March 2020

Student Final Report No. 61110037

The prevalence of lameness and diversity of serogroups of *Dichelobacter nodosus* in sheep in England

Naomi Prosser[1], Kevin Purdy[1] and Laura Green[2]

[1]School of Life Sciences, Gibbet Hill Campus, The University of Warwick, Coventry, CV4 7AL

[2]College of Life and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT

Supervisor: Lis King and Liz Genever

This is the final report of a PhD project (AHDB Beef and Lamb Ref: 61110037 that ran from October 2015 to September 2019. The work was funded by AHDB Beef and Lamb and BBSRC.

CONTENTS

BEEF & LAMB

1. Abstract

Lameness in sheep is a significant problem in the UK, and footrot, caused by *Dichelobacter nodosus*, is the most common cause. A recent reduction in the prevalence of lameness between 2004 and 2013 was associated with increased use of best practice managements by farmers. A prevalence of < 2% lameness is achievable with current best practice managements and is the target for national prevalence of lameness set by the Farm Animal Welfare Council for 2021. Vaccination is a preferred management by farmers, and ideally would be fully effective, preventing lameness and saving farmer time. The commercial multivalent vaccine reduces the prevalence of lameness by 20%. Mono-/bivalent vaccines are more effective, but only against homologous serogroups.

A cohort of 164 English sheep farmers completed questionnaires regarding the prevalence of, and managements for, lameness in their flock. There was an increase in the prevalence of lameness in ewes in England in 2015 compared to 2013, which was associated with a decrease in the proportion of farmers treating all lame sheep within three days, despite the increased uptake of other flock-level best practice managements. Population attributable fraction indicated that if all farmers moved to prompt treatment (≤ 3 days) of all lame sheep this would have the greatest impact on reducing the national prevalence of lameness in England. Farmers also submitted interdigital skin swabs from eight sheep. All serogroups A – I were detected across English flocks, at different prevalence, distributed randomly between flocks, but clustered within flocks creating heterogenous communities between flocks. Consequently, is unlikely that one bivalent footrot vaccine tailored to the two most common serogroups in England would be more effective than the commercial vaccine, because many flocks would not be protected. Tailored flock-specific vaccines may be more effective, but their feasibility in England will have to investigated. In conclusion, whilst an improved vaccine is likely to be readily accepted by farmers, the current focus of knowledge transfer needs to be on prompt (≤ 3 days) and appropriate treatment of all lame sheep.

2. Introduction

2.1. Production and welfare effects of lameness in sheep

Lame sheep are present in almost every flock in England (Winter et al., 2015) and cause considerable concern for sheep farmers (Goddard et al., 2006, O'Kane et al., 2017). Being lame is a response to pain (Ley et al., 1989), and lameness is therefore a welfare issue. Lameness results in decreased body condition of the sheep (Smith et al., 2014, Wassink et al., 2010b), which leads to a reduction in the productivity of sheep: lame ewes give birth to fewer lambs and their lambs take longer to finish (Wassink et al., 2010b), and lame lambs also take longer to reach their slaughter weight (Nieuwhof et al., 2008). The principle cause of lameness in the UK, footrot, is estimated to cost the UK sheep industry up to £80 million per annum in treatments and lost production (Wassink et al., 2010b).

2.2. Prevalence of lameness in sheep in England

Most lameness in sheep in the UK is caused by the infectious diseases footrot and contagious ovine digital dermatitis (CODD) (Winter et al., 2015). Footrot is the most common cause of lameness in sheep in the UK (Winter et al., 2015), and is caused by *Dichelobacter nodosus* (Beveridge, 1941), with *Fusobacterium necrophorum* as a secondary invader and involved in disease severity (Beveridge, 1941, Witcomb et al., 2014). Footrot primarily affects the epidermal tissue of the hoof (Witcomb et al., 2015). It is thought that prior damage to the interdigital skin, for example due to long grass or wet conditions, is necessary for entry of *D. nodosus* (Beveridge, 1941). The disease has two clinical presentations. It initiates as an interdigital dermatitis, often termed scald or strip, where the interdigital skin is inflamed and there is loss of hair, a white or grey pus, and a foul rotting smell (Winter, 2008). This sometimes progresses to severe footrot, where there is underrunning of the hoof horn, causing it to separate from the living dermis (Egerton et al., 1969), often accompanied by a foul smelling grey pus (Winter, 2008). The load of *F. necrophorum* increases as disease progresses from interdigital dermatitis to severe footrot (Witcomb et al., 2014). Transmission of *D. nodosus* occurs between sheep via the pasture, and *D. nodosus* has been detected in both straw bedding and soil samples (Muzafar et al., 2015). The survival of *D. nodosus* in soil is influenced by soil type, temperature and moisture (Muzafar et al., 2016), with clay soils, lower temperatures and higher moisture content all associated with longer survival times (Cederlof et al., 2013, Muzafar et al., 2016). The risk of footrot is also increased in lambs (Angell et al., 2018) and with increasing ewe age (Kaler et al., 2010b).

Footrot is endemic in the UK due to mild and wet environmental conditions suitable for year-round transmission (Smith et al., 2014, Green and George, 2008). Footrot was present in over 90% of

flocks in England in 2004 (Kaler and Green, 2008a), with a mean prevalence of 6.5% and 3.1% for interdigital dermatitis and severe footrot lesions respectively (Kaler and Green, 2009), but the prevalence of interdigital dermatitis and severe footrot lesions within a flock can be as high as 50% and 20% respectively (Kaler and Green, 2009). Footrot is present in almost all flocks and accounts for 68% of foot lesions (Winter et al., 2015). The relative proportion of lameness caused by footrot fell from over 80% to approximately 70% between 2004 and 2013 (Kaler and Green, 2009, Winter et al., 2015).

CODD initiates as a lesion at the coronary band, with or without hair loss, which progresses to downward underrunning of the hoof wall from the underlying live tissue, sometimes resulting in the avulsion of the entire hoof capsule, and has much more blood associated with it than footrot (Angell et al., 2015). The causal agent is not known, however treponemes have been implicated in many studies (e.g. Moore et al., 2005b, Naylor et al., 1998) and the disease is likely to have originated from digital dermatitis in cattle because the treponemes associated with CODD are highly associated with the phylogroups in bovine digital dermatitis (Sullivan et al., 2015). However, treponemes are often involved in polymicrobial lesions. *D. nodosus* and *F. necrophorum* are also associated with CODD lesions (Sullivan et al., 2015, Moore et al., 2005b). CODD lesions often appear on feet with severe footrot lesions and vaccination against footrot lowers new infection rates of CODD, but not recovery rates (Duncan et al., 2012) which indicates a role of *D. nodosus* in the pathogenesis of CODD.

CODD is not present in as many flocks as footrot, but since its identification in 1997 it has been increasing in the proportion of flocks that it infects, present in a recently estimated 35% – 58% of flocks (Angell et al., 2014, Dickins et al., 2016, Harwood et al., 1997), and is contributing to an increased proportion of the lameness in ewes nationally (Winter et al., 2015). The mean prevalence of CODD in infected flocks is 2.3% in England (Dickins et al., 2016), but up it can affect up to 50% of sheep in a flock (Angell et al., 2014). Anecdotally, CODD causes epidemics of lameness when first introduced into a flock before becoming endemic, and this is supported by the distribution of between flock prevalence of CODD (Dickins et al., 2016).

Between 2004 and 2013 the period prevalence of all sheep lameness fell from 10.2% to 4.9%, according to estimates from random samples of English sheep farmers, and the geometric mean prevalence of lameness fell from 5.4% to 3.5% (Kaler and Green, 2009, Winter et al., 2015). The recent reduction of the prevalence of lameness in ewes in the UK can be attributed to change in the management of lameness towards antibiotics and vaccination, and away from foot trimming (Winter et al., 2015). The Farm Animal Welfare Council has set a target for the prevalence of lameness to be ≤ 2% by 2021, with an interim target of ≤ 5% by 2016 (FAWC, 2011). The target of

≤ 5% for 2016 was achieved in 2013 (Winter et al., 2015). A prevalence of ≤ 2% is already achievable with prompt treatment of lame sheep within three days with parenteral and topical antibiotics (Wassink et al., 2010b).

2.3. Prevention and treatment of lameness in sheep

Cases of infectious causes of lameness, footrot and CODD, should be treated promptly (within three days) with a long-acting parenteral antibiotic, and topical antibiotic spray on all four feet, which is sufficient to reduce the flock prevalence of lameness to 2% or less (Kaler et al., 2010a, Wassink et al., 2010b). These recommended treatments reduce the load of the infectious agents in the flock and improve the resilience of sheep to disease. Other managements that can further reduce the prevalence of lameness are separating lame sheep from the rest of the flock, vaccinating against footrot, culling sheep that have been lame twice or more in a year and only selecting breeding replacements from parents that have never been lame (Winter et al., 2015, Witt and Green, 2018). Traditionally farmers would routinely trim overgrown hooves, and trim excess hoof horn in cases of footrot; however, recent evidence has demonstrated that there is no reduction in the prevalence of lameness from routine foot trimming, that foot trimming that causes bleeding in the flock increases the prevalence of lameness, and that trimming feet with footrot lesions delays healing (Winter et al., 2015, Kaler et al., 2010a).

2.4. Serogroups of *Dichelobacter nodosus*

D. nodosus can be subdivided into ten serogroups (A – I and M) which differ in their fimbrial antigens (Claxton et al., 1983). Serogroups are not associated with endemic disease (Moore et al., 2005a); however, the immunological response of the sheep is serogroup specific and there is no cross immunity between serogroups therefore vaccination will only confer protection against homologous serogroups (Stewart, 1978). Most serogroups of *D. nodosus* contain several serotypes; however, there is cross immunity across serotypes in the same serogroup, so an immune response against one serotype will protect against all other serotypes within that serogroup (Claxton et al., 1983).

Strains of *D. nodosus* also differ in their pathogenicity but this is independent of serogroup. More virulent strains of *D. nodosus* show increased twitching motility, form larger colonies and have stable proteases (Depiazzi and Richards, 1985). *D. nodosus* has diverse virulence factors, however the acidic protease AprV2, which degrades host tissue such as keratin, is essential for virulence (Kennan et al., 2010).

2.5. The diversity of serogroups of *Dichelobacter nodosus* in the UK

All serogroups (A – I, and M) have been detected in the UK (Thorley and Day, 1986, Hindmarsh and Fraser, 1985). In the most recent study of the serogroups of *D. nodosus* in the UK in 2005 serogroups A – I were detected on 39 non-randomly selected farms (Moore et al., 2005a). The study did not investigate serogroup M, however, five isolates from three sheep were not typed as serogroups A – I and so were possibly serogroup M (Moore et al., 2005a). Serogroup H was the most prevalent serogroup, and B and F were the next two most frequently detected serogroups. In previous studies serogroup B was the most prevalent (Hindmarsh and Fraser, 1985, Kingsley et al., 1986).

It is common for several serogroups of *D. nodosus* to be detected on a sheep, and within a flock concurrently. In the UK, > 1 serogroup was detected from 10% of sheep and 60% of flocks, with up to four serogroups detected per flock using culture methods (Hindmarsh and Fraser, 1985, Moore et al., 2005a, Kingsley et al., 1986). The median number of serogroups per flock is two and at least 22.9% of flocks have more than two serogroups (Moore et al., 2005a). The actual number of flocks with more than two serogroups is likely to be higher if direct PCR methods are used which are more sensitive than culture (McPherson et al., 2018).

2.6. Methods of detection of *Dichelobacter nodosus* serogroups

In order to conduct research into the serogroups and serotypes of *D. nodosus* present in flocks, several methods to detect serogroups have been developed. Originally slide agglutination reactions using unabsorbed antisera were used to determine serogroup, and tube agglutination reactions, also using unabsorbed antisera, were used to determine serotype. Samples would initially have to be cultured, which is a difficult and lengthy process because a complex media is required. PCR has been developed using the *fimA* gene to detect the serogroups and is more sensitive than culture methods (McPherson et al., 2018). Furthermore, multiplex PCR has also been developed (Dhungyel et al., 2002) to allow the detection of nine of the serogroups (A – I) in samples in as few as two reactions; however, to date, there is no published primer for detection of serogroup M using PCR. The multiplex PCR is unable to detect a serogroup when it is in very low quantities in a mixed serogroup sample, but this is when the difference in concentration is 1000-fold (Dhungyel et al., 2002), so it is unlikely to be an issue. These advances have made the detection of *D. nodosus* serogroups much faster with a greater likelihood of all the serogroups present in a sample being detected.

2.7. Multivalent vaccination against *Dichelobacter nodosus*

Although treatment of interdigital dermatitis or severe footrot with antibiotics is generally successful (e.g. Kaler et al., 2010a), becoming lame is a welfare issue and sheep remain susceptible to footrot once they have recovered and may need treating again in the future. Therefore, protection against footrot is highly desirable. Despite the poor natural immune response of sheep to footrot, vaccination can be effective (e.g. Egerton and Burrell, 1970), and could be an important management tool. Monovalent vaccines do not provide protection against heterologous serogroups of *D. nodosus* because antibodies are serogroup specific (Hunt et al., 1994), therefore vaccination is ineffective if it targets different serogroups to those present in the flock (e.g. Dhungyel et al., 2015). Because multiple serogroups are often present in flocks, bivalent and multivalent vaccines with two to several antigens to fimbrial serogroups have been created to cover the serogroups found in the field (Egerton, 1973). Multivalent vaccines against *D. nodosus* confer lower immunity per strain compared with monovalent vaccines because of antigenic competition (Schwartzkoff et al., 1993). There are significantly lower antibody titres in sheep vaccinated with multivalent vaccines compared with monovalent vaccines, and titres decline faster, therefore the sheep has both lower protection and for a shorter duration (Schwartzkoff et al., 1993). The exact mechanism for the antigenic competition is unknown; however, vaccine antigens are not compromised in their efficacy pre-injection, therefore the reduced efficacy of multivalent footrot vaccine must occur in the sheep (Schwartzkoff et al., 1993). Individual sheep differ in their response to the vaccine, both in the total amount of antibody produced and the relative proportion of antibodies against each serogroup in the vaccine (Hunt et al., 1994, Raadsma et al., 1994). Antigens also differ in their immunodominance (Dhungyel and Whittington, 2010, Raadsma et al., 1994).

2.8. Tailored bivalent vaccines against *Dichelobacter nodosus*

Sequential application of mono- or bivalent vaccines with different antigens can negate the effects of antigenic competition and therefore could be used to eliminate footrot from a flock. However, full elimination of footrot in flocks with > 2 serogroups using sequential bivalent vaccination is yet to be achieved in practice. There has been success with eliminating more extensive interdigital lesions, with or without underrunning of the hoof wall, (defined as virulent footrot in Australia) with sequential (12 monthly) application of tailored mono- and bivalent vaccines in 4 out of 12 flocks in Australia, and disease reduction was achieved in a further 7 flocks (Dhungyel et al., 2013). Pre-vaccination, 2 – 8 serogroups were detected in each flock. After the application of 1 – 5 vaccines over 1.5 – 5 years, the incidence of footrot with a score of ≥ 2 was eliminated in four flocks, fell in seven flocks and rose in one flock. On each occasion, all the feet of 30% – 50% of the sheep were investigated. Feet were scored using a 4-point scoring system described by Egerton and Roberts (1971), however score 1, 'limited mild interdigital lesions', were ignored in this study and

eradication was considered achieved when there were no lesions of score 2 – 4 (more extensive interdigital lesions with or without underrunning of the hoof wall) present in the flock, therefore it was not true footrot eradication. Only the serogroups with virulent strains (defined using the Gelatin gel and Elastase tests) detected in the flock were considered for inclusion in the mono- or bivalent vaccines, which were given in two 1ml doses one month apart. There was a one-year interval between doses of different vaccines. The disadvantage of this approach is that the serogroups responsible for footrot and their proportional prevalence within a particular flock have to be identified (Hill et al., 2010) and the vaccination program tailored to each flock. This is expensive and time consuming.

An inter-vaccine interval of three months with different antigens was sufficient to avoid antigenic competition with two sequential bivalent vaccines targeting distinct serogroups (Dhungyel and Whittington, 2010). With ten serogroups of *D. nodosus*, sequentially administering vaccines against every serogroup would take a minimum of fifteen months. It is unknown whether three-month intervals are enough to avoid antigenic competition between > 2 sequential bivalent vaccines. This needs to be tested but is unlikely given that sequential targeted bivalent vaccination is less successful the greater the number of serogroups in the flock (Dhungyel et al., 2013). Short inter-vaccination intervals would be preferable to reduce the risk of some serogroups to recrudesce or be reintroduced into a flock after the targeted serogroup immunity wanes.

2.9. Vaccination as a footrot control tool in the UK

Currently the only form of vaccination against footrot that is available to farmers in the UK is the licensed commercial vaccine, Footvax™, which contains nine of the ten serogroups (A – I), including two serotypes of the most diverse serogroup, B (B1 and B2) (MSD Animal Health), in order to provide protection against almost all the serogroups of *D. nodosus*. Footvax™, has varying efficacy between flocks; in a recent observational study of 1,260 flocks in England (Winter et al., 2015) it reduced the proportional prevalence of lameness by an average of 20%, an absolute mean reduction of 1% lameness. In other studies, Footvax™ reduced the prevalence of footrot lesions by 80% in two flocks in Germany (Ennen et al., 2009) and reduced the odds of a sheep developing footrot by 62% in one flock in the UK (Duncan et al., 2012). The differing response from individual sheep may explain the varying between-flock level of protection against footrot from the vaccine. Due to the vaccine not providing complete protection from developing footrot, and with a duration of protection of only 4 – 5 months, the manufacturer of Footvax™ recommends vaccination together with other managements for treatment and control of footrot such as the Five Point Plan (MSD Animal Health, 2014) (which encourages farmers to Vaccinate to give immunity, Cull to improve resilience in their flock, and Avoid, Treat and Quarantine to reduce the disease challenge

(Clements and Stoye, 2014)) and that vaccination is conducted either biannually to provide year-round protection, or annually to coincide with the expected peak period of disease spread. Furthermore, a particularly harsh adjuvant is used in Footvax™ to increase the stimulation of the immune system of the sheep to produce antibodies; mineral oil is used as an adjuvant which causes lumps from reactions at the injection site. This does cause some concern amongst farmers and discourages them from using the vaccine.

Most vaccination studies have been conducted in Australia where sheep flocks are managed differently to in the UK and face different environmental conditions. In many parts of Australia footrot stops transmitting in the dry season, making it an ideal time for disease control, and elimination attempts have had success (Green and George, 2008). Elimination was less successful in areas with higher rainfall and a shorter, wetter, and cooler dry period (reviewed in Green and George, 2008). *D. nodosus* persists for longer in the soil with higher moisture content and at 5°C rather than 25°C (Muzafar et al., 2016), and transmission is associated with temperature and rainfall (Smith et al., 2014). The more temperate climate of the UK is ideal for footrot transmission year-round (Smith et al., 2014), leading it to be endemic here, with frequent epidemics within a flock (Green et al., 2007). Dry spells are also far less predictable than in Australia, giving no time suited to a concerted elimination effort. Furthermore, Australian flocks are much larger but more closed, in contrast to the regular sheep movements in the UK (Green and George, 2008). Flocks with footrot in Australia may not sell to other flocks, but only directly to slaughter, in contrast to the UK where it is almost impossible to purchase sheep from a footrot-free flock (Green and George, 2008). Flock-specific vaccination is likely to be more difficult in the UK given that multiple serogroup infections in a flock are common, footrot is endemic, biosecurity is poor, and it is very difficult to identify flocks free from footrot to purchase footrot-free sheep.

2.10. Objectives

The objectives of the project were to:

- investigate the prevalence of lameness and change in management of lameness in ewes in England monitored from 2013 to 2015
- estimate the population attributable fractions that would inform on key management practices that could reduce the national global flock prevalence of lameness
- identify if all serogroups (A – I) of *D. nodosus* are still present in England
- identify if there are regional differences in serogroup diversity and distribution in *D. nodosus* in England
- identify if serogroup diversity and distribution are associated with biosecurity practices, including vaccination

- identify if the prevalence, diversity and distribution of serogroups are such that a vaccine with fewer serogroups could be used nationally, and this may offer better protection than the current commercial vaccine against footrot

The work for the first two objectives described in this report has been published as:
PROSSER, N. S., PURDY, K. J. & GREEN, L. E. 2019. Increase in the flock prevalence of lameness in ewes is associated with a reduction in farmers using evidence-based mangement of prompt treatment: A longitudinal observational study of 154 English sheep flocks 2013-2015. *Preventive Veterinary Medicine,* 173**,** 104801.

3. Materials and methods

3.1. Ethical approval

Approval for the work in this thesis was granted by The University of Warwick Biomedical and Scientific Research Ethics Committee (REGO-2016-1758 AMO1) and Animal Welfare and Ethical Review Body (AWERB.24/15-16) on 23/02/16 and 08/04/16 respectively.

3.2. Selection of farmers to the study

The author invited 722 farmers to participate in the study. Each farmer had completed detailed questionnaires on lameness in their flock in 2013 (Winter et al., 2015) and most in 2014 (Grant et al., 2018), and had indicated that they were willing to participate in further research. Invitation letters and reply slips were posted to farmers. The invitation letter also included a summary of a paper published from the 2013 questionnaire results (Winter et al., 2015); which was sent in order to encourage participation. Most farmers were invited by letter in February 2016 (Table 3.1). A further 12 farmers, who had footrot in their flock in 2013 but not 2014, were invited in April 2016 after initially being omitted from inclusion with the intention to include them in a different study (Table 3.1).

Table 3.1 The invitation of 722 English sheep farmers to the study.

Date	Number of farmers	Method	
09/02/2016	27	In person	Attended a University of Warwick "Update on Lameness in Sheep" event where they could discuss participation and read a poster about the project.
10/02/2016	683	Post	Did not attend the "Update on Lameness in Sheep" event.
05/04/2016	12	Post	Initially omitted from inclusion with the intention to include them in another study.

A total of 192/722 (26.6%) farmers agreed to participate in the study. Replies were received from farmers from 09/02/2016 – 30/06/2016.

3.3. Questionnaire design

A three-page questionnaire on the average period prevalence of, and management practices for, lameness in sheep was developed for the calendar year 2015 (Appendix 1). The questions were closed and semi-closed and were selected from those used in more detailed questionnaires completed by the same farmers in 2013 (Winter et al., 2015) and 2014 (Grant et al., 2018). As in the 2013 and 2014 studies, the prevalence of lameness estimated by farmers was the average percentage of ewes lame at any one time over the period, this was validated in King and Green (2011). The time to treatment of lame sheep was the longest time that any sheep was left lame before treatment, categorised into ≤ 3 days (i.e. all sheep treated within 3 days), ≤ 1 week, ≤ 2 weeks and > 2 weeks (i.e. some sheep were not treated within 2 weeks of onset of lameness). Farmers were asked if they practised routine foot trimming, and if they did what percent of feet bled at a routine foot trimming event. This was categorised into 0 – 2% and ≥ 5%. Farmers were asked if they culled sheep that had been lame, whether they vaccinated ewes against footrot and the number of years they had used the vaccine, and if their sheep mixed with other sheep at planned events e.g. sheep shows or unplanned e.g. insecure boundaries between farms. The questionnaire was internally reviewed by the research team for consistency and ease of completion, it took approximately ten minutes to complete.

3.4. Postage and packaging of swab samples and questionnaires

Packaging was designed and sourced by the author for the return postage of the swabs to be compliant with UN3373 packaging requirements (for category B biological substances). Additional requirements from the University of Warwick Health and Safety Department were that farmers were provided with instructions on how to use the UN3373 packaging correctly and that the

packages were signed for on receipt at the University of Warwick. For return postage, the swabs were stored in charcoal amies transport media in plastic tubes, placed and sealed in absorbing tissue and bubble wrap lined plastic bags, and placed in a carboard box. Each package was placed in a UN3373 labelled prepaid return envelope and handed in at a Post Office counter for delivery. A Special Delivery Guaranteed Returns Service was set up by the author with the Royal Mail, with guaranteed delivery by 1pm the next day to minimise the time that the swabs were stored at room temperature before freezing.

Upon the receipt of a reply, each of the 192 farmers was sent a swabbing kit. The swabbing kit contained a cover letter, eight sterile swabs with charcoal amies gel transport media, illustrated instructions explaining how to take the swabs to ensure that farmers used the same technique as each other and as used in other research at The University of Warwick (Appendix 2) and a sheet to collect information on the sheep and foot swabbed. The swabbing kit also contained the questionnaire (Appendix 1) on the prevalence of lameness and managements used in 2015.

In order to increase the number of flocks that could be included in the study, participants were asked to self-swab their sheep. The participants were asked to swab the interdigital skin of one foot of each of eight sheep, ideally all lame with footrot (if eight lame sheep were not present the farmer was asked to complete the sampling with healthy sheep), that had not received treatment in the last two weeks. Eight swabs were the maximum number that could be transported in UN3373 compliant packaging to comply with the total volume of samples. Farmers were asked to place each swab in its charcoal amies transport media container, complete the information sheet and the 2015 questionnaire and pack and post the swabs. Swabs were posted immediately or refrigerated on farm overnight and then posted. They were delivered at room temperature within 24 hours of posting. On arrival, the swabs were immediately transferred to -20°C where they were stored until processed.

Three weeks after the swabs were sent to the farmers, or after the time period that the farmer had indicated they would take samples, the author sent out reminder emails/letters. In June 2016, all farmers who had not returned their samples received a final reminder with a deadline for the return of the swabs and questionnaires of the end of July 2016. A total of 146 farmers returned swabs, of these 144 also returned the questionnaire.

3.5. Swab sample collection from the clinical trial

Swabs and questionnaire data were sourced from an additional 18 flocks where the farmers had responded to the 2013 and 2014 lameness questionnaires and were participating in another

project (Witt and Green, 2018). As part of that project, feet from up to 15 diseased sheep were swabbed between November 2015 and August 2016. Eight swabs per flock were selected for use in this project using the same criteria as in section 3.4. Post-swabbing, sheep were selected by the author in the following order of preference: lame with severe footrot or interdigital dermatitis (locomotion score ≥ 2 (Kaler et al., 2009)), lame with another lesion (locomotion score ≥ 2), not lame (locomotion score < 2). Feet were selected in the following order of preference: severe footrot lesion, interdigital dermatitis lesion, another lesion, no lesion. Swabs were stored in phosphate buffered saline. The liquid was spun off with a centrifuge and split into two aliquots, with one aliquot given to the author and used for this project.

