Combining genetics and epidemiology:

a model of footrot in sheep

By

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Declaration

I declare that apart from advice and assistance acknowledged the work reported in this thesis is my own and has not been submitted for any other degree.

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Summary

The interaction between host genetics and epidemiological processes in ovine footrot was investigated using a combination of data analysis and simulation modelling. The study's aims were to determine the potential for genetic selection to be used to reduce the prevalence of footrot in the UK and to assess different strategies for use of conventional epidemiological interventions.

A stochastic simulation model was developed, incorporating host genetics for traits controlling footrot resistance, bacterial population dynamics, sheep population dynamics and epidemiological processes. Sensitivity analysis of the model showed survival time of *Dichelobacter nodosus* in the environment and infection rate were the key determinants of disease outcomes.

Antibiotics were predicted to be the most effective conventional control method, reducing prevalence of footrot to 1-2% when administered promptly. Pasture rotation, selective culling and vaccination were all predicted to reduce prevalence but to a lower extent.

Analysis of field data confirmed the likely role for some degree of host genetic control of footrot resistance, i.e. resistance appears to be lowly to moderately heritable. Using the simulation model it was then shown that genetic selection could be effective at reducing footrot prevalence. In combination with antibiotic treatment or pasture rotation elimination of footrot from an individual flock could be achieved. Genetic selection was predicted to be effective at reducing prevalence and improving resistance but the choice of selection criteria impacts the results seen. It is likely that progress would be slower in field situations because footrot traits would be diluted by simultaneous selection for other traits affecting profitability.

Field studies are required to determine optimal combinations of interventions and genetic selection and to validate modelling outcomes. Combined data from longitudinal disease observations, genetic information and bacterial samples are necessary to address current knowledge gaps and to further advance understanding of host and disease processes in ovine footrot.

Chapter 1. Introduction

1.1 - Lameness in sheep

1.1.1 - Footrot and interdigital dermatitis

Footrot is an infectious bacterial disease of sheep in which infection is transmitted between animals via contaminated pasture (Beveridge, 1941). Clinical signs include lameness and foot lesions which start in the interdigital space and can progress to cause separation of the hoof horn from the sensitive dermis (Beveridge, 1941). The disease is common, with a within-flock prevalence of 8 – 10% in England (Kaler and Green, 2009), detrimental to production (Wassink *et al.,* 2010) and reduces both animal health and welfare (Fitzpatrick *et al.*, 2006). In a survey conducted in 2001, sheep farmers rated it as the second highest threat to animal health and welfare, after sheep scab (Morgan-Davies *et al.*, 2006).

The primary aetiological agent of footrot is *Dichelobacter nodosus* (Beveridge, 1941) although other species of bacteria are also associated with disease. Amongst these, *Fusobacterium necrophorum* is thought to play an important role in disease pathogenesis (Egerton *et al.*, 1969; Roberts & Egerton, 1969), although the exact mechanisms of the infection process have not yet been elucidated. Recent work from Witcomb (2012) reinforces Beveridge's 1941 work that *D. nodosus* is the necessary agent for footrot and *F. necrophorum* a secondary invader. Footrot is also closely linked to interdigital dermatitis (sometimes referred to as scald) which presents as irritated and inflamed interdigital skin in the feet of sheep. In 1941, Beveridge suggested that this was the result of early colonisation with *D. nodosus,* an idea supported by the microbial analysis undertaken by Witcomb (2012), although for many years it was believed to be caused by *F. necrophorum.* It is now thought that *F.*

necrophorum aids in the progression of disease only once the lesions have advanced to a state where separation of the hoof and horn has occurred as it is at this point that *F. necrophorum* is seen to multiply (Witcomb, 2012).

Footrot can present within individual sheep many times in their lifetime; there is no long-term immunity. Challenge trials suggest that immunity lasts for up to 12 weeks, and for a much shorter period of time in many cases (Egerton and Roberts, 1971). This contributes to the recurring patterns of footrot seen in the UK because when sheep recover from an episode of footrot they quickly become susceptible again and may be reinfected after only a short period of time. This also maintains a high level of *D. nodosus* on the pasture, where the bacteria may survive for 7 to 10 days (Beveridge, 1941; Whittington, 1995), due to continued shedding from infected sheep.

Footrot occurs globally, with documented studies in countries including the UK (e.g. Nieuwhof *et al.*, 2008a and 2008b; Wassink *et al.*, 2010), New Zealand (e.g. Skerman *et al.*, 1988), Australia (e.g. Beveridge, 1941; Raadsma *et al.*, 1994), India (e.g. Wani *et al.*, 2007), Bhutan (e.g. Gurung *et al.*, 2006), Nepal (e.g. Ghimire *et al.*, 1998) and Germany (Zhou *et al.*, 2009). It is present all year round in much of the UK but countries with more seasonal variation in temperatures, for example in parts of Australia, experience footrot as seasonal epidemics. In Australia the reliably hot and dry summer period prevents the bacteria from surviving on pasture and thus disease transmission is nil in the summer months, with some areas reporting transmission for only 6 weeks of the year (Green and George, 2008).

1.1.2 - Treatment and control strategies

The most effective treatment for footrot is prompt use of parenteral and topical antibiotics which significantly reduce footrot prevalence and incidence (Wassink *et al.*, 2010; Kaler *et al.*, 2010; Green *et al.*, 2007; Wassink *et al.*, 2003). A recent study by Wassink *et al.* estimated that the prompt use of antibiotics improved income by approximately £6.30 per mated ewe in a UK sheep flock (Wassink *et al.*, 2010). This strategy is a complete change to the original management recommended and has only recently been regarded as the optimal approach to management of ovine footrot.

Foot trimming was recommended as a method of treating and preventing footrot (Beveridge, 1941), but recent studies have shown this is detrimental to recovery (Kaler *et al.*, 2010; Kaler and Green, 2009; Green *et al.*,2007). It is hypothesised that trimming of the hoof horn may damage foot integrity and thus make the hoof more susceptible to bacterial invasion. Footbathing using copper sulphate or formalin compounds (Winter, 2009) may kill off bacteria on the hoof but use of this practice is also associated with higher prevalence of lameness (Kaler and Green, 2009), perhaps due to the gathering of infected sheep in close proximity to each other.

Because *Dichelobacter nodosus* has a short lifespan on the field (Beveridge, 1941; Whittington, 1995), pasture rotation is another option for reducing prevalence of disease. If a pasture has been free from sheep for over a week then it may be free from contamination. Moving sheep to clean fields reduces their exposure to *D. nodosus* until contamination levels rise again due to shedding from previously infected sheep.

There is a commercially available footrot vaccine, Footvax, which has been used in a number of field studies. Footvax is a multivalent vaccine containing ten strains of *D.*

nodosus in an oil-based adjuvant, which is advised to be administered in two doses around 6 weeks apart (MSD Animal Health). Field trials have shown the vaccine to cause a high number of local reactions and at least one study concluded that the vaccine should not be recommended for use because of this welfare concern (Ennen *et al.,* 2009). There is no cross-protection between different serotypes of *D. nodosus* and antibody response to different serotypes is highly variable (Raadsma *et al.,* 1996). The commercial vaccine is multivalent but it is important that the correct serotypes of bacteria are targeted in order for a vaccine to be effective, meaning that better results are seen when a vaccine is designed for an individual flock where prevalent strains are limited and have been identified (Dhungyel *et al.,* 2008).

In a recent UK study a vaccine efficacy of 62% against footrot was estimated for the Footvax vaccine in a field trial (Duncan *et al.,*2011), although this was achieved in a study that also administered antibiotics to all affected sheep. Other studies have reported a wide range of efficacies using different vaccines. Dhungyel *et al.* (2008) conducted pilot studies in two Australian sheep flocks where only a single strain of *D.nodosus* was present. In each flock a single strain vaccine was used to target the specific strain present, and this resulted in elimination of footrot from these flocks when combined with culling of the sheep that failed to respond to the vaccine. An American study by Lewis *et al.*(1989), carried out over a two year period, demonstrated a reduction of footrot incidence by 61% in year one, and 45% in year two when using a commercially available ten-strain vaccine (not specified). Moore *et al.* (2001) used a live vector vaccine using a modified *Corynebacterium pseudotuberculosis* to deliver *D. nododus* protease antigens. Although this did elicit an immune response it failed to protect against further infections with *D. nodosus* (no efficacy value given), but there was some evidence that disease progression was

slowed. Similarly, in 1971 Egerton and Roberts published the results of vaccine trials using *D. nodosus* (then *Fusiformis nodosus*) which showed that mild infections still developed but did not progress to severe clinical signs in vaccinated sheep, and recovery was more rapid than in non-vaccinated animals.

One reason for the limited efficacy of the vaccine may be the timing of vaccination. Ideally it would be given immediately prior to the expected peak of disease prevalence, but this falls just after lambing and due to safety concerns the vaccine must not be administered to ewes between 4 weeks prior to and 4 weeks following lambing (MSD Animal Health). It is hypothesised that the result of this earlier vaccination time is that at the time of peak prevalence of footrot, immunity has already waned considerably in vaccinated sheep due to the short total duration of immunity.

