of becoming lame without contact with lame sheep.

Model averaged terms suggested lambs were more likely to become lame through contact with other sheep compared to ewes. Lambs are born without *D. nodosus* on their feet (Muzafar et al., 2015), and *D. nodosus* persists on diseased feet (Clifton et al., 2019), so it is most likely that ewes were a reservoir of infection that led to the infection in lambs, with which they have close contact (Table 8.4). Lambs were first lame at around two weeks old (chapter 4), which is consistent with the time take to develop clinical signs of foot lesions after birth when *D. nodosus* positive ewes are kept with naïve lambs (Kuhnert et al., 2019).

Twin lambs were less likely to become lame than single lambs (Table 8.3, Table 8.5, Table 8.6, Table 11). This is consistent with results in chapter 4 and in Kaler et al., (2010b). A biological explanation for this difference has been proposed, that single born-lambs tend to be heavier, which could increase susceptibility to footrot because of greater physical contact with the pasture - lambs that are heavier are more likely to become lame than those that don't become lame (Lima et al., 2020c). However, the current study provides an alternative explanation. Twin lambs spent less time with their mother than single lambs (Table 8.4) in our study, assuming this difference in contact is consistent from birth, as suggested by other studies (Broster et al., 2010), twin lambs might be less likely to become lame because they had less contact with infectious ewes.

That both ewes and lambs are more likely to become lame if a member of their family is already lame has been reported previously (Kaler et al., 2010b, Wassink et al., 2010b). Sheep have stronger associations with family members than other sheep (Table 8.4, Morgan and Arnold, 1974), and it is possible that only certain interactions, e.g. those that occur predominately within family groups, such as suckling, bring sheep into sufficiently close contact of sufficient duration to be infected with bacteria from an infectious sheep via the environment. Since association indexes were much weaker between non-family members (Table 8.4), these weak connections may add noise rather than information about the order of acquisition of lameness, which could explain why social transmission was not detected in the spatial proximity network (Table 8.7). Weak connections are usually thought to accelerate spread of disease across networks by providing "short-cuts" across sub-groups (Centola and Macy, 2007). However, here, weak connections in the network may not bring sheep into sufficient contact to actually transmit bacteria. Other explanations for transmission being most likely within family groups include genetics, where some families are more susceptible to footrot, or interactions between host genetics and the environment (Russell et al., 2013), which were not explored in this study.

The behavioural effects that result from lameness could also be why social transmission was not detected in the spatial proximity network (Table 8.7). Lame sheep spend less time in contact with non-related sheep (Table 7.8) and this reduction in time spent with non-related sheep could mean that lame sheep are less likely to transmit *D. nodusus* outside of their family group. Lame ewes may stay spatially close to their lambs to protect and feed them, whilst lame lambs may reduce other parts of their daily time budget – such as playing with non-family members. There is evidence that footrot alters sheep time budgets – lambs with footrot lie down more frequently but for shorter periods than healthy lambs (Härdi-Landerer et al., 2017).

The pasture was wet during the study (Table 5.6) and since moisture allows *D. nodosus* to survive for a few days in the environment (Clifton et al., 2019), the pasture was likely to be contaminated with *D. nodosus*. Therefore, homogenous networks (where all sheep were set to have the same connection to each other), were included in the multi-network NBDA to approximate the role of transmission that occurred solely through the environment. However, these networks received low Akaike Weight, indicating that they were not a good fit compared to other models – suggesting that either the pasture was not homogenously infectious or sheep did not contact the pasture homogenously due to having preferences for certain areas, or both.

Gathering sheep was associated with an increase in the rate of asocial transmission of lameness (Table 8.11), which could be a result of bringing all animals into a small space and therefore increasing the risk of transmission of *D. nodosus* between sheep from pasture. However, since sheep were gathered when the farmer deemed "enough" sheep were lame to be worth the time for treatment it is possible this association is explained by increased numbers of lame sheep increasing the force of infection as more sheep were shedding bacteria into the environment, since load of *D. nodosus* is highest during episodes of ID (Witcomb et al., 2014). Waiting until several sheep in a group are lame before treating is associated with higher prevalence of lameness at the flock level (Kaler and Green, 2008b, Winter et al., 2015). Using the Gamma and Weibull baselines allowed for systematic increases/decreases in the baseline rate of acquisition - since the constant baseline models received higher Akaike Weight, this may suggest that it is the act of gathering the sheep together that causes the increase in the rate of asocial transmission of lameness.

The two-week study period was defined by the battery life of sensors and was relatively short compared to the incubation period of footrot. The incubation period for footrot is one to two weeks (Egerton et al., 1969, Kuhnert et al., 2019) and at the start of the study ewes were at different stages of infection and disease and this variability would have added noise to the order of acquisition of lameness, and therefore the role of social connections. Lameness is also not a perfect proxy for footrot infection, although lame sheep are likely to have footrot lesions, some non-lame sheep would have had lesions (Kaler et al., 2011). Sheep were treated during the study and so some foot lesions may have healed before the study end when feet were checked. Some sheep had non-infectious potential causes of lameness (Table 6.4) but less is known about how these lesions correlate to the severity of lameness, and they were not associated with lameness in ewes in this study (Table 6.5).

Simulation-based approaches could have helped to validate the analytical approach by testing hypotheses about pathogen transmission. Examples of simulations that would have helped to provide further insight into the spread of *D. nodosus* could have been a similar approach to Sah et al. (2017), looking at how different estimates of pathogen transmissibility are related to disease spread in combination with the observed network structure. Approaches such as exponential random graph models allow approaches such as compartmental models to be combined with the network structure, which would have also increased insight into the relationship between the network and disease spread.

8.5 Conclusion

The NBDA identified that there is social transmission of lameness within family groups and provided further evidence that waiting until “enough” sheep in a group are lame before gathering and treating is associated with increased incidence of lameness. Ewes and lambs become lame through different routes, with lambs more likely to become lame from contact with lame sheep whilst some lameness in ewes was asocial and possibly because of prior infection. It is most likely that lambs acquire infection from their mothers, and since twins spend less time with their mother compared to singles, this may be why twins acquired lameness at a slower rate in the study.

Chapter 9 General discussion, conclusions and future research

9.1 Key findings

1. Separate analysis of data for ewes and lambs increases insight into good management practices – for example footbathing to treat ID is associated with high prevalence of lameness in lambs, while footbathing to treat severe footrot is associated with high prevalence of lameness in ewes. Since lambs rarely develop SFR, risks for lambs with ID may equate to those associated with SFR in ewes.
2. Triangulation of results from multiple model types identified robust sets of predictor variables associated with lameness in ewes and lambs, which are the most likely to have the most reliable impact on sheep farmers
3. Lameness affects sheep behaviour, with lame sheep spending less time with non-related sheep.
4. Single and twin lambs have different social patterns, which could equate to different risks of transmission of *D. nodosus* throughout the flock or acquiring *D. nodosus* themselves, assuming spatial proximity is sufficient for transmission of bacteria between sheep.
5. Over the two week-study period, there was social transmission of lameness within family groups. Since ewes were lame prior to lambing, it is most likely that the ewes in the flock were a reservoir of infection that led to infection in their lambs.

9.2 Discussion of key findings

The aim of this thesis was to determine the role of lambs, time, and space in persistence of *D. nodosus*, the causative agent of footrot. There were two aspects to this – questionnaire data was used to determine if there are specific risk factors for lameness in lambs and how management of lambs impacts prevalence of lameness in both the ewe and lamb flock, and a longitudinal observational study using biologgers was used to evaluate how social contact patterns in a flock of ewes and their lambs could be related to disease spread.

The questionnaire data, particularly from 2018, suggested there is a benefit to separate analysis of risk factors for ewes and lambs. Although farmers are likely to manage their lambs in a similar way to their ewes, managements can have different effects since disease presents differently – for example, while footbaths are generally not recommended as an effective treatment for ewes (Wassink et al., 2010b) they can be associated with lower prevalence of lameness if used to treat ID (Witt and Green, 2018). However, for lambs use of footbaths to treat ID is associated with higher prevalence of lameness (Table 3.3), likely because for lambs, ID is the common presenting sign of footrot - overturning the paradigm that footbaths are beneficial for treatment of footrot in lambs.

Techniques to manage large numbers of explanatory variables are likely to become increasingly important in animal health research as datasets become larger. The advantage of the combination of regularised regression and bootstrap samples is that it provides a platform to rank the importance of covariates (Lima et al., 2020a, Lima et al., 2020b). In practice, many management practices have a small impact on prevalence of lameness in sheep flocks which makes use of a ranking system to discriminate their likely importance particularly useful.

Multiple model triangulation highlighted that there is uncertainty in selection of predictors associated with prevalence of lameness between methods. Triangulation of results from multiple models reduce the number of "false positive" associations – where predictors have been selected due to over-fitting. Some management practices (treating lame sheep within three days, stopping routine foot trimming) are associated with large population attributable fractions (Prosser et al., 2019) and would therefore prevent many lame sheep, which illustrates that it is more beneficial to focus messaging to farmers on the practices that are likely to have the widest application.

In the flock of Poll Dorset sheep, the proportion of lame lambs was less than ewes (Table 6.3) which is typical for a farm in England, according to questionnaire data (Table 2.1, Table 3.1). The NBDA suggested the most likely route of social transmission of lameness is within family groups, and since lambs are born without *D. nodosus* on their feet (Muzafar et al., 2015), at this stage of the lamb's life, transmission most likely occurs from infectious ewes to their lambs. The questionnaire data also suggested low prevalence of infectious foot lesions in ewes was associated with low prevalence of lameness in lambs (Table 2.4). Combined, this may explain why some management practices that are only used on ewes and cause high prevalence of lameness in ewes are also associated with high prevalence of lameness in lambs, as they contribute to keeping a reservoir of infection in the ewe flock that leads to infection in lambs when they are born.

There are several possible reasons that social transmission of lameness occurred mostly within family groups and did not appear to have a significant role in the spatial proximity network. The first could be due to the division of ewes and lambs into social communities (Figure 7.2) - highly modular networks can trap disease within sub-groups (Sah et al., 2017). The increased time spent with family members may be why sheep in the same family group are likely to become lame, but it is also possible that only certain interactions bring sheep into close enough contact to pick up sufficient amounts of bacteria to cause disease, and these may be interactions such as feeding that only occur within family groups. Lameness is associated with a change in social behaviour, with sheep making fewer out of family connections when they are lame compared to not lame, which could also explain why social transmission was not detected in the NBDA with the spatial proximity networks, but in the family network. Establishing how the first sheep in the family group becomes lame would likely need to be studied over a longer time period – but results in chapter 7 suggest single lambs are most likely to make out of community contacts, and therefore may be most likely to introduce disease into their family group, if their mother is not already infectious.

Being a twin lamb, compared to a single was highlighted as both a trait-based feature (once birth-weight and age were controlled for, Table 6.7), and in the NBDA, as protective against development of lameness (Table 8.6, 8.11). If the modular structure of the network does influence the risk of sheep becoming lame, the reason why twins have a different risk of becoming lame compared to singles, could be due to their preferential attachment to their twin (Table 8.4, (Broster et al., 2012a),which results in less time spent with their mother compared to singles (Table 8.4, (Broster et al., 2010). This could mean twins are less likely to acquire *D. nodosus* via the environmental contamination from their dam.

Farmers manage their flock differently at different stages of the production cycle – for example, farmers may not treat ewes for lameness when they are late in pregnancy (O'Kane et al., 2017), lame ewes with lambs at foot might not be caught and treated in case the family group become separated (Witt and Green, 2018) and concerns over lambs being trampled may prevent farmers gathering the flock (Witt and Green, 2018). Prompt treatment of individual sheep is key to lowering flock prevalence of lameness (Kaler et al., 2010a, Prosser et al., 2019, Winter et al., 2015, Wassink et al., 2010b), with separation of lame ewes is also associated with lower flock prevalence of lameness (Wassink et al., 2004, Witt and Green, 2018). Both of these most likely lower flock prevalence of lameness by reducing the force of infection in the field, as fewer sheep are shedding bacteria. The current study suggests that it could be beneficial to farmers to not turn lame ewes and their lambs out after lambing with the rest of flock – since lambs are most likely infected from their mother keeping the whole family group where the ewe is lame separate could help prevent the epidemics of ID that often are seen in lambs (Wassink et al., 2004), particularly if a lamb to lamb transmission pathway becomes more important as the lambs age. While social transmission of lameness between unrelated lambs was not detected in this analysis, possibly because of the short time period of the study relative to the incubation period of footrot or because outside the family transmission mostly occurs due to picking bacteria up from areas of the pasture that are not used homogenously, such as by water troughs or hedge rows, it is still possible that there is a role of spatial co-location in transmission between older lambs, which form ‘play gangs’ and spend less time with their mothers (Morgan and Arnold, 1974) and this contributes to the epidemics seen by farmers.

9.3 Limitations of this study

Many assumptions had to be made about how *D. nodosus* is transmitted between sheep. *D. nodosus* is found transiently on pasture (Clifton et al., 2019, Graham and Egerton, 1968, Whittington, 1995) but persists on diseased feet (Clifton et al., 2019), and it would therefore seem likely that sheep become infected by picking up bacteria that are shed into the environment from diseased sheep. The social network analysis assumed that contacts made within 1.0-1.5m (the approximate body length of an adult ewe) and a temporal resolution of 20 seconds would be sufficient for transmission, but it is possible that this threshold is too large or the time too short, which may be why there was only evidence for social transmission of lameness within family groups.

Lameness was assumed to be a suitable proxy for infection with *D. nodosus*, and ability to transmit *D. nodosus* to other sheep. Most lameness in sheep in England is caused by footrot (Winter et al., 2015) and sheep that are lame are likely to have footrot – although false positives can occur since sheep are lame from other causes - non-infectious foot lesions (white line and fibroma) were seen on ewes in the flock (Table 6.4) but were not associated with lameness (Table 6.5). Lameness is known to be less than 100% sensitive as a diagnostic test for footrot – as mild footrot lesions can be found on sheep that are not lame (Kaler et al., 2011). It would not have been practical, or ethical, to check sheep for foot lesions on a daily basis and swab the lesions to determine the bacterial load but bacterial load has been found to be highest during episodes of ID (Witcomb et al., 2014). It also takes time to develop clinical signs of footrot following infection – foot lesions can be seen at 14 days post infection in lambs (Kuhnert et al., 2019) and the study period was limited by the battery life of the sensors. Given the limits on what is known about the epidemiological parameters for transmission of *D. nodosus*, this thesis focused on the use of social network analysis approaches that answer the question of whether there <u>could</u> be a role of social contact in transmission of *D. nodosus* between sheep.

9.4 Conclusions

In conclusion, this thesis has contributed to the understanding of the dynamics of the spread of lameness though sheep flocks. Use of management practices that are detrimental to control of lameness in ewes contributes to high prevalence of lameness in ewes that leads to lambs acquiring disease from their mothers. Twins spend less time with their mothers, which may explain why they became lame at a slower rate than singles over the study period.

9.5 Future work

Since the study took place very early in the lambs life, and likely over their first experience of footrot, it would be interesting to collect contact data from a flock with older lambs, to see if there are changes in disease spread, as a result of lambs no longer being naïve to disease. Since lameness affects sheep behaviour, further work could explore which behaviours are affected, using either data from either observation or devices such as accelerometers, which can be used to predict whether sheep are grazing, walking, lying or standing. These could then be linked to performance indicators such as finishing weight, to create a full picture of the impact of lameness on lambs.

Carrying out a study over a longer time period may increase the power to detect any social transmission between non-related sheep. Additionally, social contact patterns in lambs change as they age (Norton et al., 2012), and carrying out studies at different points in the production cycle would determine if changes in contact patterns relate to any changes in disease spread.

Further work could also explore how other factors, such as variation in bacterial load or severity of foot lesion, relate to the probability of transmission between sheep.