3.6. Questionnaire data input

The responses to the questionnaire for the farmers participating in the clinical trial (Witt and Green, 2018) were retrieved from a more detailed questionnaire conducted as part of that study. The questionnaire responses for both the postal and clinical trial farmers were typed into Microsoft Excel and data entry was rechecked once to ensure accuracy.

3.7. Farmer subsets for analysis

Two farmers did not return questionnaires, and eight farmers did not give the flock size and prevalence of lameness in ewes. These ten farmers were omitted from analysis into the risk factors for the prevalence of lameness in 2015 leaving 154 farmers. All 164 farmers were included in analysis of the serogroups present in the flocks.

3.8. Representativeness of respondents from invitees

The representativeness of the 154 respondents to the 2015 questionnaire was compared with all 740 (722 plus 18) farmers invited to participate in the 2015 study by geographical location. T-tests (Crawley, 2013) were used to test for a difference in the prevalence of lameness and flock size in 2013 between the 154 respondents and the 740 invitees, and the 154 respondents and the total 1,260 respondents to the 2013 questionnaire.

3.9. Changes in percentage lameness and managements between 2013, 2014 and 2015

The prevalence of lameness in ewes and the ewe flock size in 2013, 2014 and 2015 were compared using log-transformed data in multilevel models (Dohoo et al., 2003) (Table 4.1) with no assumed correlation structure using the nlme package (version 3.1-137) (Pinheiro et al., 2018). Year was a fixed effect and flock a random effect in all models. The analysis was conducted with

both the 128 flocks who gave data on flock size and prevalence of lameness for all three years, and all 154 respondents to the 2015 questionnaire. Post-hoc analysis was conducted with Tukey HSD (Crawley, 2013) using the multcomp package (Hothorn et al., 2008). For the 154 farmers who answered the 2015 questionnaire, the percentage of farmers that practised managements associated with lameness in 2013 (Winter et al., 2015) were compared with the percentage of farmers who practised those managements in 2014 and 2015 using chi-squared tests and Fisher's exact tests (Crawley, 2013).

3.10. Identification of management practices associated with flock prevalence of lameness in 2015

The flock prevalence of lameness had an overdispersed distribution, the dispersion parameter of the models (residual deviance divided by the residual degrees of freedom) was greater than one, in both negative binomial models, which assume a negative binomial distribution, and overdispersed Poisson (quasi-Poisson) models, which leave the dispersion parameter unrestricted, which were investigated to identify the model with the best fit (Ver Hoef and Boveng, 2007). Best fit was tested by ranking the predicted number of lame sheep per flock in deciles and comparing with the observed number of lame sheep from each model. A multivariable quasi-Poisson regression model was the best fit and so this was used.

The model took the form:

$$\text{Observed number of lame ewes}_j \sim \alpha + \text{offset} + \beta_j X_j + e_j$$

Where ~ is a natural log link, α is the intercept, the offset is the natural log of the expected number of lame ewes (calculated internally from the flock size), β_j are coefficients for a vector of X_j farmer managements which vary by farm j and e_j is the residual random error.

Each variable was tested in a univariable model and the multivariable model was then built using a manual forward stepwise procedure adding the term with the greatest decrease in AIC at each iteration. Once the addition of further variables no longer improved model fit, all the variables were retested in the model to check for residual confounding (Cox and Wermuth, 1996). The model fit was tested by comparing the predicted and observed number of lame sheep per flock ranked in deciles and visually assessed. The model was re-run including only the 128 farmers who provided data on flock size and prevalence of lameness for all three years, and excluding the 18 farms with data from the clinical trial.

3.11. Population attributable fractions of managements associated with the prevalence of lameness

The percentage of the 154 farmers using the management practices (risk factors) in the multivariable model for 2015 were compared with the percentage of the same farmers practising those managements in 2013 and 2014 using chi-squared tests (Crawley, 2013).

In addition, for each risk factor in the multivariable model, the attributable fraction (AF) in the exposed flocks (where the risk factor was present) and the population attributable fraction (PAF) (the proportion of the national lameness attributable to the risk factor) were calculated. Using:

$$AF = (RR - 1) / RR \text{ and } PAF = AF (a_1 / m_1)$$

Where RR is the risk ratio for a risk factor, a_1 is the number of flocks exposed to the risk factor and m_1 is the total number of flocks in the model (Dohoo et al., 2003).

3.12. DNA extraction from swabs

Swabs were randomised for processing by assigning a random number stratified by flock. Random numbers were generated in Microsoft Excel 2016. DNA extraction was performed using a Nucleospin® Tissue kit (Macherey-Nagel) with modifications. Swabs were thawed at room temperature, transferred to microcentrifuge tubes and lysis buffer T1 (400µl) and proteinase K (40µl) were added. Samples were vortexed for five seconds and incubated for 10 minutes at 56°C. Post-incubation, lysis buffer B3 (400µl) was added. Each sample was vortexed for 5 seconds and incubated at 70°C for 5 minutes. Samples were cooled at room temperature for 5 minutes before being centrifuged at 11,350rpm for 1 minute. Supernatant was added to 100% ethanol (400µl) and centrifuged at 10,850rpm for 1 minute. Supernatant was loaded onto spin columns and centrifuged at 11,000rpm for 1 minute. Flow-through was discarded and BW wash buffer (500µl) was added to the spin columns and centrifuged at 10,850rpm for 1 minute. Flow-through was discarded and B5 wash buffer (600µl) was added to the spin columns and centrifuged at 10,850rpm for 1 minute. Flow-through was discarded and spin columns were centrifuged for an additional 1 minute at 10,850rpm to dry the membrane. Spin columns were placed into microcentrifuge tubes and BE elution buffer (45µl) heated to 70°C was added and allowed to stand for 2 minutes. Tubes were spun at 10,850rpm for 1 minute to elute DNA and extracted DNA was stored at -20°C.

3.13. Screening for *Dichelobacter nodosus* with a qPCR

Each DNA sample was tested in triplicate for the presence of *D. nodosus* using a qPCR that detected the load of the gene *aprV2* and *aprB2* using the method published by Frosth et al. (2015) with some modifications. Modifications were made to the probe labelling (*aprV2*probe 6FAM-BHQ1, *aprB2*probe TxRd-BHQ2), Klearkall master mix (LGC Group) was used and only 1μl of template DNA was used. The initial denaturation step was extended to 15 minutes as required by the Klearkall master mix (LGC Group). *D. nodosus* strain VCS1703A was used as a positive control for *aprV2*, and strain C305 was used as a positive control for *aprB2* (Table 3.2). Samples that were 3/3 positive for either *aprV2* or *aprB2* were classed as positive for *D. nodosus.* Load was measured as a log load, defined as $\log_{10}$(number of genome copies per μl).

3.14. Serogroup identification of *Dichelobacter nodosus* positive samples using PCR

Samples positive for *D. nodosus* by qPCR were tested for nine serogroups (A – I) using single serogroup PCRs. Single serogroup PCRs were used because they were more sensitive than multiplex serogroup PCRs (Appendix 3). The primers and program used were published by Dhungyel et al. (2002). Each reaction (25μl) contained MyTaq Red Mix (Bioline) (12.2μl), the forward and reverse primers (0.5μM each), and bovine serum albumin (0.5mg/ml). PCR products were electrophoresed on 3% agarose gels stained with ethidium bromide and visualised under ultraviolet light. Positive controls were normalised to 10ng/μl and sources of positive controls are in Table 3.2. During the sensitivity testing of the multiplex and single PCRs, the PCRs were tested for specificity against all other positive controls but not against other bacterial species. However, the PCR results from the swab samples were clean, with bands of the correct size, making it unlikely that the PCRs were amplifying anything other than *D. nodosus*.

Table 3.2 The strain and source of the positive controls used in the D. nodosus serogroup specific PCRs.

D. nodosus strain	Serogroup positive control	Source
V2_3LR	A	Giebel (2017)
12	B	Muzafar et al. (2015)
C305	C	Australia
20	D	Muzafar et al. (2015)
E	E	Bristol University
JIR_3568	F	Australia
VCS1703A	G	Australia
0107 -EDH	H	Smith et al. (2017)
0108 -EDI	I	Smith et al. (2017)

3.15. Data analysis of the diversity of serogroups of *Dichelobacter nodosus*

All data analysis was conducted in R statistical software (version 3.5.1) (R Core Team, 2017) and by the author. All tests were conducted in base R unless a package is cited. Post hoc analysis of chi-square tests and Fisher's exact tests were conducted with the fifer (version 1.1) (Fife, 2017) and rcompanion (version 2.1.1) (Mangiafico, 2019) packages respectively.

Three datasets were created: one contained all 1,150 swabs from 164 flocks, one the 566 *D. nodosus* positive swabs from footrot-affected feet from 138 flocks, and one was a simulated dataset of the same 566 *D. nodosus* positive swabs from footrot-affected feet from 138 flocks, with serogroups randomly assigned to swabs. In order to create the simulated dataset, the results from the 566 *D. nodosus* positive swabs from footrot lesions from 138 flocks were simulated 1,000 times assuming a random distribution of serogroups present within and between flocks. To do this, each swab was assigned as positive or negative for each serogroup (A – I) weighted by the observed proportion of swabs positive for that serogroup nationally. Only swabs from footrot lesions were used in the simulations to remove the heterogeneity in detection of *D. nodosus* from feet with other lesions, and because the majority of *D. nodosus* positive swabs were from footrot lesions.

Analysis into the diversity of serogroups is analysis into whether each serogroup was detected. Analysis into the number of serogroups is analysis into how many serogroups were detected per foot/flock.

3.16. Factors associated with the number of swabs submitted

Kruskal-Wallis tests were used to investigate whether flock size, the prevalence of lameness or the prevalence of severe footrot in ewes, were associated with the number of swabs submitted and the number of swabs that were *D. nodosus* positive per flock.

3.17. Lesion status of swabbed feet

All swabbed feet were categorised into four lesion categories (Table 3.3). A chi-square test was used to investigate differences in the proportion of *D. nodosus* positive swabs by lesion state.

Table 3.3 Disease status categories of the swabbed feet.

Lesion	Description
Footrot	Footrot lesion (either severe footrot or interdigital dermatitis) present on swabbed foot
Other	One or more lesions that did not include footrot present on swabbed foot
Unknown	Lesion status of swabbed foot unknown
Healthy	No lesion present on swabbed foot

3.18. aprV2/B2 strains of Dichelobacter nodosus

A t-test was used to compare the loads of a*prV2* and *aprB2* strains of *D. nodosus* within feet, and a Wilcoxon signed-rank test was used to compare the loads between flocks. Fisher's exact tests were used to compare the lesion status of the foot and the diversity of the serogroups between feet with a higher load of *aprB2* than *aprV2* and a higher load of *aprV2* than *aprB2*, and between feet that were *aprB2* positive and *aprB2* negative. This analysis was repeated for the diversity of serogroups at flock-level.

3.19. *Dichelobacter nodosus* load by number of serogroups detected

A t-test was used to compare the load of *D. nodosus* in samples where at least one serogroup was detected and samples where no serogroup was detected. A Kruskal-Wallis test was used to investigate the load of *D. nodosus* with different numbers of serogroups detected per swab. An equality of coefficients of variation test was used to investigate variation in the log load of *D. nodosus* on swabs by number of serogroups using the cvequality package (version 0.2.0) (Marwick and Krishnamoorthy, 2019).

3.20. Prevalence and diversity of serogroups on feet and in flocks

The prevalence of each serogroup per foot and flock was calculated and exact binomial tests were used to determine the 95% confidence intervals for the proportion of flocks that were positive for each serogroup. Difference in the prevalence of each serogroup in flocks between the observed and simulated datasets was tested with a chi-square test.

3.21. Number of serogroups detected on feet and in flocks

The number of serogroups detected per foot and flock were calculated. Difference in the number of serogroups in feet and flocks between the observed and simulated datasets were tested with Fisher's exact tests.

3.22. Disease state on the serogroups detected on feet

Fisher's exact tests were used to investigate both the serogroups detected and the number of serogroups detected on feet by lesion status.

3.23. Number of swabs on the serogroups detected in flocks

Exact binomial tests were used to determine the 95% confidence intervals for the proportion of flocks that were positive for each serogroup by number of *D. nodosus* positive swabs, and differences in these proportions between serogroups were tested with Fisher's exact tests. Differences between the observed and simulated data were tested with chi-square tests.

3.24. Multinomial model of disease state, number of swabs and biosecurity managements on the number of serogroups detected

Unordered multinomial logistic regression models (Dohoo et al., 2003) were used to investigate whether the number of swabs submitted/analysed and the biosecurity managements in the flock were associated with the number of serogroups detected per flock. The nnet package (version 7.3-12) (Venables and Ripley, 2002) was used to fit the model. The models were built using a manual forward stepwise procedure by sequentially testing each term using variables with the lowest Akaike's Information Criterion (AIC) at each iteration. Variables were retained in the final multivariable models if $p \leq 0.05$ and more than one flock was in each significantly different category. All remaining variables were retested in the final model (Cox and Wermuth, 1996). Chi-square tests and Fisher's exact tests were used to test for correlation between the biosecurity terms in the final model and all other tested biosecurity terms. The models took the form:

$$logit(\pi_{1k}/p_{i0k}) = \beta_{0k} + \Sigma\beta_0 X + e_k$$
$$logit(\pi_{2k}/p_{i0k}) = \beta_{1k} + \Sigma\beta_1 X + e_k$$

Where $logit(\pi_{1k}/p_{i0k})$ is the probability of having 1 – 2 serogroups detected versus ≥ 3 and $logit(\pi_{2k}/p_{i0k})$ is the probability of having 0 serogroups detected versus ≥ 3, β_{0k} and β_{1k} are constants for 1 – 2 serogroups and 0 serogroups, $\beta_0 X$ and $\beta_1 X$ are coefficients for number of swabs and biosecurity variables X for 1 – 2 serogroups and 0 serogroups, and e_k is the residual random error that follows a binomial distribution.

3.25. Vaccination on serogroups in a flock

Fisher's exact tests were used to investigate the diversity of serogroups detected between flocks vaccinated against footrot for > 0 – 5 years, > 5 years, and flocks that did not vaccinate. These timeframes were selected from the results in section 4.3.

3.26. Spatial analysis of the diversity of serogroups of *Dichelobacter nodosus* in England

Raup-Crick analysis of community diversity was conducted using null models using the method described by Chase et al. (2011). This analysis measured the dissimilarity in the communities of serogroups between flocks. Principal coordinates analysis (Gower, 1966) of the Raup-Crick analysis was used to visually assess for geographical clusters using the ape package (version 5.2) (Paradis and Schliep, 2018).

3.27. Investigation of the association between the number of samples taken per flock and the number of serogroups detected

In the current study there were up to eight swabs per flock. To investigate whether the number of serogroups detected per flock were limited by the number of swabs, or the number of swabs that came from footrot-affected feet, three sources of swabs were compared:

I) Flocks (n = 11) in the current study where 8 swabs were selected from the clinical trial (Witt and Green, 2018)

II) All pooled swabs from the same 11 flocks in the clinical trial (Witt and Green, 2018) where each foot of 15 sheep was swabbed

III) Flock A where 390 cultures had detected 4 serogroups (Smith et al., 2017) and a further 411 DNA analyses had also detected the same 4 serogroups (Zoë Willis, in prep)

3.28. Comparison of serogroups detected from eleven flocks in the clinical trial from eight and more than eight swabs (datasets I and II)

In the clinical trial (Witt and Green, 2018), swabs within a flock were pooled by footrot severity score (Appendix 4). Presence of serogroups was investigated using the same PCRs described in the methods (section 3.14) (Dr Emma Monaghan, in prep). Samples were not tested for *D. nodosus* prior to pooling. For 11 flocks, 8 swabs were analysed separately for this thesis and > 8 swabs (range = 16 – 60) were pooled for serogroup analysis in the clinical trial. The serogroups detected within flocks when eight (the current study) and more than eight swabs (clinical trial) were compared.

3.29. Simulation of data from Flock A with four known serogroups (Dataset III)

In the current study the median number of *D. nodosus* positive swabs per flock was four and the number of swabs requested per flock was eight. *D. nodosus* positive swabs from four and eight sheep lame with footrot lesions respectively was simulated 1,000 times each from Flock A. Flock A had 99 ewes with 789 swabs taken from footrot-affected feet over a 10-month period (Smith et al., 2017). Serogroups B, H, I and D were detected from culture (Smith et al., 2017) and from extracted DNA from 411 samples in single serogroup PCRs, in 91.8%, 79.6%, 14.3% and 8.2% respectively (Zoë Willis, in prep).

The probability of detecting all four serogroups in Flock A with differing numbers of *D. nodosus* positive swabs was calculated with the formula:

$$Probability = (1 - (1 - 0.918)^n) \times (1 - (1 - 0.796)^n) \times (1 - (1 - 0.143)^n) \times (1 - (1 - 0.082)^n)$$

Where n is the number of swabs.

3.30. Minimum number of swabs required to detect serogroups of differing prevalence

The minimum numbers of swabs required to have a 95% likelihood of detection of serogroups by prevalence X was calculated using the formula:

$$Prevalence\ X = 1 - \sqrt[n]{0.05}$$

Where n is the number of swabs.

3.31. Feasibility of reducing the number of serogroups in a national vaccine

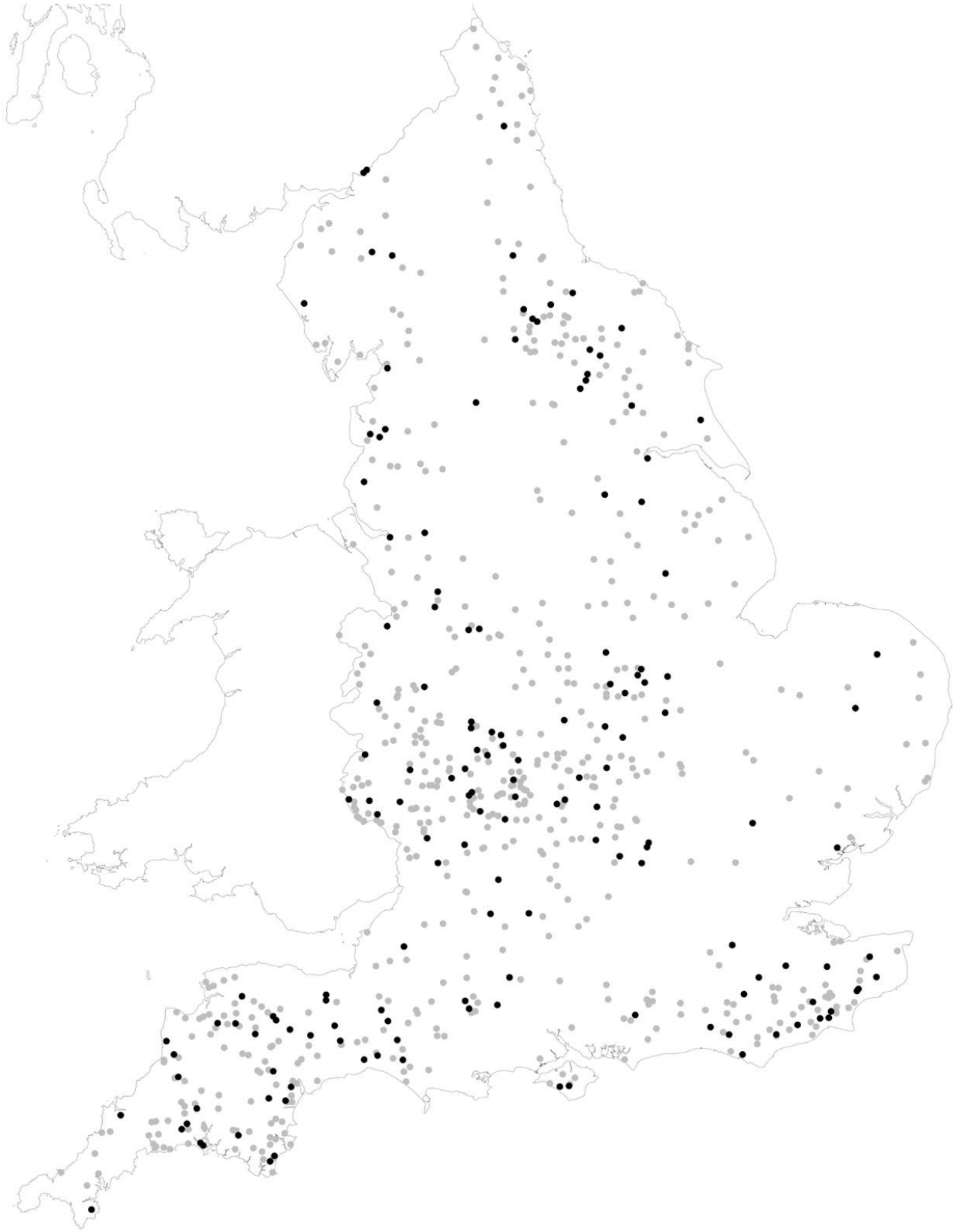
The number of times each serogroup was detected in each flock was calculated. The coverage of bivalent and multivalent vaccines targeting the most prevalent serogroups was investigated by calculating the number and proportion of flocks that would be completely protected (assuming a 100% effective vaccine), and for which the serogroups present in the vaccine were also the most prevalent serogroups (frequency of detection) in the flock.

4. Results

4.1. Representativeness of respondents

There was no significant difference in the flock geometric mean period prevalence of lameness in 2013 between the 154 participating and the 740 invited farmers (3.4% and 3.5% respectively) or the total 1,260 respondents to the 2013 questionnaire (3.5%) (Winter et al., 2015). There was also no significant difference in flock size between the participating and invited farmers and all respondents of the 2013 questionnaire. There was no difference in geographical location of the 740 farmers invited to participate in the study and the 154 participants (Figure 4.1).

Figure 4.1 Locations of 722 English sheep farmers who had completed questionnaires on sheep lameness in 2013 or 2014 and were invited to participate in the study (grey), and the 154 farmers who participated by completing a questionnaire regarding lameness in their sheep in 2015 (black).



4.2. Multilevel models of prevalence of lameness in ewes and chi-squared and Fisher's exact test of managements for lameness 2013 – 2015

The flock period prevalence of lameness in ewes for the 128 flocks that gave the prevalence of lameness and flock size in all three years was significantly higher in 2015 (4.1%) than in both 2013 (3.3%) and 2014 (3.2%) in the multilevel models (Table 4.1). This result was the same when all 154 farmers were included in the multilevel models.

Table 4.1 Multilevel models of the flock size and prevalence of lameness in ewes in all 154 participating English sheep flocks who completed questionnaires on lameness in their sheep in 2013, 2014 and 2015, and the 128 who answered all questions on the flock size and the prevalence of lameness in ewes in each year.

Variable		2013	2014	2015
154 flocks				
Ewe flock size	Median	400	400	400
	IQR	243 – 608	250 – 600	241 – 600
	Range	25 – 5,500	4 – 6,000	3 – 6,000
	Number of respondents	154	128	154
Prevalence of lameness in ewes	Geometric mean[a]	3.4%	3.1%	4.2%
	95% CI	2.9 – 3.9%	2.8 – 3.6%	3.7 – 4.7%
	Range	0.0 – 40.0%	0.4 – 25.0%	0.5 – 25.0%
	Number of respondents	153	128	154
128 flocks				
Ewe flock size	Median	400	400	400
	IQR	250 – 613	250 – 600	265 – 600
	Range	25 – 5,500	4 – 6,000	50 – 6,000
Prevalence of lameness in ewes	Geometric mean[b]	3.3%	3.1%	4.1%
	95% CI	2.8 – 3.9%	2.8 – 3.6%	3.6 – 4.6%
	Range	0.0 – 40.0%	0.4 – 25.0%	0.5 – 25.0%

[a] Arithmetic mean prevalence of lameness in ewes from multilevel model significantly different between 2013 and 2015 (p = 0.017), and 2014 and 2015 (p = 0.002). [b] Arithmetic mean prevalence of lameness in ewes from multilevel model significantly different between 2013 and 2015 (p = 0.033), and 2014 and 2015 (p = 0.005). All other pairwise comparisons not significant (p = 0.615 – 0.992).

There was a large significant reduction in the proportion of farmers that treated all sheep ≤ 3 days of onset of lameness (28.6% in 2015 compared with 50.6% in 2013 and 40.9% in 2014) (Table 4.2). There was a large, significant reduction in the proportion of farmers who practised routine foot trimming in 2015 (19.5%) compared with 2013 (55.2%) and 2014 (38.3%). The proportion of farmers who carried out routine trimming but did not cause bleeding (7.1%, 11.9%, 6.7% in 2013, 2014, 2015) did not change. Significantly more farmers culled sheep because they had been lame in 2015 than in 2013 and 2014, 81.8% compared with 49.4% and 47.4% respectively. Significantly more farmers used Footvax™, a vaccine against footrot, in 2015 (29.2%) compared with 2013 (14.3%) and 2014 (14.3%). There were no other significant changes in management between 2013 or 2014 and 2015 in this relatively small sample of 154 flocks, and no significant change in any management between 2013 and 2014.

Table 4.2 Number and percentage of 154 English sheep flocks by management practices associated with lameness from questionnaires completed in 2013, 2014 and 2015.