1.1.3 - Adverse effects on welfare and productivity

Lameness adversely affects welfare (Ley *et al.*, 1989, 1995; Fitzpatrick *et al.*, 2006; DEFRA, 2003a, 2003b) in terms of freedom from pain, injury and disease, and freedom from discomfort, which are two of the five freedoms that are deemed to be the basic welfare rights of animals (FAWC, website; Brambell, 1965). The use of treatments and preventive measures are required to reduce the prevalence and incidence of lameness in sheep and thus maintain high welfare standards. In addition, lameness in sheep greatly reduces production and profitability. In Great Britain, footrot costs the sheep industry approximately £24.4 million per year (Nieuwhof & Bishop, 2005). The costs of footrot include production losses (e.g. reduced weight gain and fleece weight (Marshall *et al.*, 1991; Stewart *et al.*, 1984; Nieuwhof *et al.*,

2008a)) and increased labour costs due to time intensive treatments and monitoring which may include examining the feet of many sheep.

1.1.4 – Host genetic aspects of resistance to footrot

Field and experimental data indicate that susceptibility to footrot is partly under genetic control. A number of studies have estimated heritability (see section 1.2.1) of footrot severity and associated lameness, but in general only on data sets with short time scales or with limited observations (e.g. Skerman *et al.*, 1988 (New Zealand); Raadsma *et al.*, 1994 (Australia); Nieuwhof *et al.*, 2008b (UK)). In the UK study by Nieuwhof *et al.* (2008b), mule ewes and Scottish Blackface (SBF) ewes and lambs were observed once (SBF ewes and lambs) or twice per year (mule ewes) for two years (2005 and 2006), scoring the sheep on a scale of 0 to 5 according to the protocol described by Egerton and Roberts (1971). Heritability estimates ranged from 0 in SBF lambs to 0.26 for severe lesions in SBF ewes. Skerman *et al.* (1988) observed sheep twice per year from 1979 to 1984 and split up the dataset for the heritability analysis into mild footrot (scald) and severe footrot, with heritabilities being estimated at 0.28 and 0.17 respectively. Using a combined score for both mild and severe footrot the heritability was estimated at 0.25.

In the study by Raadsma *et al.* (1994) in Australia, Merino sheep were challenged each year between 1989 and 1992, with scores recorded six times during the 27 weeks following challenge, which is the most comprehensive study to date. They were also exposed to natural infection after this time by being placed in fields with infected sheep. Additionally a vaccine was also used 6 to 12 weeks post-challenge. This provided a range of heritability estimates under different circumstances, but the

heritability of overall footrot liability was estimated at 0.1 to 0.29. Heritability estimates were also higher pre-vaccination compared with post-vaccination.

These estimates were obtained from repeated observations but this depth of measurement has so far not been performed in the UK, where the climate is very different from Australia and thus may yield different results. Vaccination is also a confounding factor in the Raadsma study, which may affect the overall heritability values estimated. Part of this study attempts to address this gap in knowledge by calculating heritability values for footrot phenotypes from a large longitudinal data set with many observations over a two year period, collected by Wassink *et al.* (2010).

In New Zealand, there has been some success with breeding for footrot resistance in Broomfield Corriedale sheep. The farmer managing the Broomfield Corriedale flock used sires selected specifically for footrot resistance, with one sire and its offspring being used extensively. Strict culling policies were also followed to remove affected ewes, although as improvements were seen this culling rate dropped from an initial rate of 75% in 1971, to less than 2% by 1980. The prioritised selection for footrot resistance resulted in significantly reduced clinical footrot (lower prevalence and less severe lesions) compared to that seen in other breeds when introduced to contaminated pasture in field trials (Skerman & Moorhouse, 1987). In Australia selective breeding has also been successfully used to reduce the prevalence of footrot in Western Australia, where it is estimated that only 0.7% of farms have virulent footrot (Mitchell, 2001), and in New South Wales, where fewer than 4% of flocks are now affected (Egerton *et al.*, 2004).

It may therefore be possible, in principle, to use breeding programmes to reduce disease prevalence or incidence in the UK (Conington *et al.,* 2008). However, the climatic differences between Australia and New Zealand and the UK, where long, hot summers free from transmission do not occur, could mean that breeding for resistance requires a different approach in the UK.

The drawbacks with most of the genetic studies on footrot in a natural setting are that the data sets are over short time scales or have limited observations (e.g. Skerman *et al.*, 1988 (New Zealand); Raadsma *et al.,* 1994 (Australia); Nieuwhof *et al.,* 2008b (UK)). This may be adequate for traits which are permanent or relative to a specific age (eg. coat colour or live weight at 20 weeks). However, for a recurring disease which is known to infect and re-infect animals (Beveridge, 1941) this snapshot approach is not always sufficient to accurately determine the proportion of disease controlled by host genetics, i.e. the heritability, and thus be able to make informed statements about the relative resistance of different animals.

1.1.5 - Appraisal of influential studies

In 1941, William Beveridge published a paper that is still considered, over seventy years later, to be the seminal work on footrot. It was the first time the identification of *Dichelobacter nodosus* as the causative agent had been made and although our knowledge of the disease has progressed with the advance of technologies, his early research still provides a solid foundation for any future work. While certain individual flocks may be able to eradicate footrot the disease is still endemic in the UK over 70 years after this paper was published.

Indeed it is in Australia that Herman Raadsma has conducted what are arguably the most thorough studies to date on the genetic aspects of resistance to footrot. His series of five papers on footrot (Raadsma *et al.,* 1993, 1994, 1995, 1996; Litchfield *et al.,* 1993) in sheep present a broad view of the differences and similarities in sheep responses to challenge with the disease. When considering resistance traits Raadsma *et al.* (1994) found that there was a low repeatability between foot scores taken at different time points. This indicates that one or two measurements are not sufficient to accurately determine an animal's level of resistance to footrot. His study also showed that there was considerable phenotypic variation in disease traits between sheep.

A number of vaccine trials were conducted as part of the Raadsma studies (1993, 1994, 1995, 1996) and these showed that a sheep's natural response to infection was the most important covariate determining variation in response to vaccine, with those sheep that never spontaneously healed having the lowest antibody response. Additionally, high variation was seen in antibody response to different antigens, with different serotypes of *D. nodosus* eliciting different levels of antibody production. This could impact vaccination strategies within a flock because if the prevalent serotype is one that elicits a low immune response it may not result in good protection against disease.

The study estimated heritability to a number of footrot traits including the number of feet affected and an overall score, with heritability values ranging from 0.01 to 0.57 using a least squares analysis, or 0.09 to 0.26 using a restricted maximum likelihood (REML) method. High genetic and phenotypic correlations between the different footrot traits were calculated, but the correlations between disease phenotype and antibody response were very varied.

While the Raadsma papers cover many important aspects of disease, one of their drawbacks is that most of the data collected are from artificial challenge experiments. Artificial challenge does not necessarily give the same results as would be seen in natural challenge, and indeed there were a number of differences between the natural and induced challenges within this study, e.g. different factors significant for disease traits and different heritability values. However, a challenge experiment provides a controlled environment in which responses to infection and vaccine may be carefully studied and quantified, which is not possible in a field setting, and thus more data may be obtained in this type of study.

1.2 - Quantitative genetics

Quantitative genetics is the branch of genetics that deals with the inheritance of traits that are variable i.e. they do not fall into a simple presence/absence category but may be placed on a spectrum with many degrees (Falconer and Mackay, 1996). In general, the traits analysed using quantitative genetics are influenced by many genes, often at many loci, to give a broad range of phenotypes. The degree of inheritance of these traits, for example resistance to a particular disease, litter size or body weight, may be calculated based on phenotypic data combined with knowledge of pedigrees, and used to calculate breeding values in order to be able to select for particular trait values. This information can be used to design breeding schemes to maximise performance, and it is also of interest when considering the evolution of different species and their phenotypes.

1.2.1 – Heritability

Heritability is a measure of the proportion of the phenotypic variation in a trait that is due to additive genetic variation (Falconer & Mackay, 1996). This parameter is particularly important in the analysis of complex traits, i.e. those due to the effects of many genes. Estimated heritabilities are population specific, and hence should be estimated separately for multiple populations. There are two major reasons for this. Firstly, the populations may differ genetically and hence have different levels of additive genetic variation for the trait of interest. Secondly, the environmental variance is also likely to differ if the populations inhabit different environments.

Quantitative genetics principles are used when calculating breeding values, i.e. the expected performance of the progeny of animals. The breeding value is the expected (or average) performance of progeny of an individual when that individual is mated to a random group of mates. Within a family, i.e. within progeny from the same parents, there is added between-individual variation arising from random recombination events at meiosis when gametes are formed. Therefore, genetic variation occurs both between and within families. The expected family mean is defined by the breeding values of the parents, and recombination leads to variation between sibs within a family.

The focus of animal genetics research is shifting towards more specific molecular components of inheritance such as quantitative trait loci (QTL) as genome investigation becomes a cheaper and more powerful tool, but the more general quantitative genetics principles will remain in use in breeding schemes for many years to come, and indeed will be required to interpret data arising from genome investigations. The key points and equations are outlined below.

Using the definition given above, heritability (h^2) may be written as:

$$h^2 = \frac{V_A}{V_P}$$

Where h^2 represents heritability, V_A stands for additive genetic variance and V_P is the total phenotypic variance. It may also be considered to be a regression of the breeding value on the phenotypic value (Falconer and Mackay, 1996).