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Appendices

Appendix 1 Fit statistics for the latent class models tested (2-7 classes) for models for type and frequency of treatment of lambs with interdigital dermatitis or severe footrot in 823 flocks of sheep in England, 2012-2013

Number of classes	Model 1: Treatments for lambs			
	AIC	BIC	G^2	Log likelihood
1	13028.20	13131.88	3485.89	-6492.10
2	12450.22	12662.31	2861.92	-6180.11
3	12207.37	12527.85	2573.06	-6035.69
4	12071.02	12499.90	2390.71	-5944.51
5	11970.96	12508.24	2244.65	-5871.48
6	11891.13	12536.81	2118.83	-5808.57
7	11840.86	12594.93	2022.55	-5760.43

1. AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; G^2 = likelihood/deviance statistic

Appendix 2 Fit statistics for the latent class models tested (2-7 classes) for models for type and frequency of treatment of ewes with interdigital dermatitis or severe footrot in 908 flocks of sheep in England, 2012-2013.

Number of classes	Model 2: Treatments for ewes			
	AIC	BIC	G^2	Log likelihood
1	14158.61	14264.46	3288.63	-7057.31
2	13619.21	13835.71	2703.22	-6764.60
3	13494.80	13821.97	2532.82	-6679.40
4	13370.82	13808.64	2362.84	-6594.41
5	13289.63	13838.11	2235.64	-6530.81
6	13248.76	13907.90	2148.78	-6487.38
7	13219.86	13989.66	2073.88	-6449.93

1. AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; G^2 = likelihood/deviance statistic

Appendix 3 Class conditional response probabilities that a farmer used a type and frequency of treatment for lambs with interdigital dermatitis or severe footrot, and standard errors for 823 flocks of sheep in England, 2012-2013

Treatment and use frequency	Class conditional response probability (standard error)			
	LC1	LC2	LC3	LC4
Severe footrot				
Antibiotic injection				
Never	0.44 (0.07)	0.16 (0.03)	0.38 (0.03)	0.00 (0.00)
Sometimes	0.47 (0.07)	0.54 (0.04)	0.43 (0.03)	0.50 (0.04)
Usually	0.03 (0.02)	0.25 (0.04)	0.08 (0.02)	0.19 (0.03)
Always	0.06 (0.03)	0.05 (0.02)	0.11 (0.02)	0.31 (0.04)
Foot spray				
Never	0.23 (0.07)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Sometimes	0.75 (0.07)	0.10 (0.03)	0.00 (0.00)	0.01 (0.01)
Usually	0.02 (0.04)	0.82 (0.05)	0.04 (0.02)	0.01 (0.02)
Always	0.00 (0.00)	0.08 (0.04)	0.96 (0.02)	0.98 (0.02)
Foot trimming				
Never	0.36 (0.06)	0.14 (0.03)	0.08 (0.02)	0.14 (0.03)
Sometimes	0.60 (0.06)	0.45 (0.04)	0.47 (0.03)	0.39 (0.04)
Usually	0.01 (0.01)	0.35 (0.04)	0.20 (0.03)	0.19 (0.03)
Always	0.02 (0.02)	0.06 (0.02)	0.24 (0.03)	0.28 (0.04)
Interdigital dermatitis				
Antibiotic injection				
Never	0.66 (0.07)	0.44 (0.05)	0.99 (0.01)	0.00 (0.00)
Sometimes	0.32 (0.06)	0.40 (0.04)	0.01 (0.01)	0.79 (0.03)
Usually	0.00 (0.00)	0.16 (0.03)	0.00 (0.00)	0.11 (0.02)
Always	0.02 (0.02)	0.00 (0.00)	0.00 (0.00)	0.10 (0.02)
Foot spray				
Never	0.20 (0.06)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Sometimes	0.61 (0.07)	0.15 (0.03)	0.05 (0.02)	0.02 (0.01)
Usually	0.09 (0.03)	0.69 (0.05)	0.08 (0.02)	0.03 (0.02)
Always	0.11 (0.04)	0.17 (0.04)	0.86 (0.03)	0.94 (0.02)
Foot trimming				
Never	0.65 (0.06)	0.33 (0.04)	0.37 (0.03)	0.22 (0.04)
Sometimes	0.33 (0.06)	0.49 (0.04)	0.49 (0.03)	0.52 (0.04)
Usually	0.01 (0.01)	0.16 (0.03)	0.07 (0.02)	0.13 (0.03)
Always	0.01 (0.01)	0.02 (0.01)	0.08 (0.02)	0.13 (0.03)
Time to treatment				
<1 day	0.06 (0.02)	0.06 (0.02)	0.07 (0.02)	0.08 (0.02)
1-<3 days	0.37 (0.05)	0.43 (0.04)	0.42 (0.03)	0.47 (0.03)
>3-<7 days	0.38 (0.05)	0.39 (0.04)	0.40 (0.03)	0.37 (0.03)
>7 days	0.19 (0.04)	0.12 (0.03)	0.12 (0.02)	0.08 (0.02)
None treated	0.01 (0.01)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

Appendix 4 Class conditional response probabilities that a farmer used a type and frequency of treatment on ewes with interdigital dermatitis or severe footrot, and standard error for 908 flocks of sheep in England, 2012-2013

Treatment and use frequency	Class conditional response probability (standard error)			
	LC1	LC2	LC3	LC4
Severe footrot				
Antibiotic injection				
Never	0.13 (0.06)	0.00 (0.00)	0.09 (0.02)	0.07 (0.01)
Sometimes	0.44 (0.07)	0.26 (0.05)	0.52 (0.04)	0.45 (0.03)
Usually	0.19 (0.06)	0.26 (0.04)	0.35 (0.04)	0.23 (0.02)
Always	0.24 (0.06)	0.47 (0.05)	0.05 (0.02)	0.25 (0.02)
Foot spray				
Never	0.21 (0.09)	0.01 (0.01)	0.01 (0.01)	0.00 (0.00)
Sometimes	0.66 (0.09)	0.03 (0.02)	0.11 (0.03)	0.00 (0.00)
Usually	0.08 (0.05)	0.11 (0.04)	0.77 (0.05)	0.02 (0.01)
Always	0.04 (0.05)	0.85 (0.04)	0.11 (0.05)	0.97 (0.01)
Foot trimming				
Never	0.09 (0.05)	0.14 (0.03)	0.03 (0.01)	0.00 (0.00)
Sometimes	0.41 (0.07)	0.67 (0.06)	0.26 (0.04)	0.01 (0.01)
Usually	0.25 (0.06)	0.19 (0.07)	0.51 (0.04)	0.32 (0.03)
Always	0.25 (0.06)	0.00 (0.00)	0.20 (0.04)	0.67 (0.03)
Interdigital dermatitis				
Antibiotic injection				
Never	0.53 (0.08)	0.32 (0.05)	0.56 (0.05)	0.52 (0.03)
Sometimes	0.33 (0.06)	0.48 (0.05)	0.31 (0.04)	0.34 (0.02)
Usually	0.07 (0.04)	0.12 (0.03)	0.12 (0.03)	0.08 (0.01)
Always	0.07 (0.04)	0.09 (0.03)	0.00 (0.00)	0.05 (0.01)
Foot spray				
Never	0.23 (0.08)	0.00 (0.00)	0.04 (0.02)	0.00 (0.00)
Sometimes	0.75 (0.09)	0.01 (0.02)	0.05 (0.03)	0.04 (0.01)
Usually	0.00 (0.00)	0.08 (0.03)	0.76 (0.05)	0.10 (0.02)
Always	0.02 (0.03)	0.90 (0.04)	0.15 (0.04)	0.86 (0.02)
Foot trimming				
Never	0.51 (0.09)	0.57 (0.05)	0.30 (0.05)	0.12 (0.02)
Sometimes	0.35 (0.06)	0.43 (0.05)	0.44 (0.04)	0.50 (0.03)
Usually	0.09 (0.05)	0.00 (0.00)	0.22 (0.03)	0.21 (0.02)
Always	0.06 (0.03)	0.00 (0.00)	0.03 (0.02)	0.17 (0.02)
Time to treatment				
<1 day	0.06 (0.03)	0.08 (0.03)	0.06 (0.02)	0.06 (0.01)
1-<3 days	0.28 (0.06)	0.48 (0.05)	0.32 (0.04)	0.49 (0.02)
>3-<7 days	0.45 (0.07)	0.40 (0.05)	0.46 (0.04)	0.36 (0.02)
>7 days	0.20 (0.05)	0.04 (0.02)	0.16 (0.03)	0.10 (0.01)
None treated	0.01 (0.01)	0.00 (0.00)	0.01 (0.01)	0.00 (0.00)

Appendix 5 Summary of the predictors selected in the sub-models with prevalence of lameness in lambs in 1271 flocks of sheep in England. The full sub-models (with number and % of flocks in each category) are available online in the paper Supplementary Material.

Predictor	**OR (95% CI)**		
	>2-5% LiL	**>5-10% LiL**	**>10% LiL**
a) Catching and recognising lame sheep (N = 1222)			
Locomotion score farmer recognised sheep as lame at			
1	Ref	Ref	Ref
2	1.15 (0.85-1.55)	1.25 (0.82-1.90)	1.66 (0.95-2.89)
3	0.71 (0.44-1.14)	**0.48 (0.23-1.01)**	1.80 (0.83-3.89)
4	1.26 (0.32-5.01)	**4.85 (1.23-19.19)**	**7.93 (1.49-42.33)**
Minimum locomotion score when farmer decided to treat lame sheep			
1	Ref	Ref	Ref
2	1.17 (0.83-1.64)	1.33 (0.8-2.22)	0.80 (0.44-1.45)
3	**1.49 (1.00-2.22)**	1.66 (0.93-2.98)	0.79 (0.39-1.61)
4 or more	1.47 (0.78-2.75)	**2.43 (1.06-5.56)**	0.65 (0.20-2.09)
Did not treat	3.07 (0.50-18.8)	2.23 (0.15-32.99)	**0.00 (0.00-0.00)**
Number of sheep lame treated at minimum locomotion score recognised as			
1	Ref	Ref	Ref
2-5	1.30 (0.88-1.93)	1.06 (0.58-1.94)	1.22 (0.55-2.68)
6-10	1.52 (0.94-2.46)	**2.01 (1.02-3.97)**	2.44 (1.02-5.82)
>10	**1.76 (1.07-2.89)**	**2.06 (1.02-4.16)**	3.07 (1.29-7.30)
Individuals not	0.41 (0.06-2.84)	**0.00 (0.00-0.00)**	0.00 (0.00-0.00)
Time to treatment once sheep recognised as lame			
<1 day	Ref	Ref	Ref
1-<3 days	1.55 (0.92-2.60)	2.07 (0.83-5.20)	3.92 (0.89-17.4)
>3-7 days	**1.81 (1.06-3.10)**	**3.18 (1.26-8.03)**	**4.83 (1.08-21.65)**
>7 days	1.50 (0.79-2.84)	2.69 (0.96-7.53)	3.44 (0.68-17.36)
Individuals not	0.57 (0.04-8.88)	**0.11 (0.11-0.11)**	**0.51 (0.51-0.51)**
Use central handling facility to catch lame sheep			
No	Ref	Ref	Ref
Yes	1.00 (0.76-1.31)	1.28 (0.87-1.90)	**1.95 (1.17-3.25)**
Use dog that can catch individuals to catch lame sheep			
No	Ref	Ref	Ref
Yes	1.13 (0.77-1.67)	**1.79 (1.08-2.99)**	**2.67 (1.47-4.84)**
b) Treating lambs lame with ID and SFR (N = 899)			
Treat lambs with SFR with antibiotic injection			
Always	Ref	Ref	Ref
Usually	0.81 (0.47-1.42)	0.89 (0.40-1.98)	0.56 (0.22-1.46)

Predictor	**OR (95% CI)**		
	>2-5% LiL	**>5-10% LiL**	**>10% LiL**
Sometimes	**0.61 (0.38-0.96)**	1.13 (0.59-2.15)	0.55 (0.26-1.16)
Never	**0.55 (0.33-0.93)**	0.51 (0.23-1.10)	**0.38 (0.15-0.92)**
Treat lambs with SFR with foot trim			
Always	Ref	Ref	Ref
Usually	0.86 (0.51-1.47)	0.71 (0.35-1.43)	0.97 (0.39-2.42)
Sometimes	1.04 (0.64-1.69)	0.94 (0.50-1.76)	1.53 (0.66-3.53)
Never	**0.46 (0.25-0.85)**	0.15 (0.05-0.42)	**0.19 (0.04-0.97)**
Treat lambs with ID with foot trim			
Always	Ref	Ref	Ref
Usually	0.72 (0.30-1.71)	**0.33 (0.11-0.96)**	0.39 (0.11-1.46)
Sometimes	0.63 (0.29-1.34)	**0.33 (0.14-0.81)**	0.35 (0.12-1.06)
Never	0.66 (0.30-1.47)	0.43 (0.17-1.10)	**0.22 (0.06-0.74)**
Use Lincospectin™ foot spray			
No	Ref	Ref	Ref
Yes	1.72 (1.02-2.89)	1.48 (0.72-3.04)	**3.37 (1.59-7.13)**
Use antibiotic aerosol foot spray			
No	Ref	Ref	Ref
Yes	1.09 (0.68-1.73)	1.56 (0.76-3.20)	3.79 (1.11-12.91)
c) Treating ewes lame with ID and SFR (N = 980)			
Treat ewes with SFR with antibiotic injection			
Always	Ref	Ref	Ref
Usually	0.90 (0.60-1.36)	**2.01 (1.12-3.59)**	0.98 (0.49-1.96)
Sometimes	1.13 (0.79-1.62)	1.36 (0.78-2.38)	0.84 (0.44-1.58)
Never	0.81 (0.44-1.49)	0.82 (0.3-2.22)	0.29 (0.06-1.35)
Treat ewes with ID with foot trim			
Always	Ref	Ref	Ref
Usually	1.11 (0.63-1.95)	0.99 (0.45-2.18)	0.95 (0.39-2.36)
Sometimes	1.03 (0.63-1.68)	0.87 (0.44-1.72)	0.77 (0.35-1.70)
Never	1.11 (0.65-1.89)	0.96 (0.46-2.00)	**0.33 (0.13-0.87)**
Treat ewes with SFR with foot spray			
Always	Ref	Ref	Ref
Usually	**0.78 (0.54-1.13)**	0.81 (0.49-1.36)	0.89 (0.46-1.72)
Sometimes	**0.60 (0.36-1.01)**	0.81 (0.41-1.61)	1.19 (0.52-2.71)
Never	0.30 (0.10-0.87)	0.23 (0.03-1.91)	0.87 (0.10-7.57)
Use Lincospectin™ foot spray			
No	Ref	Ref	Ref
Yes	1.27 (0.78-2.09)	1.36 (0.69-2.67)	**3.75 (1.92-7.34)**

Predictor	OR (95% CI)		
	>2-5% LiL	**>5-10% LiL**	**>10% LiL**
Use antibiotic aerosol foot spray			
No	Ref	Ref	Ref
Yes	1.27 (0.78-2.09)	1.20 (0.58-2.48)	**3.72 (1.06-13.04)**
d) Routine foot trim of the flock (N = 1206)			
Did not trim	Ref	Ref	Ref
Trimmed but no	0.69 (0.41-1.18)	0.50 (0.22-1.15)	1.10 (0.40-3.03)
Caused bleeding	1.27 (0.97-1.65)	1.00 (0.69-1.45)	**2.78 (1.68-4.58)**
e) Footbathing of the flock (N = 833)			
Footbath lambs			
No	Ref	Ref	Ref
Yes	**2.25 (1.34-3.79)**	1.47 (0.68-3.17)	1.82 (0.72-4.61)
Footbath to treat			
No	Ref	Ref	Ref
Yes	1.15 (0.82-1.61)	0.83 (0.53-1.30)	**2.08 (1.18-3.67)**
Footbath to treat ID			
No	Ref	Ref	Ref
Yes	0.98 (0.66-1.45)	**3.76 (1.90-7.43)**	1.25 (0.64-2.45)
Footbath to prevent			
No	Ref	Ref	Ref
Yes	0.78 (0.55-1.10)	0.68 (0.43-1.08)	**0.42 (0.24-0.73)**
Routine footbathing			
Did not do routinely	Ref	Ref	Ref
Once a week	2.88 (0.71-11.66)	**8.06 (1.82-**	**6.79 (0.99-46.65)**
Once a fortnight	1.18 (0.67-2.06)	1.35 (0.63-2.88)	**3.15 (1.28-7.76)**
Once a month	**1.56 (1.01-2.42)**	1.78 (0.98-3.22)	**3.32 (1.59-6.90)**
Other	1.01 (0.65-1.57)	1.12 (0.61-2.04)	**2.19 (1.05-4.56)**
Footbathing of ewes before housing			
No	Ref	Ref	Ref
Yes	0.78 (0.56-1.10)	0.83 (0.52-1.33)	0.46 (0.25-0.83)
f) Culling and replacement of ewes (N = 1135)			
Number of times sheep lame before culling			
Did not cull when	Ref	Ref	Ref
1	0.51 (0.23-1.13)	0.65 (0.22-1.93)	0.00 (0.00-0.00)
2	0.91 (0.59-1.38)	1.10 (0.62-1.95)	1.01 (0.46-2.20)
2 or more	**1.49 (1.08-2.06)**	**2.16 (1.41-3.29)**	**2.00 (1.16-3.45)**
Persistently lame	**2.24 (1.17-4.26)**	1.46 (0.55-3.89)	**3.21 (1.23-8.33)**
Use of EID ear tag to identify sheep for culling			