Variable		2013		2014		2015	
		N	%	N	%	N	%
Lowest locomotion score at which the farmer recognised sheep as lame (Kaler et al., 2009)	1	80	51.9	72	46.8	Not investigated	
	2	55	35.7	48	31.2		
	≥ 3	18	11.7	10	6.5		
Number of lame sheep at locomotion score when farmers treated them	1	19	12.3	25	16.2	Not investigated	
	2 – 5	77	50.0	65	42.2		
	6 – 10	31	20.1	25	16.2		
	> 10	25	16.2	13	8.4		
	Did not treat individuals	0	0.0	1	0.6		
Time to treatment of all lame sheep	**≤ 3 days**	**78**	**50.6**	**63**	**40.9**	**44**	**28.6**
	≤ 1 week	**55**	**35.7**	**51**	**33.1**	**74**	**48.1**
	≤ 2 weeks	**15**	**9.7**	**14**	**9.1**	**24**	**15.6**
	> 2 weeks	**3**	**1.9**	**2**	**1.3**	**10**	**6.5**
Ease of catching individual lame sheep	Easy/very easy	20	13.0	19	12.3	Not investigated	
	Neither easy or difficult	67	43.5	54	35.1		
	Difficult/very difficult	65	42.2	56	36.4		
Method of catching individual sheep: corner of field	No	99	64.3	Not investigated		Not investigated	
	Yes	55	35.7				
Method of catching lame sheep: dog that can catch individuals	No	132	85.7	Not investigated		Not investigated	
	Yes	22	14.3				
Proportion of sheep that bled during a routine foot trim, per year	**Did not trim**	**63**	**40.9**	**72**	**46.8**	**122**	**79.2**
	Zero	**6**	**3.9**	**7**	**4.5**	**2**	**1.3**
	< 1%	**6**	**3.9**	**6**	**3.9**	**2**	**1.3**
	1 – 2%	**35**	**22.7**	**29**	**18.8**	**16**	**10.4**
	> 2 – < 5%	**11**	**7.1**	**1**	**0.6**	**0**	**0.0**
	5 – < 10%	**18**	**11.7**	**11**	**7.1**	**7**	**4.5**
	≥ 10%	**9**	**5.8**	**5**	**3.2**	**3**	**1.9**
Footbath all ewes ever over the past year	No	52	33.8	50	32.5	64	41.6
	Yes	102	66.2	81	52.6	90	58.4
Footbath to treat footrot	No	98	63.6	Not investigated		Not investigated	
	Yes	56	36.4				

BOLD: significant differences in farmer practices in 2015 compared with both 2013 and 2014 (Wald's test $p<0.05$). N: number of farmers; %: percent of farmers. There were no significant differences in farmer practices between 2013 and 2014. "No response" was a category in each variable, results not shown.

Table 4.2 (continued) Number and percentage of 154 English sheep flocks by management practices associated with lameness from questionnaires completed in 2013, 2014 and 2015.

Variable		2013		2014		2015	
		N	%	N	%	N	%
Footbath to prevent ID	No	96	62.3	Not investigated		Not investigated	
	Yes	58	37.7				
Occasion footbathed: at turnout	No	150	97.4	127	82.5	Not investigated	
	Yes	4	2.6	4	2.6		
Occasion footbathed: new sheep on arrival	No	101	65.6	78	50.6	Not investigated	
	Yes	30	19.5	30	19.5		
	No new sheep	22	14.3	21	13.6		
Culled sheep previously lame	**No**	**71**	**46.1**	**56**	**36.4**	**27**	**17.5**
	Yes	**76**	**49.4**	**73**	**47.4**	**126**	**81.8**
Relied on memory to identify culls	No	151	98.1	Not investigated		Not investigated	
	Yes	3	1.9				
Avoided selling ewes for breeding from repeatedly lame mothers	No	149	96.8	Not investigated		Not investigated	
	Yes	5	3.2				
Vaccinated ewes with Footvax™	**No**	**132**	**85.7**	**109**	**70.8**	**109**	**70.8**
	Yes	**22**	**14.3**	**22**	**14.3**	**45**	**29.2**
Length of time vaccinating against footrot	> 5 years	Not investigated		Not investigated		15	9.7
	> 2 – 5 years					12	7.8
	> 1 – 2 years					12	7.8
	> 0 – 1 year					12	7.8
	Did not vaccinate					96	62.3
Checked feet of new sheep on arrival	Never	16	10.4	Not investigated		Not investigated	
	Sometimes	18	11.7				
	Usually	36	23.4				
	Always	58	37.7				
	No new arrivals	23	14.9				
Isolated new sheep on arrival	Did not isolate	10	6.5	5	3.2	Not investigated	
	Isolated for < 3 weeks	75	48.7	60	39.0		
	Isolated for ≥ 3 weeks	44	28.6	41	26.6		
	No new arrivals	23	14.9	23	14.9	23	14.9
Sheep mixed with other flocks	No	131	85.1	Not investigated		144	93.5
	Yes	17	11.0			10	6.5
	Do not know	2	1.3			0	0.0

BOLD: significant differences in farmer practices in 2015 compared with both 2013 and 2014 (Wald's test $p<0.05$). N: number of farmers; %: percent of farmers. There were no significant differences in farmer practices between 2013 and 2014. "No response" was a category in each variable, results not shown.

Table 4.2 (continued) Number and percentage of 154 English sheep flocks by management practices associated with lameness from questionnaires completed in 2013, 2014 and 2015.

Variable		2013		2014		2015	
		N	%	N	%	N	%
Sheep left farm then returned: for shows	No	148	96.1	Not investigated		148	96.1
	Yes	6	3.9			6	3.9
Sheep left farm then returned: for summer grazing	No	126	81.8	Not investigated		Not investigated	
	Yes	28	18.2				
Sheep left farm then returned: for market	No	149	96.8	Not investigated		Not investigated	
	Yes	5	3.2				
Farm location	Upland	12	7.8	Not investigated		Not investigated	
	Hill	2	1.3				
	Lowland	138	89.6				
Organic status	Not organic	143	92.9	Not investigated		Not investigated	
	Organic	9	5.8				
Production of breeding stock	No	119	77.3	Not investigated		Not investigated	
	Yes	35	22.7				

BOLD: significant differences in farmer practices in 2015 compared with both 2013 and 2014 (Wald's test $p<0.05$). N: number of farmers; %: percent of farmers. There were no significant differences in farmer practices between 2013 and 2014. "No response" was a category in each variable, results not shown.

4.3. Multivariable quasi-Poisson regression model of risk factors for lameness in sheep

The univariable model results are in Appendix 5. The dispersion parameter of the final model was 8.8. In the multivariable model, four variables were significantly associated with the prevalence of lameness in ewes in 2015 (Table 4.3). The prevalence of lameness was higher in flocks where farmers treated all lame sheep ≤ 1 week (RR 1.57, 95% CI: 1.18 – 2.13), ≤ 2 weeks (RR 1.49, 95% CI: 1.02 – 2.18) or > 2 weeks (RR 1.73, 95% CI: 1.10 – 2.65) of onset of lameness, compared with flocks where farmers treated all sheep ≤ 3 days of onset of lameness. The prevalence of lameness was greater in flocks where ≥ 5% of sheep feet bled during routine foot trimming (RR 1.79, 95% CI: 1.26 – 2.48) than in flocks where routine foot trimming was not practised. The prevalence of lameness was higher in flocks where annual vaccination against footrot had been practised for between 2 and 5 years (RR 2.05, 95% CI: 1.31 – 3.24) and ≤ 1 year (RR 2.83, 95% CI: 1.72 – 4.66), and when vaccination against footrot was not practised (RR 1.70, 95% CI: 1.20 – 2.48) compared with flocks that had been vaccinated annually for > 5 years. Flocks that mixed with other flocks (planned or accidental) had a higher prevalence of lameness (RR 1.58, 95% CI: 1.06 – 2.27) than those where sheep did not mix with other flocks. The model fit was visually good (Appendix

6). There was no change to the model results when only the 128 farmers who provided lameness data for all three years of the study were included or when the 18 farmers in the clinical trial were excluded (data not shown). The management practices significantly different between 2013 and 2015 were also significantly different between 2014 and 2015 (data not shown).

Table 4.3 Multivariable quasi-Poisson regression model of risk factors associated with the period prevalence of lameness in ewes in 154 English sheep flocks in 2015.

Variable	Number	Percent	Risk Ratio	95% CI	
Time to treatment of all lame sheep					
≤ 3 days	44	28.6	1.00		
≤ 1 week	74	48.1	**1.57**	**1.18**	**2.13**
≤ 2 weeks	24	15.6	**1.49**	**1.02**	**2.18**
> 2 weeks	10	6.5	**1.73**	**1.10**	**2.65**
Percent of sheep that bled during routine foot trimming					
No routine foot trimming	122	79.2	1.00		
0 – 2%	20	13.0	1.26	0.91	1.73
≥ 5%	10	6.5	**1.79**	**1.26**	**2.48**
Length of time vaccinating against footrot					
> 5 years	15	9.7	1.00		
> 2 – 5 years	12	7.8	**2.05**	**1.31**	**3.24**
> 1 – 2 years	12	7.8	1.11	0.67	1.84
> 0 – 1 year	12	7.8	**2.83**	**1.72**	**4.66**
Did not vaccinate	96	62.3	**1.70**	**1.20**	**2.48**
Sheep mixed with other flocks					
No	144	93.5	1.00		
Yes	10	6.5	**1.58**	**1.06**	**2.27**

BOLD: categories significantly different from the baseline (Wald's test p<0.05). CI: confidence intervals. Model coefficient: -0.957, Standard Error: 0.189. "No response" was a category in each variable, results not shown.

4.4. Population attributable fractions (PAF) of risk factors for lameness in ewes and farmer changes in management practices between 2013 and 2015

Up to 65.3% of the prevalence of lameness was explained by the model (Table 4.4). The percentage of farmers that treated all lame sheep promptly, that is, ≤ 3 days of onset of lameness, fell by 22% from 50.6% to 28.6% (Table 4.2) between 2013 and 2015 and the PAF of lameness

attributable to not treating all sheep ≤ 3 days of onset of lameness rose from 13.3% in 2013 (Grant et al., 2018) to 25.3% in 2015 (Table 4.4). Significantly fewer farmers practised routine foot trimming in 2015 compared with 2013, 19.5% and 55.2% respectively, and the proportion of flocks where ≥ 5% feet bled fell by 11% from 17.5% to 6.5%. The PAF of lameness attributable to feet bleeding during routine foot trimming fell from 9.5% to 2.9%. More farmers used Footvax™ in 2015 than in 2013; 29.2% compared with 14.3% respectively. In 2015, vaccinating against footrot annually for < 6 years had the largest PAF of 34.7%. The number of years a flock had been vaccinated against footrot was not investigated in 2013 and so a change in this specific vaccination behaviour could not be investigated (Table 4.2), however the PAF for not vaccinating ewes at all in 2013 compared with vaccinating once per year (regardless of duration of vaccination) was 3.3%. There was no significant change in the percentage of farmers whose sheep did not mix with other flocks (85.1% in 2013 and 93.5% in 2015) and the PAF for this practice was 2.4% in 2015. Attending sheep shows had a PAF of 1.3% in 2013.

Table 4.4 Attributable fractions and population attributable fractions of four management practices associated with the prevalence of lameness in ewes in 154 English sheep flocks in 2015.

Variable	Farmers (%)	RR	AF (%)	PAF (%)
Time to treatment of all lame sheep: ≤ 3 days	28.6	1.00	0.0	0.0
Time to treatment of all lame sheep: ≤ 1 week	48.1	1.57	36.4	17.5
Time to treatment of all lame sheep: ≤ 2 weeks	15.6	1.49	32.8	5.1
Time to treatment of all lame sheep: > 2 weeks	6.5	1.73	42.1	2.7
No routine foot trimming	79.2	1.00	0.0	0.0
≥ 5% sheep bled during routine foot trimming	6.5	1.79	44.1	2.9
Vaccinating > 5 years	9.7	1.00	0.0	0.0
Did not vaccinate	62.3	1.70	41.3	25.7
Vaccinating ≤ 1 year	7.8	2.83	64.7	5.0
Vaccinating > 2 – ≤ 5 years	7.8	2.05	51.3	4.0
Sheep not mixed with other flocks	93.5	1.00	0.0	0.0
Sheep mixed with other flocks	6.5	1.58	36.7	2.4

RR: Risk ratio; AF: Attributable fraction (exposed); PAF: Population attributable fraction.

4.5. Swab sample collection and DNA extraction

In total 1,144 swabs were collected from 146 flocks by post and 144 swabs from the 18 flocks in the clinical trial. Eleven farmers returned less than eight swabs (range = 3 – 7). A total of 138 samples were excluded from the study due to contamination during DNA extraction. The median usable number of swabs per flock was seven (range = 3 – 8). Out of the 1,150 samples, 687 (59.7%) were positive for *D. nodosus* with a median number of *D. nodosus* positive swabs per flock of four (range = 0 – 8). Therefore, there was heterogeneity in the number of swabs analysed per flock.

4.6. Factors associated with the number of swabs submitted per flock

The number of *D. nodosus* positive swabs per flock was not associated with the prevalence of lameness or severe footrot in ewes, or flock size, in either dataset (*D. nodosus* positive swabs or all swabs) in Kruskal-Wallis tests (Table 4.5 and Table 4.6). Therefore, flocks which had low prevalence of lameness, or few sheep, did not submit samples that were less likely to be *D. nodosus* positive.

Table 4.5 Kruskal-Wallis tests for associations with the number of D. nodosus positive swabs submitted per 164 English sheep flocks.

Factor	Test statistic	p value
Prevalence of lameness in ewes in 2015	7.55	0.479
Prevalence of severe footrot in ewes in 2015	3.56	0.895
Ewe flock size in 2015	10.43	0.236

Table 4.6 Kruskal-Wallis tests for associations with the number of swabs submitted per 164 English sheep flocks.

Factor	Test statistic	p value
Prevalence of lameness in ewes in 2015	4.87	0.301
Prevalence of severe footrot in ewes in 2015	2.54	0.637
Ewe flock size in 2015	4.96	0.292

4.7. Lesion status of swabbed feet

A total of 861 (74.9%) swabs were from feet with footrot and 78 (6.8%) were from healthy feet (Table 4.7). A total of 65.7% of swabs from feet with footrot were *D. nodosus* positive, and this was significantly higher than swabs from healthy feet (23.1% were *D. nodosus* positive) ($p < 0.001$ in post-hoc analysis of a chi-square test) (Table 4.7 and Table 4.8). A total of 156 (95.1%) flocks submitted ≥ 1 swab from a foot with footrot and 153 (93.3%) flocks had ≥ 1 *D. nodosus* positive

swab. There were a total of 566 *D. nodosus* positive swabs from footrot-affected feet that came from 138 (84.1%) flocks, and the percentage of swabs that were *D. nodosus* positive from the post (60.0%) or from the clinical trial (58.0%) were similar (Table 4.7 and Table 4.8). Therefore, most farmers submitted swabs from feet with footrot, and the majority of flocks had swabs that were *D. nodosus* positive that could be investigated for presence of serogroups.

Table 4.7 The number and percentage of swabs analysed by disease state obtained from 146 postal flocks and 18 clinical trial flocks.

Foot disease status	All swabs (post)		All swabs (clinical trial)		All swabs	
	N	%	N	%	N	%
Footrot	740	72.6	121	92.4	861	74.9
Healthy	78	7.7	0	0.0	78	6.8
Other	125	12.3	10	7.6	135	11.7
Unknown	76	7.5	0	0.0	76	6.6
Total	1,019		131		1,150	

Table 4.8 The number and percentage of D. nodosus positive swabs analysed by disease state obtained from 146 postal flocks and 18 clinical trial flocks.

Foot disease status	*D. nodosus* positive swabs (post)		*D. nodosus* positive swabs (clinical trial)		*D. nodosus* positive swabs	
	N	%	N	%	N	%
Footrot	493	80.7	73	96.1	566	82.4
Healthy	18	2.9	0	0.0	18	2.6
Other	58	9.5	3	3.9	61	8.9
Unknown	42	6.9	0	0.0	42	6.1
Total	611		76		687	

4.8. aprV2/B2 strains of Dichelobacter nodosus

All 687 *D. nodosus* positive swabs were positive for *aprV2*, and 74 (10.8%) were also positive for *aprB2*. All 138 *D. nodosus* positive flocks were positive for *aprV2* and 37 (24.2%) were also positive for *aprB2*. There was a higher load of *aprV2* than *aprB2* strains of *D. nodosus* on feet with a t-test ($t = 2.75$, $p = 0.007$) (Figure 4.2) and in flocks with a Wilcoxon signed-rank test ($V = 691$, $p < 0.001$) (Figure 4.3) when both strains were present. There was a higher total load of *aprB2* than *aprV2* on 22 (3.2%) *D. nodosus* positive swabs (from 16 flocks) and 2 (1.3%) *D. nodosus* positive

flocks. *aprV2* strains of *D. nodosus* were more prevalent than *aprB2* strains at both foot and flock level.

Figure 4.2 The log load of aprV2 and aprB2 strains of D. nodosus on 74 swabs, with a trendline at aprB2 = aprV2.

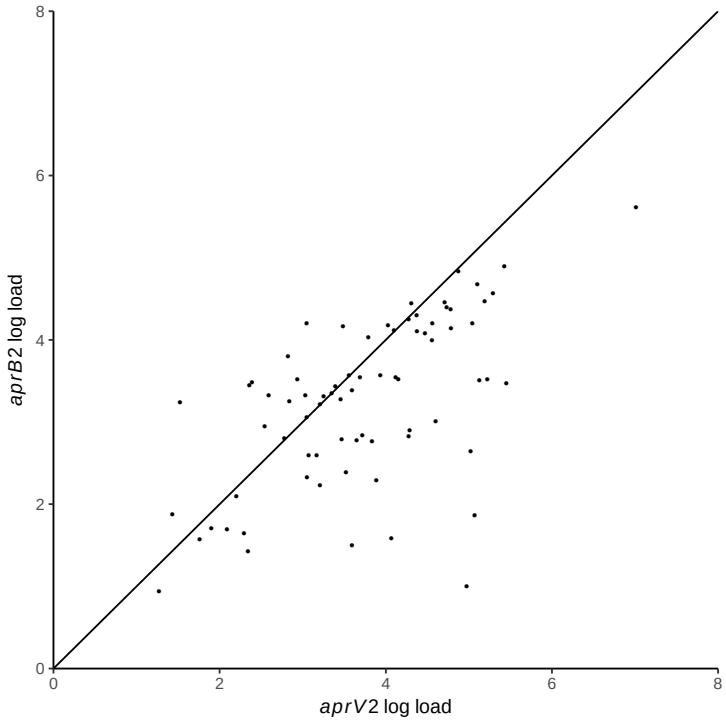
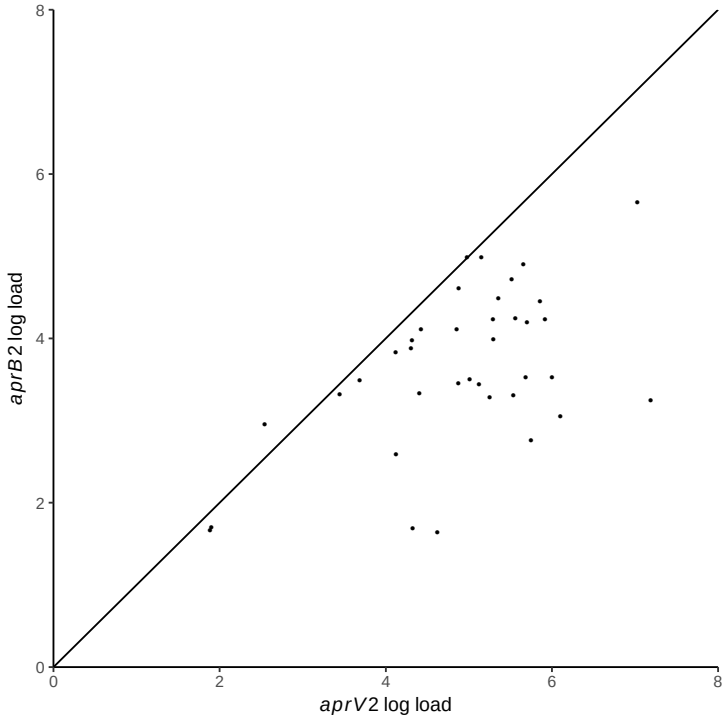


Figure 4.3 The total log load of aprV2 and aprB2 strains of D. nodosus in 37 flocks, with a trendline at aprB2 = aprV2.



There was a significant difference in the lesion status of the foot between feet that had a higher log load of *aprB2* strains of *D. nodosus*, and feet that had a higher log load of *aprV2* strains (Fisher's exact test, p = 0.010). However, all adjusted p-values were > 0.05 in a post-hoc test (p = 0.077 – 1.000). A Fisher's exact test for lesion status with *aprB2* presence or absence was marginally non-significant (p = 0.064) (Table 4.9). Therefore, there is no consistent association between lesion and *aprV2* or *aprB2* strains of *D. nodosus*, and further study will be needed to test this with more samples.

Table 4.9 The lesion status of feet with a higher log load of aprB2 than aprV2 strains of D. nodosus, a higher log load of aprV2 than aprB2 strains of D. nodosus, positive for aprB2 strains of D. nodosus, and negative for aprB2 strains of D. nodosus.

Lesion status	Higher *aprB2* log load	Higher *aprV2* log load	*aprB2* positive	*aprB2* negative
Footrot	14	552	54	512
Healthy	3	57	4	14
Other	4	57	8	53
Unknown	1	41	8	34

There was a significant difference in the diversity of the serogroups detected from the swabs with a higher load of *aprB2* than a higher log load of *aprV2* (Fisher's exact test, p < 0.001), and from swabs that were positive for *aprB2* compared with negative for *aprB2* (Fisher's exact test, p = 0.001) (Table 4.10). Serogroup C was more commonly detected in both feet with a higher load of *aprB2* than *aprV2*, and feet that were *aprB2* positive (Table 4.10). Of the two flocks with a higher log load of *aprB2* than *aprV2* strains of *D. nodosus*, serogroup C was detected from one flock, and no serogroup was detected from the other. There was no difference in the serogroups detected in *aprB2* positive and *aprB2* negative flocks (Fisher's exact test, p = 0.404). Therefore, *aprV2* strains of *D. nodosus* were ubiquitous in English sheep flocks and were almost always more prevalent on feet and in flocks than *aprB2* strains of *D. nodosus*. Serogroup C was associated with *aprB2* on feet, but not in flocks, which could be due to the dominance of *aprV2* strains diluting any effect.

Table 4.10 The serogroups detected from feet with a higher log load of aprB2 than aprV2 strains of D. nodosus, a higher log load of aprV2 than aprB2 strains of D. nodosus, positive for aprB2 strains of D. nodosus, and negative for aprB2 strains of D. nodosus.

Serogroup	Higher *aprB2* log load	Higher *aprV2* log load	*aprB2* positive	*aprB2* negative
A	0	94	5	89
B	1	229	22	208
C	11	106	26	91
D	0	33	1	32
E	1	16	5	12
F	0	8	1	7
G	1	35	4	32
H	5	277	33	249
I	0	22	0	22
Number of swabs	22	665	74	613

Table 4.11 The serogroups detected from flocks with a higher log load of aprB2 than aprV2 strains of D. nodosus, a higher log load of aprV2 than aprB2 strains of D. nodosus, positive for aprB2 strains of D. nodosus, and negative for aprB2 strains of D. nodosus.

Serogroup	Higher *aprB2* log load	Higher *aprV2* log load	*aprB2* positive	*aprB2* negative
A	0	52	11	41
B	0	101	24	77
C	1	53	21	33
D	0	18	5	13
E	0	8	4	4
F	0	4	1	3
G	0	18	4	14
H	0	106	26	80
I	0	14	4	10
Number of flocks	2	151	37	116

4.9. *Dichelobacter nodosus* load by number of serogroups detected

In the 687 *D. nodosus* positive swabs, there was a significantly lower mean log load of *D. nodosus* in serogroup negative swabs than serogroup positive swabs, 3.13 genome copies/μl compared with 4.50 genome copies/μl ($t = 15.52$, $p < 0.001$) (Figure 4.4), which explains that low loads lead to low detection of serogroups. There was a significantly different median log load of *D. nodosus*

between samples with different numbers of serogroups detected in a Kruskal-Wallis test (H = 187.88, p < 0.001) (Figure 4.5), the mean log loads of *D. nodosus* of swabs with 0, 1, 2, 3 and 4 serogroups were 3.13, 4.39, 4.58, 4.94 and 4.49 respectively. The range of log loads of *D. nodosus* was wide with overlap and the variance in log load was different between the number of serogroups detected, reducing from 31.50 to 11.89 as the number of serogroups detected increased from 0 to 4 (coefficient of variation test statistic = 63.00, p < 0.001) (Figure 4.5). There were many samples with high log loads of *D. nodosus* (> 4) for which there were no or few serogroups detected. Therefore, not all serogroups were detected, and some serogroups may have been present at a very high load on feet. It is also possible that some of the samples had serogroup M present.

Figure 4.4 The log load of genome copies of D. nodosus per µl for 687 D. nodosus positive swabs with and without a serogroup detected.