In the data analysis outlined in chapter 2 of this thesis, heritability estimates are derived using three different methods, from a study population comprising lambs with known dams but unknown sires. The first uses the observational component, i.e. the dam phenotype comprising repeated observations made over time on disease phenotypes including lesions and lameness scores. A between-dam variance (σ^2_D), i.e. how much variation in the trait of interest is seen between the different dams in the population, is calculated and from this the heritability is estimated using the following equation:

$$h^2 = \frac{4\sigma_D^2}{V_P}$$

This equation may be derived by considering that an individual expresses half of the genotype ($0.5a_i$) of its dam. Using the expectation: variance(kx) = k^2variance(x), then the expected value of the dam variance (σ_D^2) (i.e. variance($0.5a_i$)) is $0.25\sigma_a^2$. Thus the additive genetic variance is $4\sigma_D^2$ and the heritability is as shown. Two biases can occur. Firstly, in the case of several lambs per ewe (e.g. litters of twins), common environmental effects such as ewe milk production or maternal ability, may result in an upwards bias in the estimated heritability, if these environmental factors affect the trait of interest. Secondly, the mating structure, i.e. which dams are mated to which

sires, may affect the estimates of the variance components. The extent of this possible bias is explored in Chapter 2.

The second method uses regression, calculating the regression of offspring phenotype on dam phenotype, which is expected to be an estimate of half the heritability, i.e:

$$b = \frac{1}{2}h^2$$

This equation is based on having data from only one parent (sire or dam), which matches the data we have. If both parents were known then an average (mid-parent) value would be used and the regression coefficient would be an estimate of the heritability. Consider the case of regressing lamb performance on dam performance. The denominator of the regression is simply the phenotypic variance for the trait. To define the numerator, let *p*, *a* and *e* represent the phenotype, additive genetic term and environmental term, and subscripts $_D$ and $_P$ refer to the dam and progeny. The numerator is then $\text{cov}(p_{Di}, p_{pi}) = \text{cov}(a_{Di} + e_{Di}, a_{pi} + e_{pi})$. Assuming that the environmental terms are uncorrelated with each other and with the genetic components, then this equation reduces to $\text{cov}(a_{Di}, a_{pi}) = \text{cov}(a_{Di}, \frac{1}{2}a_{Di})$. Following the arguments given above, this is $\frac{1}{2}\sigma_a^2$, and the regression coefficient therefore estimates $\frac{1}{2}h^2$. Clearly, this estimate may be biased upwards if there is an environmental covariance between ewes and lambs, e.g. they face the same level of challenge.

A third method is the 'animal model', a mixed effects model that calculates genetic parameters based on the use of pedigree information, i.e. it relates the similarity between phenotypes to the expected genetic covariance between animals, and thus

estimates the genetic variance (Lynch and Walsh, 1998). This method requires complete pedigree information, i.e. both sire and dam are known.

1.2.2 - Genetic selection

Genetic selection can be used in a wide range of situations to improve production and health traits in sheep (Simm, 2000). Estimated breeding values (EBVs) may be combined to give a total score calculated using a weighting system based on the economic importance of each trait, with the combined score being an index. Different indexes may be produced for different breeds with different breeding goals. Traits included in a combined EBV for sheep breeders may include fat depth, muscle composition, live weight, average litter size, maternal ability and growth rate (Simm, 2000). In structured breeding programmes, selection is usually based on these combined breeding values.

Traits under selection are often polygenic i.e. they are controlled by a number of genes, and their values lie on continuous spectra so there is a large range of values seen between different sheep. The rate of improvement is dependent on heritability, trait variability, selection intensity and generation interval. For optimal improvement rates the trait would have a high heritability, high variability (so that selected breeding animals are much better than the average for the selected trait), and there would be a short generation interval and high selection intensity.

In terms of breeding for disease resistance, there is potential application to many different diseases including bacterial diseases (e.g. mastitis and footrot), helminth infections (e.g. from *Haemonchus* and *Teladorsagia* species), viral diseases (e.g. Marek's disease) and transmissible spongiform encephalopathies (e.g. scrapie)

(Bishop *et al.*,2010). As with all long term control methods, breeding for disease resistance must be considered in terms of feasibility, sustainability and desirability (Stear *et al.,* 2001; Gamborg and Sandoe, 2005). In other words, genetic progress should be possible within a reasonable amount of time. The progress should be sustainable and not detrimental to other traits, animal welfare, or the species and ecosytem diversity. Finally, there should be a need or desire for improvements to be made that cannot be achieved using conventional methods i.e. treatment or short term management strategies.

A case that highlights the potential of genetic selection is the scrapie eradication programme that was carried out in the UK (Dawson *et al.,* 2008). In this case, resistance to (classical) scrapie was known to be largely controlled by variation in the PrP gene, with significant polymorphisms at three specific codons and which may be easily genotyped in sheep (Hunter, 2007). Rams with the resistant genotype were used to breed and any with the highly susceptible genotype were slaughtered. This was an effective programme that achieved a rapid change in PrP genotypes in the national flock, but it took place under a very specific set of circumstances.

It was at a time when fear of prion diseases was high - following the BSE outbreak from infected beef cattle and the subsequent link between new-variant CJD in humans, there was a high level of concern about prion diseases. Transmission of BSE to sheep via the oral route had been experimentally demonstrated and there was concern that scrapie might mask the signs of BSE, thus increasing the risk of future transmission to the human population. Because of this situation, scrapie control in infected flocks became compulsory following EU guidelines, and implemented as the National Scrapie Plan (NSP) in the UK (Dawson *et al.,* 2008). Rams were genotyped before breeding was permitted, with those deemed most susceptible being castrated

or culled. Control orders were also put on infected flocks to minimise further spread and compensation was given to farmers for the loss of sheep culled as part of the programme (State Veterinary Service, 2006). Negative selection was applied to the most susceptible allele (VRQ) and positive selection to the optimal allele (ARR) which resulted in a 60% decrease in the frequency of the VRQ allele and a 36.5% increase in the ARR allele between 2002 and 2006 (Dawson *et al.,* 2008).

This mandatory process and an easily measurable resistance genotype provided ideal conditions for an effective selection programme and as such resulted in a large change in allele frequencies in the UK sheep population. However, there are other forms of prion disease, in particular atypical scrapie, which have different resistance profiles. Consequently selection applied for resistance to classical scrapie will not be effective at controlling other forms of the disease (Hunter, 2007).

For footrot there is no mandatory selection programme, and there is no known risk of disease in humans. Even if similar public or governmental pressures did exist, accurately determining the resistance genotype is not a simple task.

There is a footrot gene marker test commercially available in New Zealand, although the efficacy of this test is subject to debate. This marker is based on alleles at the DQA2 gene, which is part of the major histocompatibility complex (Hickford, 2000), a component of the immune system. However, studies to date have shown that results from this test have little or no correlation with disease outcomes in the UK sheep population (Genever, 2009). This suggests that while it might be possible to create a marker test to identify susceptible and resistant sheep, it would have to be tailored to the individual populations to be effective. There might also be interactions between different D. nodosus strains / types and different markers.

1.2.3 - Interaction of genetics and epidemiology

Scrapie has a distinct genotype associated with resistance, but for most diseases the susceptibility must be calculated according to phenotype. This can cause problems when using genetic selection especially when the trait under selection is a binary outcome (e.g. healthy vs. disease), as different heritabilities to disease traits are seen at different prevalence of infection (Nieuwhof *et al.*, 2008b; Bishop and Woolliams, 2010). When starting a selection programme, if disease is at high prevalence then good progress may be made (assuming the host genotype controls variation in resistance) initially. As disease prevalence is reduced selection may become more difficult because there are fewer differences between individuals and thus the 'best' individuals are difficult to identify. Part of this study attempts to quantify this phenomenon for footrot, using antibiotic treatment to reduce prevalence and then comparing selection based on rams in the low prevalence flock with selection using rams from a high prevalence (no treatment) flock.

Another factor that must be considered is the environmental component of disease. If genetic selection is successful in improving the resistance of the sheep population it may reduce disease prevalence. The reductions of this are due to two factors. Firstly, there is the direct effect of having less susceptible sheep, viz. the genetic component. Secondly, because the prevalence is reduced due to this genetic improvement, the levels of infectious agent will also be reduced, meaning lower exposure to disease and thus resulting in a further decrease in prevalence. Therefore, reductions in prevalence following genetic selection are expected to be greater than predicted using genetics alone because of the added effects of reduced pathogen in the environment. This has previously been demonstrated for nematode infections in sheep (Bishop and Stear, 1997). This is explored in this study by comparing genetic

selection in situations where the pathogen level is kept constant with models where the pathogen level is allowed to vary as it would in natural situation, with the aim of quantifying the direct genetic versus indirect environmental effects of genetic selection.

1.3 - Mathematical modelling

Mathematical modelling is the application of mathematical techniques to simulate real-world events. For example, transmission of a simple disease in a population might be represented by a series of differential equations describing the rates at which individuals become infected and recover from infection. More complex models can be developed to mimic more complex real-world situations, such as the model presented in this study which includes expressions to account for individuals' disease patterns along with host genetics, flock dynamics and bacterial population dynamics. These models may be used to aid understanding of particular systems or to predict future outcomes and can be extremely powerful tools.

1.3.1 - Mathematical models of disease

Mathematical modelling is a tool that is applied to infectious diseases for one of three primary purposes (Keeling and Rohani, 2008; Green and Medley, 2002):

- Understanding - e.g. of mechanisms of spread, key reservoirs of disease or the effects of variation in key parameters such as number of contacts per infected individual.