Predictor	OR (95% CI)		
	>2-5% LiL	**>5-10% LiL**	**>10% LiL**
No	Ref	Ref	Ref
Yes	**0.53 (0.29-1.00)**	0.88 (0.29-1.00)	0.47 (0.14-1.59)
Did not breed replacement ewes			
No	Ref	Ref	Ref
Yes	**1.41 (1.03-1.92)**	1.15 (0.74-1.78)	1.17 (0.67-2.05))
g) Vaccination of whole flock with FootVax™			
No sub-model built.			
h) Whole flock antibiotic treatment (N = 1271)			
Use of oxytetracycline LA for whole flock antibiotic injection			
No	Ref	Ref	Ref
Yes	1.27 (0.78-2.07))	1.64 (0.88-3.05)	**2.36 (1.20-4.66)**
i) Farm biosecurity (N = 1252)			
Feet of new sheep checked before purchase			
Never	Ref	Ref	Ref
Sometimes	0.95 (0.59-1.51)	0.98 (0.50-1.91)	1.19 (0.57-2.51)
Usually	1.13 (0.73-1.74)	1.05 (0.56-1.96)	1.29 (0.65-2.6)
Always	**0.59 (0.39-0.89)**	0.73 (0.41-1.31)	0.51 (0.25-1.04)
Did not purchase	**0.57 (0.37-0.88)**	0.82 (0.45-1.49)	0.49 (0.23-1.04)
Having sheep that did not return to the farm			
No	Ref	Ref	Ref
Yes	1.01 (0.78-1.32)	0.76 (0.53-1.09)	**0.60 (0.39-0.93)**
Mixing sheep with neighbouring flocks			
Yes	Ref	Ref	Ref
No	**0.54 (0.29-0.99)**	**0.46 (0.22-0.97)**	1.92 (0.44-8.44)
Unknown	0.48 (0.09-2.44)	**0.00 (0.00-0.00)**	0.00 (0.00-
j) Farm and farmer characteristics (N = 1189)			
Bought in			
No	Ref	Ref	Ref
Yes	**1.52 (1.18-1.97)**	1.01 (0.71-1.45)	1.65 (1.04-2.60)
Ewe stocking rate			
<4 ewes/acre	Ref	Ref	Ref
4-8 ewes/acre	0.88 (0.68-1.14)	1.02 (0.71-1.47)	**1.62 (1.01-2.59)**
>8 ewes/acre	0.80 (0.42-1.51)	0.67 (0.25-1.82)	1.83 (0.70-4.83)

1. N = number of flocks in the model, OR: odds ratio, CI: confidence interval. Odds ratios significantly different from the baseline (according to Wald's test for significance) are marked in bold, LiL = prevalence of lameness in lambs. Significance was defined when p≤0.05.

Appendix 6 Summary of the predictors selected in the sub-models with prevalence of lameness in lambs in 1271 flocks of sheep in England. The full sub-models (with number and % of flocks in each category) are available online in the paper Supplementary Material.

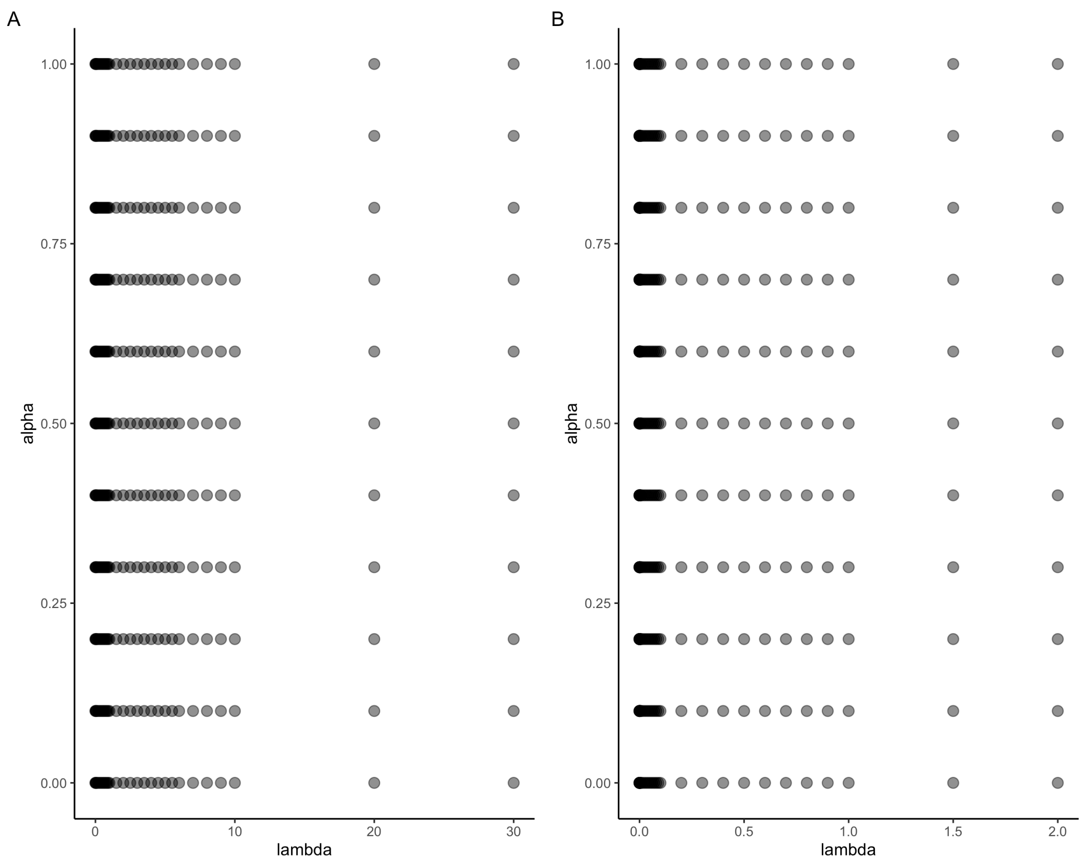
Predictor	Association with lame sheep in sub-model		
	>2-5% LiE	>5-10% LiE	>10% LiE
a) Catching and recognising lame sheep (N = 1233)			
Number of sheep lame treated at minimum locomotion score recognised as lame			
1	Ref	Ref	Ref
2-5	**1.53 (1.44-2.24)**	**3.01 (1.55-5.83)**	0.90 (0.38-2.13)
6-10	**2.15 (1.31-3.52)**	**5.76 (2.74-12.11)**	**4.16 (1.71-10.11)**
>10	**2.42 (1.44-4.06)**	**5.51 (2.55-11.91)**	**5.78 (2.36-14.15)**
Individuals not	1.36 (0.21-8.62)	5.45 (0.78-38.15)	0.00 (0.00-0.00)
Time to treatment once sheep recognised as lame			
<1 day	Ref	Ref	Ref
1-<3 days	**1.94 (1.14-3.30)**	1.04 (0.51-2.14)	3.33 (0.74-14.95)
>3-7 days	**2.85 (1.63-4.96)**	**2.45 (1.19-5.04)**	**5.85 (1.29-26.44)**
>7 days	**2.59 (1.32-5.07)**	1.74 (0.73-4.11)	**8.03 (1.66-38.79)**
Individuals not	**0.00 (0.00-0.00)**	**0.00 (0.00-0.00)**	0.21 (0.21-0.21)
Minimum locomotion score when farmer decided to treat lame sheep			
1	Ref	Ref	Ref
2	**1.31 (0.97-1.76)**	**1.63 (1.12-2.38)**	**1.98 (1.16-3.39)**
3	**1.29 (0.81-2.05)**	1.65 (0.92-2.97)	**2.36 (1.13-4.92)**
4 or more	**1.40 (0.40-4.94)**	2.44 (0.62-9.69)	3.92 (0.78-19.58)
Use central handling facility to catch lame sheep			
No	Ref	Ref	Ref
Yes	1.28 (0.98-1.69)	**1.52 (1.06-2.18)**	**1.75 (1.05-2.92)**
Use dog to gather the flock			
No	Ref	Ref	Ref
Yes	**1.42 (1.06-1.91)**	1.37 (0.93-2.01)	0.98 (0.56-1.71)
b) Treatment of ewes with ID and SFR (N = 1003)			
Treat ewes with SFR with antibiotic injection			
Always	Ref	Ref	Ref
Usually	1.62 (1.06-2.48)	**1.98 (1.15-3.40)**	1.96 (0.86-4.47)
Sometimes	1.31 (0.9-1.9)	1.58 (0.97-2.57)	**2.03 (0.99-4.18)**
Never	**0.38 (0.20-0.74)**	0.71 (0.32-1.57)	0.38 (0.08-1.79)
Treat ewes with SFR			
Always	Ref	Ref	Ref

Predictor	Association with lame sheep in sub-model		
	>2-5% LiE	**>5-10% LiE**	**>10% LiE**
Usually	0.81 (0.57-1.15)	1.20 (0.78-1.86)	0.71 (0.38-1.29)
Sometimes	0.68 (0.45-1.01)	0.89 (0.53-1.47)	**0.35 (0.15-0.80)**
Never	0.22 (0.09-0.51)	**0.16 (0.04-0.70)**	**0.00 (0.00-0.00)**
Treat ewes with ID			
Always	Ref	Ref	Ref
Usually	0.89 (0.62-1.29)	1.15 (0.74-1.80)	1.14 (0.59-2.18)
Sometimes	1.11 (0.67-1.85)	1.09 (0.57-2.07)	1.60 (0.69-3.7-)
Never	**0.42 (0.18-0.99**)	0.84 (0.33-2.17)	0.00 (0.00-0.00)
c) Treatment of lambs with ID and SFR (N = 899)			
Treat lambs with SFR with antibiotic injection			
Always	Ref	Ref	Ref
Usually	0.81 (0.47-1.42)	0.89 (0.4-1.98)	0.56 (0.22-1.46)
Sometimes	**0.61 (0.38-0.96)**	1.13 (0.59-2.15)	0.55 (0.26-1.16)
Never	**0.55 (0.33-0.93)**	0.51 (0.23-1.10)	**0.38 (0.15-0.92)**
Treat lambs with SFR with foot trim			
Always	Ref	Ref	Ref
Usually	0.86 (0.51-1.47)	0.71 (0.35-1.43)	0.97 (0.39-2.42)
Sometimes	1.04 (0.64-1.69)	0.94 (0.50-1.76)	1.53 (0.66-3.53)
Never	**0.46 (0.25-0.85)**	**0.15 (0.05-0.42)**	**0.19 (0.04-0.97)**
Treat lambs with ID with foot trim			
Always	Ref	Ref	Ref
Usually	0.72 (0.30-1.71)	0.33 (0.11-0.96)	0.39 (0.11-1.46)
Sometimes	0.63 (0.29-1.34)	**0.33 (0.14-0.81)**	0.35 (0.12-1.06)
Never	0.66 (0.30-1.47)	**0.43 (0.17-1.10)**	**0.22 (0.06-0.74)**
Use of			
No	Ref	Ref	Ref
Yes	**1.72 (1.02-2.89)**	1.48 (0.72-3.04)	3.37 (1.59-7.13)
Use of antibiotic			
No			
Yes	1.09 (0.68-1.73)	1.56 (0.76-3.2)	**3.79 (1.11-12.91)**
d) Routine foot trim of the flock (N = 1206)			
Did not trim	Ref	Ref	Ref
Trimmed but no			
Caused bleeding	**1.46 (1.11-1.92)**	**2.43 (1.70-3.48)**	**4.81 (2.74-8.44)**
e) Footbathing of the flock (N = 827)			

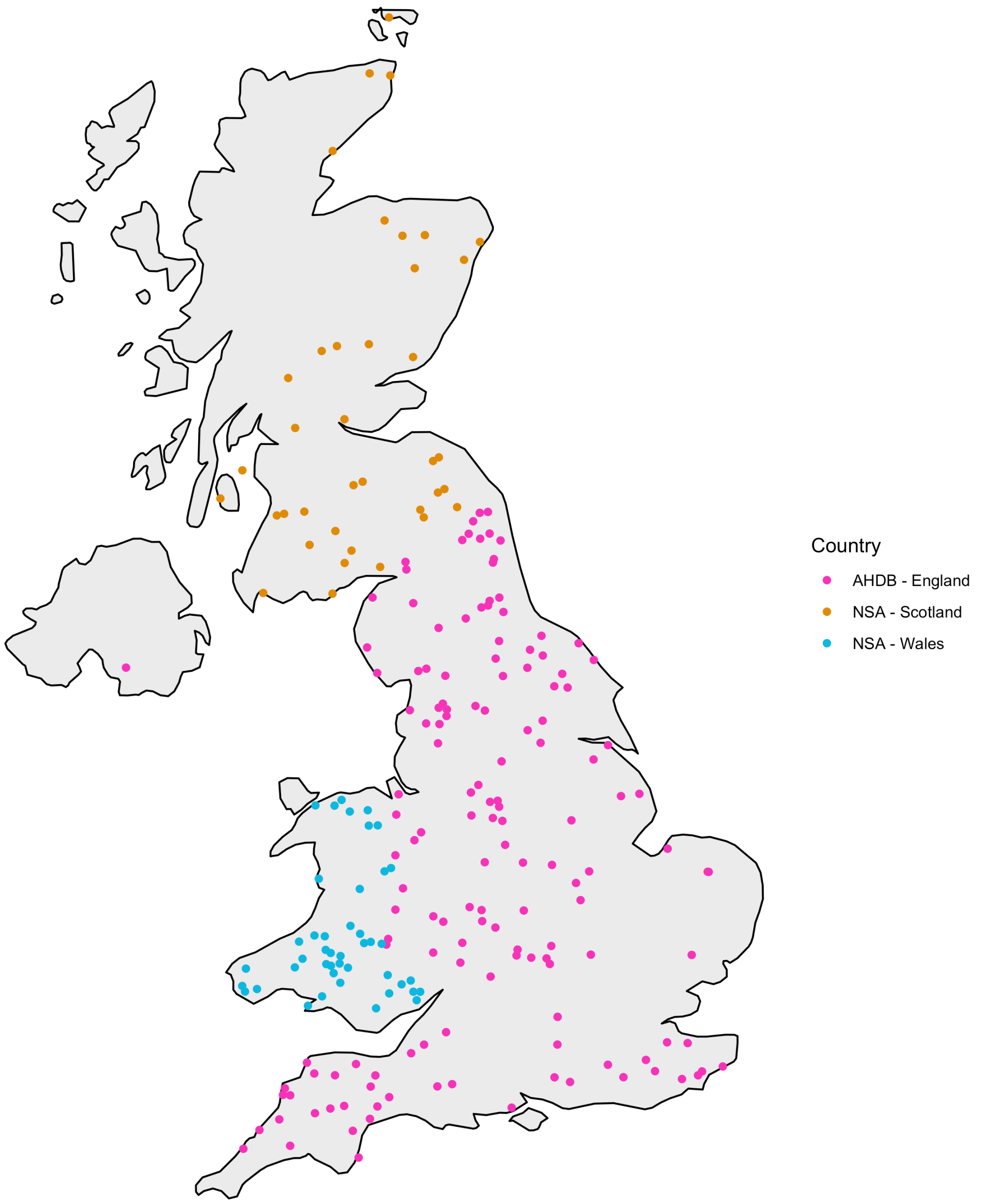
Predictor	Association with lame sheep in sub-model		
	>2-5% LiE	**>5-10% LiE**	**>10% LiE**
Footbath to treat SFR			
No	Ref	Ref	Ref
Yes	**1.60 (1.14-2.25)**	**2.24 (1.46-3.44)**	**2.82 (1.52-5.20)**
Footbath to prevent			
No	Ref	Ref	Ref
Yes	1.00 (0.70-1.42)	0.93 (0.59-1.44)	**0.35 (0.19-0.66)**
Footbath sheep when moving field			
No	Ref	Ref	Ref
Yes	1.38 (0.93-2.05)	**1.88 (1.18-3.02)**	1.66 (0.88-3.12)
Footbath sheep when sheep return to farm			
No	Ref	Ref	Ref
Yes	0.87 (0.51-1.47)	**1.80 (1.02-3.20)**	0.78 (0.31-1.96)
Footbath lambs at turnout			
No	Ref	Ref	Ref
Yes	**0.28 (0.12-0.68)**	0.51 (0.2-1.29)	0.62 (0.16-2.38)
Frequency of use of footbath for ewes at pasture			
Once a week or	Ref	Ref	Ref
Once a month	0.62 (0.32-1.21)	1.20 (0.54-2.66)	1.48 (0.48-4.62)
Other	**0.51 (0.26-0.98)**	0.60 (0.26-1.39)	0.80 (0.25-2.59)
Did not do routinely	**0.43 (0.22-0.83)**	0.75 (0.33-1.70)	0.46 (0.14-1.54)
f) Culling and replacement of ewes (N = 1135)			
Number of times			
Did not cull when	Ref	Ref	Ref
1	**0.38 (0.17-0.83)**	**0.28 (0.08-0.95)**	0.55 (0.12-2.44)
2	1.18 (0.77-1.80)	1.01 (0.58-1.76)	1.09 (0.47-2.51)
2 or more	**2.20 (1.56-3.10)**	**1.93 (1.26-2.96)**	**2.31 (1.29-4.17)**
Persistently lame	**2.91 (1.39-6.09)**	**2.51 (1.04-6.01)**	2.74 (0.88-8.53)
Avoidance of selection of replacement ewes from ewes repeatedly lame mothers			
Yes	Ref	Ref	Ref
No	**1.04 (0.76-1.43)**	**1.71 (1.14-2.57)**	**1.94 (1.05-3.59)**
Breeding of			
Yes	Ref	Ref	Ref
No	1.21 (0.83-1.74)	1.46 (0.90-2.36)	**2.52 (1.30-4.90)**
g) Vaccination with FootVax™			
No sub-model built			