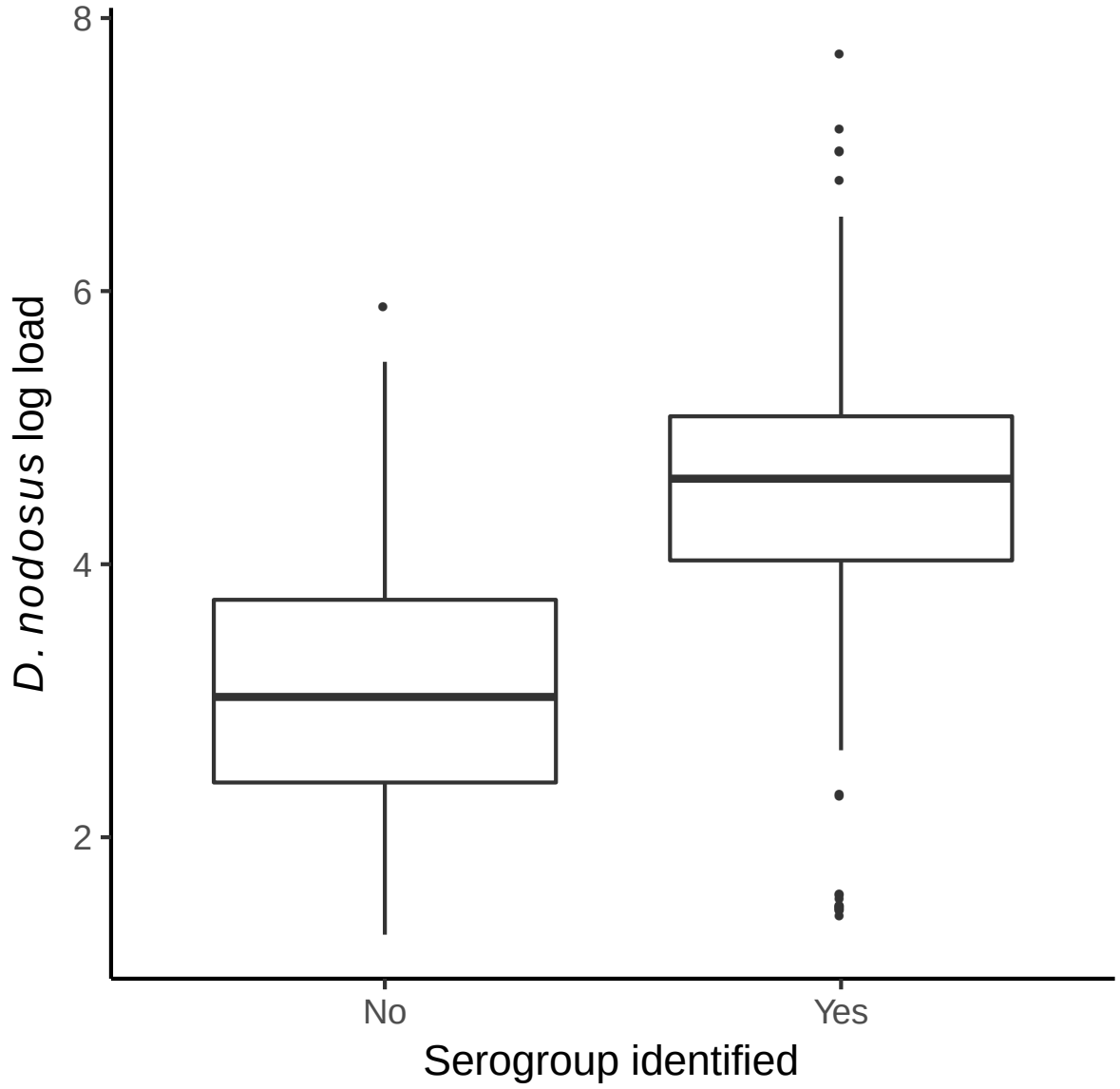
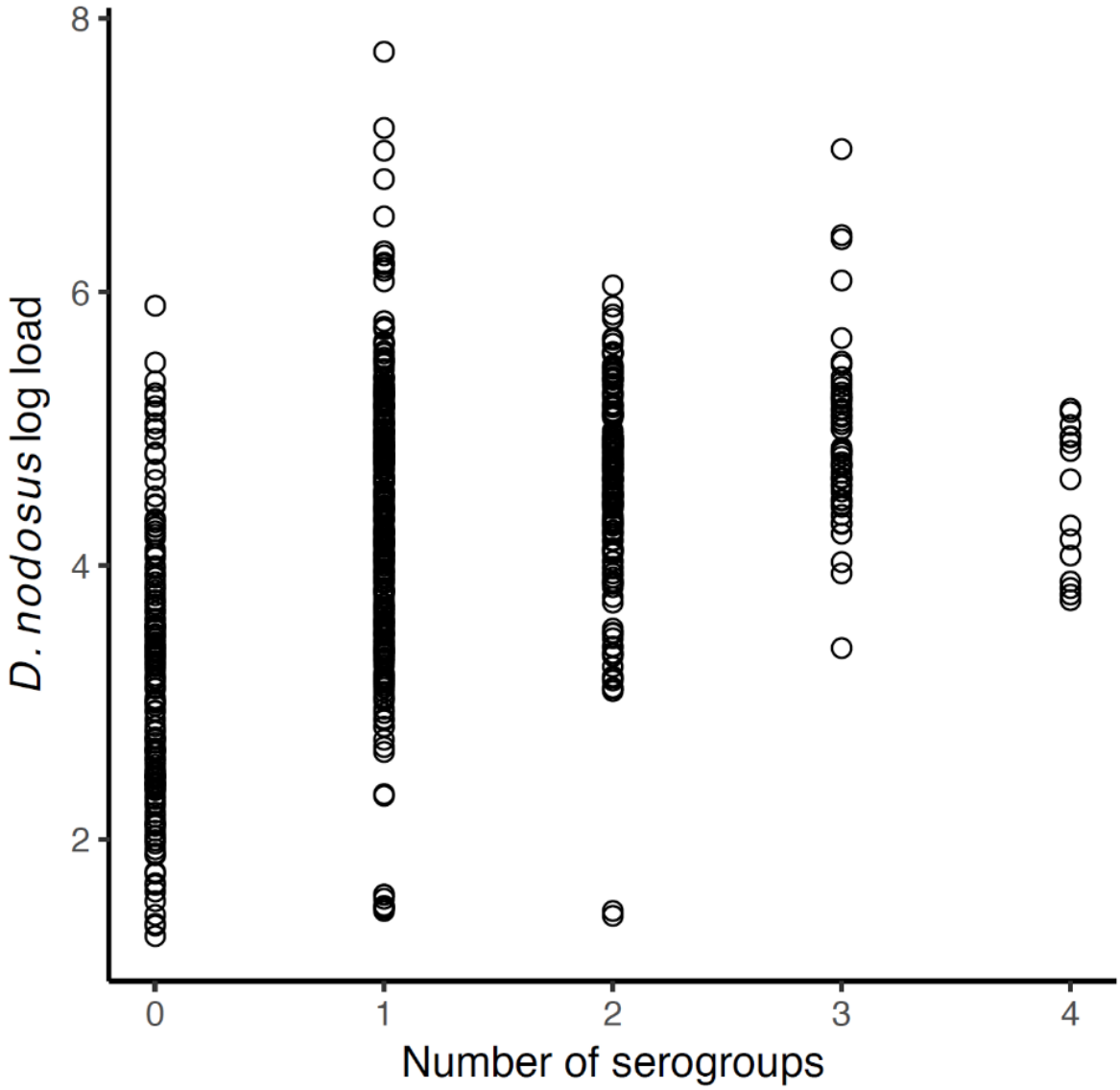


Figure 4.5 The log load of genome copies of D. nodosus per µl for 687 D. nodosus positive swabs with 0 – 4 serogroups detected.



4.10. Prevalence and diversity of serogroups on feet and in flocks

The most prevalent serogroup on feet was H (Figure 4.6 and Figure 4.7). Serogroups H and B were the only serogroups present in > 30% of *D. nodosus* positive feet. Serogroups C and A had a prevalence of ~ 15% on *D. nodosus* positive feet. Serogroups G and D were present in 5% of *D. nodosus* positive feet. Serogroups I, E and F were the rarest three serogroups, detected in < 4% of feet. The serogroups were not equally prevalent on feet, but differed in their prevalence, with some serogroups far more prevalent than others.

Figure 4.6 The percentage and number (above the bar) of serogroups detected in all 687 D. nodosus positive swabs from 164 flocks.

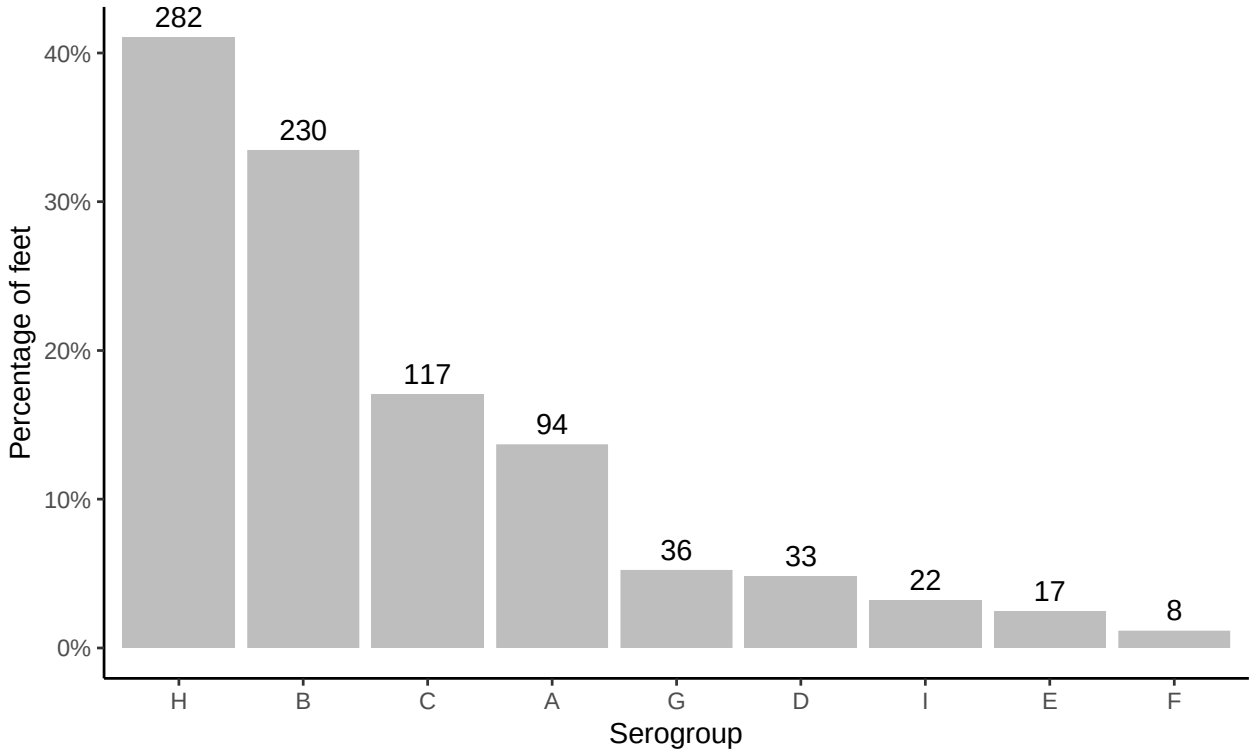
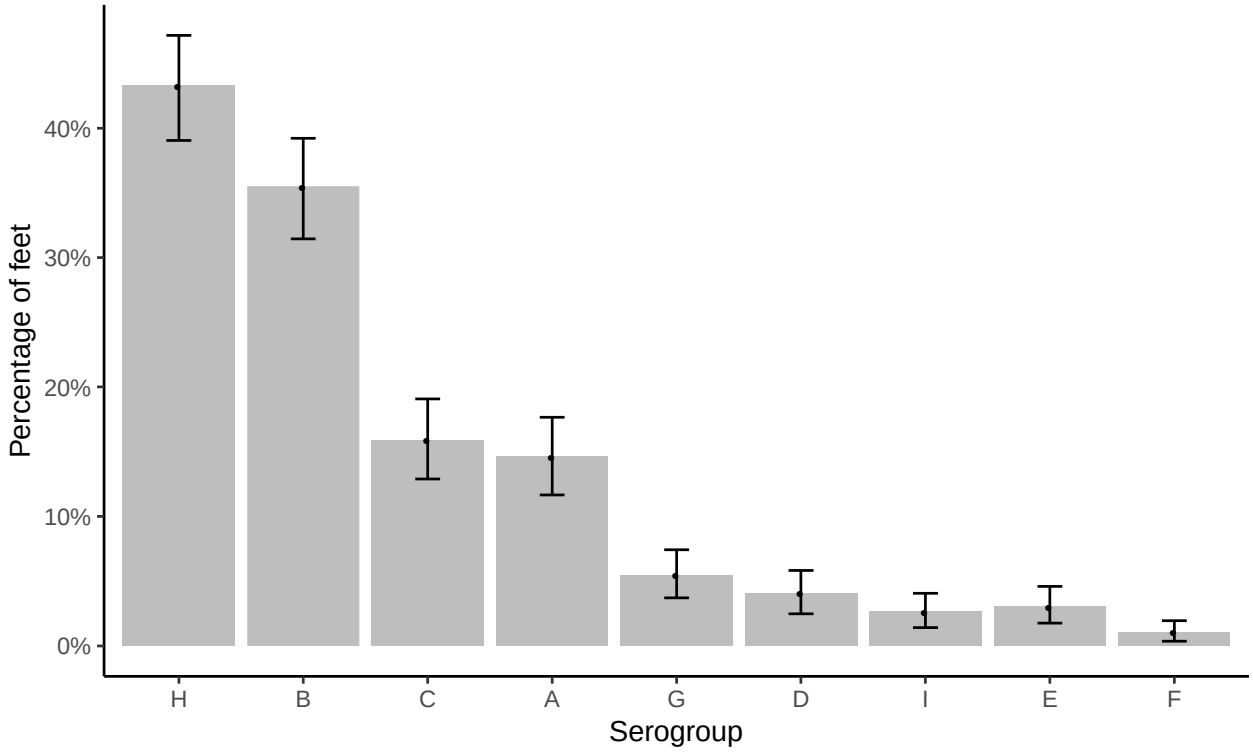


Figure 4.7 Observed (bar) and simulated (point) distribution of serogroups with 95% error bars from 566 D. nodosus positive swabs from footrot-affected feet from 138 flocks.



The same order of prevalence of serogroups was observed in flocks as in feet when all swabs were analysed, with each serogroup present at a higher prevalence in flocks than in feet (Figure 4.8 and Figure 4.9 compared with Figure 4.6 and Figure 4.7). Serogroups H and B were the only serogroups present in > 50% of flocks. Serogroups C and A were present in ~ 35% of flocks. Serogroups G and D were present in 12% of flocks. Serogroups I, E and F were the rarest three serogroups and detected in < 10% of flocks. The proportions of flocks positive for each serogroup and exact 95% confidence intervals are shown in Table 4.12. Serogroup A was marginally more prevalent than serogroup C in feet with footrot (Figure 4.9). The same order of prevalence of serogroups on feet and in flocks indicate that the distribution of serogroups in flocks and on feet were similar i.e. there was no serogroup that was present in many flocks but on few feet, or in few flocks but on many feet when present. There was no difference between the observed and expected relative proportions of flocks with each serogroup ($X^2 = 4.52$, p = 0.807), however fewer flocks were positive for each serogroup than expected (Figure 4.9) indicating that serogroups were clustered within flocks.

Figure 4.8 The percentage and number (above the bar) of serogroups detected in all 153 flocks from 687 D. nodosus positive swabs.

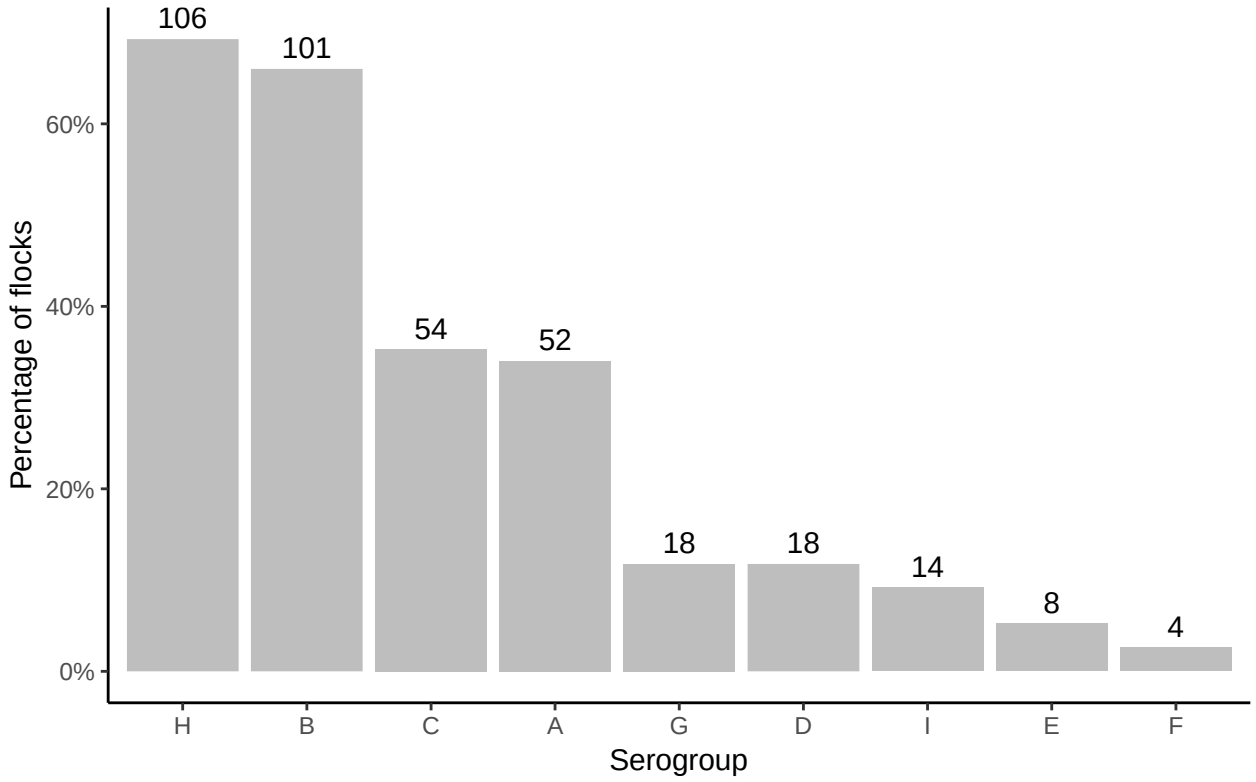


Figure 4.9 Observed (bar) and simulated (point) distribution of serogroups with 95% error bars from 138 flocks from 566 D. nodosus positive swabs from feet with footrot.

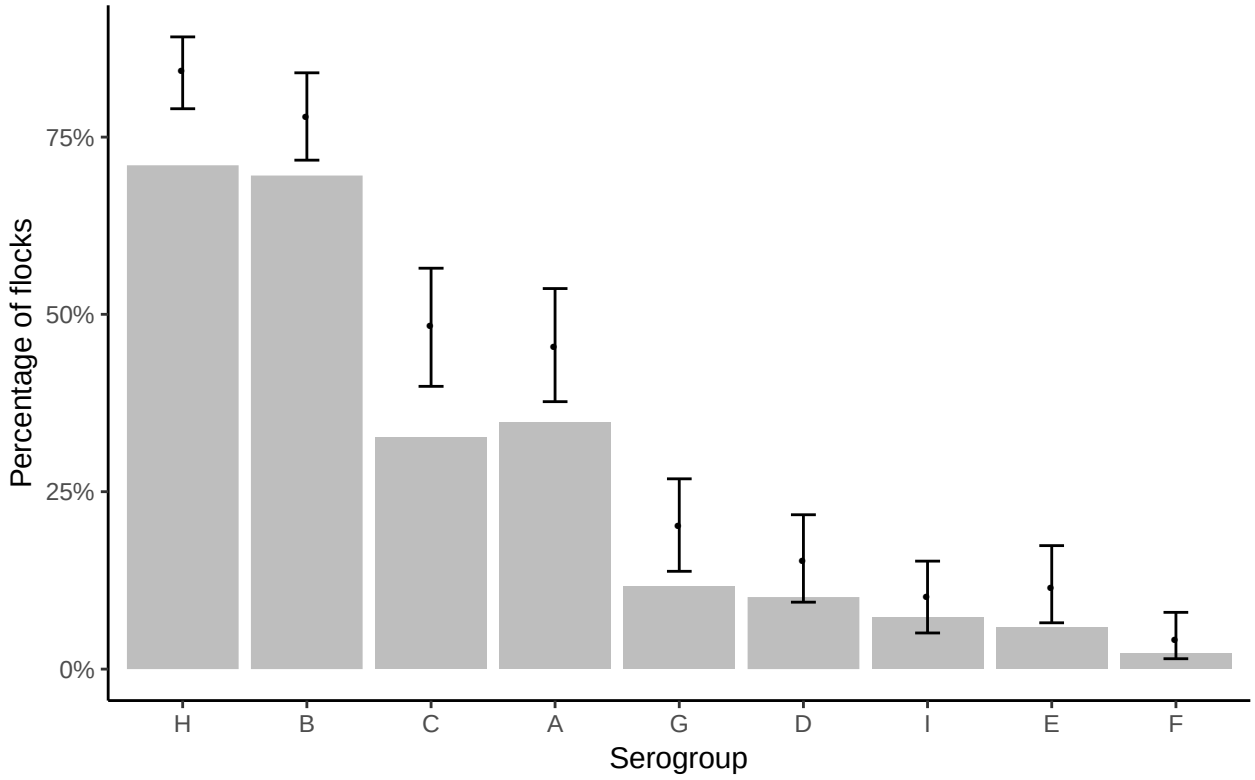


Table 4.12 The prevalence of each serogroup in 153 flocks with at least one D. nodosus positive swab (range 1 – 8) and 138 flocks with at least one D. nodosus positive swab from footrot-affected feet (range 1 – 8).

Serogroup	All lesions		Footrot-affected feet	
	Proportion of flocks	95% CI	Proportion of flocks	95% CI
H	0.69	0.61 – 0.76	0.71	0.63 – 0.78
B	0.66	0.58 – 0.73	0.70	0.61 – 0.77
C	0.35	0.28 – 0.43	0.33	0.25 – 0.41
A	0.34	0.27 – 0.42	0.35	0.27 – 0.43
G	0.12	0.07 – 0.18	0.12	0.07 – 0.18
D	0.12	0.07 – 0.18	0.10	0.06 – 0.16
I	0.09	0.05 – 0.15	0.07	0.04 – 0.13
E	0.05	0.02 – 0.10	0.06	0.03 – 0.11
F	0.03	0.01 – 0.07	0.02	0.00 – 0.06

4.11. Number of serogroups detected on feet and in flocks

A total of 40.3% of feet were *D. nodosus* negative. The distribution of the number of serogroups on both the 687 *D. nodosus* positive feet and the 566 *D. nodosus* positive footrot-affected feet were

positively skewed with a median of one serogroup (range = 0 – 4) (Figure 4.10 and Figure 4.11). There was > 1 serogroup detected from 33.0% (227) of all *D. nodosus* positive feet and 34.5% (195) of footrot lesion positive feet. There was no difference between the observed and expected (simulated) number of serogroups on feet with footrot (Fisher's exact test, p = 0.550). This suggests that the number of serogroups per foot is not clustered but is randomly distributed.

Figure 4.10 The percentage and number (above the bar) of 0 – 4 serogroups detected in all 1,150 swabs from 164 flocks.

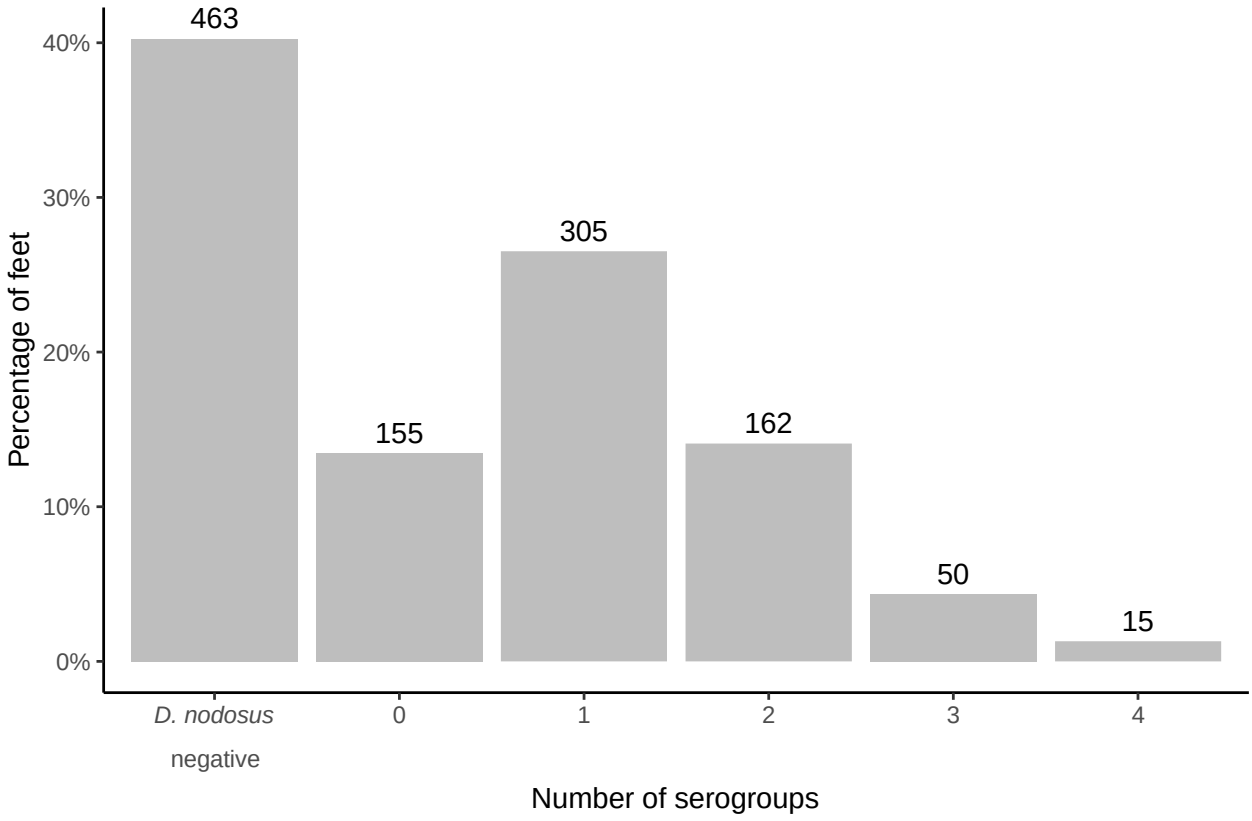
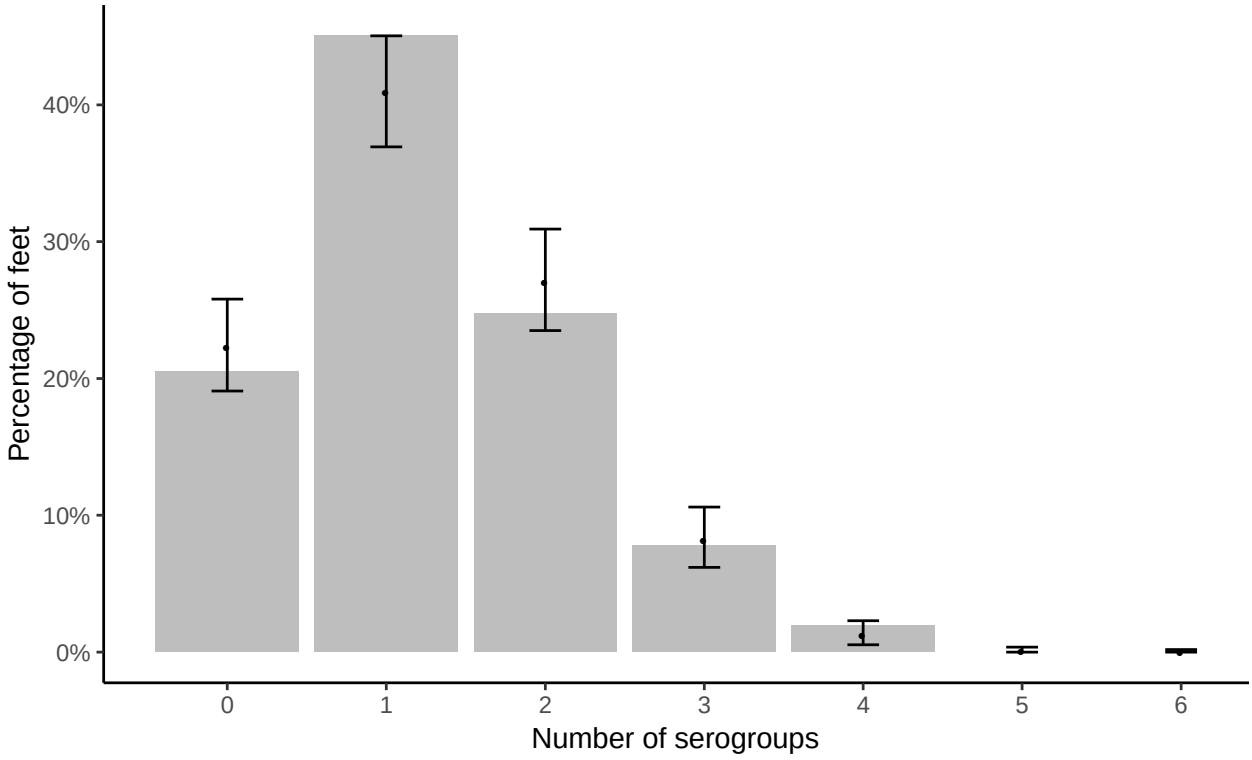


Figure 4.11 Observed and simulated distribution of 0 – 6 serogroups with 95% error bars from 566 D. nodosus positive swabs from footrot-affected feet from 138 flocks.