- Prediction - e.g. what would happen if we applied X control measures? How large is an epidemic likely to get if X happens? Can we reduce X by doing Y?
- To identify things that are unknown about transmission - e.g. if all parameters are known but disease patterns from the model do not match observation then there may be unidentified properties of the transmission process that need to be determined

Models can be used to answer many different questions but outcomes are always subject to some level of debate due to the simplifications made in models and the ways in which individuals may choose to approach their construction. Outcomes are only useful if the model can (reasonably) accurately mimic the way in which the disease behaves in natural populations. For this reason when constructing a model it is important to be able to obtain the majority of parameter values from real world data (Keeling and Rohani, 2008), although in many cases values for some parameters may be unknown.

Sensitivity analysis is a process of determining how variation in either individual or combined parameter values affects the outputs from a model. This approach is particularly important for parameters whose values are unknown. In the footrot models developed for this study, sensitivity analysis was used to examine the effects of parameters for which no data were available, to see how much impact they have on the model system.

Models that are used to aid understanding have relatively low data requirements as they are often used to generate hypotheses to be tested in an experimental setting. Predictive models have high data requirements as they need to be fitted to current

data very accurately in order to make useful predictions that can be used to inform policy or disease management strategies. The model developed in this study is a combination of these two types of model. There are limited data available on the genetic aspects of footrot resistance. These are represented in a simplified way in the model to obtain a better understanding of how different disease processes and underlying host genetics might interact. Disease outcomes are fitted to field data collected in previous studies and used in the prediction of outcomes using novel strategies such as genetic selection and the differing effects of individual management strategies. In these predictive aspects of the model it is important to have data available to validate as many outcomes as possible, and to use for parameter inputs.

1.3.2- Limitations of modelling

Modelling can be a very useful tool, but it does have limitations. Most biological systems are extraordinarily complex and to incorporate every detail of these systems into a model is impractical. Not only would parameters be difficult (in some cases impossible) to obtain, but the processing power required to replicate the full biological system does not exist. For this reason, mathematical models are necessarily ‘inaccurate’ in some aspects. However, what is important in mathematical models is to generate data that closely match data that are observed in the field, and this can often be achieved in a greatly simplified model of disease. For this reason it is vital to have field data with which to validate models. Models do not, in themselves, answer questions, but are useful tools to aid development of understanding.

1.3.3 - Stochastic versus deterministic models

Stochastic and deterministic models differ primarily in their treatment of chance effects and variability. The deterministic model will give the same results every time it is run and contains no elements of chance that are expected in real life situations. In other words, it assumes that for a given set of starting values, the outcome is fixed. With a deterministic approach the results are point estimates for outcomes, which may be (but are not always) equivalent to the mean of outcomes that might be seen using a stochastic approach. These point outcomes may be well suited to high prevalence disease in large populations (Keeling and Rohani, 2008) or in cases where all parameters are clearly defined and not subject to fluctuation.

A stochastic model allows for variation in outcomes because with each model-run different events occur, this being a closer reflection of the random nature of events in real life than a deterministic model. Stochastic models incorporate the use of random sampling from pre-defined probability distributions to determine the events within a model, such as when a disease event (e.g. infection, recovery, death) will occur, the type of event that occurs after each time step and which individual will be affected by that event. This allows for possibilities of extinction of disease and is particularly useful at low prevalence and/or small numbers of hosts where there is a high probability of extinction or large times between events. A stochastic model is suited to situations where the variability of outcomes is particularly important. In the current study a realistic flock size of 200 ewes is used, which is a relatively small number, and the differences between flocks and their individual outcomes is important, so a stochastic model is used.

This is also useful when considering risks versus rewards of adopting control strategies. If there is high variability in outcomes then farmers may be less willing to adopt a new strategy as the risk of not achieving desired outcomes would be much higher than using a strategy with a low variability about the mean. While in high variability situations there is a chance of a result significantly greater than the mean, there is an equal chance of achieving a result that is much lower than the mean, resulting in a high uncertainty with regard to outcomes. The models developed in this study will attempt to quantify some of the variation associated with different control strategies for dealing with footrot, which are currently unknown, so that better information is available on the risks associated with each management strategy.

1.3.4 - Individual based models or population level models

If a model has a homogenous population with no variation between individuals then a population level model may be ideally suited to modelling that disease as each individual reacts in the same way to disease exposure and it is number of individuals in each disease state rather than the state of each individual that matters. In diseases where variation between individuals is important in terms of desired model outcomes, for example where differing levels of susceptibility between animals is of interest for genetic selection purposes as in this study, a population level model will not capture the variation and so an individual based model may be more appropriate. An individual based model allows for different behaviours so that different sheep, in this case, could react differently to disease and thus progress through disease states in a variety of ways. This is particularly important for this study because it is hypothesised that variation between sheep could be used to form the basis of a

genetic selection programme to reduce the prevalence of footrot in UK sheep flocks, a hypothesis which will be tested using the models developed in this study.

1.3.5 - The real world….

Mathematical models provide a useful tool to study infections and the ways in which they might be expected to behave in populations. They may also be useful in determining optimal control strategies for disease management. However, it is important to also have studies conducted in the field to validate the data and strategies planned from the model. It is also important to make sure that strategies tested in the model are practical, for example, culling a high proportion of sheep every year may reduce disease very rapidly but the probability that farmers would follow such a protocol would be extremely low. It is vital to keep a real-world perspective when utilising mathematical models so that any outcomes are not only effective, but also practical and acceptable to the affected populations.

1.3.6 - Previous models

Mathematical models have previously been used to analyse and predict data for a wide range of infectious diseases in animal populations including such diverse infections and diseases as scrapie (Sabatier *et al.,* 2004), *Corynebacterium pseudotuberculosis* (O'Reilly *et al.,* 2010), mastitis (Schukken *et al.,* 2010), heartwater (O'Callaghan *et al.,* 1998) and contagious bovine pleuropneumonia (Mariner *et al.,* 2006). These models have diverse purposes, use different types of data and are constructed in different ways. Due to the wide range of models available

in the literature, just a few examples will be given below. These examples represent models that cover some of the aspects that need to be addressed in a model of footrot, including dual populations of pathogen and host, genetic differences in resistance of hosts and the effects of parameters in the model for which data are unavailable.

Sabatier's model of scrapie (2004) incorporates host resistance genotype and models three scenarios to determine the effects of different genotypes on disease resistance/susceptibility. It may be considered a model used to aid understanding of the underlying biology of scrapie susceptibility. It is a deterministic model giving expected outcomes for an 'average sheep flock', using a series of difference equations to model state transitions through four states, and outputs are time-series data for disease states, genotype frequencies and number of cases. This model allows an estimate of average effects but does not incorporate variation, which means it is difficult to estimate the likelihood of achieving those average effects. However, it is successful at reproducing data corresponding to three different types of outbreak seen in real cases and suggests that the type of outbreak observed may be due to difference in the genetic composition of the flock. It therefore fulfilled its purpose of obtaining greater understanding of the role of host genetics in disease outbreaks. The effect of a mixed population is one of the questions that the model of footrot in this study attempts to quantify, along with the potential for the use of host genetics in disease control.

O'Reilly's 2010 model of *Corynebacterium pseudotuberculosis* in sheep flocks was developed for the purpose of evaluating control measures rather than aiding in the understanding of the underlying biology of disease transmission. It is a compartmental model in which sheep may be in one of eight disease states, and uses a series of differential equations to model transitions through these states. The

population is homogenous and the rate equations are deterministic with outcomes assessed as proportions of the population in each disease state at the end of the model. The results showed the improvements made with different control strategies and presented the probability of eradication of disease under a range of endemic and epidemic scenarios. The model was able to show benefit using all control methods, and identify the scenarios under which eradication was likely. It also highlighted the need for greater understanding of the disease before using recommendations based on the model because outcomes were sensitive to changes in parameters whose values were unknown. This is relevant to footrot because there are several aspects of the disease process (e.g. carrier sheep) for which values are unavailable from published data.

In the heartwater model developed by O'Callaghan *et al.* (1998) the situation modelled is more complex as there are two populations that need to be considered - the affected host and the disease vector (tick) population. These two populations both have individual dynamics and each interacts with the other to give the resulting disease patterns seen in the field. This is another aspect that needed to be incorporated into footrot models because footrot patterns arise from a combination of the sheep population and bacterial population interacting.

A model of footrot incorporating genetic selection has already been published by Nieuwhof *et al.*(2009) but it left certain questions unanswered. Nieuwhof's model was a deterministic model in a homogenous sheep population and as such the variation in outcomes was not addressed, nor were the effects of a mixed population with differing degrees of susceptibility. The model showed that there was potential for the reduction of footrot prevalence using genetic selection and suggested an extra effect would be seen from reduction in pathogen burden along with improved genetic

resistance. The effects of mixed population, pathogen dynamics and effects of control measures are not addressed in Nieuwhof's model and these are incorporated into the models developed in this study to enhance our understanding of footrot and the potential for genetic selection.

1.4 - Scope of this thesis

This thesis may be broken down into two distinct components, data analysis and simulation modelling, which are outlined below.

1.4.1 - Analysis of field disease data to estimate genetic parameters

To date, no UK study has considered lameness, interdigital dermatitis lesions and footrot lesions together to determine heritability, correlation and covariance between footrot traits. It was hypothesised that the three traits are correlated and that lameness may be used as a proxy measure to monitor footrot instead of the more time-consuming process of checking for lesions in the whole flock. To test this, field data were analysed as described below.