Predictor	Association with lame sheep in sub-model		
	>2-5% LiE	>5-10% LiE	>10% LiE
h) Whole flock antibiotic treatment			
No sub-model built			
i) Farm biosecurity (N= 1222)			
New sheep isolated on arrival			
Never	Ref	Ref	Ref
Sometimes	0.64 (0.34-1.19)	1.14 (0.55-2.36)	0.39 (0.13-1.21)
Usually	0.74 (0.43-1.28)	1.25 (0.65-2.40)	0.56 (0.23-1.37)
Always	**0.61 (0.39-0.96)**	**0.56 (0.31-0.99)**	0.60 (0.30-1.21)
No new sheep	0.81 (0.31-2.09)	0.54 (0.16-1.85)	**0.18 (0.04-0.76)**
Feet of new sheep checked before			
Never	Ref	Ref	Ref
Sometimes	1.60 (0.98-2.63)	1.31 (0.72-2.39)	1.16 (0.53-2.57)
Usually	**2.28 (1.42-3.64)**	**1.85 (1.06-3.22)**	1.37 (0.64-2.92)
Always	1.04 (0.68-1.59)	0.59 (0.34-1.02)	0.55 (0.27-1.15)
No new sheep	0.85 (0.34-2.15)	1.15 (0.35-3.77)	1.95 (0.50-7.65)
j) Farm and farmer characteristics (N = 1229)			
Ewe stocking rate			
<4 ewes/acre	Ref	Ref	Ref
4-8 ewes/acre	1.06 (0.81-1.38)	1.28 (0.91-1.80)	**1.96 (1.18-3.26)**
>8 ewes/acre	0.74 (0.37-1.47)	1.25 (0.56-2.76)	**2.68 (1.02-7.02)**
Home-bred			
No	Ref	Ref	Ref
Yes	**0.73 (0.55-97)**	**0.60 (0.42-0.85)**	0.62 (0.38-1.01)
Housed ewes			
No	Ref	Ref	Ref
Yes	**1.53 (1.14-2.05)**	1.39 (0.96-2.03)	1.20 (0.71-2.01)

1. N = number of flocks in the model, OR: odds ratio, CI: confidence interval, LiE = prevalence of lameness in ewes. Odds ratios significantly different from the baseline (according to Wald's test for significance) are marked in bold. Significance was defined when p≤0.05.



Appendix 7 The distribution of values of alpha and lambda used for hyperparameter tuning in the elastic net models for A) Poisson models and B) Gaussian models



Appendix 8 Location of the 227 flocks who supplied a valid postcode, coloured by the source of the questionnaire (AHDB – pink, NSA sent to Scotland – orange, NSA sent to Wales – blue).

Appendix 9 The number of flocks affected by each type of foot lesion (interdigital dermatitis, severe footrot, contagious ovine digital dermatitis, granuloma, shelly hoof and white line abscess), the overall geometric mean prevalence of each foot lesion in Great Britain (October 2017-September 2018), and the geometric mean prevalence in flocks only where the foot lesion was reported.

Foot lesion	Farms affected (%)	Overall GM prevalence (%)	N	GM prevalence in farms where foot lesion present (%)	N
Interdigital dermatitis	87.8				
Ewes		0.41 (0.26-0.63)	408	2.53 (2.27-2.82)	348
Lambs		0.50 (0.32-0.79)	418	3.66 (3.28-4.08)	353
Severe footrot	75.3				
Ewes		0.09 (0.06-0.16)	430	1.90 (1.69-2.13)	324
Lambs		0.01 (0.00-0.01)	389	2.32 (2.04-2.64)	200
Contagious ovine digital dermatitis	36.9				
Ewes		0.00 (0.00-0.00)	426	1.64 (1.38-1.96)	159
Lambs		0.00 (0.00-0.00)	408	2.36 (1.84-3.03)	90
Granuloma	46.9				
Ewes		0.00 (0.00-0.00)	428	0.81 (0.70-0.94)	200
Lambs		0.00 (0.00-0.00)	381	0.84 (0.47-1.49)	20
Shelly hoof	58.7				
Ewes		0.02 (0.01-0.03)	421	2.04 (1.76-2.36)	253
Lambs		0.00 (0.00-0.00)	351	1.51 (1.18-1.93)	79
White line abscess	30.7				
Ewes		0.00 (0.00-0.00)	401	1.10 (0.94-1.29)	127
Lambs		0.00 (0.00-0.00)	376	1.21 (0.89-1.64)	46

1. N = number of flocks, GM = geometric mean, % = percentage of flocks using the denominator of 450 useable responses
2. Overall geometric mean prevalence calculated as including flocks where prevalence of the foot lesion was given as 0, adding 0.00001 to allow log transformation. Geometric mean prevalence in farms where foot lesion present was calculated as prevalence where the prevalence of the foot lesion was >0

Appendix 10 Sub-models for the Quasi-Poisson generalized linear models for management practices associated with the proportion of lame ewes in 310 sheep flocks in Great Britain, October 2017-September 2018 for the four sections (treatment of ewes and lambs with footrot, management of lameness, culling and replacement of sheep, the farm and flock environment)

Predictor	N	%	RR	LCI	UCI	P-value
1. Treatment of ewes and lambs with footrot						
(Intercept)			0.02	0.01	0.03	<0.01
Flock size (number of ewes)						
101-500	91	29.4				
1-100	118	38.1	1.99	1.33	2.91	<0.01
501-1000	72	23.2	0.95	0.75	1.20	0.67
>1000	29	9.4	1.00	0.79	1.27	0.99
Country						
England	219	70.6				
Scotland	**43**	**13.9**	**0.68**	**0.54**	**0.85**	**<0.01**
Wales	**48**	**15.5**	**0.73**	**0.55**	**0.93**	**0.02**
Time to treatment of ewes with footrot						
0-3 days	165	53.2				
>3 days	**141**	**45.5**	**1.26**	**1.05**	**1.50**	**0.01**
None to treat	4	1.3	0.04	0.00	0.48	0.11
Footbath used to treat severe footrot						
No	230	74.2				
Yes	**80**	**25.8**	**1.53**	**1.27**	**1.84**	**<0.01**
Lambs with footrot treated with antibiotic injection						
Always	109	35.2				
Never	**65**	**21.0**	**1.46**	**1.05**	**2.01**	**0.02**
Sometimes	136	43.9	1.22	0.96	1.56	0.10
Ewes with footrot treated with antibiotic injection						
Always	51	16.5				
Never	**97**	**31.3**	**0.62**	**0.39**	**0.96**	**0.04**
Sometimes	162	52.3	0.87	0.67	1.12	0.27
Lambs with footrot treated with foot spray						
Always	239	77.1				
Never	**11**	**3.5**	**1.53**	**1.03**	**2.21**	**0.03**
Sometimes	**60**	**19.4**	**1.47**	**1.15**	**1.85**	**<0.01**
Footbath used to treat interdigital dermatitis						
No	170	54.8				
Yes	**140**	**45.2**	**1.27**	**1.03**	**1.58**	**0.03**
2. Management of lameness						

Predictor	N	%	RR	LCI	UCI	P-value
(Intercept)			0.03	0.03	0.04	<0.01
Flock size (number of ewes)						
101-500	91	29.4				
1-100	**118**	**38.1**	**1.62**	**1.12**	**2.30**	**<0.01**
501-1000	72	23.2	0.89	0.72	1.10	0.27
>1000	29	9.4	0.86	0.67	1.11	0.24
Country						
England	219	70.6				
Scotland	**43**	**13.9**	**0.73**	**0.58**	**0.91**	**<0.01**
Wales	48	15.5	0.82	0.64	1.04	0.12
% sheep feet bleeding at routine foot trim						
Did not foot trim	115	37.1				
0	**50**	**16.1**	**0.61**	**0.41**	**0.87**	**<0.01**
>0-<5	104	33.5	1.02	0.84	1.25	0.81
5-100	**41**	**13.2**	**1.80**	**1.43**	**2.25**	**<0.01**
Years FootVax™ used						
Did not vaccinate	219	70.6				
<1 year	**10**	**3.2**	**5.86**	**2.74**	**11.41**	**<0.01**
1-<2 years	**19**	**6.1**	**3.19**	**1.52**	**6.06**	**<0.01**
2-<5 years	**32**	**10.3**	**2.63**	**1.31**	**4.73**	**<0.01**
>5 years	30	9.7	1.64	0.80	3.03	0.14
Rams vaccinated with FootVax™						
No	244	78.7				
Yes	**66**	**21.3**	**0.71**	**0.53**	**0.95**	**0.02**
Frequency sheep vaccinated with FootVax™						
Did not vaccinate	221	71.3				
Once a year	**76**	**24.5**	**0.42**	**0.22**	**0.87**	**0.01**
Twice a year	6	1.9	0.71	0.33	1.65	0.41
Before footrot expected	**7**	**2.3**	**0.31**	**0.12**	**0.77**	**0.01**
3. Culling and replacement of sheep						
(Intercept)			0.02	0.02	0.03	<0.01
Flock size (number of ewes)						
101-500	91	29.4				
1-100	**118**	**38.1**	**1.74**	**1.13**	**2.60**	**<0.01**
501-1000	72	23.2	0.96	0.76	1.23	0.75
>1000	29	9.4	0.97	0.75	1.27	0.84
Country						
England	219	70.6				

Predictor	N	%	RR	LCI	UCI	P-value
Scotland	43	13.9	0.82	0.63	1.05	0.12
Wales	48	15.5	0.88	0.65	1.16	0.36
Quarantine of sheep returning to farm for >3 weeks						
Always	60	19.4				
Sometimes	**49**	**15.8**	**1.60**	**1.19**	**2.17**	**<0.01**
Never	**94**	**30.3**	**1.70**	**1.29**	**2.25**	**<0.01**
Missing	107	34.5	1.06	0.78	1.45	0.69
Culling of lame sheep						
Never	77	24.8				
Lame twice per year	**34**	**11.0**	**1.66**	**1.19**	**2.34**	**<0.01**
Lame three times per year	28	9.0	1.08	0.75	1.56	0.67
After persistently lame	164	52.9	0.92	0.70	1.24	0.59
Other	7	2.3	0.57	0.18	1.37	0.27
4. The farm and flock environment						
(Intercept)			0.03	0.03	0.04	<0.01
Flock size (number of ewes)						
101-500	91	29.4				
1-100	**118**	**38.1**	**1.71**	**1.11**	**2.54**	**0.01**
501-1000	72	23.2	1.00	0.79	1.28	1.00
>1000	29	9.4	1.08	0.84	1.40	0.53
Country						
England	219	70.6				
Scotland	**43**	**13.9**	**0.80**	**0.63**	**1.02**	**0.09**
Wales	**48**	**15.5**	**0.77**	**0.57**	**1.01**	**0.07**
Predominant soil type - peat						
No	265	85.5				
Yes	**45**	**14.5**	**0.56**	**0.43**	**0.73**	**<0.01**
Flock mixed with others via shared grazing						
No	285	91.9				
Yes	25	8.1	1.25	0.96	1.60	0.10

1. Dispersion parameters taken to be 7.5 (Sub-model 1), 6.4 (Sub-model 2), 8.6 (Sub-model 3), 8.7 (Sub-model 4).
2. RR = risk ratio, LCI = lower confidence interval, UCI = upper confidence interval, N = number of flocks
3. Variables, risk ratios and confidence intervals highlighted in bold where p<0.1 (Wald's test of significance).

Appendix 11 Multivariable Quasi-Poisson generalised linear model for the management practices associated with the proportion of lame ewes in 310 sheep flocks in Great Britain, October 2017-September 2018.