The median number of serogroups per flock was two (range = 0 – 6) for the 164 flocks (Figure 4.12) with 77.1% (118) of flocks with > 1 serogroup. Similarly to feet, the number of serogroups in flocks was also positively skewed, but with more serogroups detected in flocks than on feet. Results were similar when swabs from footrot-affected feet only were analysed with 76.1% (105) of flocks with > 1 serogroup (Figure 4.13). There were fewer than expected (simulated) serogroups per flock (Fisher's exact test, p = 0.002), confirming the earlier finding (section 4.10) that serogroups were clustered within flocks when present.

Figure 4.12 The percentage and number (above the bar) of 0 – 6 serogroups detected in all 164 flocks from 1,150 swabs.

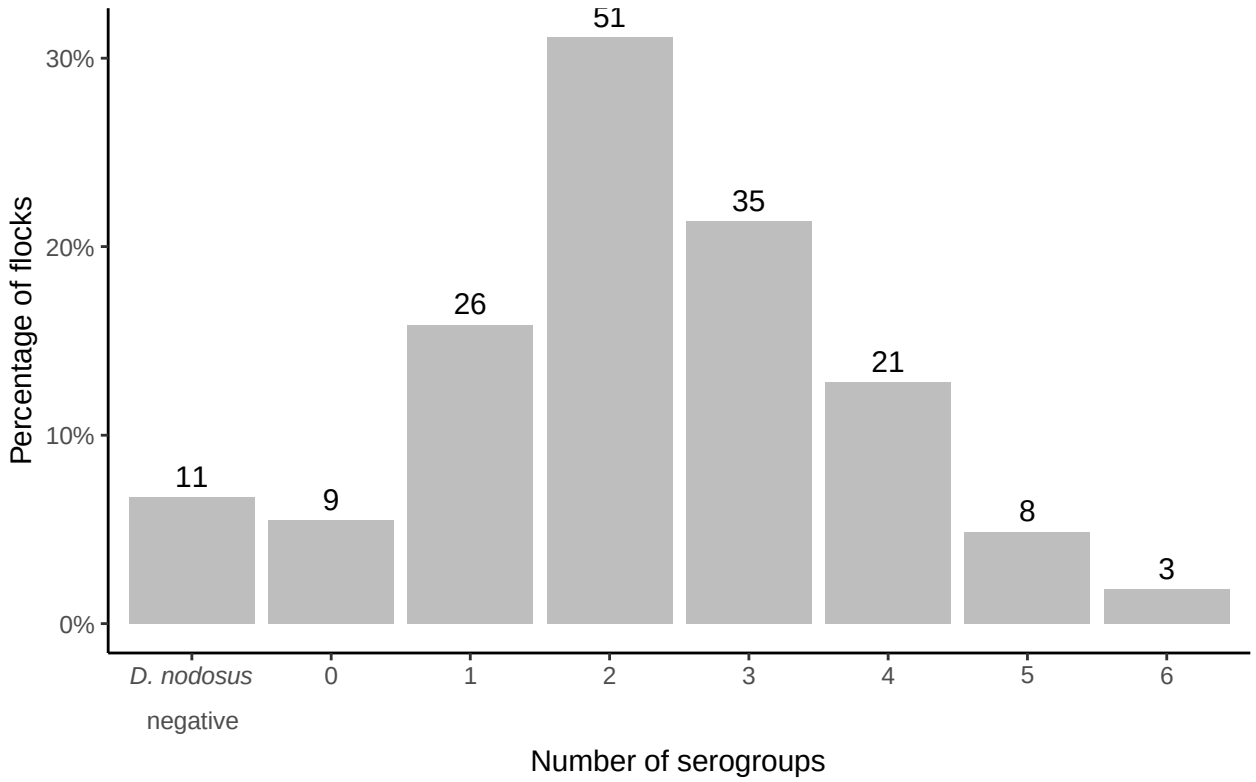
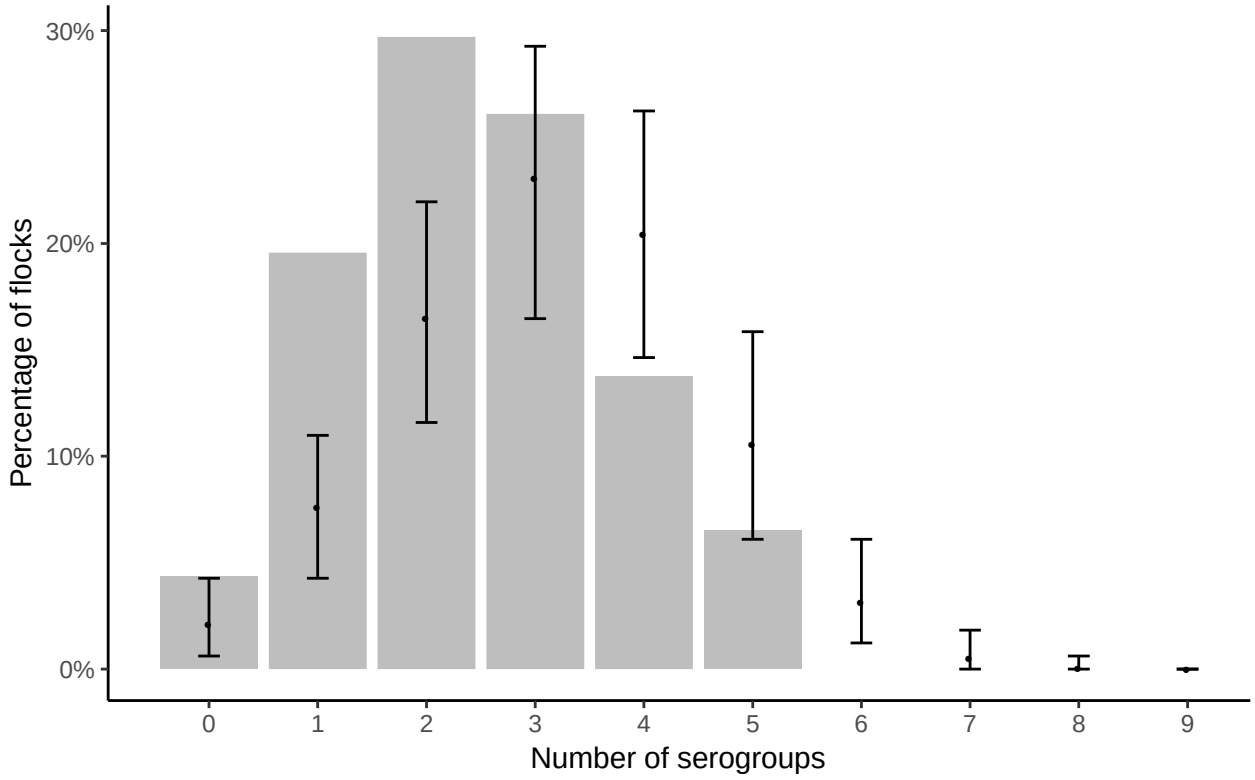


Figure 4.13 Observed (bar) and simulated (point) distribution of 0 – 9 serogroups with 95% error bars from 138 flocks from 566 D. nodosus positive swabs from footrot-affected feet.



4.12. Disease state on the serogroups detected on feet

The diversity of serogroups detected was not associated with the disease status of the foot (Fisher's exact test, p = 0.091) (Table 4.13). This was close to being significant, therefore the rarest five serogroups (G, D, I, E and F) were grouped together to test whether this result was due to small numbers, and there was no significant difference (Fisher's exact test, p = 0.333). The median number of serogroups detected per foot was one for all disease states, with a range of 0 – 4, except for healthy feet, which had a range of 0 – 3 (Table 4.14). Lesion status was close to significantly associated with the number of serogroups detected on a swab (Fisher's exact test, p = 0.068), and in a post-hoc test there was a marginally significant difference between the number of serogroups detected between feet with footrot and feet with another lesion only (p = 0.045), with more serogroups detected from footrot-affected feet. There were few non-footrot samples in the dataset, therefore the association may have become significant with increased power. Therefore, feet with footrot may have more serogroups than feet without footrot but having a footrot lesion was not associated with the presence or absence of a specific serogroup, at least for the four most common serogroups.

Table 4.13 The number and percentage of 687 D. nodosus positive swabs positive for each serogroup by disease state from 164 flocks

Serogroup	Footrot		Healthy		Other		Unknown	
	N	%	N	%	N	%	N	%
H	245	43.3	5	27.8	16	26.2	16	38.1
B	201	35.5	3	16.7	14	23.0	12	28.6
C	90	15.9	4	22.2	13	21.3	10	23.8
A	83	14.7	1	5.6	7	11.5	3	7.1
G	31	5.5	1	5.6	1	1.6	3	7.1
D	23	4.1	0	0.0	5	8.2	5	11.9
E	17	3.0	0	0.0	0	0.0	0	0.0
I	15	2.7	1	5.6	3	4.9	3	7.1
F	6	1.1	0	0.0	2	3.3	0	0.0
Total	566		18		61		42	

N = number of swabs, % = percent of swabs

Table 4.14 The number and percentage of 687 D. nodosus positive swabs with 0 – 4 serogroups by disease state from 164 flocks

Number of serogroups	Footrot		Healthy		Other		Unknown	
	N	%	N	%	N	%	N	%
0	116	20.5	6	33.3	23	37.7	10	23.8
1	255	45.1	10	55.6	22	36.1	18	42.9
2	140	24.7	1	5.6	12	19.7	9	21.4
3	44	7.8	1	5.6	1	1.6	4	9.5
4	11	1.9	0	0.0	3	4.9	1	2.4
Total	566		18		61		42	

N = number of swabs, % = percent of swabs

4.13. Number of swabs on the serogroups detected in flocks

Generally, there was an increasing probability of detecting a serogroup the greater the number of *D. nodosus* positive swabs from a flock in both the observed and simulated (expected) data (Figure 4.14). The exact binomial confidence intervals were wide for eight swabs because only two flocks had eight *D. nodosus* positive swabs. There were no differences between the observed and expected (simulated) proportions of flocks positive for each serogroup with increasing numbers of swabs (chi-square tests, p = 0.999 – 1.000, Figure 4.14). The study was restricted to eight swabs per flock for postage (section 3.4) but these results show that eight swabs were insufficient to detect all serogroups in a flock, therefore each serogroup is likely to be present in more flocks than were detected, and the proportion of flocks with > 1 serogroups higher than reported in this study. There was no difference between serogroups in the likelihood of being detected by number of *D. nodosus* positive swabs per flock, whether common or rare (Fisher's exact test, p = 0.733). This adds evidence to the earlier findings (sections 4.10 and 4.11) that each serogroup is clustered within a flock. There is also no evidence that nationally rare serogroups, when present, are any less prevalent within a flock than nationally common serogroups.

Figure 4.14 The percentage of flocks positive for each serogroup by number of D. nodosus positive swabs submitted and the exact binomial 95% confidence intervals of the mean.

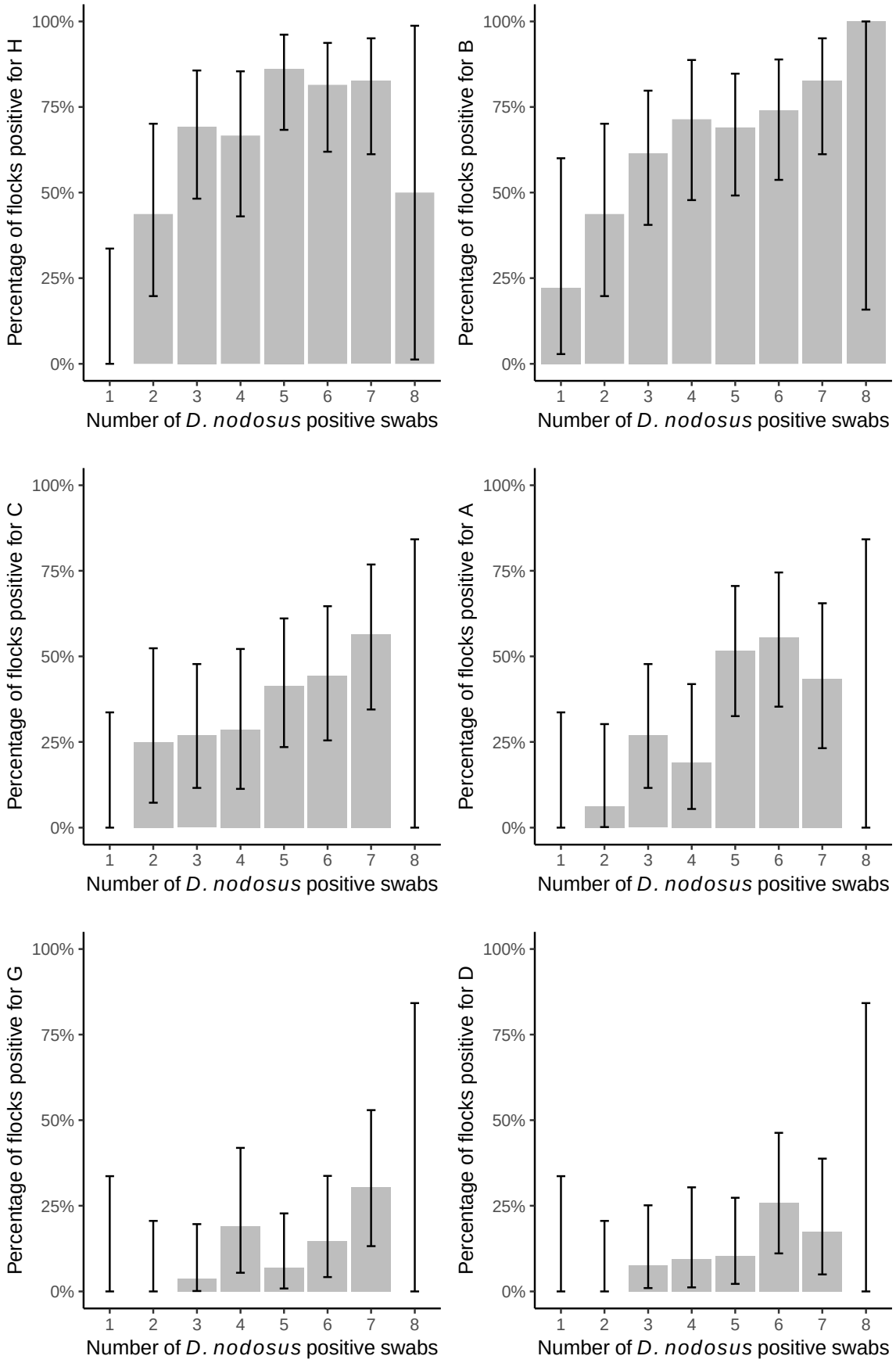


Figure 4.14 (continued) The percentage of flocks positive for each serogroup by number of D. nodosus positive swabs submitted and the exact binomial 95% confidence intervals of the mean.

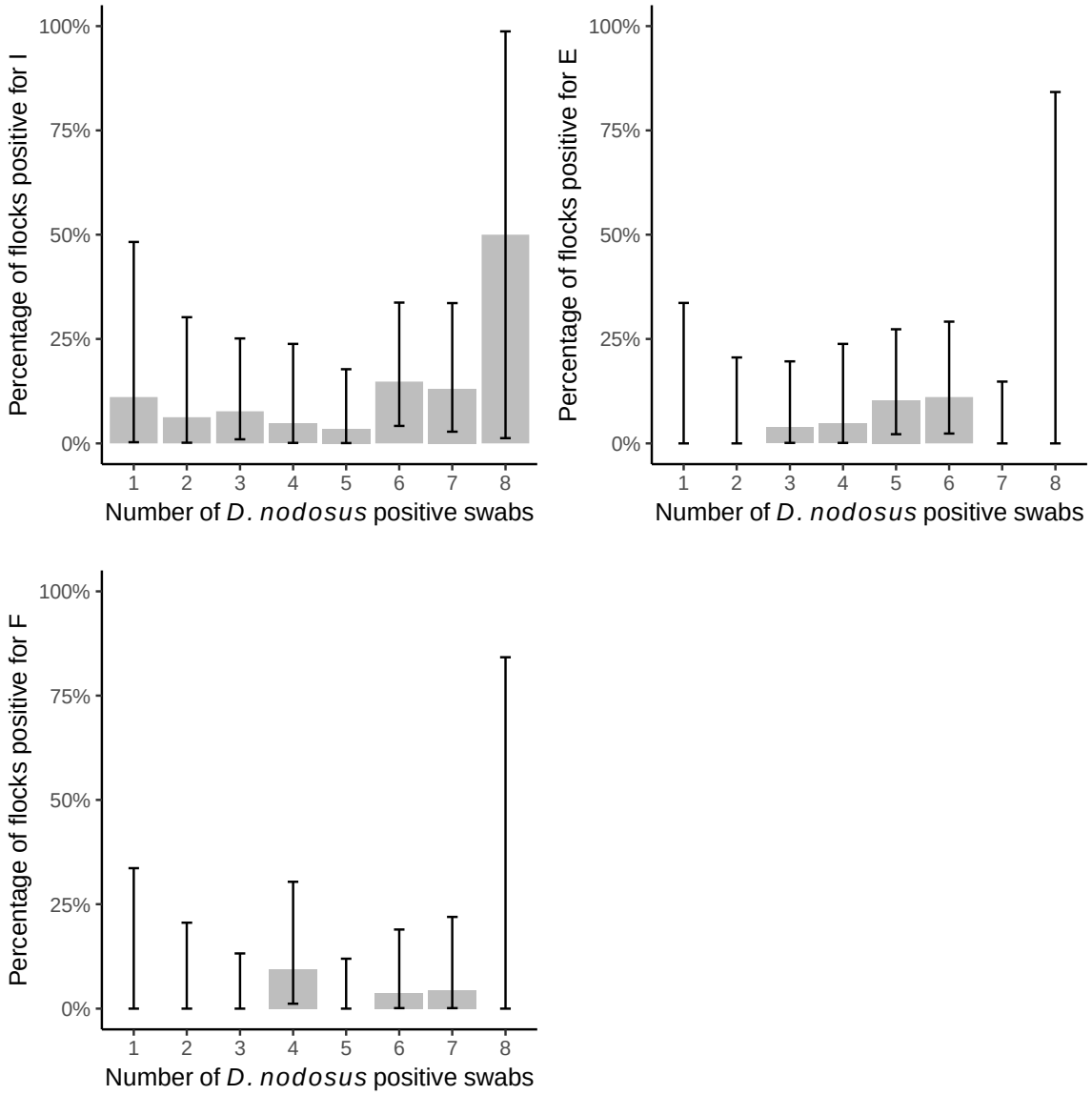


Figure 4.15 Observed (bar) and mean simulated (point) distribution of the percentage of flocks positive for each serogroup by number of D. nodosus positive swabs submitted from footrot-affected feet and 95% error bars of the simulated flocks.