This part of the study considers the factors affecting footrot in lambs and ewes, using data collected by Wassink *et al.* (2010). The study animals were observed in the field as least twice a week for nearly two years (ewes and two cohorts of lambs for the six month periods before being sent for slaughter) (Wassink *et al.*, 2010). This provides us with a detailed picture of disease over time both in the flock and individuals and potentially allows us to determine which animals are truly susceptible or resistant. The period of immunity to footrot is believed to be up to 12 weeks (from challenge

trials) so this would be far exceeded in the two year period for the ewes. In lambs, as we are following them from birth there can be no immunity from past infections. It is possible that some maternal immunity may be shared with the offspring but there are no data on this at present.

Data analysis in this study focuses on the relationship between disease in mothers and their offspring. This relationship is made up of three components. The first is the genetic material passed on from mother (and father) to the offspring, which plays a role in determining the level of susceptibility to footrot in sheep. This component is largely represented by heritability, which is the primary area of interest in this study as it is one of the main parameters determining the effectiveness of breeding programmes. Heritability values have been calculated for footrot (Skerman *et al.*, 1988 (New Zealand); Raadsma *et al.*, 1994 (Australia); Nieuwhof *et al.*, 2008b (UK)) but this is the first study where extensive repeated measurements on individuals are used in a UK setting. It is also the first case where the three footrot disease phenotypes (lameness, footrot lesions and interdigital dermatitis lesions) have been considered together in detail, which is important in understanding the complete effects of footrot. In Chapter 2 an estimation of heritability values for disease traits is made, which provides an underlying basis for inclusion of heritability in the simulation models developed in later chapters, and also for future breeding programmes.

The second part of the data analysis comprises analysis of the non-genetic factors which affect disease presentation in lambs only. Some studies have already looked at factors affecting presentation of footrot in a flock (Kaler and Green, 2010; Wassink *et al.*, 2003, 2004) but these have focused on farm level factors such as management protocols, and farm level outcomes, i.e. prevalence and incidence of disease within

the flock. In the current study the focus is on individuals and so the factors and outcomes analysed are on an individual level, using recordings and observations of individual sheep. This group of factors includes ewe related factors such as ewe nutrition, age, body condition score and disease status during pregnancy and weaning. A mixed models analysis was performed to determine whether the condition of the ewe during pregnancy and weaning, when the development of the lamb is dependent on the mother, contributes to lamb disease presentation. Other non-genetic factors are those that are directly concerning the lamb, such as its sex and birth weight, and the analysis attempts to allocate significance to each of these factors in terms of contribution to disease seen in lambs. The results from this are also presented in Chapter 2.

The final component of the disease relationship in families is the shared environment. Lambs and their mothers co-grazing on fields must spend time spatially close together as the lambs rely on their mother for milk and security. They will therefore be more likely to be exposed to the same areas of pasture with the same levels of disease contamination than other families. Studies on sheep contact networks have shown that most sheep have close spatial proximity to each other in a flock although actual physical contact is much more likely to be seen between ewes and their young lambs (Schley *et al.*, 2012). This shared environment is likely to lead to family members sharing the same risks for disease and is also addressed in the mixed models presented in Chapter 2.

1.4.2 - Development of an individual based stochastic simulation model of footrot in a UK sheep flock, along with testing of model parameters and comparison with field disease data.

Footrot is a recurring problem in UK sheep flocks and benefits from control and treatment methods can have both short- and long-term effects. To explore different options in a field setting would require long-term study flocks with large numbers of sheep to test different hypotheses. An alternative method is to use a mathematical model to predict outcomes of different approaches and part of this study involved the development of such a model to test the effects of different control and prevention strategies. This model expands on the modelling work done by Nieuwhof *et al.* (2009) in order to answer further questions about footrot patterns that may be expected in the field following different interventions.

The model is individual based because it is key that each individual sheep is unique in terms of its genetic makeup and disease history. It is also stochastic to allow the range of possible outcomes to be analysed and not solely a mean value, although the mean value remains useful for comparison of multiple strategies. The model includes a wide range of variables and depicts not only disease spread but also flock demographics including annual births and deaths, bacterial transmission and survival in the environment and host genotypes that are passed on from parents to offspring.

The model structure was designed so that field estimates were available for as many parameters as possible. However, for some values no data were available and for these a sensitivity analysis was conducted. The sensitivity analysis allowed identification of the factors that play the largest role in disease presentation within a flock, and also the estimation of parameters that gave the closest results to the field

data used to validate the model. Chapter 3 gives the full methods regarding the model structure and sensitivity analysis and also provides the results from that analysis and from baseline scenarios before disease control measures are applied.

The model makes it possible to test a number of different strategies for the control and prevention of footrot. The individual effects of current methods including pasture rotation, antibiotic treatment, selective culling of the worst affected sheep and vaccination are examined initially, with the results presented in Chapter 4. While field work to date has used a number of different treatment and control measures, little work has been done on quantifying the effects of individual methods, with the exception of limited vaccine trials (e.g. Duncan *et al.,* 2012). There are also no data available on different protocols for each method. For example, pasture rotation may be done at different time intervals, but the effects of longer or shorter times between rotation has not been explored. Administration of antibiotics has been seen in field studies to significantly reduce prevalence when administered promptly to lame sheep (Wassink *et al.,* 2010) but there has been little quantification of the difference seen when treating all lame sheep versus treating only severely affected sheep. This may be considered both in terms of the number of doses administered and also the effects on prevalence and incidence of disease.

Selective culling is also a control strategy that is subject to variation because the percentage of female sheep culled may have a significant effect on the improvements seen within the flock. A comparison of different protocols used within each method, for example different time intervals between pasture rotation and treatments, and culling different percentages of sheep is examined using the model developed in this study. Its aims are to quantify the effects of different protocols for using individual treatment methods and the results are presented in Chapter 4.

Genetic selection is an appealing prospect for use in reducing the burden of footrot in the UK as it has been clearly seen that there is a genetic component to resistance. Genetic selection schemes have been successfully used in New Zealand (Skerman and Moorhouse, 1987) but the UK has a very different climate and presents footrot in a distinct way. This study investigates the possibility of using genetic selection based on phenotypic observations of individual sheep. This is centred on ram selection as this provides much greater genetic progress than selection on ewes due to the small number of rams compared with ewes used to breed in a flock, hence much greater selection intensities. Disease observations are thoroughly recorded within the model and observations of the number of episodes and number of lame days a sheep experiences are used as the basis for selection of the 'best' rams according to different selection criteria. These data are then used to explore the potential for genetic selection in the UK in terms of reducing disease levels and lowering susceptibility in the UK sheep population, presented in Chapter 5.

As genetic selection is a long term approach, it would need to be used in combination with conventional short term control methods such as antibiotic treatment and pasture rotation. After looking at genetic selection alone, its effects when combined with different control and prevention methods are also considered.

It is also important to see how selection of the 'best' animals may be affected when disease prevalence is artificially reduced by the use of effective treatment, which is necessary to maintain healthy sheep and provide high standards of animal welfare. It has been demonstrated that heritability varies with disease prevalence (Nieuwhof *et al.,* 2008b; Bishop and Woolliams, 2010) and the effects of this on selection for disease resistance may not be insignificant. This study addresses this issue by comparing progress made with rams selected under low prevalence conditions

(caused by treatment with antibiotics) with the use of rams selected from higher prevalence flocks where no treatment is administered. The results of this and the other genetic selection experiments are presented in Chapter 5.

A discussion of all results obtained in this study along with potential areas for further investigation is then provided in Chapter 6.

Chapter 2: Using mixed statistical models to estimate heritability and repeatability values for three foot disease phenotypes in a UK sheep flock.

2.1 - Introduction

Currently available treatments and control measures only provide temporary reductions in levels of footrot and can be expensive and time-consuming to implement. There is no long term immunity to the disease (Beveridge, 1941) and treatments must be continued to maintain a low prevalence of footrot in a flock (Wassink *et al.,* 2010). Therefore new strategies for the control of footrot are desired, in particular methods that provide a permanent reduction.

It is known that susceptibility to footrot is partly under genetic control (Raadsma *et al.,* 1994; Skerman *et al.,* 1988; Nieuwhof *et al.,* 2008b; Skerman & Moorhouse, 1987) hence it may be possible, in principle, to use breeding programmes to reduce the incidence or severity of footrot. One point not yet fully resolved is the most appropriate test upon which to base genetic selection. A reliable measure of disease phenotype is needed, upon which to base selection of disease resistant animals for use in breeding programmes. The use of hoof lesions scored on a five point scale has been suggested by Conington *et al.* (2008) as one possible method for establishing the disease phenotype of an animal. Raadsma *et al.* (1995) considered the use of antibody titre as an alternative measure of disease status but concluded that it was '*at best…less efficient than the use of clinical foot scores'*.

In the current study, three distinct disease phenotypes are considered for use in a breeding programme– footrot lesions, interdigital dermatitis (ID) lesions and lameness (locomotion score (Kaler *et al.,* 2009)). These phenotypes may present together or independently and a number of scoring systems have been developed to

accurately record each of them (Kaler *et al.*, 2009 - locomotion; Egerton & Roberts, 1971 – footrot/ID lesions; Raadsma in Bishop *et al.* (2010) – footrot lesions), allowing an animal's disease status to be followed over time. The data used here are the first where all three phenotypes have been observed and recorded together, allowing comparisons between them and analysis of their covariance.