Predictor	N	%	RR	LCI	UCI	P-value
(Intercept)			0.02	0.02	0.03	<0.01
Country						
England	219	70.6	1.00			
Scotland	43	13.9	0.85	0.72	1.00	0.05
Wales	**48**	**15.5**	**0.76**	**0.64**	**0.90**	**<0.01**
Flock size (number of ewes)						
101-500	118	38.1	1.00			
1-100	91	29.4	1.26	0.98	1.60	0.07
501-1000	72	23.2	0.90	0.78	1.04	0.15
>1000	29	9.4	0.91	0.77	1.08	0.26
Problem flock (Decile 10 - ≥7.14% lameness)						
No	279	90.0	1.00			
Yes	**31**	**10.0**	**3.12**	**2.67**	**3.62**	**<0.01**
% sheep feet bleeding at routine foot trim						
Did not foot trim	115	37.1	1.00			
0	50	16.1	0.82	0.63	1.05	0.12
>0-<5	104	33.5	0.90	0.78	1.03	0.12
5-100	**41**	**13.2**	**1.31**	**1.13**	**1.51**	**<0.01**
Years FootVax™ used						
Did not vaccinate	219	70.6	1.00			
<1	**10**	**3.2**	**1.56**	**1.27**	**1.89**	**<0.01**
1-<2	**19**	**6.1**	**1.31**	**1.07**	**1.61**	**0.01**
2-5	32	10.3	0.90	0.77	1.06	0.22
>5	**30**	**9.7**	**0.75**	**0.60**	**0.92**	**<0.01**
Predominant soil type - peat						
No	265	85.5	1.00			
Yes	**45**	**14.5**	**0.77**	**0.65**	**0.90**	**<0.01**
Footbath to treat SFR						
No	230	74.2	1.00			
Yes	**80**	**25.8**	**1.27**	**1.12**	**1.42**	**<0.01**
Time to treatment of ewes with SFR						
0-3 days	165	53.2	1.00			
>3 days	141	45.5	0.86	0.73	1.01	0.06
None to treat	**4**	**1.3**	**0.07**	**0.00**	**0.41**	**0.03**
Quarantine sheep returning to farm for >3 weeks						
Always	60	19.4	1.00			

Predictor	N	%	RR	LCI	UCI	P-value
Sometimes	**49**	**15.8**	**1.25**	**1.04**	**1.51**	**0.02**
Never	**94**	**30.3**	**1.27**	**1.07**	**1.50**	**<0.01**
(Missing)	107	34.5	0.94	0.78	1.14	0.53
Time to treatment of lambs with SFR						
0-3 days	161	51.9	1.00			
>3 days	**131**	**42.3**	**1.27**	**1.07**	**1.50**	**<0.01**
None to treat	18	5.8	0.97	0.53	1.62	0.90
Stocking density						
<4 ewes/acre	164	52.9	1.00			
4-8 ewes/acre	**132**	**42.6**	**1.15**	**1.01**	**1.30**	**0.03**
>8 ewes/acre	14	4.5	1.27	0.99	1.62	0.06

1. RR = risk ratio, LCI = lower confidence interval, UCI = upper confidence interval, N = number of flocks, SFR = severe footrot
2. Variables highlighted in bold where $p<0.05$ (Wald's test of significance)
3. Dispersion parameter taken to be 2.81

Appendix 12 Sub-models for the negative binomial generalised linear models for management practices associated with the proportion of lame ewes in 310 sheep flocks in Great Britain, October 2017-September 2018 for the four sections (treatment of ewes and lambs with footrot, management of lameness, culling and replacement of sheep, the farm and flock environment)

Predictor	N	%	RR	LCI	UCI	P-value
1. Treatment of ewes and lambs with footrot						
(Intercept)			0.03	0.02	0.03	<0.01
Flock size (number of ewes)						
101-500	91	29.4				
1-100	**118**	**38.1**	**1.75**	**1.37**	**2.23**	**<0.01**
501-1000	72	23.2	0.94	0.76	1.17	0.59
>1000	29	9.4	0.88	0.66	1.18	0.37
Country						
England	219	70.6				
Scotland	43	13.9	0.86	0.67	1.12	0.25
Wales	48	15.5	0.82	0.64	1.05	0.11
Footbath used to treat SFR						
No	230	74.2				
Yes	**80**	**25.8**	**1.61**	**1.33**	**1.95**	**<0.01**
Time to treatment of ewes with SFR						
0-3 days	165	53.2				
>3 days	141	45.5	1.14	0.95	1.36	0.16
None to treat	**4**	**1.3**	**0.05**	**0.01**	**0.21**	**<0.01**
2. Management of lame ewes and lambs						
(Intercept)			0.02	0.02	0.03	<0.01
Flock size (number of ewes)						
101-500	91	29.4				
1-100	**118**	**38.1**	**1.62**	**1.26**	**2.07**	**<0.01**
501-1000	72	23.2	0.89	0.72	1.10	0.29
>1000	29	9.4	0.79	0.59	1.07	0.13
Country						
England	219	70.6				
Scotland	**43**	**13.9**	**0.79**	**0.62**	**1.02**	**0.06**
Wales	48	15.5	0.87	0.68	1.11	0.25
% sheep feet bleeding at routine foot trim						
Did not foot trim	115	37.1				
0	**50**	**16.1**	**0.67**	**0.50**	**0.89**	**<0.01**
>0-<5	104	33.5	1.06	0.87	1.30	0.56
5-100	**41**	**13.2**	**1.73**	**1.32**	**2.26**	**<0.01**
Years FootVax™ used						
Did not vaccinate	219	70.6				

Predictor	N	%	RR	LCI	UCI	P-value
<1 year	**10**	**3.2**	**1.78**	**1.17**	**2.82**	**<0.01**
1-<2 years	19	6.1	1.18	0.82	1.72	0.38
2-<5 years	32	10.3	0.85	0.64	1.14	0.25
>5 years	**30**	**9.7**	**0.57**	**0.43**	**0.77**	**<0.01**
Footbath used as routine practice						
No	209	67.4				
Yes	**101**	**32.6**	**0.82**	**0.68**	**0.98**	**0.03**
Formalin used in footbaths						
Did not footbath	66	21.3				
Always	**85**	**27.4**	**1.54**	**1.15**	**2.08**	**<0.01**
Sometimes	**79**	**25.5**	**1.51**	**1.11**	**2.04**	**<0.01**
Never	**80**	**25.8**	**1.34**	**1.00**	**1.78**	**0.05**
3. Culling and replacement of ewes						
(Intercept)			0.05	0.03	0.09	<0.01
Flock size (number of ewes)						
101-500	91	29.4				
1-100	**118**	**38.1**	**1.60**	**1.23**	**2.08**	**<0.01**
501-1000	72	23.2	1.07	0.85	1.35	0.58
>1000	29	9.4	1.05	0.77	1.44	0.77
Country						
England	219	70.6				
Scotland	43	13.9	0.94	0.73	1.24	0.67
Wales	48	15.5	0.83	0.65	1.09	0.18
Quarantine of new sheep for >3 weeks						
Always	162	52.3				
Did not purchase	**34**	**11.0**	**0.50**	**0.27**	**0.90**	**0.02**
Sometimes	**58**	**18.7**	**1.37**	**1.07**	**1.75**	**0.01**
Never	56	18.1	1.16	0.89	1.51	0.29
Open flock						
No	47	15.2				
Yes	**263**	**84.8**	**0.43**	**0.25**	**0.71**	**<0.01**
Source of replacement sheep						
Homebred	164	52.9				
Purchased	42	13.5	1.25	0.94	1.67	0.14
Homebred + purchased	**94**	**30.3**	**1.30**	**1.04**	**1.62**	**0.02**
Not applicable	10	3.2	1.19	0.68	2.17	0.55
4. The farm and flock environment						
(Intercept)			0.03	0.03	0.04	<0.01
Flock size (number of ewes)						

Predictor	N	%	RR	LCI	UCI	P-value
101-500	91	29.4				
1-100	**118**	**38.1**	**1.58**	**1.23**	**2.03**	**<0.01**
501-1000	72	23.2	0.99	0.79	1.25	0.95
>1000	29	9.4	0.92	0.68	1.27	0.62
Country						
England	219	70.6				
Scotland	43	13.9	0.88	0.68	1.15	0.32
Wales	**48**	**15.5**	**0.77**	**0.59**	**1.01**	**0.05**
Predominant soil type - peat						
No	265	85.5				
Yes	**45**	**14.5**	**0.69**	**0.53**	**0.90**	**<0.01**
Flock mixed with others via shared grazing						
No	285	91.9				
Yes	**25**	**8.1**	**1.52**	**1.09**	**2.15**	**0.01**

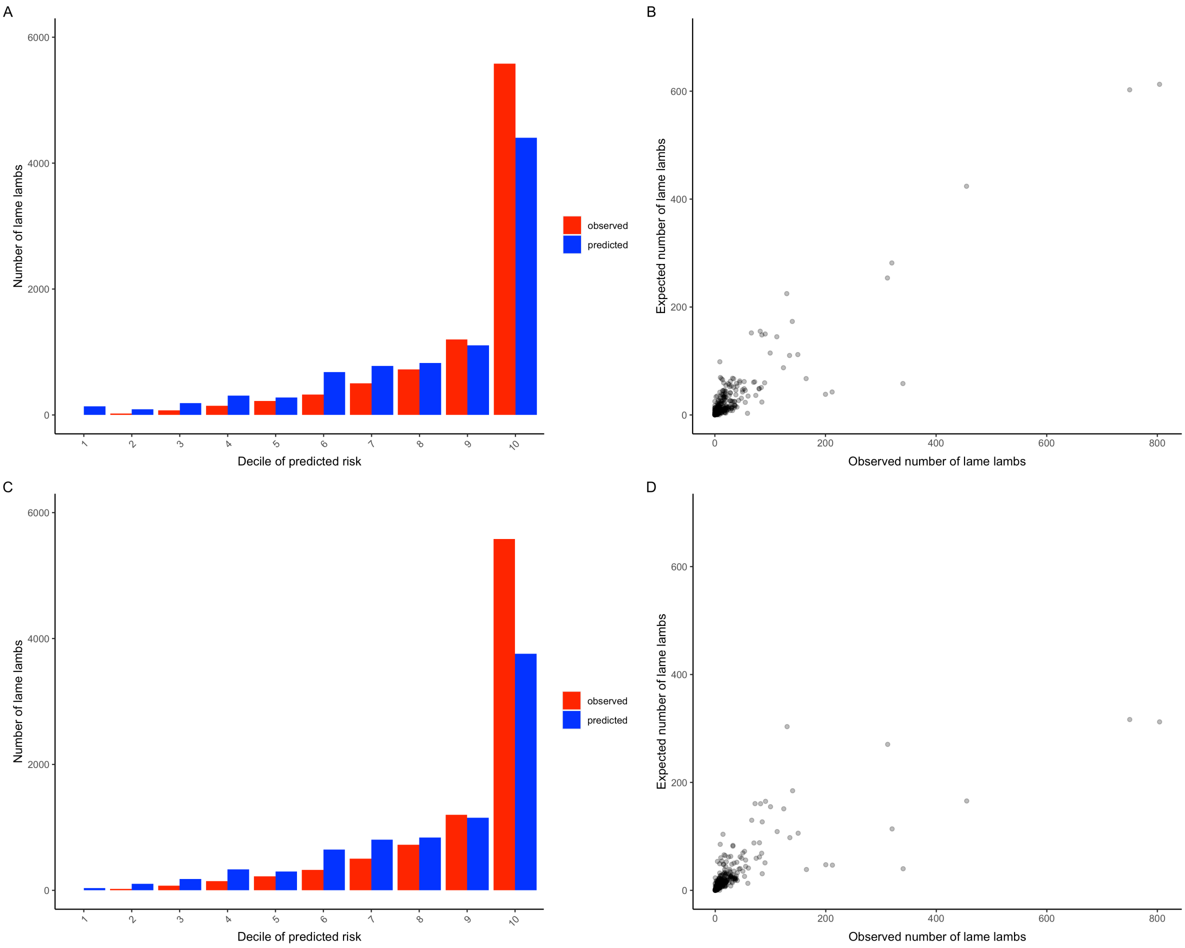
1. Theta taken to be 2.3 (Sub-model 1), 2.6 (Sub-model 2), 2.0 (Sub-model 3) and 2.0 (Sub-model 4).
2. RR = risk ratio, LCI = lower confidence interval, UCI = upper confidence interval, N = number of flocks
3. Variables, risk ratios and confidence intervals highlighted in bold where p<0.1 (Wald's test of significance).

Appendix 13 Multivariable negative binomial generalised linear model for the management practices associated with the proportion of lame ewes in 310 sheep flocks in Great Britain, October 2017-September 2018.

Predictor	N	%	RR	LCI	UCI	P-value
(Intercept)			0.02	0.02	0.03	<0.01
Country						
England	219	70.6	1.00			
Scotland	43	13.9	0.86	0.71	1.03	0.10
Wales	48	15.5	0.83	0.69	1.01	0.06
Flock size (number of ewes)						
101-500	118	38.1	1.00			
1-100	91	29.4	1.20	0.96	1.50	0.11
501-1000	72	23.2	0.93	0.79	1.09	0.36
>1000	29	9.4	0.93	0.74	1.16	0.50
Problem flock (Decile 10 - ≥7.14% lameness)						
No	279	90.0	1.00			
Yes	**31**	**10.0**	**3.72**	**2.99**	**4.65**	**<0.01**
% sheep feet bleeding at routine foot trim						
Did not foot trim	115	37.1	1.00			
0	50	16.1	0.89	0.70	1.13	0.33
>0-<5	104	33.5	1.02	0.87	1.19	0.85
5-100	**41**	**13.2**	**1.32**	**1.07**	**1.62**	**<0.01**
Years FootVax™ used						
Did not vaccinate	219	70.6	1.00			
<1	10	3.2	1.33	0.97	1.85	0.08
1-<2	19	6.1	1.23	0.93	1.65	0.15
2-5	32	10.3	0.86	0.69	1.07	0.16
>5	**30**	**9.7**	**0.72**	**0.57**	**0.90**	**<0.01**
Footbath to treat SFR						
No	230	74.2	1.00			
Yes	**80**	**25.8**	**1.17**	**1.01**	**1.36**	**0.04**
Time to treatment of ewes with SFR						
0-3 days	165	53.2	1.00			
>3 days	141	45.5	1.03	0.90	1.17	0.71
None to treat	**4**	**1.3**	**0.08**	**0.01**	**0.29**	**<0.01**
Predominant soil type - peat						
No	265	85.5	1.00			
Yes	**45**	**14.5**	**0.79**	**0.65**	**0.95**	**0.01**
Quarantine new sheep for >3 weeks						
Always	162	52.3	1.00			

Predictor	N	%	RR	LCI	UCI	P-value
Did not purchase	34	11	0.88	0.65	1.18	0.39
Sometimes	58	18.7	1.13	0.95	1.35	0.16
Never	**56**	**18.1**	**1.28**	**1.06**	**1.55**	**0.01**
Formalin used in footbaths						
Did not footbath	66	21.3	1.00			
Always	**85**	**27.4**	**1.36**	**1.07**	**1.73**	**0.01**
Sometimes	79	25.5	1.20	0.94	1.54	0.13
Never	80	25.8	1.23	0.97	1.56	0.09

1. RR = risk ratio, LCI = lower confidence interval, UCI = upper confidence interval, N = number of flocks, SFR = severe footrot
2. Variables highlighted in bold where $p<0.05$ (Wald's test of significance)
3. Theta estimated as 5.77



Appendix 14 Visual assessment of model fit for the quasi-Poisson (A, B) and negative binomial models (C, D) for associations between management practices and the number of lame ewes in 310 sheep flocks in Great Britain, October 2017-September 2018. Plots A and C show the observed and expected numbers of lame sheep ranked into ten deciles by the observed numbers of lame sheep, while plots B and D show a scatterplot of observed and expected numbers of lame sheep.

Appendix 15 Variables with a stability of >80% and p-value of <0.05 in the bootstrap Poisson elastic net model for the management practices associated with the proportion of lame ewes in 310 sheep flocks in Great Britain, October 2017-September 2018.

Predictor	N	%	RR	LCI	UCI	P-value
Country: Scotland	43	13.9	0.85	0.67	1.00	0.03
Flock size: 1-100 ewes	91	29.4	1.12	1.00	1.34	0.02
Footbath to treat ID: yes	140	45.2	1.13	1.01	1.26	<0.01
Footbath to treat SFR: yes	80	25.8	1.13	1.00	1.38	0.01
Formalin used in footbaths: always	85	27.4	1.12	1.01	1.23	0.01
Maximum altitude sheep grazed at: >230-500m above sea level	52	16.8	0.84	0.68	0.98	0.01
Minimum altitude sheep grazed at: >700-1200m above sea level	38	12.3	0.87	0.67	1.01	0.04
Problem flock (Decile 10 - ≥7.14% lameness): yes	31	10.0	2.89	2.25	4.06	<0.01
Quarantine new sheep to farm for >3 weeks: never	56	18.1	1.17	1.02	1.42	<0.01
Quarantine sheep returning to farm for >3 weeks: sometimes	49	15.8	1.13	1.00	1.40	0.01
Quarantine of sheep returning to farm for >3 weeks: never	94	30.3	1.17	1.03	1.38	<0.01
Lame sheep separated when gathered: yes	43	13.9	1.17	1.02	1.38	<0.01
Predominant soil type – peat: yes	45	14.5	0.82	0.66	0.98	<0.01
Time to treatment of ewes with SFR: none to treat	4	1.3	0.43	0.15	0.83	<0.01
% sheep feet bleeding at routine trim: 5-100	41	13.2	1.36	1.17	1.60	<0.01
Years FootVax™ used: <1	10	3.2	1.42	1.09	1.84	<0.01
Years FootVax™ used: >5	30	9.7	0.84	0.69	0.99	0.02

1. Variables shown with a bootstrap p-value of <0.05 and stability of >80%.
2. RR = risk ratio, LCI = lower confidence interval, UCI = upper confidence interval, N = number of flocks, SFR = severe footrot, ID = interdigital dermatitis
3. Dummy variables were used, therefore for categorical variables with >2 levels, % indicates the percentage performing the relevant category of the management out of the 310 flocks used for modelling.