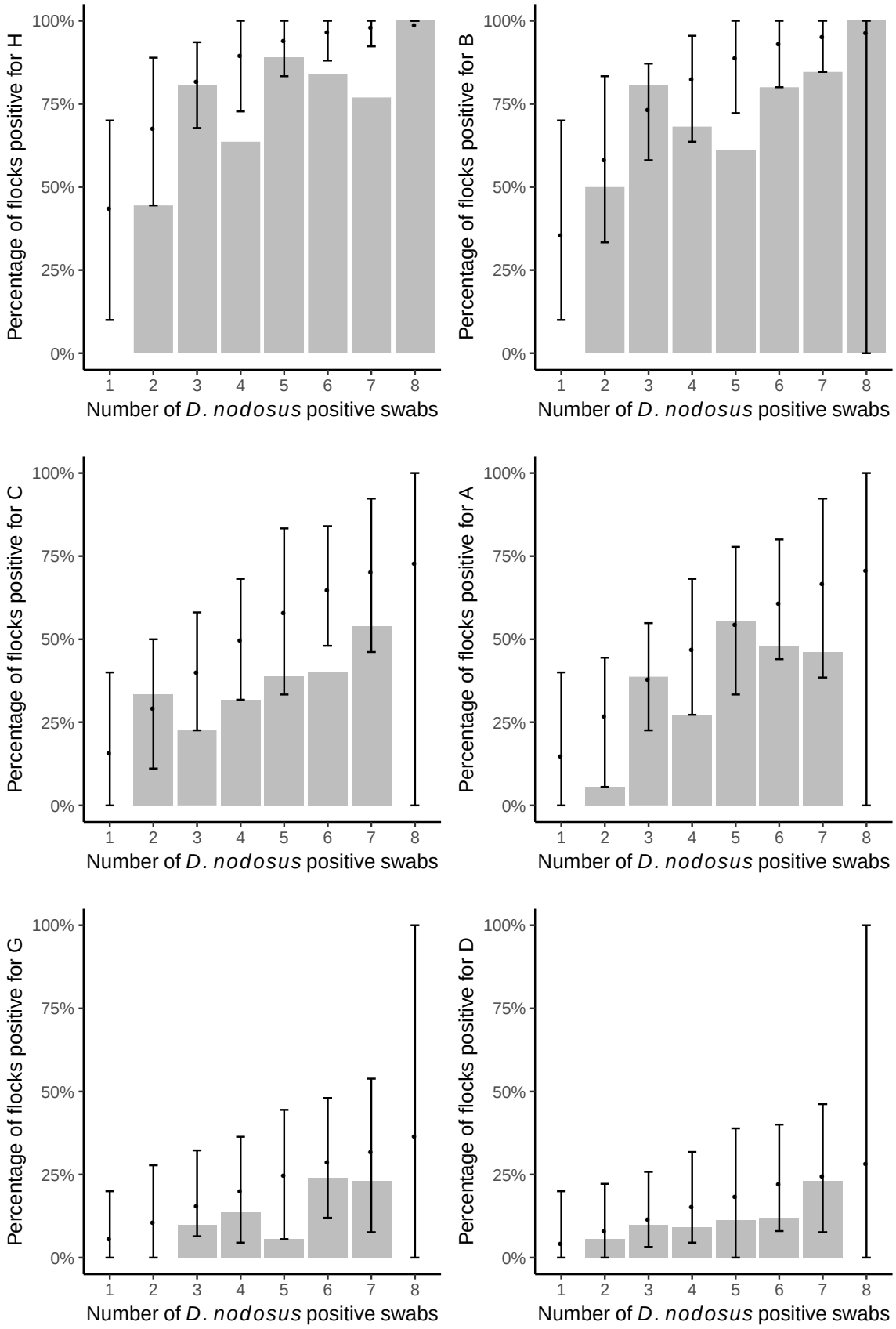
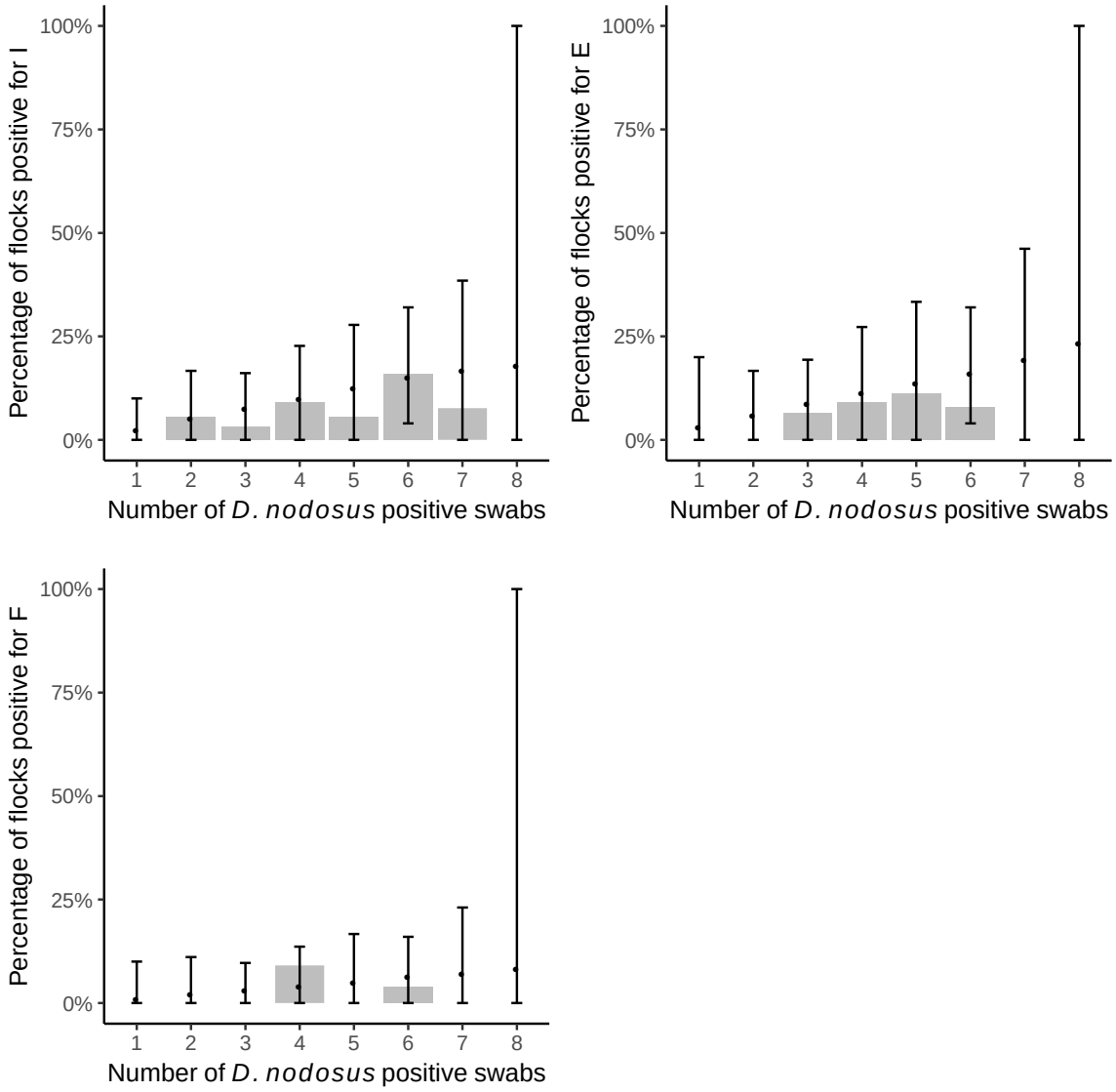
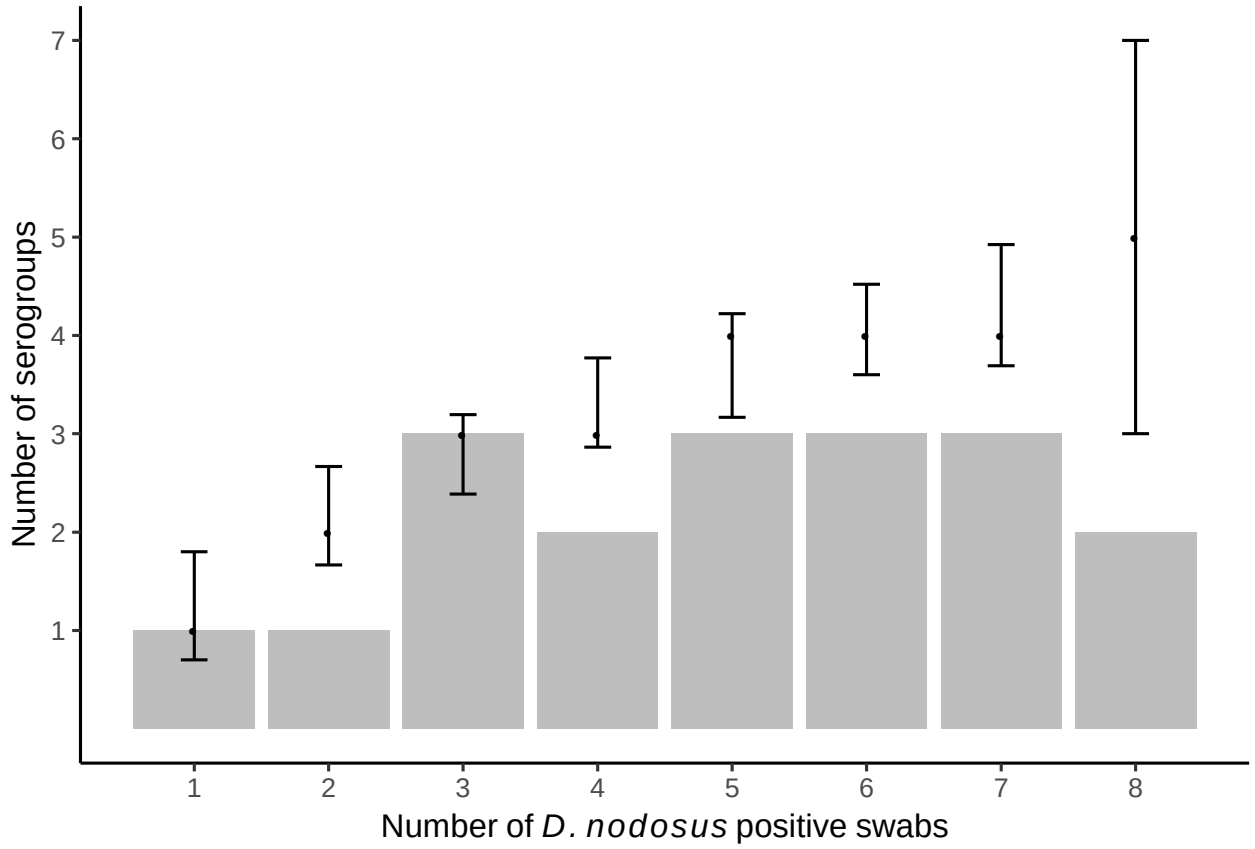


Figure 4.15 (continued) Observed (bar) and mean simulated (point) distribution of the percentage of flocks positive for each serogroup by number of D. nodosus positive swabs submitted from footrot-affected feet and 95% error bars of the simulated flocks.



There was no difference (Fisher's exact test, p = 0.996) in the number of serogroups detected with increasing number of swabs from footrot-affected feet per flock between the observed and simulated (expected) data, although an equal or smaller number of serogroups were observed than simulated for all numbers of swabs per flock (Figure 4.16). Initially more serogroups were detected with additional swabs, but the trend flattens with each additional swab. Therefore, although some serogroups may have been undetected, most serogroups would have been detected and this also adds to the evidence that serogroups were clustered within flocks (sections 4.10 and 4.11).

Figure 4.16 The mean (to one significant figure) observed (bar) and simulated (point) number of serogroups detected with 95% error bars from 1 – 8 D. nodosus positive swabs from footrot-affected feet for 138 English sheep flocks.



4.14. Multinomial model of disease state, number of swabs and biosecurity on the number of serogroups detected

A multivariable multinomial model was built to investigate whether the number of swabs analysed, foot lesions or biosecurity managements, explained the number of serogroups detected per flock when analysed together. The univariable model results of variables considered for inclusion are shown in Appendix 7.The only variables that remained in the multivariable model were the number of *D. nodosus* positive swabs and stocking rate (Table 4.15). Flocks with a greater number of *D. nodosus* positive swabs analysed were more likely to have ≥ 3 serogroups detected than 1 – 2 serogroups, and flocks which were kept at a stocking density of ≥ 4 ewes/acre were more likely to have ≥ 3 serogroups than 1 – 2 serogroups. No biosecurity variables were associated with the number of serogroups detected in the flock in the simulated dataset (data not shown), and no other biosecurity variable was correlated with stocking density (Appendix 21). This adds evidence to the earlier finding (section 4.13) that the number of *D. nodosus* positive swabs per flock collected in this study was not enough to detect all the serogroups in all the flocks, with the novel finding that more serogroups were present in flocks kept at a higher stocking density.

Table 4.15 Multivariable multinomial model results of the number of serogroups detected from 164 flocks explained by number of swabs analysed and biosecurity factors. Terms where p < 0.05 are in bold. AIC = 235.38.

Variable	Number of serogroups (ref = ≥ 3)	OR	95% CI	p value
Number of *D. nodosus* positive swabs				
	1 – 2	**0.61**	**0.49 – 0.77**	**< 0.001**
	0	**0.12**	**0.06 – 0.26**	**< 0.001**
Stocking rate (ref = < 4 ewes/acre)				
≥ 4 ewes/acre	**1 – 2**	**0.40**	**0.19 – 0.85**	**0.017**
	0	0.92	0.18 – 4.74	0.922
No response	1 – 2	0.26	0.05 – 1.39	0.116
	0	0.46	0.00 – 51.47	0.748

OR = odds ratio, CI = confidence interval

4.15. Vaccination on serogroups in a flock

There was no difference in the diversity of serogroups between the 15 flocks that had been vaccinating against footrot for > 5 years, 37 flocks that had been vaccinating for < 5 years and 101 flocks that did not vaccinate for all swabs (Table 4.16) and footrot-affected feet only swabs (Table 4.17) (Fisher's exact test, p = 0.681 and 0.796 respectively). This indicates that vaccination did not select for or against specific serogroups in a flock.

Table 4.16 The number and percentage of 153 flocks with known Footvax™ history positive for each serogroup.

Serogroup	Vaccinated > 5 years		Vaccinated < 5 years		Did not vaccinate	
	N	%	N	%	N	%
H	11	73.3	23	62.2	64	63.4
B	10	66.7	21	56.8	65	64.4
C	4	26.7	16	43.2	30	29.7
A	2	13.3	7	18.9	41	40.6
G	2	13.3	3	8.1	12	11.9
D	0	0.0	5	13.5	11	10.9
I	1	6.7	4	10.8	8	7.9
E	1	6.7	2	5.4	5	5.0
F	0	0.0	0	0.0	4	4.0
Number of flocks	15		37		101	

N = number of flocks, % = percent of flocks

Table 4.17 The number and percentage of 130 flocks with known Footvax™ history positive for each serogroup from footrot-affected feet.

Serogroup	Vaccinated > 5 years		Vaccinated < 5 years		Did not vaccinate	
	N	%	N	%	N	%
H	11	78.6	20	69.0	60	69.0
B	9	64.3	20	69.0	63	72.4
C	4	28.6	14	48.3	24	27.6
A	2	14.3	7	24.1	37	42.5
G	2	14.3	2	6.9	12	13.8
D	0	0.0	4	13.8	9	10.3
I	1	7.1	2	6.9	7	8.0
E	1	7.1	2	6.9	5	5.7
F	0	0.0	0	0.0	3	3.4
Number of flocks	14		29		87	

N = number of flocks, % = percent of flocks

4.16. Spatial patterns in serogroup diversity

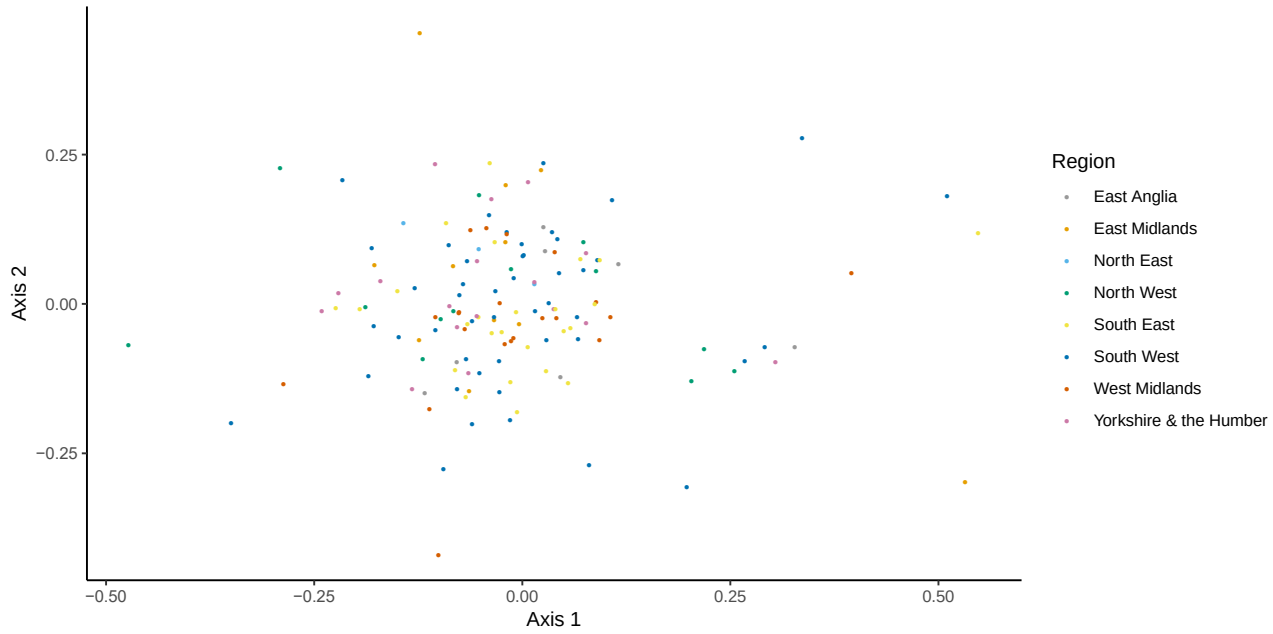
There were no patterns in the spatial location of serogroups (Figure 4.17), however the rare serogroups were too rare for any pattern in distribution to be observed. The distribution of each serogroup therefore is likely to be random.

Figure 4.17 The locations of the flocks positive for each serogroup.

4.17. Raup-Crick analysis of community dissimilarity between flocks

Raup-Crick pairwise distances (β_{RC}) were calculated for every pair of flocks to investigate serogroup community structure. Out of 10,296 pairwise comparisons, only 7 had communities that were more different than would be expected by chance ($\beta_{RC} > 0.95$). There were no pairs of communities that were more similar than would be expected by chance ($\beta_{RC} < -0.95$). The mean β_{RC} for all pairwise comparisons was -0.16 (range = -0.93 – 0.99) therefore community assembly was highly stochastic. There were no regional clusters in a principal coordinates analysis of the data (Figure 4.18). Therefore, not only does each serogroup not have a geographical distribution, but the communities of serogroups within flocks is also random.

Figure 4.18 The first two axes of a principal coordinates analysis of the β_{RC} distance metric for each of 144 flocks with ≥ 1 serogroup split into all regions.



4.18. Investigation of the number of swabs needed to detect serogroups of differing prevalence

Using the formula in section 3.30, any serogroup present in 32% and 53% of samples had a 95% likelihood of being detected with eight swabs and four swabs (the median number of *D. nodosus* positive swabs per flock in this study) respectively (Table 4.18). A total of 29 swabs would be needed for a serogroup present in 10% of samples to have a 95% chance of detection in a flock (Table 4.18). Therefore, serogroups with a prevalence of 50% or more from *D. nodosus* positive feet in a flock would have been detected in almost every flock in the current study, whereas rarer serogroups will not always have been detected. These results also mathematically confirm the

finding that additional swabs are less likely to detect additional serogroups as the number of swabs increases (Table 4.18).

Table 4.18 The minimum prevalence of a serogroup on swabs for a 95% likelihood of detection of the serogroup from 1 – 299 swabs.

Number of swabs	Prevalence
1	0.95
2	0.78
3	0.64
4	0.53
5	0.46
6	0.40
7	0.35
8	0.32
9	0.29
10	0.26
11	0.24
12	0.23
13	0.21
14	0.20
15	0.19
16	0.18
17	0.17
18	0.16
19	0.15
20	0.14
22	0.13
24	0.12
26	0.11
29	0.10
32	0.09
36	0.08
42	0.07
49	0.06
59	0.05
74	0.04
99	0.03
149	0.02
299	0.01

In the analysis of the serogroups detected from the pooled swabs (datasets I and II), a mean of one extra serogroup was detected from an extra 8 – 53 swabs per flock (Table 4.19). A total of 11

additional serogroups were identified from 8 (72.7%) of the flocks, 1 fewer serogroup was identified in 1 (9.1%) flock, and identical serogroups were detected in 3 (27.3%) flocks (Table 4.19). This confirms the finding that most serogroups were detected with the eight swabs collected from the farmers, but that a few will not have been detected.

Table 4.19 Serogroups detected in 11 flocks in the clinical trial (Witt and Green, 2018) from 8 single swabs (current study) and pooled samples with 16 – 60 swabs per sample.

Flock ID	Number of pooled samples	Number of swabs (current study)	Number of *D. nodosus* positive swabs (current study)	Serogroups detected
1	16	8	2	C
2	20	7	6	BCEhi
3	25	7	3	aBcH
4	29	7	4	aH
5	34	7	4	F
6	36	8	4	BdeHi
7	54	8	6	ABHi
8	55	7	6	ABcEH
9	55	8	5	AcH
10	60	7	6	ABH
11	60	8	8	aBH

Serogroups detected from both studies are black and upper case, from pooled swabs are blue and lower case, and from the current study are orange and lower case.

For the 1,000 simulations of Flock A with four and eight *D. nodosus* positive swabs no simulation detected 0 or 1 serogroups. All four serogroups were detected in 34.7% of simulations from eight swabs and 14.2% of simulations from four swabs, and three serogroups were detected in 82.4% of simulations from eight swabs and 57.6% of simulations from four swabs (Appendix 22). Serogroups B and H, the most common serogroups that were detected in 91.8% and 79.6% of swabs respectively, were detected in 100% of simulations from eight swabs, and 100.0% and 99.9% of simulations respectively from four swabs. Serogroups I and D which were detected from 14.3% and 8.2% of swabs were detected in 66.2% and 50.9% of simulations respectively from eight swabs and 41.8% and 30.1% of simulations respectively from four swabs (Appendix 23). A total of 37 swabs provided a 95% probability of detecting all four serogroups (using the formula in section 3.29). These results add to the evidence that the common serogroups of each flock will have been detected in almost all the flocks in the current study. Serogroups that were present in

over 80% of feet in a flock would have been detected with both four or eight *D. nodosus* positive swabs, however rare serogroups in a flock would not always have been detected.

4.19. Investigation of the feasibility of reducing the number of serogroups in a national vaccine

A bivalent vaccine with serogroups H and B would protect up to 27.1% of flocks (flocks for which H and B were the only serogroups present), and would target the two most common serogroups in a further 11.1% of flocks (Table 4.20). A multivalent vaccine with the four most common serogroups (H, B, C and A) would potentially protect 65.3% of flocks (Table 4.20).

Table 4.20 The number and percentage of 144 English sheep flocks for which the serogroups in a bivalent vaccines were also the only, or the most prevalent, serogroups detected in the flock.

	Only serogroups		Most prevalent serogroups	
Bivalent vaccines	Number	Percent	Number	Percent
HB	39	27.1	55	38.2
HB + CA	94	65.3	96	66.7
HB + CA + GD	120	83.3	120	83.3
HB + CA + GD + IE	140	97.2	140	97.2
HB + CA + GD + IE + F	144	100.0	144	100.0

5. Discussion

Key findings from this project were:

1. The prevalence of lameness in ewes in England was higher in 2015 than the previous two years and was associated with fewer farmers treating all lame sheep ≤ 3 days
2. Prompt treatment of lame sheep, ≤ 3 days of becoming lame, is the change in practice that would have the highest immediate impact on reducing the prevalence of lameness
3. All serogroups were detected nationally, serogroups were distributed with random heterogeneity in flocks, with serogroups H and B the most prevalent
4. Serogroups were clustered within flocks with fewer than expected flocks positive for each serogroup
5. The number of serogroups present in a flock is higher than previously reported, with more serogroups present in flocks with a stocking density of > 4 ewes/acre
6. A vaccine with fewer serogroups would be unlikely to be more efficacious nationally than that currently available based on the assumptions in this thesis

One aim of this project was to update the estimate of the prevalence of lameness in ewes in England and to identify changes in managements needed to further reduce the prevalence of lameness to 2% by 2021 in line with the FAWC (2011) ambition.

A second aim was to investigate whether it was possible to use a vaccine with fewer serogroups, and consequently greater efficacy, nationally by identifying the most prevalent serogroups, and the diversity of the serogroups, of *D. nodosus* in England, and whether vaccination with Footvax™ or other biosecurity managements influenced the diversity of serogroups in flocks.

The prevalence of lameness in ewes in England was higher in 2015 than in the previous two years and this was associated with a change in management practices by farmers towards flock-based managements and away from individual prompt (≤ 3 days) treatment of lame sheep. In 2015 the prevalence of lameness in 154 flocks was 4.1% which was significantly higher than 3.3% in 2013 and 3.2% in 2014. The reduction in the prevalence of lameness in ewes in the UK between 2004 and 2013 was associated with change in practice by farmers towards recommended managements of use of vaccination and antibiotics and stopping foot trimming (Winter et al., 2015). However, this project reports an increase in the prevalence of lameness in 2015 when compared with 2013 and 2014 in a cohort of farmers, and that although there had been an increased uptake in flock-level best management practices of culling lame sheep, vaccinating against footrot and not routine foot trimming, there had been a detrimental change with fewer farmers treating lame sheep promptly (≤ 3 days). The increased uptake of multiple flock-level managements was not enough to counteract the effect of neglecting this highly effective management for footrot. The increased prevalence of lameness is a negative finding for sheep welfare and for achieving the target prevalence of lameness in sheep of ≤ 2% (FAWC, 2011). Furthermore, knowledge transfer to farmers of evidence-based best practice managements for lameness has continued over this timeframe but has been ineffective in increasing the percentage of farmers treating lame sheep promptly. A preference of farmers to use vaccination to manage footrot over prompt treatment had already been identified (Wassink et al., 2010a), and Wassink et al. (2010a) recommended that knowledge transfer would have to be done carefully in order to emphasise the importance of prompt treatment. Catching lame sheep is difficult (Grant et al., 2018), but, despite this, farmers who practice prompt treatment are more likely to be satisfied with the managements they use for lameness (Wassink et al., 2010a) and best practice managements are associated with reduced treatment and production costs incurred from lameness (Winter and Green, 2017, O'Kane et al., 2017).

Prompt treatment (≤ 3 days) of all lame sheep would have the greatest immediate impact on reducing the prevalence of lameness in ewes in England. The population attributable fraction

(PAF) of lameness from not treating all lame sheep ≤ 3 days was 25.3% and was the highest PAF for any management associated with lameness in 2015 that would have an immediate impact. Recent studies identified lack of handling facilities, reluctance to treat ewes during mating, when heavily pregnant or when lambs are young, reluctance to treat lambs that are close to finishing, and lack of time as barriers to prompt treatment of lame sheep cited by farmers (Witt and Green, 2018). Identifying both the reasons why some farmers stopped treating promptly and practical methods to make prompt treatment easier will be useful in encouraging farmers to treat sheep promptly. Research has shown that one to one meetings with farmers are the most effective form of knowledge transfer for bringing about a reduction in lameness, followed by group meetings, and the least effective method is information via the post (Grant et al., 2018). One conclusion from this project is that future knowledge transfer needs to highlight the importance of prioritising prompt treatment (≤ 3 days) of the individual with antibiotics, which is highly effective and has a robust evidence base (Winter et al., 2015, Kaler et al., 2010a, Wassink et al., 2003, Kaler and Green, 2008b) and will have a major benefit compared with other behavioural changes, such as vaccination. Prompt treatment alone is sufficient to reduce the prevalence of lameness in a flock to < 2% (Wassink et al., 2010b). Farmers who were already using best practice managements, except prompt treatment, had a lower prevalence of lameness after knowledge transfer than farmers using traditional managements, indicating that farmers already using best practice managements but slow to treat were more receptive to the importance of prompt treatment than those not using best practice managements and changed their behaviour (Grant et al., 2018). Therefore, improved messaging targeted at the importance of prompt treatment could be effective at changing practice. An increased adoption of prompt treatment of lame sheep will be required in order to reach the target for the prevalence of lameness to be ≤ 2% by 2021 (FAWC, 2011).

The current study has identified that the proportion of farmers using Footvax™ has increased. Vaccination has previously been identified as a preferred management for lameness by farmers, along with concerns of lack of efficacy (Wassink et al., 2010a). A biologically effective vaccine is therefore highly desirable, because it would reduce the prevalence of lameness and so improve sheep welfare, with footrot the main cause of lameness in sheep in the UK. It would also be attractive to those farmers reluctant to treat lame sheep promptly because it would reduce the number of lame sheep requiring treatment.

The analysis in this thesis is the largest and most representative study to date of the diversity of serogroups of *D. nodosus* in England and contributes important knowledge into the serogroups of this endemic disease. One important finding is that there is random heterogeneity in the communities of serogroups between flocks and that serogroups were clustered within flocks.

Serogroups were not homogenously spread throughout flocks, but each flock had a distinct population of *D. nodosus* serogroups. Whilst serogroups H and B were the most prevalent, they were dominant in only 38.2% of flocks and so a vaccine with fewer serogroups would be unlikely to be effective nationally. This dissimilarity in communities of serogroups between flocks was stochastic, with no geographical patterns. The high variability in communities of serogroups between flocks will make tailoring the multivalent vaccine difficult because very few flocks will be completely protected by whichever combination of serogroups are included. One or two bivalent vaccines targeting the two and four most prevalent serogroups would only protect up to 27.1% and 65.3% of flocks respectively.

The presence of multiple serogroups in a flock was more common than previously reported, and the number of serogroups in a flock is associated with stocking density. The increase in the number of serogroups detected per flock is probably due to the increased sensitivity in the detection methods used here. The association between the number of serogroups present in a flock and stocking density is a novel finding. It could be due to increased transmission of serogroups of *D. nodosus* between sheep, ensuring that a population of each serogroup is more likely to be maintained. Further research will be needed to understand this, and the effect of long-term vaccination seen in the footrot-affected feet dataset, on the number of serogroups detected.