Lameness can be observed without the need to physically handle the sheep, whereas lesions require examination of all feet, a time-consuming process. If lameness and lesions are highly correlated, as seen in an observational study by Kaler *et al.* (2011), then it may be possible to use lameness as the phenotype under selection, reducing labour time and costs while still improving the incidence, prevalence and severity of lesions.

The purpose of this study was to identify genetic and non-genetic factors influencing the presentation of lameness, ID and footrot in lambs and ewes. This information will potentially inform future breeding strategies and also provide parameters for future epidemiological models of the disease.

Specifically, the following were explored:

1) Heritabilities for each of the three disease phenotypes: - footrot lesions, interdigital dermatitis lesions and locomotion score.

2) The genetic and phenotypic associations between the three phenotypes.

3) Non-genetic and environmental factors that may have an effect on disease phenotypes.

4) Repeatability of scores between measurements.

2.2 - Data

Data were collected from sheep on an Oxfordshire farm in 2005 and 2006 (Wassink *et al.*, 2010). The three disease phenotypes observed were lameness, footrot lesions and interdigital dermatitis lesions (scoring systems described in '*trait definitions*'). Foot lesions were recorded for all ewes present on the farm at four time points (March 2005, September 2005, March 2006 and October 2006). Affected sheep were also fully examined when that sheep's locomotion score was greater than 1 in the treatment groups (described below) and when the shepherd treated a sheep in the control groups. All observations were made by trained technicians.

Sheep were monitored for lameness at least once a week by driving a quad bike through the field and observing the movement of the sheep, and included 947 ewes and their lambs (2433), whose sires were unknown, over the course of the study. Lambing occurred between March and May each year, with the date of birth of each lamb being recorded. Additional information – breed, body condition score at start of study, age of ewes by dentition, birth weight of lambs, dam identity (lambs only) and sex of lambs – was also recorded.

The ewes were split into four groups, two in a treatment protocol (T) for the first period of the study (May – September 2005) and two in a control protocol (C) for the first period of the study. At the start of the second period (late September 2005) two of the groups were swapped over making four groups TT, TC, CT and CC for the two periods of the study (Wassink *et al.*, 2010). The treatment protocol consisted of topical and parenteral antibiotics administered as soon as footrot or ID lesions were diagnosed, along with foot trimming (2005 only) and footbathing when the control groups were footbathed. Prompt treatment as soon as lameness score ≥ 2 was

observed was the protocol in the treatment groups. Control groups were managed according to the farm's usual protocol which involved trimming the hoof and spraying with a topical antibiotic when footrot or ID was diagnosed and footbathing groups when lameness prevalence increased. Sheep in the control group were generally left until lameness was more pronounced, approximately locomotion score 4 (Hawker, 2007), before being caught, inspected and treated. For full details of the study protocols see Hawker (2007) and Wassink *et al.* (2010).

2.2.1 - Trait Definitions

Footrot lesions were scored on a scale of 0 (clean digit with no lesions) to 4 (active footrot lesion with complete under-running of wall hoof horn of the digit (may include under-running of the sole)) (Table 2.1).

Table 2.1. Footrot lesion scoring system.

Lesion score	Description of lesion
0	Clean digit with no lesions
1	Active footrot lesion with slight degree of separation of sole/wall of the digit
2	Active footrot lesion with marked degree of separation of sole/wall of the digit
3	Active footrot lesion with extensive under-running of the wall hoof horn of digit (may include under-running of the sole)
4	Active footrot lesion with complete under-running of wall hoof horn of the digit (may include under-running of the sole)

Interdigital dermatitis lesions were also scored from 0 (clean interdigital space with no dermatitis lesion or fetid smell) to 4 (severe interdigital dermatitis with fetid smell (>25% affected)) (Table 2.2).

Table 2.2 . Interdigital dermatitis lesion scoring system.

Lesion score	Description of lesion
0	Clean interdigital space with no dermatitis lesion or fetid smell
1	Slight interdigital dermatitis, irritation of skin but dry
2	Slight interdigital dermatitis with fetid smell (<5% affected)
3	Moderate interdigital dermatitis with fetid smell (5-25% affected)
4	Severe interdigital dermatitis with fetid smell (>25% affected)

Table 2.3. Locomotion scoring system.

Locomotion Score	Locomotion Description
0	Clinically sound
1	Mildly lame slightly uneven gait and slight shortening of stride
2	Moderately lame noticeable nodding of head, uneven gait, shortened stride
3	Badly lame excessive nodding, holds up affected limb(s) while standing and obvious discomfort putting foot to ground when moving
4	Severely lame holding up affected limb when standing and moving, excessive nodding
5	Severely lame with more than one limb affected (so cannot hold up), reluctance to move
6	Severely lame with shaking of affected limb(s), reluctance to rise when lying, extreme difficulty moving when standing

Source: Kaler *et al.*, 2009

Locomotion was scored between 0 (clinically sound) and 6 (severely lame with shaking of affected limb(s), reluctant to rise when lying, extreme difficulty moving when standing) (Table 2.3). Finally, a combined foot score was calculated from the two lesion scores, resulting in a score of 0 (no footrot lesion, no interdigital dermatitis lesion) to 8 (footrot lesion score 4) (Table 2.4).

Table 2.4. Combined foot score scoring scale.

Combined foot score (CFS)	0	1	2	3	4	5	6	7	8
ID score	0	1	2	3	4	any	any	any	any
FR score	0	0	0	0	0	1	2	3	4

2.3 - Materials and methods

2.3.1 - Statistical models

Data on lambs and ewes were collated and analysed in a number of different ways to obtain estimates for heritability in lambs, repeatability of measurements in ewes (within-year and between-year) and lambs (within-year only), contribution of non-genetic effects to disease phenotype variation and covariation between disease phenotypes.

All models were implemented using ASReml software (Gilmour *et al.*, 1996). This is a software package designed to fit linear mixed models to large data sets in order to estimate variance components, particularly for cases where data structures are complex and unbalanced. Its calculations are based on a restricted maximum likelihood method and the algorithms it uses have been optimised specifically for estimation of genetic parameters such as heritabilities and genetic correlations.

To assist in the interpretation of disease resistance, the maximum score ever achieved by an animal for a specific trait within a specified time period was chosen as the indicator of its susceptibility for use in models. For basic models the maximum score per year was used, while for repeated measures models, maximum values for each disease trait for each of three consecutive months (June to August) were used. The models were run using both binary and scaled outcomes for 2005 and 2006 together and separately.

Scaled outcome models used variables as recorded in the field. Binary outcomes were classified as 1 for disease (i.e. any score greater than 0) and 0 for no disease and were analysed using a logit link function.

The basic fixed effects model for lambs was:

$$Y_{ijklmnop} = Year_i + breed_j + b1.DOB + sex_k + TG_l + LS_m + b2.BW + Dage_n + DBCS_o + b3.Year.DOB + r_{ijklmnop}$$

Where Year is the i^{th} year of measurement, breed is the j^{th} breed of the dam, DOB is the day of birth (days from January 1) fitted as a covariate with b1 being the regression of the trait measurement on DOB, sex is the k^{th} sex of the lamb, TG is the l^{th} treatment group to which the lamb was allocated, LS is the m^{th} litter size into which the lamb was born, BW is the lamb's birth weight with b2 being the regression of the trait measurement on BW, Dage is the n^{th} age of the dam (measured by dentition), DBCS is the dam's body condition score (o levels) at the start of the

study, Year.DOB is an interaction of year by day of birth with b3 being the year-specific regressions on DOB and r is the residual term.

Random effects were fitted alongside these fixed effects, in several analyses as follows.

1. To estimate heritabilities using a dam model, the basic fixed effects model was extended to include random effects of dam (i.e. the dam identifier) and litter (coded as Year.dam), with the statistical significance of including litter tested using a likelihood ratio test.

2. To estimate heritabilities using the animal model, the basic fixed effects model was extended to include random effects of lamb, linked to a pedigree matrix containing dam and with sire set to unknown.

3. To estimate across-time repeatabilities in datasets containing multiple records per lamb, lamb was fitted as a random effect.

Estimation of heritabilities and repeatabilities from the variance components obtained from these analyses is described below. For the models described above, bivariate models were also developed to calculate correlations between traits. Further, the disease traits described above were also analysed as binary outcomes, coded 0 or 1, using generalised linear mixed models with the same fixed and random effects and using a logit link function to normalise residuals. The combined foot score in lambs was also analysed as a binary outcome with the classification of diseased (1) shifting from a CFS $\geq$1 to a CFS of 8 in gradations of 1 to examine the variation in heritability of different severities of disease.

Regressions of lamb phenotype on dam phenotype were obtained using the fixed effects model described above and adding the dam disease phenotype (DDP) as a covariate in the analyses. In this case, DDP was defined as the maximum score in dams for the phenotype under analysis in the lambs.

The basic model for ewes was:

$$Y_{ijklmnop} = breed_i + age_j + BCS_k + TG_l + LS_m + year_n + ewe_o + r_{ijklmnop}$$

Where breed is the breed of the ewe, age is its age as measured by dentition, BCS is the ewe's body condition score at the start of the study, TG is the treatment group to which the ewe was assigned, LS is the litter size born to the ewe, year is the year in which the measurement was taken (there are two measurements per ewe in most models, one for each year of the study), ewe is the identifier for the ewe fitted as a random effect, accounting for the fact that many ewes had repeated measures across years, and r is the residual term. For analyses where repeated measurements per ewe within a year were analysed, a further random term of year.ewe was fitted to allow estimation of within-year repeatabilities.