Appendix 16 Variables with a stability of >80% and p-value of <0.05 in the bootstrap Gaussian elastic net model for the management practices associated with the proportion of lame ewes in 310 sheep flocks in Great Britain, October 2017-September 2018.

Predictor	N	%	Coef	LCI	UCI	P-value
Country: Scotland	43	13.9	-0.07	-0.16	0.00	<0.01
Foot spray used to treat ewes with SFR: sometimes	39	12.6	-0.09	-0.19	-0.01	<0.01
Flock size: 1-100 ewes	91	29.4	0.24	0.16	0.33	<0.01
Flock size: 501-1000 ewes	72	23.2	-0.05	-0.14	0.00	0.04
Flock size: >1000 ewes	29	9.4	-0.07	-0.17	-0.01	<0.01
Footbath used to treat ID: yes	140	45.2	0.08	0.01	0.13	<0.01
Formalin used in footbaths: always	85	27.4	0.04	0.00	0.09	0.02
Set stocked grazing system used: yes	147	47.4	-0.03	-0.11	0.00	0.04
Maximum altitude sheep grazed at: >230-500m above sea level	52	16.8	-0.09	-0.16	-0.03	<0.01
Sheep mixed at shows: yes	23	7.4	0.08	0.00	0.18	0.02
Open flock: yes	263	84.8	-0.06	-0.18	0.00	0.02
Problem flock (Decile 10 - ≥7.1% lameness): yes	31	10.0	0.42	0.33	0.49	<0.01
Sheep purchased from market: yes	200	64.5	-0.05	-0.12	0.00	<0.01
Sheep purchased from private sale: yes	110	35.5	-0.05	-0.13	0.00	0.01
Quarantine new sheep to farm for >3 weeks: never	56	18.1	0.07	0.00	0.14	0.02
Quarantine sheep returning to farm for >3 weeks: never	94	30.3	0.05	0.01	0.12	0.02
Source of replacement sheep: purchased + homebred	94	30.3	0.04	0.00	0.09	0.02
Selection of replacements from never lame ewes: no	87	28.1	0.05	0.00	0.15	0.02
Selection of replacements from never lame ewes: NA (none replaced)	38	12.3	0.07	0.00	0.18	0.01
Lame sheep separated when gathered: yes	43	13.9	0.11	0.02	0.22	<0.01
Predominant soil type - peat: yes	45	14.5	-0.08	-0.16	0.00	0.03
Time to treatment of ewes with SFR: none to treat	4	1.3	-0.49	-0.86	-0.27	<0.01

Predictor	N	%	Coef	LCI	UCI	P-value
% sheep feet bleeding at routine trim: 5-100	41	13.2	0.11	0.04	0.19	<0.01
Years FootVax™ used: <1	10	3.2	0.17	0.07	0.37	<0.01

1. Variables shown with a bootstrap p-value of <0.05 and stability of >80%.
2. Coef = coefficient, LCI = lower confidence interval, UCI = upper confidence interval, N = number of flocks, SFR = severe footrot, ID = interdigital dermatitis
3. Dummy variables were used, therefore for categorical variables with >2 levels, % indicates the percentage performing the relevant category of the management out of the 310 flocks used for modelling.

Appendix 17 Sub-models for the Quasi-Poisson generalised linear models for management practices associated with the proportion of lame lambs in 310 sheep flocks in Great Britain, October 2017-September 2018 for the four sections (treatment of ewes and lambs with footrot, management of lameness, culling and replacement of sheep, the farm and flock environment)

Predictor	N	%	RR	LCI	UCI	P-value
1. Treatment of ewes and lambs with footrot						
(Intercept)			0.04	0.02	0.06	<0.01
Flock size (number of ewes)						
101-500	91	29.4	1.00			
1-100	118	38.1	1.29	0.62	2.43	0.45
501-1000	72	23.2	0.77	0.54	1.10	0.15
>1000	29	9.4	1.06	0.75	1.50	0.76
Country						
England	219	70.6	1.00			
Scotland	**43**	**13.9**	**0.43**	**0.28**	**0.62**	**<0.01**
Wales	**48**	**15.5**	**0.59**	**0.37**	**0.89**	**0.02**
Footbath to treat ID						
No	170	54.8	1.00			
Yes	**140**	**45.2**	**1.71**	**1.29**	**2.30**	**<0.01**
Foot trim to treat ewes with SFR						
Never	51	16.5	1.00			
Sometimes	**97**	**31.3**	**2.25**	**1.19**	**4.27**	**0.01**
Always	**162**	**52.3**	**2.84**	**1.69**	**4.78**	**<0.01**
Antibiotic injection to treat ewes with SFR						
Always	164	52.9	1.00			
Never	**48**	**15.5**	**0.54**	**0.26**	**1.02**	**0.08**
Sometimes	98	31.6	0.98	0.73	1.30	0.88
Foot trim to treat lambs wth SFR						
Never	60	19.4	1.00			
Sometimes	**140**	**45.2**	**0.54**	**0.34**	**0.89**	**0.01**
Always	**110**	**35.5**	**0.65**	**0.43**	**1.00**	**0.05**
Time to treatment of lambs with SFR						
0-3 days	161	51.9	1.00			
>3 days	**131**	**42.3**	**1.46**	**1.12**	**1.91**	**<0.01**
None to treat	18	5.8	0.03	NA	0.88	0.30
Separate lambs with SFR from flock when treated						
Always	30	9.7	1.00			
Never	**144**	**46.5**	**0.53**	**0.38**	**0.74**	**<0.01**
Sometimes	**136**	**43.9**	**0.48**	**0.34**	**0.69**	**<0.01**

Predictor	N	%	RR	LCI	UCI	P-value
2. Management of lameness						
(Intercept)			0.02	0.01	0.04	<0.01
Flock size (number of ewes)						
101-500	91	29.4	1.00			
1-100	118	38.1	1.26	0.56	2.53	0.55
501-1000	72	23.2	0.97	0.66	1.44	0.88
>1000	29	9.4	1.33	0.87	2.06	0.19
Country						
England	219	70.6	1.00			
Scotland	**43**	**13.9**	**0.37**	**0.23**	**0.58**	**<0.01**
Wales	**48**	**15.5**	**0.52**	**0.31**	**0.84**	**0.01**
% sheep feet bleeding at routine foot trim						
Did not foot trim	115	37.1	1.00			
0	50	16.1	0.57	0.25	1.12	0.13
>0-<5	104	33.5	1.17	0.82	1.66	0.40
5-100	41	13.2	1.39	0.91	2.13	0.13
Years FootVax™ used						
Did not vaccinate	219	70.6	1.00			
<1 year	**10**	**3.2**	**3.45**	**0.99**	**10.29**	**0.04**
1-<2 years	19	6.1	2.28	0.73	5.90	0.12
2-<5 years	32	10.3	2.10	0.70	5.14	0.14
>5 years	30	9.7	3.39	1.17	7.92	0.01
Vaccinate ewes with FootVax™						
No	242	78.1	1.00			
Yes	**68**	**21.9**	**2.06**	**1.17**	**3.85**	**0.02**
Frequency sheep vaccinated with FootVax™						
Did not vaccinate	221	71.3	1.00			
Once a year	**76**	**24.5**	**0.20**	**0.07**	**0.64**	**<0.01**
Twice a year	**6**	**1.9**	**0.27**	**0.07**	**1.11**	**0.06**
Before footrot expected	**7**	**2.3**	**0.27**	**0.06**	**1.13**	**0.07**
Vaccinate sheep with SFR with FootVax™						
No	306	98.7	1.00			
Yes	**4**	**1.3**	**2.18**	**1.01**	**4.32**	**0.04**
Formalin used in footbaths						
Did not footbath	66	21.3	1.00			
Always	**85**	**27.4**	**2.76**	**1.45**	**5.79**	**<0.01**
Sometimes	79	25.5	1.78	0.92	3.75	0.11
Never	80	25.8	1.52	0.76	3.32	0.26

Predictor	N	%	RR	LCI	UCI	P-value
3. Culling and replacement of ewes						
(Intercept)			0.01	0.01	0.03	<0.01
Flock size (number of ewes)						
101-500	91	29.4	1.00			
1-100	118	38.1	1.54	0.76	2.87	0.20
501-1000	72	23.2	0.97	0.69	1.36	0.84
>1000	**29**	**9.4**	**1.72**	**1.22**	**2.45**	**<0.01**
Country						
England	219	70.6	1.00			
Scotland	**43**	**13.9**	**0.54**	**0.36**	**0.80**	**<0.01**
Wales	**48**	**15.5**	**0.51**	**0.32**	**0.77**	**<0.01**
Selection of replacements from ewes that were never lame						
Yes	86	27.7	1.00			
No	**87**	**28.1**	**1.65**	**1.17**	**2.34**	**<0.01**
Unknown	**99**	**31.9**	**1.36**	**0.97**	**1.92**	**0.08**
Not applicable	**38**	**12.3**	**0.37**	**0.19**	**0.67**	**<0.01**
Source of replacement sheep						
Homebred	164	52.9	1.00			
Purchased	**42**	**13.5**	**2.75**	**1.89**	**3.96**	**<0.01**
Homebred + purchased	**94**	**30.3**	**1.37**	**0.98**	**1.89**	**0.06**
Not applicable	**10**	**3.2**	**5.29**	**1.90**	**13.35**	**<0.01**
Culling of lame sheep						
Never	77	24.8				
Lame twice per year	34	11.0	0.98	0.56	1.74	0.95
Lame three times per year	28	9.0	1.50	0.88	2.60	0.14
After persistently lame	164	52.9	1.00	0.64	1.60	0.99
Other	**7**	**2.3**	**4.11**	**1.99**	**8.11**	**<0.01**
Open flock*						
No	47	15.2	1.00			
Yes	**263**	**84.8**	**1.83**	**0.95**	**3.87**	**0.09**
4. The farm and flock environment						
(Intercept)			0.04	0.02	0.07	<0.01
Flock size (number of ewes)						
101-500	91	29.4	1.00			
1-100	118	38.1	1.03	0.49	1.95	0.94
501-1000	72	23.2	1.07	0.75	1.52	0.72
>1000	**29**	**9.4**	**1.55**	**1.07**	**2.27**	**0.02**

Predictor	N	%	RR	LCI	UCI	P-value
Country						
England	219	70.6	1.00			
Scotland	**43**	**13.9**	**0.45**	**0.29**	**0.68**	**<0.01**
Wales	**48**	**15.5**	**0.58**	**0.36**	**0.91**	**0.02**
Maximum altitude flock was grazed at (m above sea level)						
0-230	52	16.8	1.00			
>230-500	**52**	**16.8**	**0.53**	**0.33**	**0.83**	**<0.01**
>500-850	**61**	**19.7**	**0.50**	**0.32**	**0.78**	**<0.01**
>850-1200	**56**	**18.1**	**0.48**	**0.31**	**0.74**	**<0.01**
>1200-3400	**42**	**13.5**	**0.69**	**0.45**	**1.05**	**0.08**
Missing	47	15.2	0.78	0.47	1.26	0.32
Predominant soil type - peat						
No	265	85.5	1.00			
Yes	**45**	**14.5**	**0.43**	**0.26**	**0.66**	**<0.01**
Crops used as forage						
No	233	75.2	1.00			
Yes	**77**	**24.8**	**1.30**	**0.96**	**1.77**	**0.09**
Use of set stocked grazing system						
No	163	52.6	1.00			
Yes	**147**	**47.4**	**1.65**	**1.18**	**2.29**	**<0.01**
Use of rotational grazing system						
No	124	40	1.00			
Yes	**186**	**60**	**1.51**	**1.08**	**2.12**	**0.02**

1. Dispersion parameters taken to be 32.5 (Sub-model 1) 39.3 (Sub-model 2) 30.7 (Sub-model 3) and 33.5 (Sub-model 4)
2. RR = risk ratio, LCI = lower confidence interval, UCI = upper confidence interval, N = number of flocks, SFR = severe footrot, ID = interdigital dermatitis
3. Variables highlighted in bold where p <0.10 (Wald's test of significance)

Appendix 18 Multivariable Quasi-Poisson generalized linear model for the management practices associated with the proportion of lame lambs in 310 sheep flocks in Great Britain, October 2017-September 2018.

Predictor	**N**	**%**	**RR**	**LCI**	**UCI**	**P-value**
(Intercept)			0.04	0.02	0.07	<0.01
Country						
England	219	70.6				
Scotland	**43**	**13.9**	**0.52**	**0.35**	**0.75**	**<0.01**
Wales	**48**	**15.5**	**0.62**	**0.41**	**0.93**	**0.03**
Flock size (number of ewes)						
101-500	118	38.1				
1-100	91	29.4	1.04	0.54	1.85	0.91
501-1000	72	23.2	0.98	0.70	1.37	0.90
>1000	29	9.4	1.22	0.89	1.69	0.22
Footbath to treat ID						
No	170	54.8				
Yes	**140**	**45.2**	**1.64**	**1.25**	**2.17**	**<0.01**
Foot trim to treat lambs with SFR						
Never	60	19.4				
Sometimes	**140**	**45.2**	**0.52**	**0.33**	**0.84**	**<0.01**
Always	**110**	**35.5**	**0.59**	**0.39**	**0.91**	**0.02**
Foot trim to treat ewes with SFR						
Never	51	16.5				
Sometimes	97	31.3	1.73	0.95	3.20	0.08
Always	**162**	**52.3**	**2.13**	**1.24**	**3.68**	**<0.01**
Lambs with SFR separated from flock at treatment						
Always	30	9.7				
Sometimes	**136**	**43.9**	**0.55**	**0.38**	**0.79**	**<0.01**
Never	**144**	**46.5**	**0.63**	**0.45**	**0.89**	**<0.01**
% sheep feet bleeding at routine foot trim						
Did not foot trim	115	37.1				
0	50	16.1	0.64	0.33	1.16	0.17
>0-<5	104	33.5	1.11	0.78	1.58	0.57
5-100	**41**	**13.2**	**1.91**	**1.34**	**2.72**	**<0.01**
Selection of replacements from ewes that were never lame						
Yes	86	27.7				
No	**87**	**28.1**	**2.07**	**1.47**	**2.92**	**<0.01**
Unknown	**99**	**31.9**	**1.61**	**1.15**	**2.27**	**<0.01**
Not applicable	38	12.3	1.18	0.70	1.94	0.53

Predictor	N	%	RR	LCI	UCI	P-value
Predominant soil type – peat						
No	265	85.5				
Yes	**45**	**14.5**	**0.53**	**0.35**	**0.78**	**<0.01**
Predominant soil type - clay						
No	141	45.5				
Yes	**169**	**54.5**	**1.38**	**1.06**	**1.81**	**0.02**
Maximum altitude flock was grazed at (m above sea level)						
0-230	52	16.8				
>230-500	**52**	**16.8**	**0.49**	**0.31**	**0.78**	**<0.01**
>500-850	**61**	**19.7**	**0.54**	**0.35**	**0.82**	**<0.01**
>850-1200	56	18.1	0.72	0.48	1.10	0.13
>1200-3400	**42**	**13.5**	**0.46**	**0.31**	**0.68**	**<0.01**
Missing	47	15.2	0.79	0.51	1.22	0.30

1. RR = risk ratio, LCI = lower confidence interval, UCI = upper confidence interval, N = number of flocks, SFR = severe footrot, ID = interdigital dermatitis
2. Variables highlighted in bold where p<0.05 (Wald's test of significance)
3. Dispersion parameter taken to be 25.48

Appendix 19 Sub-models for the negative binomial generalised linear models for management practices associated with the proportion of lame lambs in 310 sheep flocks in Great Britain, October 2017-September 2018 for the four sections (treatment of ewes and lambs with footrot, management of lameness, culling and replacement of sheep, the farm and flock environment)