Creating flock-specific vaccines is another option of improving vaccination against footrot, and is currently the method of vaccination in Australia, however the prevalence of flocks with multiple serogroups, and the frequent movement of sheep between flocks will make flock-specific vaccination more challenging than it is in Australia. There are many unknowns to the feasibility of tailored vaccination in England, such as the impact of vaccination on non-target serogroups and whether UK flocks are too open for flock-specific vaccines to be effective and this should be an area of future research, in addition to developing PCR methods for the detection of serogroup M. It is clear that there will not be a fully effective vaccination against *D. nodosus* in the near future, therefore it is important that emphasis is placed on the importance of prompt treatment of lame sheep, and finding ways to help farmers achieve this in their flock.

In conclusion, the prevalence of lameness in ewes in England increased in 2015 compared with 2013, and this is associated with a decrease in the proportion of farmers treating lame sheep within three days, despite the increased uptake of flock-level best practice managements. Delayed treatment of lame sheep (> 3 days from onset of lameness) increased in the proportion of lameness that it was attributed to in the national flock since 2013, and prompt treatment (≤ 3 days) of all lame sheep in all flocks will have the greatest immediate impact on reducing the prevalence of lameness in sheep in England. Farmers increased their uptake of vaccination against footrot in

2015 compared with 2013, therefore improving the efficacy of vaccination is desirable. All serogroups A – I were detected, at different prevalence but distributed randomly between flocks, and there was heterogeneity in the serogroup communities between flocks. This makes a tailored vaccine against *D. nodosus* for England unlikely to be more effective than the current commercial vaccine, as many flocks will not be protected. Tailored flock-specific vaccines may be more effective, but their feasibility in England will have to investigated. An improved vaccine is likely to be readily accepted by farmers, but the emphasis of knowledge transfer needs to be on the importance of prompt treatment (≤ 3 days) of all lame sheep.

6. Industry messages

A group of 164 English sheep farmers completed questionnaires regarding the prevalence of, and managements for, lameness in their flock in 2015, and submitted interdigital swabs from the feet of eight sheep. There was an increase in the prevalence of lameness in ewes in England in 2015 compared to 2013, which was associated with a decrease in the proportion of farmers treating all lame sheep within three days. The proportion of the national lameness that that was attributed to delayed treatment of lame sheep also increased from 13% in 2013 to 25% in 2015. This study found that prompt treatment (≤ 3 days) of all lame sheep would have the greatest immediate impact on reducing the prevalence of lameness in sheep in England.

This study also investigated the diversity of serogroups of *D. nodosus* (the cause of footrot) in England and investigated whether a vaccine against *D. nodosus* with fewer serogroups could protect the national flock. Vaccines against footrot that target one or two serogroups are more effective, but only against the serogroups they contain. All serogroups A – I were detected, each at a different prevalence, but the serogroups were distributed randomly between flocks. However, a vaccine targeting the two most prevalent serogroups in England would only protect up to 27% of flocks.

7. References

ANGELL, J. W., BLUNDELL, R., GROVE-WHITE, D. H. & DUNCAN, J. S. 2015. Clinical and radiographic features of contagious ovine digital dermatitis and a novel lesion grading system. *Veterinary Record,* 176**,** 9.

ANGELL, J. W., DUNCAN, J. S., CARTER, S. D. & GROVE-WHITE, D. H. 2014. Farmer reported prevalence and factors associated with contagious ovine digital dermatitis in Wales: A questionnaire of 511 sheep farmers. *Preventive Veterinary Medicine,* 113**,** 132-138.

ANGELL, J. W., GROVE-WHITE, D. H. & DUNCAN, J. S. 2018. Sheep and farm level factors associated with footrot: a longitudinal repeated cross-sectional study of sheep on six farms in the UK. *Veterinary Record,* 182**,** 7.

BEVERIDGE, W. I. B. 1941. *Foot-Rot in Sheep: A Transmissable Disease due to Infection with Fusiformis nodosus (n. sp.) Studies on its Cause, Epidemiology and Control,* Melbourne, Commonwealth of Australia Council for Scientific and Industrial Research.

CEDERLOF, S. E., HANSEN, T., KLAAS, I. C. & ANGEN, O. 2013. An evaluation of the ability of *Dichelobacter nodosus* to survive in soil. *Acta Veterinaria Scandinavica,* 55**,** 4.

CHASE, J. M., KRAFT, N. J. B., SMITH, K. G., VELLEND, M. & INOUYE, B. D. 2011. Using null models to disentangle variation in community dissimilarity from variation in alpha-diversity. *Ecosphere,* 2**,** 1-11.

CLAXTON, P. D., RIBEIRO, L. A. & EGERTON, J. R. 1983. Classification of *Bacteroides nodosus* by agglutination tests. *Australian Veterinary Journal,* 60**,** 331-334.

CLEMENTS, R. H. & STOYE, S. C. 2014. The 'Five Point Plan': a successful tool for reducing lameness in sheep. *Veterinary Record,* 175**,** 225.

COX, D. R. & WERMUTH, N. 1996. *Multivariate dependencies: models, analysis and interpretation,* London, Chapman & Hall.

CRAWLEY, M. J. 2013. *The R Book,* Chichester, United Kingdom, John Wiley & Sons, Ltd.

DEPIAZZI, L. J. & RICHARDS, R. B. 1985. Motility in relation to virulence of *Bacteroides nodosus*. *Veterinary Microbiology,* 10**,** 107-116.

DHUNGYEL, O., SCHILLER, N., EPPLESTON, J., LEHMANN, D., NILON, P., EWERS, A. & WHITTINGTON, R. 2013. Outbreak-specific monovalent/bivalent vaccination to control and eradicate virulent ovine footrot. *Vaccine,* 31**,** 1701-1706.

DHUNGYEL, O., SCHILLER, N. & WHITTINGTON, R. 2015. Identification and characterization of serogroup M *Dichelobacter nodosus* from sheep with virulent footrot. *Veterinary Microbiology,* 176**,** 378-381.

DHUNGYEL, O. P. & WHITTINGTON, R. J. 2010. Modulation of inter-vaccination interval to avoid antigenic competition in multivalent footrot (*Dichelobacter nodosus*) vaccines in sheep. *Vaccine,* 28**,** 470-473.

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

DICKINS, A., CLARK, C. C. A., KALER, J., FERGUSON, E., O'KANE, H. & GREEN, L. E. 2016. Factors associated with the presence and prevalence of contagious ovine digital dermatitis: A 2013 study of 1136 random English sheep flocks. *Preventive Veterinary Medicine,* 130**,** 86-93.

DOHOO, I., MARTIN, W. & STRYHN, H. 2003. *Veterinary Epidemiologic Research,* Prince Edward Island, Canada, AVC Inc.

DUNCAN, J. S., GROVE-WHITE, D., MOKS, E., CARROLL, D., OULTRAM, J. W., PHYTHIAN, C. J. & WILLIAMS, H. W. 2012. Impact of footrot vaccination and antibiotic therapy on footrot and contagious ovine digital dermatitis. *Veterinary Record,* 170**,** 462.

EGERTON, J. R. 1973. Surface and somatic antigens of *Fusiformis nodosus*. *Journal of Comparative Pathology,* 83**,** 151-159.

EGERTON, J. R. & BURRELL, D. H. 1970. Prophylactic and therapeutic vaccination against ovine foot-rot. *Australian Veterinary Journal,* 46**,** 517-522.

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

EGERTON, J. R., ROBERTS, D. S. & PARSONSON, I. M. 1969. The aetiology and pathogenesis of ovine foot-rot I. A histological study of the bacterial invasion. *Journal of Comparative Pathology,* 79**,** 207-216.

ENNEN, S., HAMANN, H., DISTL, O., HICKFORD, J., ZHOU, H. & GANTER, M. 2009. A field trial to control ovine footrot via vaccination and genetic markers. *Small Ruminant Research,* 86**,** 22-25.

FAWC 2011. Opinion on Lameness in Sheep. London: Farm Animal Welfare Council.

FIFE, D. 2017. A Biostatisticians Toolbox for Various Activities, Including Plotting, Data Cleanup, and Data Analysis. version 1.1 ed.

FROSTH, S., KÖNIG, U., NYMAN, A.-K., PRINGLE, M. & ASPÁN, A. 2015. Characterisation of *Dichelobacter nodosus* and detection of *Fusobacterium necrophorum* and *Treponema* spp. in sheep with different clinical manifestations of footrot. *Veterinary Microbiology,* 179**,** 82-90.

GIEBEL, K. 2017. *Persistence of Dichelobacter nodosus, the causal agent of ovine footrot.* PhD, University of Warwick.

GODDARD, P., WATERHOUSE, T., DWYER, C. & STOTT, A. 2006. The perception of the welfare of sheep in extensive systems. *Small Ruminant Research,* 62**,** 215-225.

GOWER, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika,* 53**,** 325-338.

GRANT, C., KALER, J., FERGUSON, E., O'KANE, H. & GREEN, L. E. 2018. A comparison of the efficacy of three intervention trial types: postal, group, and one-to-one facilitation, prior management and the impact of message framing and repeat messages on the flock prevalence of lameness in sheep. *Preventive Veterinary Medicine,* 149**,** 82-91.

GREEN, L. E. & GEORGE, T. R. N. 2008. Assessment of current knowledge of footrot in sheep with particular reference to *Dichelobacter nodosus* and implications for elimination or control strategies for sheep in Great Britain. *Veterinary Journal,* 175**,** 173-180.

GREEN, L. E., WASSINK, G. J., GROGONO-THOMAS, R., MOORE, L. J. & MEDLEY, G. F. 2007. Looking after the individual to reduce disease in the flock: A binomial mixed effects model investigating the impact of individual sheep management of footrot and interdigital dermatitis in a prospective longitudinal study on one farm. *Preventive Veterinary Medicine,* 78**,** 172-178.

HARWOOD, D. G., CATTELL, J. H., LEVIS, C. J. & NAYLOR, R. 1997. Virulent foot rot in sheep. *Veterinary Record,* 140**,** 687-687.

HILL, A. E., DHUNGYEL, O. P. & WHITTINGTON, R. J. 2010. Diagnostic sampling strategies for virulent ovine footrot: Simulating detection of *Dichelobacter nodosus* serogroups for bivalent vaccine formulation. *Preventive Veterinary Medicine,* 95**,** 127-136.

HINDMARSH, F. & FRASER, J. 1985. Serogroups of *Bacteroides nodosus* isolated from ovine footrot in Britain. *Veterinary Record,* 116**,** 187-188.

HOTHORN, T., BRETZ, F. & WESTFALL, P. 2008. Simultaneous inference in general parametric models. *Biometrical Journal,* 50**,** 346-363.

HUNT, J. D., JACKSON, D. C., BROWN, L. E., WOOD, P. R. & STEWART, D. J. 1994. Antigenic competition in a multivalent foot rot vaccine. *Vaccine,* 12**,** 457-464.

KALER, J., DANIELS, S. L. S., WRIGHT, J. L. & GREEN, L. E. 2010a. Randomized clinical trial of long-acting oxytetracycline, foot trimming, and flunixine meglumine on time to recovery in sheep with footrot. *Journal of Veterinary Internal Medicine,* 24**,** 420-425.

KALER, J. & GREEN, L. E. 2008a. Naming and recognition of six foot lesions of sheep using written and pictorial information: A study of 809 English sheep farmers. *Preventive Veterinary Medicine,* 83**,** 52-64.

KALER, J. & GREEN, L. E. 2008b. Recognition of lameness and decisions to catch for inspection among sheep farmers and specialists in GB. *BMC Veterinary Research,* 4**,** 41.

KALER, J. & GREEN, L. E. 2009. Farmers' practices and factors associated with the prevalence of all lameness and lameness attributed to interdigital dermatitis and footrot in sheep flocks in England in 2004. *Preventive Veterinary Medicine,* 92**,** 52-59.

KALER, J., MEDLEY, G. F., GROGONO-THOMAS, R., WELLINGTON, E. M. H., CALVO-BADO, L. A., WASSINK, G. J., KING, E. M., MOORE, L. J., RUSSELL, C. & GREEN, L. E. 2010b. Factors associated with changes of state of foot conformation and lameness in a flock of sheep. *Preventive Veterinary Medicine,* 97**,** 237-244.

KALER, J., WASSINK, G. J. & GREEN, L. E. 2009. The inter- and intra-observer reliability of a locomotion scoring scale for sheep. *Veterinary Journal,* 180**,** 189-194.

KENNAN, R. M., WONG, W., DHUNGYEL, O. P., HAN, X., WONG, D., PARKER, D., ROSADO, C. J., LAW, R. H. P., MCGOWAN, S., REEVE, S. B., LEVINA, V., POWERS, G. A., PIKE, R. N., BOTTOMLEY, S. P., SMITH, A. I., MARSH, I., WHITTINGTON, R. J., WHISSTOCK, J. C., PORTER, C. J. & ROOD, J. I. 2010. The subtilisin-like protease AprV2 is required for virulence and uses a novel disulphide-tethered exosite to bind substrates. *Plos Pathogens,* 6**,** e1001210.

KING, E. M. & GREEN, L. E. 2011. Assessment of farmer recognition and reporting of lameness in adults in 35 lowland sheep flocks in England. *Animal Welfare,* 20**,** 321-328.
KINGSLEY, D. F., HINDMARSH, F. H., LIARDET, D. M. & CHETWIN, D. H. 1986. Distribution of serogroups of *Bacteroides nodosus* with particular reference to New Zealand and the United Kingdom. Footrot in ruminants: proceedings of a Workshop, 1986 1986 Melbourne. CSIRO Division of Animal Health.
LEY, S. J., LIVINGSTON, A. & WATERMAN, A. E. 1989. The effect of chronic clinical pain on thermal and mechanical thresholds in sheep. *Pain,* 39**,** 353-357.
MANGIAFICO, S. 2019. rcompanion: Functions to Support Extension Education Program Evaluation. 2.1.1 ed.
MARWICK, B. & KRISHNAMOORTHY, K. 2019. cvequality: Tests for the equality of Coefficients of Variation from Multple Groups. 0.2.0 ed.
MCPHERSON, A. S., DHUNGYEL, O. P. & WHITTINGTON, R. J. 2018. Detection and serogrouping of *Dichelobacter nodosus* infection by use of direct PCR from lesion swabs to support outbreak-specific vaccination for virulent footrot in sheep. *Journal of Clinical Microbiology,* 56**,** e01730-17.
MOORE, L. J., WASSINK, G. J., GREEN, L. E. & GROGONO-THOMAS, R. 2005a. The detection and characterisation of *Dichelobacter nodosus* from cases of ovine footrot in England and Wales. *Veterinary Microbiology,* 108**,** 57-67.
MOORE, L. J., WOODWARD, M. J. & GROGONO-THOMAS, R. 2005b. The occurrence of treponemes in contagious ovine digital dermatitis and the characterisation of associated *Dichelobacter nodosus*. *Veterinary Microbiology,* 111**,** 199-209.
MSD ANIMAL HEALTH. *Footvax* [Online]. Available: http://www.msd-animal-health.co.uk/products_public/footvax/overview.aspx [Accessed 26/10/2015 2015].
MSD ANIMAL HEALTH 2014. Stamp Out Lameness.
MUZAFAR, M., CALVO-BADO, L. A., GREEN, L. E., SMITH, E. M., RUSSELL, C. L., GROGONO-THOMAS, R. & WELLINGTON, E. M. H. 2015. The role of the environment in transmission of *Dichelobacter nodosus* between ewes and their lambs. *Veterinary Microbiology,* 179**,** 53-59.
MUZAFAR, M., GREEN, L. E., CALVO-BADO, L. A., TICHAUER, E., KING, H., JAMES, P. & WELLINGTON, E. M. H. 2016. Survival of the ovine footrot pathogen *Dichelobacter nodosus* in different soils. *Anaerobe,* 38**,** 81-87.
NAYLOR, R. D., MARTIN, P. K., JONES, J. R. & BURNELL, M. C. 1998. Isolation of spirochaetes from an incident of severe virulent ovine footrot. *Veterinary Record,* 143**,** 690-691.
NIEUWHOF, G. J., BISHOP, S. C., HILL, W. G. & RAADSMA, H. W. 2008. The effect of footrot on weight gain in sheep. *Animal,* 2**,** 1427-1436.
O'KANE, H., FERGUSON, E., KALER, J. & GREEN, L. 2017. Associations between sheep farmer attitudes, beliefs, emotions and personality, and their barriers to uptake of best practice: The example of footrot. *Preventive Veterinary Medicine,* 139**,** 123-133.
PARADIS, E. & SCHLIEP, K. 2018. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics,* 35**,** 526-528.
PINHEIRO, J., BATES, D., DEBROY, S., SARKAR, D. & TEAM, R. C. 2018. nlme: Linear and Nonlinear Mixed Effects Models. 3.1-137 ed.
R CORE TEAM 2017. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
RAADSMA, H. W., OMEARA, T. J., EGERTON, J. R., LEHRBACH, P. R. & SCHWARTZKOFF, C. L. 1994. Protective antibody titres and antigenic competition in multivalent *Dichelobacter nodosus* fimbrial vaccines using characterised rDNA antigens. *Veterinary Immunology and Immunopathology,* 40**,** 253-274.
SCHWARTZKOFF, C. L., EGERTON, J. R., STEWART, D. J., LEHRBACH, P. R., ELLEMAN, T. C. & HOYNE, P. A. 1993. The effects of antigenic competition on the efficacy of multivalent footrot vaccines. *Australian Veterinary Journal,* 70**,** 123-126.
SMITH, E. M., GILBERT, A., RUSSELL, C. L., PURDY, K. J., MEDLEY, G. F., MUZAFAR, M., GROGONO-THOMAS, R. & GREEN, L. E. 2017. Within-flock population dynamics of *Dichelobacter nodosus*. *Frontiers in Veterinary Science,* 4**,** 1-6.

SMITH, E. M., GREEN, O. D. J., CALVO-BADO, L. A., WITCOMB, L. A., GROGONO-THOMAS, R., RUSSELL, C. L., BROWN, J. C., MEDLEY, G. F., KILBRIDE, A. L., WELLINGTON, E. M. H. & GREEN, L. E. 2014. Dynamics and impact of footrot and climate on hoof horn length in 50 ewes from one farm over a period of 10 months. *The Veterinary Journal,* 201**,** 295-301.

STEWART, D. J. 1978. The role of various antigenic fractions of *Bacteroides nodosus* in eliciting protection against foot-rot in vaccinated sheep. *Research in Veterinary Science,* 24**,** 14-19.

SULLIVAN, L. E., CLEGG, S. R., ANGELL, J. W., NEWBROOK, K., BLOWEY, R. W., CARTER, S. D., BELL, J., DUNCAN, J. S., GROVE-WHITE, D. H., MURRAY, R. D. & EVANS, N. J. 2015. High-level association of bovine digital dermatitis *Treponema* spp. with contagious ovine digital dermatitis lesions and presence of *Fusobacterium necrophorum* and *Dichelobacter nodosus*. *Journal of Clinical Microbiology,* 53**,** 1628-1638.

THORLEY, C. M. & DAY, S. E. J. 1986. Serotyping survey of 1296 strains of *Bacteroides nodosus* isolated from sheep and cattle in Great Britain and western Europe. *In:* STEWART, D. J., PETERSON, J. E., MCKERN, N. M. & EMERY, D. L., eds. Footrot in Ruminants, Proceedings of a Workshop, 1986. CSIRO Australia and Australian Wool Corporation, 135-142.

VENABLES, W. N. & RIPLEY, B. D. 2002. *Modern Applied Statistics with S.,* New York, Springer.

VER HOEF, J. M. & BOVENG, P. L. 2007. Quasi-poisson vs. negative binomial regression: How should we model overdispersed count data? *Ecology,* 88**,** 2766-2772.

WASSINK, G. J., GEORGE, T. R. N., KALER, J. & GREEN, L. E. 2010a. Footrot and interdigital dermatitis in sheep: Farmer satisfaction with current management, their ideal management and sources used to adopt new strategies. *Preventive Veterinary Medicine,* 96**,** 65-73.

WASSINK, G. J., GROGONO-THOMAS, R., MOORE, L. J. & GREEN, L. E. 2003. Risk factors associated with the prevalence of footrot in sheep from 1999 to 2000. *Veterinary Record,* 152**,** 351-358.

WASSINK, G. J., KING, E. M., GROGONO-THOMAS, R., BROWN, J. C., MOORE, L. J. & GREEN, L. E. 2010b. A within farm clinical trial to compare two treatments (parenteral antibacterials and hoof trimming) for sheep lame with footrot. *Preventive Veterinary Medicine,* 96**,** 93-103.

WINTER, A. C. 2008. Lameness in sheep. *Small Ruminant Research,* 76**,** 149-153.

WINTER, J. R. & GREEN, L. E. 2017. Cost-benefit analysis of management practices for ewes lame with footrot. *Veterinary Journal,* 220**,** 1-6.

WINTER, J. R., KALER, J., FERGUSON, E., KILBRIDE, A. L. & GREEN, L. E. 2015. Changes in prevalence of, and risk factors for, lameness in random samples of English sheep flocks: 2004-2013. *Preventive Veterinary Medicine,* 122**,** 121-128.

WITCOMB, L. A., GREEN, L. E., CALVO-BADO, L. A., RUSSELL, C. L., SMITH, E. M., GROGONO-THOMAS, R. & WELLINGTON, E. M. H. 2015. First study of pathogen load and localisation of ovine footrot using fluorescence in situ hybridisation (FISH). *Veterinary Microbiology,* 176**,** 321-327.

WITCOMB, L. A., GREEN, L. E., KALER, J., UL-HASSAN, A., CALVO-BADO, L. A., MEDLEY, G. F., GROGONO-THOMAS, R. & WELLINGTON, E. M. H. 2014. A longitudinal study of the role of *Dichelobacter nodosus* and *Fusobacterium necrophorum* load in initiation and severity of footrot in sheep. *Preventive Veterinary Medicine,* 115**,** 48-55.

WITT, J. & GREEN, L. 2018. Development and assessment of management practices in a flock-specific lameness control plan: A stepped-wedge trial on 44 English sheep flocks. *Preventive Veterinary Medicine,* 157**,** 125-133.

8. Appendix 1 - 2015 questionnaire on lameness in sheep

Questionnaire

Section 1. Causes of lameness

In the table below, please read the description in the left column and look at the picture and then answer questions 1 and 2 for each lesion.

What you might notice when you look at the foot	Example picture of the lesion	1. What did you call this lesion? *Please circle one answer in each box below*	2. Did this lesion cause lameness in your flock between January 2015 and December 2015? *Please circle one answer and fill in % for each box below*
☐ Red, wet interdigital space ☐ Foul smell ☐ May be grey pasty scum ☐ Loss of hair in interdigital space		**Footrot** **Scald** **CODD** **Toe granuloma** **Other** *(please state)* _____	**Yes** ***If yes,* what percentage of ewes was lame from this lesion?** _____% **No** **Do not know**
☐ Some separation of horn from underlying live foot ☐ Foul smelling blackish slimy dead tissue		**Footrot** **Scald** **CODD** **Toe granuloma** **Other** *(please state)* _____	**Yes** ***If yes,* what percentage of ewes was lame from this lesion?** _____% **No** **Do not know**
☐ Abnormal at coronary band (top of foot) ☐ Loss of hair above coronary band ☐ There may be complete detachment of hoof		**Footrot** **Scald** **CODD** **Toe granuloma** **Other** *(please state)* _____	**Yes** ***If yes,* what percentage of ewes was lame from this lesion?** _____% **No** **Do not know**
☐ Strawberry-like growth at the toe ☐ Sometimes hidden under overgrown horn ☐ Bleeds when handled		**Footrot** **Scald** **CODD** **Toe granuloma** **Other** *(please state)* _____	**Yes** ***If yes,* what percentage of ewes was lame from this lesion?** _____% **No** **Do not know**

BEEF & LAMB

Section 2. Treatment of lame sheep

3. Between January 2015 and December 2015 which of the following did you use to treat footrot in your flock?
(Please circle all that apply)

Ewes:	**Foot trimming**	**Antibiotic injection**	**Foot spray**	**Separate lame individuals**	**Other treatments** *(please specify what you did)* _____
Lambs:	**Foot trimming**	**Antibiotic injection**	**Foot spray**	**Separate lame individuals**	**Other treatments** *(please specify what you did)* _____

4. Between January 2015 and December 2015, when you saw lame sheep how soon did you treat them?
(Please circle one)

The first day you saw them	**Within three days**	**Within one week**	**Within two weeks**	**Longer than two weeks**	**Did not treat any lame sheep**

Section 3. Routine flock managements

5. Between January 2015 and December 2015 which of the following did you use to manage footrot in your flock?
(Please circle all that apply)

Routine foot trimming	**Routine foot bathing**	**Vaccination**	**Separation of lame sheep**	**Other** *(please specify)* _____

6. Approximately what percentage of sheep bled when you did a routine trim? _____ % **Not applicable**

7. Did you quarantine sheep arriving on your farm for at least 3 weeks? *(Please circle one)*

Always	**Sometimes**	**Never**

8. When did you cull sheep that had been lame? *(Please circle one)*

Never	**After 1 bout of lameness**	**After 2 bouts of lameness**	**When persistently lame**	**Other** *(please specify)* _____

Section 4: Vaccination against footrot

9. Which of your sheep did you vaccinate with Footvax between January 2015 and December 2015?
(*Please circle all that apply*)

None	**Ewes**	**Rams**	**Sheep with footrot**	**Bought-in sheep**	**Other** *(please specify)* _____

If None, please go to ***Section 5***

10. How often did you use Footvax? *(Please circle one)*

Never	**Once a year**	**More often than once a year**

11. When did you start vaccinating against footrot? *(Please circle one)*

I don't vaccinate	**Within the last year**	**Within the last 2 years**	**Within the last 5 years**	**More than 5 years ago**	

Section 5. Purchasing sheep

12. Did you purchase any sheep between January 2015 and December 2015? *(Please circle one)*

Yes **No**

If No, please go to question 16

13. Where did you purchase your sheep between January 2015 and December 2015? *(Please circle all that apply)*

Market **Private Farm Sale** **Other** *(Please specify)*
_____

14. Which regions did your purchased sheep come from? *(Please circle all that apply)*

North West **North East** **Midlands** **East Anglia** **Other** *(please specify)*
South West **South East** **Scotland** **Wales**
_____

15. Over the past five years, how frequently have you purchased sheep? *(Please circle one)*

More than once a year **Once a year** **Other** *(please specify)*
_____

Section 6. Your flock

16. Between January 2015 and December 2015…
    a. How many ewes did you have in your breeding flock? _____ **ewes**
    b. What was the average level of lameness in ewes in your flock? _____ **%**
    c. What was the average level of lameness in lambs in your flock? _____ **%**
    d. What was the highest level of lameness in your ewe flock? _____ **%**
    e. What was the highest level of lameness in your lamb flock? _____ **%**

17. Approximately what stocking rate did you use for ewes between January 2015 and December 2015? (*Please circle one*)

Less than 4 ewes per acre **4 – 8 ewes per acre** **More than 8 ewes per acre**

18. Did your flock mix with other flocks? *(Please circle all that apply)*

No **Yes, shared grazing** **Yes, at shows** **Yes, other** *(please specify)*
_____

19. Did your flock share grazing with cattle? *(Please circle one)*

Yes **No** **Don't know**

20. Did you avoid selecting replacement ewes from mothers that were repeatedly lame? *(Please circle one)*

Yes **No**

PLEASE RETURN YOUR COMPLETED QUESTIONNAIRE IN THE PREPAID PACKAGE PROVIDED

BEEF & LAMB

9. Appendix 2 - Interdigital swabbing instructions

Illustrated by Zoë Willis

Foot Swabbing Instructions

Important Information

- Please read through these instructions carefully before taking swab samples.
- Only take samples from animals that you have NOT treated during the last 2 weeks so that the bacteria on the foot are undisturbed.
- DO NOT remove dirt from the skin before swabbing, as this will remove some of the bacteria we are interested in.
- DO NOT touch the swab end of the stick (see step 5 below), as this will contaminate it with other bacteria.