As for lambs, bivariate models were used to calculate correlations between traits, and all phenotypes were analysed both as continuous and binary variables, fitting a logit link function for the binary analyses.

The number of sheep in each category for factors included in the models are given in Tables 2.5 – 2.10.

Table 2.5. Number (percentage) of sheep by litter sizes.

Litter Size	2005		2006	
	Lambs	Ewes	Lambs	Ewes
0	NA	194 (20.3%)	NA	114 (15.7%)
1	233 (17.2%)	233 (24.3%)	180 (16.7%)	179 (24.7%)
2	931 (68.7%)	466 (48.7%)	816 (75.8%)	406 (55.9%)
3	192 (14.2%)	64 (6.7%)	81 (7.5%)	27 (3.7%)
Total:	1356	957	1077	726

Table 2.6. Number (percentage) of sheep in different treatment groups.

Group	Ewes 2005	Ewes 2006	Lambs 2005	Lambs 2006
TC	224 (23.4%)	179 (24.7%)	364 (26.8%)	270 (25.1%)
TT	233 (24.3%)	174 (24.0%)	323 (23.8%)	286 (26.6%)
CT	242 (25.3%)	185 (25.5%)	357 (26.3%)	250 (23.2%)
CC	244 (25.5%)	174 (24.0%)	312 (23.0%)	246 (22.8%)
Unknown	14 (1.5%)	14 (1.9%)	0	25 (2.3%)

Table 2.7. Number (percentage) of lambs of each sex born in 2005 and 2006.

Sex	2005	2006
Male	686 (50.6%)	523 (48.6%)
Female	670 (49.4%)	551 (51.2%)
Unknown	0	3 (0.3%)

Table 2.8. Number (percentage) of ewes of different ages and the number of lambs born to them in each year.

Age	Ewes 2005	Ewes 2006	Lambs (dam age) 2005	Lambs (dam age) 2006
2 tooth	34 (3.6%)	75 (10.3%)	59 (4.4%)	43 (4.0%)
4 tooth	69 (7.2%)	28 (3.9%)	107 (7.9%)	94 (8.7%)
6 tooth	260 (27.2%)	164 (22.6%)	448 (33.0%)	320 (29.7%)
Full mouth	293 (30.6%)	280 (38.6%)	553 (40.8%)	289 (26.8%)
Broken mouth	106 (11.1%)	62 (8.5%)	185 (13.6%)	35 (3.2%)
Unknown	195 (20.4%)	117 (16.1%)	4 (0.3%)	296 (27.5%)

Table 2.9. Number (percentage) of ewes with each body condition score (BCS) and the number of lambs they had each year.

BCS	Ewes 2005	Ewes 2006	Lambs (dam BCS) 2005	Lambs (dam BCS) 2006
1	12 (1.3%)	5 (0.7%)	21 (1.5%)	8 (0.7%)
1.5	56 (5.9%)	62 (8.5%)	108 (8.0%)	40 (3.7%)
2	125 (13.1%)	104 (14.3%)	235 (17.3%)	100 (9.3%)
2.5	206 (21.5%)	161 (22.2%)	385 (28.3%)	194 (18.0%)
3	208 (21.7%)	163 (22.5%)	360 (26.5%)	239 (22.2%)
3.5	109 (11.4%)	73 (10.1%)	176 (13.0%)	135 (12.5%)
4	44 (4.6%)	43 (5.9%)	65 (4.8%)	61 (5.7%)
4.5	4 (0.4%)	3 (0.4%)	6 (0.4%)	6 (0.6%)
5	0	0	0	0
Unknown	193 (20%)	112 (15.4%)	0	294 (27.3%)

Table 2.10. Number (percentage) of ewes of different breeds and the number of lambs born to them in 2005 and 2006.

Breed	Ewe 2005	Ewe 2006	Lamb (dam) 2005	Lamb (dam) 2006
Mule	542 (56.6%)	386 (53.2%)	974 (71.8%)	576 (53.5%)
Hartline	171 (17.9%)	116 (16.0%)	306 (22.6%)	169 (15.7%)
Other	244 (25.5%)	224 (30.9%)	76 (5.6%)	332 (30.8%)

2.3.2 - Estimation of Genetic Parameters

Full pedigree information was not available, so estimated additive genetic variances (σ^2_A) in this study are approximations. In the lamb model σ^2_A was approximated by the dam variance components and in the ewe model from the repeatability effects. The dam variance components, σ^2_D, are a measure of the between-dam variances and have expectation $¼\sigma^2_A + \sigma^2_M$, where σ^2_M is the (non-estimated) maternal variance component. Repeatability effects represent the consistency of trait scores between measurements at different time points. The covariance of phenotypes across time, σ^2_R, has expectation $\sigma^2_G + \sigma^2_{PE}$, where σ^2_G is the genetic variance component σ^2_{PE} is the permanent environment variance component.

For the animal model genetic relatedness between animals, inferred from the pedigree structure, was used to estimate σ^2_A. In these analyses, unknown sires were represented in two ways, to cover the extreme possibilities of the (unknown) mating design used by the farmer. Firstly all sires were set to be 'missing', which represents a situation where all lambs had a different sire, i.e. the sire genotype would be assumed different for each lamb. Secondly all sires were set to be the same, so that the sire genetic values would be the same for every lamb. These two situations provide upper and lower bound estimates for the heritability respectively, based on

different ram usage within the flock, as any ram usage pattern by the farmer must fall within these two extreme scenarios.

Heritability is defined as the ratio of additive genetic variance to phenotypic variance (Falconer & Mackay, 1996), i.e.:

$$h^2 = \frac{\sigma^2{}_A}{\sigma^2{}_P}$$

Where h^2 represents heritability, $\sigma^2{}_A$ is for additive genetic variance and $\sigma^2{}_P$ is the total phenotypic variance. In principle, phenotypic variance is the mean of the squared deviations from the mean observed values for each trait. With several random effects fitted, it is the sum of the fitted variance components, e.g.

$$\sigma^2_p = \sigma^2_D + \sigma^2_r$$

When using the between-dam variance (σ^2_D) heritability is calculated using the following equation:

$$h^2 = \frac{4\sigma^2{}_D}{\sigma^2{}_P}$$

This estimate will be biased upwards if $\sigma^2{}_M$ is non-trivial. From the animal model, the heritability was constructed as:

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2}$$

Using the regression of offspring phenotype on dam phenotype, the expected value of the regression coefficient (b) is half the heritability (Falconer & Mackay, 1996). Hence the heritability was estimated as:

$$h^2 = 2b$$

This regression will result in a biased heritability only if there is an environmental covariance between the maximum trait values observed in the lamb and in the dam, as described in Chapter 1.

All heritabilities presented in this study are within-breed heritabilities. Breed differences are accounted for as fixed effects in the model and will be examined solely as factors affecting disease phenotypes.

The genetic correlation (r_A) is defined as (Falconer & Mackay, 1996):

$$r_A = \frac{\sigma_{AX,AY}}{\sigma_{AX}\sigma_{AY}}$$

Where $\sigma_{AX,AY}$ is the genetic covariance of the two traits (X and Y) under examination and σ_{AX} and σ_{AY} are the genetic standard deviations of traits X and Y respectively. Biases in the genetic covariances are, in principle, the same as those in the genetic variances and impact on correlations is likely to be trivial.

Similarly, the phenotypic correlation (r_p) is defined (Falconer & Mackay, 1996) as:

$$r_P = \frac{\sigma_{PX,PY}}{\sigma_{PX}\sigma_{PY}}$$

Where $\sigma_{PX,PY}$ is the phenotypic covariance of traits X and Y and σ_{PX} and σ_{PY} are the phenotypic standard deviations of traits X and Y respectively. These correlations will be unbiased.

2.4 - Results

2.4.1 - Description of data

More ewes than lambs had non-zero values for each disease phenotype in both years and non-zero phenotypes were more prevalent in 2005 than 2006 (Table 2.11).

Table 2.11. Numbers (percentages) of ewes and lambs observed to have positive disease observations for each of three disease phenotypes in 2005 and 2006.

	Ewes		**Lambs**	
	2005	**2006**	**2005**	**2006**
Number of animals	957	726	1356	1077
Locomotion score > 0	627 (65.5%)	505 (69.6%)	538 (39.7%)	227 (21.1%)
Interdigital dermatitis lesion >0	269 (28.1%)	185 (25.5%)	276 (20.4%)	85 (7.9%)
Footrot lesion>0	251 (26.2%)	117 (16.1%)	32 (2.4%)	23 (2.1%)

The most common non-zero maximum locomotion score was 2 in lambs and ewes (Table 2.12). Despite the fact that over the two years examined in the study over 80% of ewes showed a positive locomotion score at some point, there are 569 cases where zero was the maximum observed score for a particular year, indicating that sheep lame in year one were not always lame in year two and *vice versa*.

Table 2.12. Frequency distribution of the maximum locomotion scores observed in ewes and lambs for 2005 and 2006 [*].