Predictor	N	%	RR	LCI	UCI	P-value
1. Treatment of ewes and lambs with footrot						
(Intercept)			14.32	9.03	23.43	<0.01
Flock size (number of ewes)						
101-500	91	29.4				
1-100	**118**	**38.1**	**0.33**	**0.24**	**0.46**	**<0.01**
501-1000	**72**	**23.2**	**2.27**	**1.65**	**3.14**	**<0.01**
>1000	**29**	**9.4**	**8.19**	**5.36**	**12.83**	**<0.01**
Country						
England	219	70.6				
Scotland	43	13.9	0.79	0.55	1.15	0.18
Wales	**48**	**15.5**	**0.70**	**0.51**	**0.99**	**0.03**
Time to treatment of lambs with SFR						
0-3 days	161	51.9				
>3 days	**131**	**42.3**	**1.53**	**1.08**	**2.16**	**0.02**
None to treat	**18**	**5.8**	**0.03**	**0.01**	**0.10**	**<0.01**
Footbath used to treat ID						
No	170	54.8				
Yes	**140**	**45.2**	**1.48**	**1.14**	**1.92**	**<0.01**
Antibiotic injection to treat lambs with SFR						
Always	109	35.2				
Never	**65**	**21.0**	**0.66**	**0.47**	**0.93**	**0.01**
Sometimes	136	43.9	0.95	0.72	1.25	0.70
Lambs with footrot separated from flock at treatment						
Always	30	9.7				
Never	**144**	**46.5**	**0.69**	**0.44**	**1.05**	**0.07**
Sometimes	**136**	**43.9**	**0.63**	**0.39**	**0.98**	**0.03**
Time to treatment of ewes with SFR						
0-3 days	165	53.2				
>3 days	**141**	**45.5**	**1.41**	**1.00**	**2.00**	**0.05**
None to treat	4	1.3	0.34	0.05	2.63	0.30
2. Management of lameness						
(Intercept)			10.06	6.96	14.75	<0.01
Flock size (number of ewes)						
101-500	91	29.4				

Predictor	N	%	RR	LCI	UCI	P-value
1-100	**118**	**38.1**	**0.26**	**0.19**	**0.37**	**<0.01**
501-1000	**72**	**23.2**	**2.26**	**1.63**	**3.15**	**<0.01**
>1000	**29**	**9.4**	**6.24**	**4.08**	**9.86**	**<0.01**
Country						
England	219	70.6				
Scotland	**43**	**13.9**	**0.64**	**0.44**	**0.94**	**0.02**
Wales	48	15.5	0.78	0.56	1.12	0.16
Formalin used in footbaths						
Did not footbath	66	21.3				
Always	**85**	**27.4**	**2.54**	**1.71**	**3.75**	**<0.01**
Sometimes	**79**	**25.5**	**1.49**	**0.99**	**2.23**	**0.05**
Never	80	25.8	1.19	0.80	1.76	0.38
% sheep feet bleeding at routine foot trim						
Did not foot trim	115	37.1				
0	**50**	**16.1**	**0.50**	**0.34**	**0.74**	**<0.01**
>0-<5	104	33.5	0.92	0.69	1.24	0.59
5-100	**41**	**13.2**	**1.47**	**0.99**	**2.23**	**0.06**
Sheep separated from flock when persistently lame						
No	191	61.6				
Yes	**119**	**38.4**	**1.29**	**1.00**	**1.67**	**0.05**
3. Culling and replacement of ewes						
(Intercept)			8.72	5.92	13.01	<0.01
Flock size (number of ewes)						
101-500	91	29.4				
1-100	**118**	**38.1**	**0.27**	**0.19**	**0.38**	**<0.01**
501-1000	**72**	**23.2**	**2.34**	**1.68**	**3.29**	**<0.01**
>1000	**29**	**9.4**	**11.30**	**7.09**	**18.62**	**<0.01**
Country						
England	219	70.6				
Scotland	43	13.9	0.75	0.51	1.13	0.14
Wales	**48**	**15.5**	**0.72**	**0.50**	**1.05**	**0.07**
Selection of replacements from ewes that were never lame						
Yes	86	27.7				
No	**87**	**28.1**	**1.83**	**1.27**	**2.62**	**<0.01**
Unknown	99	31.9	1.06	0.74	1.51	0.76
Not applicable	38	12.3	1.04	0.53	2.00	0.91
Source of replacement sheep						
Homebred	164	52.9				
Purchased	**42**	**13.5**	**1.70**	**1.02**	**2.96**	**0.04**

Predictor	N	%	RR	LCI	UCI	P-value
Homebred + purchased	**94**	**30.3**	**1.82**	**1.33**	**2.50**	**<0.01**
Not applicable	10	3.2	1.87	0.86	4.61	0.17
Sheep purchased from private sale						
No	200	64.5				
Yes	**110**	**35.5**	**1.27**	**0.96**	**1.69**	**0.09**
4. The farm and flock environment						
(Intercept)			12.14	8.71	17.20	<0.01
Flock size (number of ewes)						
101-500	91	29.4				
1-100	**118**	**38.1**	**0.24**	**0.17**	**0.33**	**<0.01**
501-1000	**72**	**23.2**	**2.47**	**1.77**	**3.48**	**<0.01**
>1000	**29**	**9.4**	**7.46**	**4.69**	**12.26**	**<0.01**
Country						
England	219	70.6				
Scotland	43	13.9	0.86	0.59	1.29	0.44
Wales	48	15.5	0.79	0.55	1.15	0.20
Predominant soil type - peat						
No	265	85.5				
Yes	**45**	**14.5**	**0.47**	**0.33**	**0.69**	**<0.01**
Predominant soil type - sand						
No	254	81.9				
Yes	**56**	**18.1**	**1.45**	**1.04**	**2.07**	**0.03**
Crops used as forage						
No	233	75.2				
Yes	**77**	**24.8**	**1.53**	**1.09**	**2.14**	**<0.01**
Use of set stocked grazing system						
No	163	52.6				
Yes	**147**	**47.4**	**1.34**	**1.04**	**1.74**	**0.02**
Resowing of pastures						
Mixed frequency	173	55.8				
All permanent	134	43.2	1.12	0.82	1.52	0.46
All resown	**3**	**1**	**0.23**	**0.07**	**1.20**	**0.04**

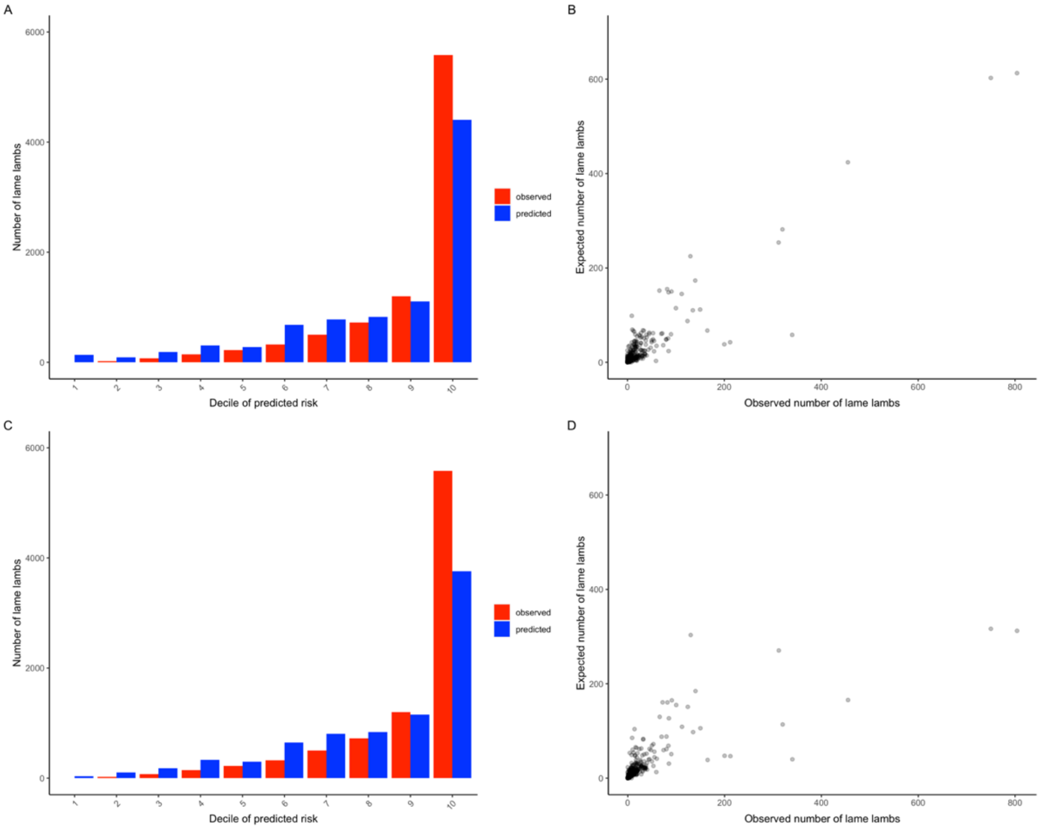
1. Theta taken to be 1.1 (Sub-model 1), 0.9 (Sub-model 2), 0.8 (Sub-model 3) and 0.9 (Sub-model 4)
2. RR = risk ratio, LCI = lower confidence interval, UCI = upper confidence interval, N = number of flocks, SFR = severe footrot, ID = interdigital dermatitis
3. Variables highlighted in bold where p <0.10 (Wald's test of significance)

Appendix 20 Multivariable negative binomial generalised linear model for the management practices associated with the proportion of lame lambs in 310 sheep flocks in Great Britain, October 2017-September 2018.

Predictor	N	%	RR	LCI	UCI	P-value
(Intercept)			0.16	0.03	0.78	0.02
Country						
England	219	70.6				
Scotland	**43**	**13.9**	**0.71**	**0.52**	**0.96**	**0.02**
Wales	48	15.5	0.84	0.64	1.12	0.23
Flock size (number of ewes)						
101-500	91	29.4				
1-100	118	38.1	1.34	0.99	1.82	0.05
501-1000	72	23.2	0.90	0.69	1.18	0.42
>1000	29	9.4	1.32	0.91	1.95	0.13
Time to treatment of lambs with SFR						
0-3 days	161	51.9				
>3 days	**131**	**42.3**	**1.51**	**1.22**	**1.87**	**<0.01**
None to treat	**18**	**5.8**	**0.04**	**0.01**	**0.12**	**<0.01**
Footbath to treat ID						
No	170	54.8				
Yes	**140**	**45.2**	**1.35**	**1.09**	**1.68**	**<0.01**
Antibiotic injection to treat lambs with SFR						
Always	109	35.2				
Sometimes	136	43.9	0.99	0.78	1.26	0.92
Never	**65**	**21.0**	**0.71**	**0.53**	**0.95**	**0.02**
Foot trim to treat lambs with SFR						
Never	60	19.4				
Sometimes	**140**	**45.2**	**0.58**	**0.39**	**0.86**	**0.01**
Always	110	35.5	0.74	0.49	1.10	0.15
Lambs with SFR separated from flock at treatment						
Always	30	9.7				
Sometimes	**136**	**43.9**	**0.64**	**0.44**	**0.93**	**0.01**
Never	**144**	**46.5**	**0.71**	**0.49**	**1.00**	**0.04**
Foot trim to treat ewes with SFR						
Never	51	16.5				
Sometimes	**97**	**31.3**	**1.70**	**1.09**	**2.63**	**0.03**
Always	**162**	**52.3**	**1.95**	**1.26**	**3.01**	**<0.01**
% sheep feet bleeding at routine foot trim						
Did not foot trim	115	37.1				
0	50	16.1	0.74	0.52	1.06	0.08

Predictor	N	%	RR	LCI	UCI	P-value
>0-<5	104	33.5	0.90	0.69	1.18	0.43
5-100	**41**	**13.2**	**1.48**	**1.07**	**2.07**	**0.02**
Selection of replacements from ewes that were never lame						
Yes	86	27.7				
No	**87**	**28.1**	**1.77**	**1.34**	**2.34**	**<0.01**
Unknown	**99**	**31.9**	**1.38**	**1.04**	**1.84**	**0.02**
Not applicable	38	12.3	1.06	0.64	1.74	0.81
Source of replacement sheep						
Homebred	164	52.9				
Purchased	42	13.5	1.40	0.95	2.09	0.09
Homebred + purchased	**94**	**30.3**	**1.55**	**1.21**	**1.97**	**<0.01**
Not applicable	10	3.2	1.84	0.93	3.80	0.09
Predominant soil type - peat						
No	265	85.5				
Yes	**45**	**14.5**	**0.64**	**0.48**	**0.87**	**<0.01**
Maximum altitude flock was grazed at (m above sea level)						
0-230	52	16.8				
>230-500	**52**	**16.8**	**0.69**	**0.48**	**0.99**	**0.04**
>500-850	61	19.7	0.74	0.52	1.05	0.08
>850-1200	56	18.1	0.88	0.60	1.28	0.48
>1200-3400	**42**	**13.5**	**0.63**	**0.42**	**0.95**	**0.03**
(Missing)	47	15.2	1.12	0.78	1.63	0.53
Pasture used as forage						
No	2	0.6				
Yes	**308**	**99.4**	**0.16**	**0.03**	**0.74**	**0.01**
Flock mixed with others at shows						
No	287	92.6				
Yes	**23**	**7.4**	**0.63**	**0.43**	**0.96**	**0.03**

1. RR = risk ratio, LCI = lower confidence interval, UCI = upper confidence interval, N = number of flocks, SFR- severe footrot, ID = interdigital dermatitis
2. Variables highlighted in bold where p<0.05 (Wald's test of significance)



Appendix 21 Visual assessment of model fit for the quasi-Poisson (A, B) and negative binomial models (C, D) for associations between management practices and the number of lame lambs in 310 sheep flocks in Great Britain, October 2017-September 2018. Plots A and C show the observed and expected numbers of lame sheep ranked into ten deciles by the observed numbers of lame sheep, while plots B and D show a scatterplot of observed and expected numbers of lame sheep.

Appendix 22 Variables with a stability of >80% and p-value of <0.05 in the bootstrap Poisson elastic net model for the management practices associated with the proportion of lame ewes in 310 sheep flocks in Great Britain, October 2017-September 2018.

Predictor	**N**	**%**	**RR**	**LCI**	**UCI**	**P-value**
Country: Scotland	43	13.9	0.84	0.66	0.97	<0.01
Antibiotic injection to treat ewes with SFR: never	48	15.5	0.87	0.79	1.00	0.02
Foot spray to treat ewes with SFR: never	10	3.2	0.79	0.39	0.98	0.01
Foot trim to treat ewes with SFR: always	162	31.3	1.12	0.98	1.25	0.03
Flock size: 501-1000 ewes	72	23.2	0.90	0.77	1.01	0.04
Footbath to treat ID: yes	140	45.2	1.22	1.07	1.57	<0.01
Formalin used in footbaths: always	85	27.4	1.14	1.02	1.31	<0.01
Flock moved to fresh pasture: other frequency	153	49.4	0.89	0.76	1.00	0.02
Antibiotic injection to treat lambs with SFR: never	65	21.0	0.92	0.71	1.00	0.04
Maximum altitude sheep grazed at: >230-500ft above sea level	52	16.8	0.86	0.59	0.98	0.02
Sheep did not mix with other flocks: yes	247	79.7	1.14	1.01	1.51	0.01
Problem flock (Decile 10 - ≥8.5% lameness): yes	31	10.0	3.63	2.61	5.53	<0.01
Sheep purchased from other source: yes	13	4.2	0.80	0.50	0.97	<0.01
Quarantine sheep returning to farm for >3 weeks: missing	107	34.5	0.91	0.78	1.02	0.04
Source of replacement sheep: purchased + homebred	94	30.3	1.12	0.98	1.26	0.05
Selection of replacements from never lame ewes: no	87	28.1	1.25	1.06	1.60	<0.01
Sheep separated from flock when persistently lame: yes	119	38.4	1.14	1.01	1.27	0.02
Predominant soil type – peat: yes	45	14.5	0.84	0.68	0.98	0.01
Time to treatment of ewes with SFR: >3 days	141	45.5	1.19	1.02	1.36	<0.01
Time to treatment of ewes with SFR: none to treat	4	1.3	0.64	0.28	0.96	<0.01
Time to treatment of lambs with SFR: >3 days	131	42.3	1.15	1.02	1.35	0.01

Predictor	N	%	RR	LCI	UCI	P-value
Time to treatment of lambs with SFR: none to treat	18	5.8	0.66	0.12	0.95	<0.01
% sheep feet bleeding at routine trim: 5-100	41	13.2	1.19	1.01	1.48	0.01

1. Variables shown with a bootstrap p-value of <0.05 and stability of >80%.
2. RR = risk ratio, LCI = lower confidence interval, UCI = upper confidence interval, N = number of flocks, SFR = severe footrot, ID = interdigital dermatitis
3. Dummy variables were used, therefore for categorical variables with >2 levels, % indicates the percentage performing the relevant category of the management out of the 310 flocks used for modelling.