If you have any questions please do not hesitate to contact me on N.Prosser@warwick.ac.uk.

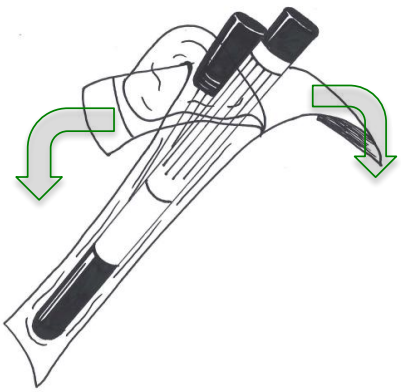
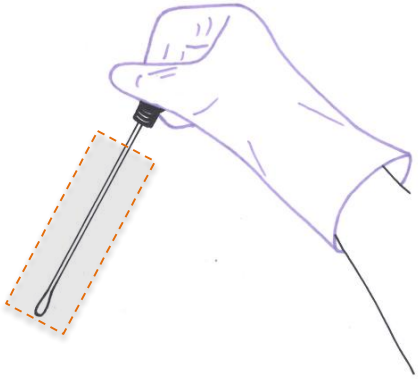
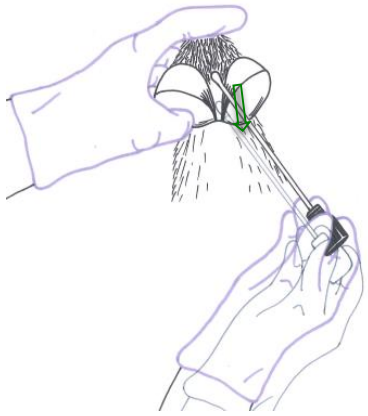
Swabbing Instructions

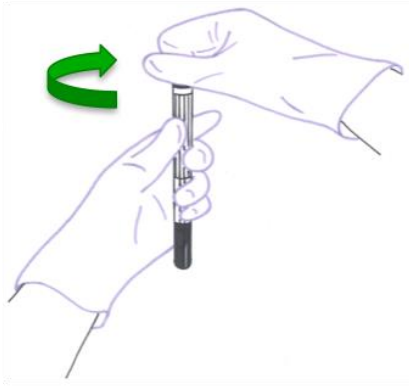
1. Select 8 lame sheep to take the samples from. If you have fewer than 8 lame sheep in your flock sample every lame sheep you have, and then sample some healthy sheep to give a total of 8.

2. Use one swab per foot.

3. Turn the sheep you are going to sample.

4-9. Follow instructions below

		
4. The plastic packaging contains a sealed tube and separate sterile swab. With clean or gloved hands peel back the sterile packaging.	5. Take the swab from the packaging holding the top. Do not touch the swab or stick below the top.	6. With one hand, open the foot to display the skin between the toes. With the other, wipe the skin between the toes with the swab 5 times from front to back. Press firmly but do not draw blood.

		
7. Take the tube from the packaging. Break the seal and open the tube	8. Place the swab inside the tube; ensure the stick (beige area) does not touch anything.	9. Push the swab cap down firmly. You may dispose of the other cap.

10. Attach a sample number from the sheet of sticky labels onto the tube.

11. Fill in the details about the sheep on the record sheet linking it to the same sample number (so we know some information about where the sample has come from).

12. Repeat for up to 7 more sheep (preferably lame with footrot or scald).

13. Please store the swabs in the fridge until posting. Follow the packaging instructions immediately before posting. Please post the swabs within a week of the first sheep swabbed.

10. Appendix 3 - Multiplex and single serogroup PCR sensitivity

Serogroup	Multiplex PCR (Genome copies)	Single PCR (Genome copies)
A	9.4×10^3	9.4×10^2
B	7.2×10^4	7.2×10^3
C	9.4×10^2	9.4×10^2
D	7.2×10^3	7.2×10^3
E	2.5×10^4	2.5×10^3
F	9.4×10^3	9.4×10^2
G	2.5×10^3	2.5×10^3
H	7.2×10^4	7.2×10^3
I	2.5×10^3	2.5×10^4

11. Appendix 4 - Footrot lesion scoring system

Interdigital dermatitis (ID) lesion classification

0 Clean interdigital foot with no dermatitis (scald) 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 the interdigital area affected).

3 Moderate interdigital dermatitis, partial/complete loss of hair, redness, pasty scum (10-50% of the interdigital area affected).

4 Severe interdigital dermatitis, partial/complete loss of hair, redness, pasty scum (>50% affected).

Footrot (SFR) lesion classification (damage to tissue at the worst affected point). Depth measurement as a comparison to the digit's width at its mid-length point

0 No under-running of the wall of the digit.

1 Depth of lesion less than 25% of the width of the digit's sole at its mid-length point. Lesion relatively dry but foul smelling.

2 Depth of lesion at or more than 25% but less than 50% of the width of the digit's sole at its mid-length point. Lesion wet and foul smelling.

3 Depth of lesion at or more than 50% but less than 75% of the width of the digit's sole at its mid-length point. Lesion wet and foul smelling.

4 Depth of lesion 100% of the width of the digit's sole at its mid-length point. Lesion wet and foul smelling.

12. Appendix 5 - The univariable model results of variables tested in the multivariable quasi-Poisson regression model for lameness in ewes in 154 English sheep flocks

Variable	Farmers		RR	95% CI	
	N	%			
Used foot trimming to treat SFR in ewes					
No	62	40.3	1.00		
Yes	89	57.8	1.10	0.86	1.42
Used antibiotic injection to treat SFR in ewes					
No	17	11.0	1.00		
Yes	134	87.0	0.70	0.46	1.12
Used foot spray to treat SFR in ewes					
No	7	4.5	1.00		
Yes	144	93.5	0.96	0.56	1.85
Separated lame individuals to treat SFR in ewes					
No	103	66.9	1.00		
Yes	48	31.2	0.93	0.72	1.19
Used another treatment to treat SFR in ewes					
No	150	97.4	1.00		
Yes	1	0.6	0.82	0.08	3.06
Used foot trimming to treat SFR in lambs					
No	109	70.8	1.00		
Yes	**41**	**26.6**	**1.34**	**1.02**	**1.74**
Used antibiotic injection to treat SFR in lambs					
No	63	40.9	1.00		
Yes	87	56.5	1.13	0.87	1.47
Used foot spray to treat SFR in lambs					
No	20	13.0	1.00		
Yes	**130**	**84.4**	**1.85**	**1.23**	**2.93**
Separated lame individuals to treat SFR in lambs					
No	126	81.8	1.00		
Yes	**24**	**15.6**	**1.43**	**1.00**	**1.98**
Used another treatment to treat SFR in lambs					
No	149	96.8	1.00		
Yes	1	0.6	0.82	0.08	3.07

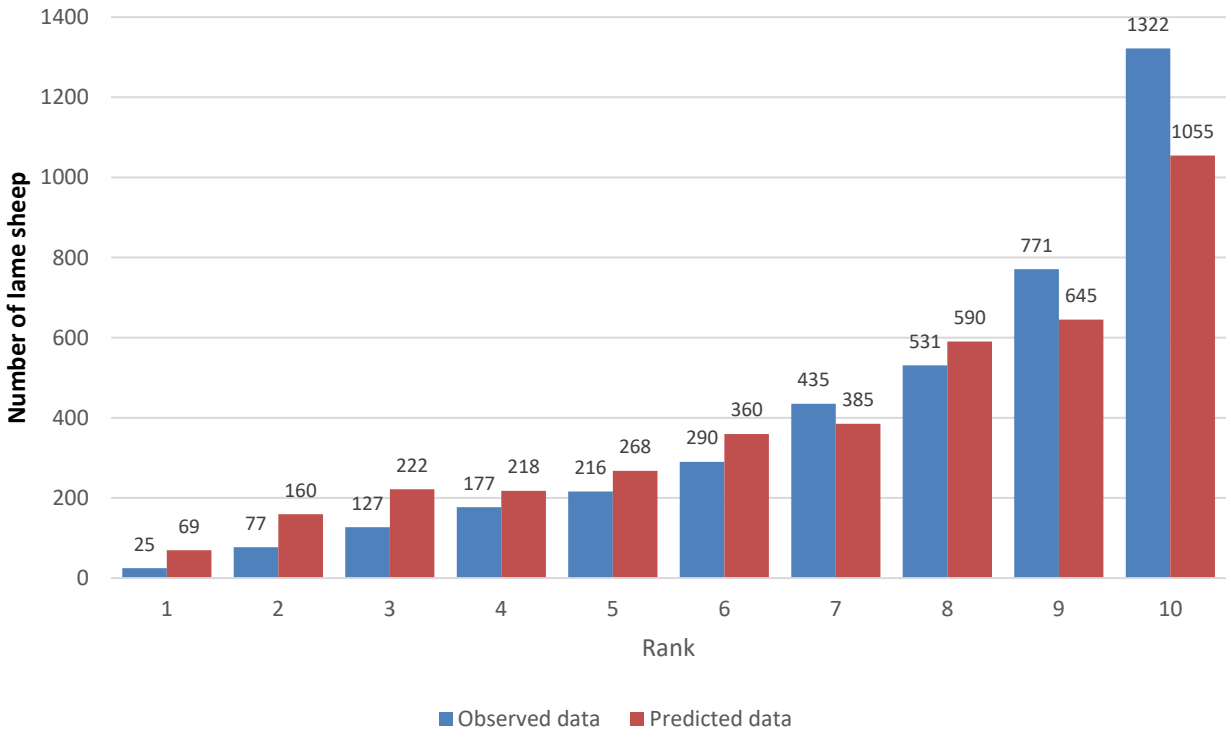
Time to treatment of all lame sheep					
≤ 3 days	44	28.6	1.00		
≤ 1 week	**74**	**48.1**	**1.85**	**1.37**	**2.54**
≤ 2 weeks	**24**	**15.6**	**1.75**	**1.17**	**2.60**
> 2 weeks	**10**	**6.5**	**1.87**	**1.14**	**2.99**
Used routine foot trimming to manage SFR					
No	126	81.8	1.00		
Yes	**28**	**18.2**	**1.37**	**1.01**	**1.81**
Used routine foot bathing to manage SFR					
No	64	41.6	1.00		
Yes	90	58.4	1.14	0.89	1.49
Used footrot vaccination to manage SFR					
No	96	62.3	1.00		
Yes	58	37.7	0.92	0.72	1.18
Separated lame sheep to manage SFR					
No	96	62.3	1.00		
Yes	58	37.7	1.04	0.82	1.33
Used another management to manage SFR					
No	151	98.1	1.00		
Yes	3	1.9	0.86	0.19	2.39
Percentage of sheep that bled during routine foot trimming					
No routine foot trimming	122	79.2	1.00		
0 – 2%	20	13.0	1.17	0.80	1.65
≥ 5%	**10**	**6.5**	**1.94**	**1.30**	**2.79**
Sheep arriving on farm quarantined for at least 3 weeks					
N/A	21	13.6	1.00		
Always	76	49.4	1.08	0.73	1.66
Sometimes	31	20.1	1.14	0.72	1.83
Never	23	14.9	1.34	0.83	2.19
Sheep that had been lame were culled					
After 1 or 2 bouts	28	18.2	1.00		
After 3 or more bouts	10	6.5	1.34	0.72	2.38
When persistently lame / severe disease / misshapen hoof	88	57.1	1.21	0.86	1.75
Never	27	17.5	1.24	0.78	1.98
Vaccinated with Footvax™					

Some sheep, but not all ewes	13	8.4	1.00		
No sheep	96	62.3	1.36	0.95	2.00
Ewes	45	29.2	1.40	0.95	2.10
Frequency of Footvax™ use					
Never	96	62.3	1.00		
Once/year	47	30.5	1.08	0.83	1.39
> once/year	**11**	**7.1**	**0.60**	**0.39**	**0.89**
Length of time vaccinating against footrot					
> 5 years	15	9.7	1.00		
Did not vaccinate	**96**	**62.3**	**1.92**	**1.33**	**2.85**
> 0 – 1 year	**12**	**7.8**	**3.38**	**2.01**	**5.64**
> 1 – 2 years	12	7.8	1.36	0.80	2.28
> 2 – 5 years	**12**	**7.8**	**2.29**	**1.44**	**3.67**
Sheep purchased					
Yes	131	85.1	1.00		
No	23	14.9	1.06	0.73	1.49
Sheep purchased from market					
No	65	42.2	1.00		
Yes	**87**	**56.5**	**1.39**	**1.09**	**1.78**
Sheep purchased from private farm sale					
No	86	55.8	1.00		
Yes	**66**	**42.9**	**0.73**	**0.57**	**0.93**
Regions sheep were purchased from					
The South East & East Anglia	21	13.6	1.00		
No purchase made	23	14.9	1.41	0.87	2.29
The North & Scotland	**32**	**20.8**	**1.56**	**1.05**	**2.38**
The Midlands & Wales	31	20.1	1.37	0.87	2.19
The South West	26	16.9	1.58	0.98	2.56
Don’t know	1	0.6	1.40	0.08	6.47
Multiple regions	17	11.0	1.07	0.64	1.77
Frequency of sheep purchases over last 5 years					
> once/year	45	29.2	1.00		
≤ once/year	83	53.9	1.01	0.77	1.32
Prevalence of lameness in lambs in the flock					
For each percent increase in prevalence of lamb lameness	**154**	**100.0**	**1.05**	**1.03**	**1.06**

Ewe stocking rate					
< 4 /acre	69	44.8	1.00		
≥ 4 /acre	82	53.2	1.12	0.87	1.43
Sheep mixed with other flocks					
No	144	93.5	1.00		
Yes	10	6.5	1.43	0.90	2.16
Flock shared grazing with cattle					
Yes	72	46.8	1.00		
No	82	53.2	0.97	0.76	1.24
Avoided selecting replacement ewes from repeatedly lame mothers					
Yes	66	42.9	1.00		
No	87	56.5	1.03	0.80	1.33

BOLD: Significant associations with lameness in ewes (Wald's test p<0.05). N: number of farmers; RR: risk ratio; CI: confidence intervals; SFR: severe footrot.

13. Appendix 6 - The predicted and observed deciles of the number of lame sheep per flock from a multivariable quasi-Poisson regression model with four explanatory variables for 154 English sheep flocks, ranked by the observed data



14. Appendix 7 - Univariable multinomial model results of the number of serogroups detected from *D. nodosus* positive swabs from 153 flocks

Terms where p < 0.05 are in bold.

Number of serogroups (ref = ≥ 3)	OR	95% CI	p value	AIC
Swabs submitted				271.89
1 – 2	0.41	0.11 – 1.48	0.174	
0	0.36	0.08 – 1.60	0.180	
Swabs submitted from footrot-affected feet				**268.77**
1 – 2	**0.82**	**0.70 – 0.97**	**0.020**	
0	0.79	0.58 – 1.06	0.112	
Contaminated swabs				275.24
1 – 2	1.04	0.65 – 1.66	0.868	
0	0.86	0.31 – 2.36	0.769	
***D. nodosus* positive swabs**				**230.83**
1 – 2	**0.61**	**0.49 – 0.77**	**< 0.001**	
0	**0.20**	**0.09 – 0.43**	**< 0.001**	
***D. nodosus* positive swabs from footrot lesions**				**243.44**
1 – 2	**0.68**	**0.57 – 0.82**	**< 0.001**	
0	**0.38**	**0.23 – 0.62**	**< 0.001**	
D. nodosus positive swabs from healthy feet				273.13
1 – 2	1.38	0.64 – 2.94	0.408	
0	< 0.01	< 0.01 – 3.65e29	0.865	
D. nodosus positive swabs from non-footrot lesions				274.62
1 – 2	0.87	0.56 – 1.34	0.518	
0	1.18	0.55 – 2.52	0.676	
D. nodosus positive swabs from unknown lesion				271.84
1 – 2	1.16	0.80 – 1.69	0.433	
0	< 0.01	< 0.01 – 6.40e20	0.809	

OR = odds ratio, CI = confidence interval, AIC = Akaike's Information Criterion

Variable	Number of serogroups (ref = ≥ 3)	Number of flocks	OR	95% CI	AIC / p value
Used routine footbathing to manage footrot					273.99
No	≥ 3	27			
	1 – 2	26			
	0	5			
Yes	≥ 3	38			
	1 – 2	51	1.39	0.70 – 2.76	0.341
	0	4	0.57	0.40 – 2.32	0.430
No response	≥ 3	2			
	1 – 2	0	-	-	-
	0	0	-	-	-
Vaccinated to manage footrot					272.48
No	≥ 3	46			
	1 – 2	43			
	0	5			

Yes	≥ 3	19			
	1 – 2	34	1.91	0.95 – 3.85	0.068
	0	4	1.94	0.47 – 8.01	0.361
No response	≥ 3	2			
	1 – 2	0	-	-	-
	0	0	-	-	-
Sheep vaccinated against footrot					274.28
None	≥ 3	46			
	1 – 2	43			
	0	5			
Ewes	≥ 3	14			
	1 – 2	26	1.99	0.92 – 4.30	0.081
	0	4	2.63	0.62 – 11.14	0.190
Not all ewes	≥ 3	5			
	1 – 2	8	1.71	0.52 – 5.64	0.377
	0	0	-	-	-
No response	≥ 3	2			
	1 – 2	0	-	-	-
	0	0	-	-	-
Frequency of Footvax™ use					274.06
Never	≥ 3	46			
	1 – 2	43			
	0	5			
Once/yr	≥ 3	16			
	1 – 2	29	1.94	0.93 – 4.06	0.079
	0	2	1.15	0.20 – 6.52	0.875
>once/yr	≥ 3	3			
	1 – 2	5	1.78	0.40 – 7.91	0.448
	0	2	6.12	0.82 – 45.85	0.078
No response	≥ 3	2			
	1 – 2	0	-	-	-
	0	0	-	-	-
Started vaccination					281.93
Did not vaccinate	≥ 3	46			
	1 – 2	43			
	0	5			
> 0 – 1 year	≥ 3	4			
	1 – 2	8	2.14	0.60 – 7.62	0.241
	0	0	-	-	-
> 1 – 2 years	≥ 3	3			
	1 – 2	7	2.50	0.61 – 10.28	0.205
	0	2	6.13	0.82 – 45.93	0.077
> 2 – 5 years	≥ 3	6			
	1 – 2	5	0.89	0.25 – 3.14	0.858
	0	0	-	-	-
> 5 years	≥ 3	4			
	1 – 2	9	2.41	0.69 – 8.39	0.168
	0	1	-	-	0.492
No response	≥ 3	4			
	1 – 2	5	1.34	0.34 – 5.31	0.680
	0	1	-	-	-
Separated lame sheep to manage footrot					272.71
No	≥ 3	45			
	1 – 2	42			
	0	5			
Yes	≥ 3	20			
	1 – 2	35	1.91	0.94 – 3.90	0.075
	0	4	1.08	0.18 – 6.37	0.936
No response	≥ 3	2			

	1 – 2	0	-	-	-
	0	0	-	-	-
Culled sheep that had been lame					275.02
After 1/2 bouts	≥ 3	11			
	1 – 2	13			
	0	1			
After 3/more bouts	≥ 3	2			
	1 – 2	7	2.96	0.51 – 17.26	0.228
	0	**3**	**16.51**	**1.09 – 250.32**	**0.043**
Persistently / severe disease / misshapen hoof	≥ 3	43			
	1 – 2	41	0.81	0.32 – 2.00	0.644
	0	5	1.28	0.14 – 12.12	0.829
Never	≥ 3	9			
	1 – 2	15	1.41	0.45 – 0.47	0.557
	0	0	-	-	-
No response	≥ 3	2			
	1 – 2	1	-	-	-
	0	0	-	-	-
Flock mixed with other flocks					274.05
No	≥ 3	63			
	1 – 2	68			
	0	9			
Yes	≥ 3	2			
	1 – 2	1	-	-	-
	0	0	-	-	-
No response	≥ 3	2			
	1 – 2	8	0.46	0.04 – 5.24	0.534
	0	0	-	-	-
Purchased sheep					277.63
No	≥ 3	12			
	1 – 2	10			
	0	1			
Yes	≥ 3	53			
	1 – 2	66	1.49	0.60 – 3.73	0.389
	0	8	1.81	0.21 – 15.90	0.592
No response	≥ 3	2			
	1 – 2	1	-	0.05 – 7.63	0.694
	0	0	-	-	-
Purchased sheep from market					277.95
No	≥ 3	27			
	1 – 2	30			
	0	5			
Yes	≥ 3	38			
	1 – 2	44	1.04	0.53 – 2.05	0.905
	0	4	0.57	0.14 – 2.31	0.430
No response	≥ 3	2			
	1 – 2	3	1.35	0.21 – 8.70	0.752
	0	0	-	-	-
Purchased sheep from private farm sale					278.08
No	≥ 3	37			
	1 – 2	43			
	0	4			
Yes	≥ 3	28			
	1 – 2	31	0.95	0.49 – 1.87	0.889
	0	5	1.65	0.41 – 6.72	0.484
No response	≥ 3	2			
	1 – 2	3	1.29	0.20 – 8.14	0.786
	0	0	-	-	-

Frequency of sheep purchases in last 5yrs					275.35
≤ once/yr	≥ 3	30			
	1 – 2	45			
	0	4			
> once/yr	≥ 3	23			
	1 – 2	18	0.52	0.24 – 1.13	0.098
	0	4	1.30	0.29 – 5.78	0.726
No response	≥ 3	14			
	1 – 2	14	0.67	0.28 – 1.60	0.363
	0	1	-	-	-
Regions sheep purchased from					285.10
No purchase	≥ 3	12			
	1 – 2	10			
	0	1			
Multiple regions	≥ 3	7			
	1 – 2	7	1.20	0.31 – 4.59	0.790
	0	3	5.14	0.44 – 59.47	0.190
The North / Scotland	≥ 3	15			
	1 – 2	16	1.28	0.43 – 3.83	0.659
	0	1	-	-	-
The Midlands / Wales	≥ 3	8			
	1 – 2	20	3.00	0.93 – 9.70	0.066
	0	2	3.00	0.23 – 38.88	-
The South East / East Anglia	≥ 3	6			
	1 – 2	11	2.20	0.60 – 8.08	0.235
	0	1	-	-	-
The South West	≥ 3	16			
	1 – 2	9	0.67	0.21 – 2.18	0.511
	0	1	-	-	-
Don't know	≥ 3	0			
	1 – 2	1	-	-	-
	0	0	-	-	-
No response	≥ 3	3			
	1 – 2	3	1.20	0.20 – 7.31	0.773
	0	0	-	-	-
Quarantined sheep arriving on farm for at least 3 weeks					282.46
Always	≥ 3	29			
	1 – 2	38			
	0	3			
Sometimes	≥ 3	14			
	1 – 2	17	0.93	0.39 – 2.18	0.862
	0	2	1.38	0.21 – 9.23	0.739
Never	≥ 3	9			
	1 – 2	12	1.02	0.38 – 2.74	0.973
	0	2	2.15	0.31 – 14.94	0.440
N/A	≥ 3	11			
	1 – 2	9	0.62	0.23 – 1.71	0.358
	0	1	-	-	-
No response	≥ 3	4			
	1 – 2	1	-	-	-
	0	1	-	-	-
Stocking rate					270.37
< 4 ewes/acre	≥ 3	20			
	1 – 2	41			
	0	4			
≥ 4 ewes/acre	≥ 3	43			
	1 – 2	**33**	**0.37**	**0.19 – 0.75**	**0.006**
	0	5	0.58	0.14 – 2.40	0.453
	≥ 3	4			

No response	1 – 2	3	0.37	0.07 – 1.79	0.215
	0	0	-	-	-
Flock shared grazing with cattle					278.03
No	≥ 3	36			
	1 – 2	39			
	0	4			
Yes	≥ 3	29			
	1 – 2	37	1.18	0.61 – 2.29	0.629
	0	5	1.55	0.38 – 6.31	0.539
No response	≥ 3	2			
	1 – 2	1	-	-	-
	0	0	-	-	-

OR = odds ratio, CI = confidence interval, AIC = Akaike's Information Criterion, - = no flocks in one of the comparison groups