Appendix 23 Variables with a stability of >80% and p-value of <0.05 in the bootstrap Gaussian elastic net model for the management practices associated with the proportion of lame ewes in 310 sheep flocks in Great Britain, October 2017-September 2018.

Predictor	N	%	Coef	LCI	UCI	P-value
Country: Scotland	43	13.9	-0.07	-0.19	-0.01	0.01
Culling management: after lame three times/year	28	9.0	0.06	0.00	0.18	0.03
Flock size: 1-100 ewes	118	38.1	0.17	0.09	0.30	<0.01
Flock size: 501-1000 ewes	72	23.2	-0.08	-0.14	-0.01	0.01
Footbath to treat ID: yes	140	45.2	0.09	0.03	0.18	<0.01
Pasture used as forage: yes	308	99.4	-0.39	-0.60	-0.06	<0.01
Formalin used in footbaths: always	85	27.4	0.05	0.00	0.12	0.02
Antibiotic injection to treat lambs with SFR: never	65	21.0	-0.05	-0.15	0.00	0.01
Maximum altitude sheep grazed at: >230-500ft above sea level	52	16.8	-0.07	-0.21	0.00	<0.01
Maximum altitude sheep grazed at: >850-1200ft above sea level	56	18.1	0.06	0.00	0.16	0.02
Problem flock (Decile 10 - ≥8.5% lameness): yes	31	10.0	0.60	0.46	0.75	<0.01
Sheep purchased from private sale: yes	110	35.5	-0.07	-0.14	0.00	<0.01
Quarantine new sheep to farm for >3 weeks: never	56	18.1	0.09	0.02	0.18	0.01
Source of replacement sheep: purchased + homebred	94	30.3	0.07	0.01	0.13	<0.01
Selection of replacements from never lame ewes: no	87	28.1	0.08	0.01	00.22	0.01
Selection of replacements from never lame ewes: unknown	99	31.9	0.05	0.00	0.15	0.04
Selection of replacements from never lame ewes: NA (none replaced)	38	12.3	0.11	0.02	0.26	<0.01
Lame sheep separated when gathered: yes	43	13.9	0.07	0.00	0.20	0.02
Predominant soil type – peat: yes	45	14.5	-0.08	-0.19	-0.01	0.02
Time to treatment of ewes with SFR: >3 days	141	45.5	0.06	0.01	0.13	<0.01
Time to treatment of ewes with SFR: none to treat	4	1.3	-0.45	-1.03	-0.07	<0.01

Predictor	N	%	Coef	LCI	UCI	P-value
Time to treatment of lambs with SFR: >3 days	131	42.3	0.06	0.01	0.15	<0.01
Time to treatment of lambs with SFR: none to treat	18	5.8	-0.37	-0.61	-0.15	<0.01
Years FootVax™ used: 1-<2	19	6.1	0.12	0.00	0.28	0.02
Years FootVax™ used: 2-5	32	10.3	-0.07	-0.17	0.00	0.01

1. Variables shown with a bootstrap p-value of <0.05 and stability of >80%.
2. Coef = coefficient, LCI = lower confidence interval, UCI = upper confidence interval, N = number of flocks, SFR = severe footrot, ID = interdigital dermatitis
3. Dummy variables were used, therefore for categorical variables with >2 levels, % indicates the percentage performing the relevant category of the management out of the 310 flocks used for modelling.

Appendix 24 Percentages of farmers using Formalin in footbaths for each type of foot lesion (interdigital dermatitis, severe footrot, contagious ovine digital dermatitis, granuloma, shelly hoof and white line abscess) in 310 flocks of sheep in Great Britain (October 2017-September 2018)

Lesion	Presence	N	Use of Formalin in footbaths			
			Did not footbath	Always	Sometimes	Never
Interdigital dermatitis*	Present	281	17.8	27.8	28.1	26.3
	Absent	22	63.6	18.2	0.0	18.2
	Unknown	0	0.0	0.0	0.0	0.0
	Missing	7	28.6	42.9	0.0	28.6
Severe footrot*	Present	242	16.9	30.2	27.3	25.6
	Absent	64	37.5	15.6	20.3	26.6
	Unknown	1	0.0	0.0	0.0	100.0
	Missing	3	33.3	66.7	0.0	0.0
Contagious ovine digital dermatitis*	Present	124	8.1	29.0	36.3	26.6
	Absent	172	29.7	27.3	17.4	25.6
	Unknown	9	22.2	11.1	44.4	22.2
	Missing	5	60.0	20.0	0.0	20.0
Granuloma*	Present	152	13.8	31.6	28.3	26.3
	Absent	147	28.6	23.1	24.5	23.8
	Unknown	3	0.0	33.3	0.0	66.7
	Missing	8	37.5	25.0	0.0	37.5
Shelly Hoof	Present	191	19.7	32.1	24.4	23.8
	Absent	104	24.0	19.2	26.9	29.8
	Unknown	7	28.6	0.0	57.1	14.3
	Missing	6	16.7	50.0	0.0	33.3
White Line Abscess	Present	97	15.5	26.8	33.0	24.7
	Absent	188	22.9	28.2	22.3	26.6
	Unknown	15	26.7	20.0	26.7	26.7
	Missing	10	40.0	30.0	10.0	20.0

1. * indicates $p < 0.05$ from a Fisher's exact test of association between the presence of a foot lesion (presence, absence, unknown, missing) and use of Formalin in footbaths (always, sometimes and never and did not footbath at all). P values obtained by 2000 Monte Carlo simulations.

Appendix 25 Relationships between the percentage of farmers using a treatment type (foot spray, foot trimming, and antibiotic injection) and the length of time taken to treat sheep following recognition of lameness in in 310 flocks of sheep in Great Britain (October 2017-September 2018)

Treatment type	Frequency of use	N	Time to treatment		
			0-3	>3	None to treat
		Ewes			
Antibiotic injection to treat ewes with SFR	Always	164	50.6	49.4	0.0
	Sometimes	98	59.2	38.8	2.0
	Never	48	50.0	45.8	4.2
Foot trim to treat ewes with SFR	Always	162	50.6	49.4	0.0
	Sometimes	97	55.7	41.2	3.1
	Never	51	56.9	41.2	2.0
Foot spray to treat ewes with SFR	Always	261	53.3	46.4	0.4
	Sometimes	39	59.0	35.9	5.1
	Never	10	30.0	60.0	10.0
		Lambs			
Antibiotic injection to treat lambs with SFR	Always	109	52.3	47.7	0.0
	Sometimes	136	52.2	36.8	11.0
	Never	65	50.8	44.6	4.6
Foot trim to treat lambs with SFR	Always	110	52.7	47.3	0.0
	Sometimes	140	45.7	42.9	11.4
	Never	60	65.0	31.7	3.3
Foot spray to treat lambs with SFR	Always	239	53.1	46.9	0.0
	Sometimes	60	46.7	26.7	26.7
	Never	11	54.5	27.3	18.2

1. N = number of flocks, SFR = severe footrot

Appendix 26 Associations between type of treatment used to treat ewes and lambs with severe footrot in 310 flocks of sheep in Great Britain, October 2017-September 2018)

Treatment of ewes and lambs with SFR	1	2	3	4	5	6	7	8
1. Time to treatment of ewes	-	0.05	0.13	0.01	<0.01	0.20	0.41	0.03
2. Antibiotic injection to treat ewes with SFR		-	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
3. Foot trim to treat ewes with SFR			-	<0.01	0.37	<0.01	<0.01	<0.01
4. Foot spray to treat ewes with SFR				-	0.22	<0.01	<0.01	<0.01
5. Time to treatment of lambs					-	<0.01	<0.01	<0.01
6. Antibiotic injection to treat lambs with SFR						-	<0.01	<0.01
7. Foot trim to treat lambs with SFR							-	<0.01
8. Foot spray to treat lambs with SFR								-

1. P-values from chi-square test of association, or Fisher's exact test (2000 Monte Carlo simulations) where assumptions for the chi square test were not met

2. Categories for type of treatment were always, sometimes and never.

3. Categories for time to treatment were 0-3 days, >3 days and no lame sheep to treat.

4. SFR = severe footrot

Appendix 27 The number and percentage of farms with <2, 2-5% and >5% prevalence of lameness in both ewes and lambs in all 450 flocks of sheep in Great Britain, October 2017-September 2018)

Prevalence of lameness	Overall		Ewes		Lambs	
	N	%	N	%	N	%
<2%	183	40.7	172	38.2	195	43.3
2-5%	169	37.6	178	39.6	150	33.3
>5%	98	21.8	100	22.2	105	23.3

1. N = number of flocks, % = percentage

Appendix 28 Data collection sheets for locomotion scoring of a) individual ewes and b) lambs in the Blackdown Lamb Deployment

a) Ewes

Locomotion **Date:** **Recorder:**

Ewe ID	Lamb ID	Locomotion score	Foot			
			LF	RF	LR	RR
1	1					
1	*					
2	2					
2	*					
3	3					
3	*					
4	4					
4	*					
5	5					
5	*					
6	6					
6	*					
7	7					
7	*					
8	8					
8	*					
9	9					
9	*					
10	10					
10	*					
11	11					
11	*					
12	12					
12	*					
13	13					
13	*					
14	14					
14	*					
15	15					
15	*					
16	16					
16	*					
17	17					
17	*					
18	18					
18	*					
19	19					
19	*					
20	20					
20	*					
21	21					
21	*					
22	22					
22	*					
23	23					
23	*					
24	24					
24	*					
25	25					
25	*					
26	26					
26	*					
27	27					
27	*					
28	28					
28	*					

LF: left fore, RF: right fore, LR: left rear, RR: right rear

Ewe ID	Lamb ID	Locomotion score	Foot			
			LF	RF	LR	RR
29	29					
29	*					
30	30					
30	*					
31	31					
31	*					
32	32					
32	*					
33	33					
33	*					
34	34					
34	*					
35	35					
35	*					
36	36					
36	*					
37	37					
37	*					
38	38					
38	*					
39	39					
39	*					
40	40					
40	*					
41	41					
41	*					
42	42					
42	*					
43	43					
43	*					
44	44					
44	*					
45	45					
45	*					
46	46					
46	*					
47	47					
47	*					
48	48					
48	*					
49	49					
49	*					
50	50					
50	*					
51	51					
51	*					
52	52					
52	*					
53	53					
53	*					
54	54					
54	*					
55	55					
55	*					
56	56					
56	*					

Ewe ID	Lamb ID	Locomotion score	Foot			
			LF	RF	LR	RR
57	57					
57	*					
58	58					
58	*					
59	59					
59	*					
60	60					
60	*					
61	61					
61	*					
62	62					
62	*					
63	63					
63	*					
64	64					
64	*					
65	65					
65	*					
66	66					
66	*					
67	67					
67	*					

b) Lambs

Locomotion **Date:** **Recorder:**

Ewe ID	Lamb ID	Locomotion score	Foot			
			LF	RF	LR	RR
1	1					
1	*					
2	2					
2	*					
3	3					
3	*					
4	4					
4	*					
5	5					
5	*					
6	6					
6	*					
7	7					
7	*					
8	8					
8	*					
9	9					
9	*					
10	10					
10	*					
11	11					
11	*					
12	12					
12	*					
13	13					
13	*					
14	14					
14	*					
15	15					
15	*					
16	16					
16	*					
17	17					
17	*					
18	18					
18	*					
19	19					
19	*					
20	20					
20	*					
21	21					
21	*					
22	22					
22	*					
23	23					
23	*					
24	24					
24	*					
25	25					
25	*					
26	26					
26	*					
27	27					
27	*					
28	28					
28	*					

Ewe ID	Lamb ID	Locomotion score	Foot			
			LF	RF	LR	RR
29	29					
29	*					
30	30					
30	*					
31	31					
31	*					
32	32					
32	*					
33	33					
33	*					
34	34					
34	*					
35	35					
35	*					
36	36					
36	*					
37	37					
37	*					
38	38					
38	*					
39	39					
39	*					
40	40					
40	*					
41	41					
41	*					
42	42					
42	*					
43	43					
43	*					
44	44					
44	*					
45	45					
45	*					
46	46					
46	*					
47	47					
47	*					
48	48					
48	*					
49	49					
49	*					
50	50					
50	*					
51	51					
51	*					
52	52					
52	*					
53	53					
53	*					
54	54					
54	*					
55	55					
55	*					
56	56					
56	*					

Ewe ID	Lamb ID	Locomotion score	Foot			
			LF	RF	LR	RR
57	57					
57	*					
58	58					
58	*					
59	59					
59	*					
60	60					
60	*					
61	61					
61	*					
62	62					
62	*					
63	63					
63	*					
64	64					
64	*					
65	65					
65	*					
66	66					
66	*					
67	67					
67	*					

LF: left fore, RF: right fore, LR: left rear, RR: right rear

Appendix 29 Sensitivity, specificity and accuracy of locomotion scoring as a proxy for having an infectious foot lesions for four conditions, sheep that were lame on 15.10.2019, lame in the three days prior to 15.2019, lame in the seven days prior to 15.2019, or in 14 days prior to 15.2019 for all 118 sheep

Classification of lameness		**Se**	**Sp**	**A**	**BA**
Lame at ≥1					
Ewes	Lame on 15.10.19	0.54	0.47	0.52	0.50
	Lame in 3 days prior to 15.10.19	0.60	0.47	0.56	0.53
	Lame in 7 days prior to 15.10.19	0.31	0.67	0.42	0.49
	Lame in 14 days prior to 15.10.19	0.06	0.87	0.30	0.46
Lambs	Lame on 15.10.19	0.88	0.12	0.59	0.50
	Lame in 3 days prior to 15.10.19	0.76	0.31	0.59	0.53
	Lame in 7 days prior to 15.10.19	0.64	0.46	0.57	0.55
	Lame in 14 days prior to 15.10.19	0.40	0.88	0.59	0.64
Lame at ≥2					
Ewes	Lame on 15.10.19	0.43	0.67	0.50	0.55
	Lame in 3 days prior to 15.10.19	0.71	0.47	0.64	0.59
	Lame in 7 days prior to 15.10.19	0.74	0.47	0.66	0.60
	Lame in 14 days prior to 15.10.19	0.57	0.47	0.54	0.52
Lambs	Lame on 15.10.19	0.71	0.46	0.62	0.59
	Lame in 3 days prior to 15.10.19	0.93	0.08	0.60	0.50
	Lame in 7 days prior to 15.10.19	0.93	0.15	0.63	0.54
	Lame in 14 days prior to 15.10.19	0.88	0.31	0.66	0.59
Lame at ≥3	3				
Ewes	Lame on 15.10.19	0.86	0.07	0.62	0.46
	Lame in 3 days prior to 15.10.19	0.89	0.07	0.64	0.48
	Lame in 7 days prior to 15.10.19	0.86	0.13	0.64	0.50
	Lame in 14 days prior to 15.10.19	0.77	0.20	0.60	0.49
Lambs	Lame on 15.10.19	0.93	0.08	0.60	0.50
	Lame in 3 days prior to 15.10.19	0.93	0.12	0.62	0.52
	Lame in 7 days prior to 15.10.19	0.90	0.19	0.63	0.55

Classification of lameness	Se	Sp	A	BA
Lame in 14 days prior to 15.10.19	0.88	0.23	0.63	0.56

1. Se = sensitivity, Sp = specificity, A= accuracy, BA = balanced accuracy