Maximum locomotion score	Ewes 2005	Ewes 2006	Lambs 2005	Lambs 2006
0	330 (34.5%)	239 (32.9%)	818 (60.3%)	850 (78.9%)
1	108 (11.3%)	64 (8.8%)	71 (5.2%)	34 (3.2%)
2	290 (30.3%)	166 (22.9%)	337 (24.9%)	102 (9.5%)
3	115 (12.0%)	158 (21.8%)	33 (2.4%)	68 (6.3%)
4	95 (9.9%)	74 (10.2%)	75 (5.5%)	21 (1.9%)
5	7 (0.7%)	24 (3.3%)	1 (0.1%)	2 (0.2%)
6	12 (1.3%)	1 (0.1%)	21 (1.5%)	0 (0%)

[*] Percentages given are out of the total number of sheep represented in each column.

Table 2.13. Frequency of maximum ID lesion scores of differing severities in ewes and lambs.

Max ID lesion score	Ewes 2005	Ewes 2006	Lambs 2005	Lambs 2006
0	688 (71.9%)	542 (74.7%)	1080 (79.6%)	992 (92.1%)
1	41 (4.3%)	21 (2.9%)	44 (3.2%)	10 (0.9%)
2	77 (8.0%)	53 (7.3%)	84 (6.2%)	14 (1.3%)
3	82 (8.6%)	50 (6.9%)	86 (6.3%)	21 (1.9%)
4	69 (7.2%)	60 (8.3%)	62 (4.6%)	40 (3.7%)

*Percentages given are out of the total number of sheep represented in each column.

There were fewer lambs with ID than with lameness (Table 2.13). However, feet were only examined for lesions when a lamb had a locomotion score >1. Therefore,

positive lesion scores are conditional upon there also being a positive locomotion score. The prevalence of lesions in the absence of lameness is unknown for all sheep.

Frequencies of maximum footrot lesion scores are shown in Table 3.14. The frequency of positive footrot lesion scores in lambs was very low, 55 / 2433 (2.3%).

Table 2.14. Frequency of maximum footrot scores of differing severities in ewes and lambs.

Max FR lesion score	Ewes 2005	Ewes 2006	Lambs 2005	Lambs 2006
0	706 (73.8%)	609 (83.9%)	1324 (97.6%)	1054 (97.9%)
1	77 (8.0%)	57 (7.9%)	14 (1.0%)	15 (1.4%)
2	103 (10.8%)	43 (5.9%)	11 (0.8%)	5 (0.5%)
3	62 (6.5%)	13 (1.8%)	5 (0.4%)	2 (0.2%)
4	9 (0.9%)	4 (0.6%)	2 (0.1%)	1 (0.1%)

* Percentages given are out of the total number of sheep represented in each column.

The combined foot score (CFS) was created out of a combination of footrot lesion scores and ID lesion scores as described in Table 2.4. Table 2.15 shows the distribution of CFS in ewes and lambs. In ewes the most frequent positive maximum CFS is 6, which corresponds to a footrot lesion score of 2 (with or without interdigital dermatitis lesions being observed). In lambs the most frequent positive maximum CFS is 3 which corresponds to an animal with an ID lesion score of 3 but no footrot lesions observed. As lesions were only observed once a positive

locomotion score was observed this is again conditional on a locomotion score of 1 or greater being present at the same time.

Table 2.15. Distribution of maximum combined foot scores in ewes and lambs.

Maximum combined foot score	Ewes 2005	Ewes 2006	Lambs 2005	Lambs 2006
0	567 (59.2%)	503 (69.3%)	1068 (79%)	990 (92%)
1	23 (2.4%)	16 (2.2%)	42 (3%)	9 (1%)
2	44 (4.6%)	37 (5.1%)	78 (6%)	11 (1%)
3	45 (4.7%)	29 (4.0%)	80 (6%)	18 (2%)
4	27 (2.8%)	24 (3.3%)	56 (4%)	26 (2%)
5	77 (8.0%)	57 (7.9%)	14 (1%)	15 (1%)
6	103 (10.8%)	43 (5.9%)	11 (1%)	5 (<1%)
7	62 (6.5%)	13 (1.8%)	5 (<1%)	2 (<1%)
8	9 (0.9%)	4 (0.6%)	2 (<1%)	1 (<1%)

* Percentages given are out of the total number of sheep represented in each column.

2.4.2 - Non-disease factors affecting presentation of disease phenotypes

Non-disease factors were analysed in the basic fixed effects models to determine whether or not they affected the presentation of disease in lambs and ewes. P-values are used to give the probability of each outcome occurring by chance alone, with the standard cut-offs of 0.05 and 0.01, representing 5% and 1% chances that the results would be obtained at random, given that the assumptions used to create the model are

true, i.e. that a linear model is a good description of the biological relationship and that residuals are independent and normally distributed. Results are presented in Tables 2.16 (ewes) and 2.17 (lambs), in terms of the significance of each effect.

Table 2.16. P values for factors affecting presentation of disease outcomes in ewes.

Disease trait	Factors					
	LS*	TG*	Breed	BCS*	Age	Year
Locomotion score	<0.01	<0.01	<0.01	0.39	<0.01	0.42
Footrot lesion score	<0.01	0.08	<0.01	0.03	0.05	<0.01
ID lesion score	<0.01	<0.01	<0.01	0.93	0.06	0.27

*LS = litter size, TG = treatment group, BCS = body condition score

Table 2.17. P values for factors affecting presentation of disease outcomes in lambs

Disease trait	Factors								
	DOB	Sex	BW	LS	TG	Breed	DBCS	Dage	Y.DOB
Locomotion score	<0.01	0.04	0.05	<0.01	<0.01	0.32	0.62	0.82	<0.01
Footrot lesion score	0.02	0.01	<0.01	<0.01	0.34	0.47	0.20	0.50	0.48
ID lesion score	0.92	<0.01	0.17	<0.01	<0.01	0.94	0.04	0.04	<0.01

*DOB = day of birth (1-365), BW = birth weight, LS = litter size, TG = treatment group, DBCS = dam body condition score at start of study, Dage = dam age (by dentition), Y.DOB = date of birth (interaction between year and day of birth).

Table 2.18. Significant factors affecting disease in ewes and the magnitude of their effects on disease phenotype ± s.e.

Factor	Factor categories	Locomotion score	ID lesion score	FR lesion score
Litter size	**0**	-1.16 ± 0.06	-0.63 ± 0.07	-0.40 ± 0.05
	1	+0.06 ± 0.06	+0.13 ± 0.07	+0.05 ± 0.05
	2	0 (baseline)	0 (baseline)	0 (baseline)
	3	-0.570 ± 0.12	-0.40 ± 0.13	-0.20 ±0.09
Breed	**Hartline**	0 (baseline)	0 (baseline)	0 (baseline)
	Mule	-0.23 ± 0.07	-0.28 ± 0.08	-0.29 ± 0.05
	Other	-0.48 ± 0.13	-0.38 ± 0.15	-0.19 ± 0.09
Treatment group	**TC**	0 (baseline)	0 (baseline)	NS
	TT	+0.18 ± 0.07	+0.84 ± 0.09	
	CT	+0.18 ± 0.07	+0.43 ± 0.08	
	CC	+0.20 ± 0.07	-0.02 ± 0.09	
	Unknown	-0.25 ± 0.22	+0.63 ± 0.26	
Age	**2 tooth**	-0.11 ± 0.16	NS	NS
	4 tooth	0 (baseline)		
	6 tooth	+0.06 ± 0.11		
	Full mouth	+0.13 ± 0.11		
	Broken mouth	-0.01 ± 0.12		
	Unknown	+0.93 ± 0.19		
Year	**2005**	NS	NS	0 (baseline)
	2006			-0.26 ± 0.04

Table 2.19. Significant factors affecting disease in lambs and the magnitude of their effects on disease phenotype ± s.e.

Factor	Factor categories	Locomotion score	ID lesion score	FR lesion score
Litter size	**1**	+0.18 ± 0.06	+0.22 ± 0.06	-0.02 ± 0.02
	2	0 (baseline)	0 (baseline)	0 (baseline)
	3	-0.37 ± 0.07	-0.22 ± 0.07	+0.06 ±0.02
Treatment group	**Purple**	0 (baseline)	0 (baseline)	NS
	Red	+0.02 ± 0.07	+0.08 ± 0.07	
	Orange	+0.22 ± 0.06	+0.90 ± 0.06	
	Green	+0.26 ± 0.07	+0.04 ± 0.07	
	Unknown	+0.08 ± 0.23	+0.36 ± 0.22	
Day of birth (DOB, 1-365)	**Continuous**	0.48 ± 0.06	NS	NS
Birth weight	**Continuous**	NS	NS	0.02 ± 0.006
Sex	**Male**	NS	0 (baseline)	NS
	Female		-0.10 ± 0.04	
	Unknown		+0.95 ± 0.59	

Consideration of the factors which were significant at the 0.01 level provides further information about the influence of these factors on presentation of disease (Tables 2.18 (ewes) and 2.19 (lambs)). Effects given are not absolute scores but are relative to the other levels of the group under analysis, e.g. -1 would be an average maximum score of 1 less than that seen in the baseline group. Where the explanatory factor is a continuous variable the regression coefficient is given**.**

2.4.3 - Heritability of disease phenotypes in lambs

Dam variance components and heritabilities derived from this variance component analysis are presented in Table 2.20, for both scaled and binary outcomes. Both binary outcomes and scaled outcomes gave similar heritability estimates for maternal effects on interdigital dermatitis and lameness. The results for footrot had greater discrepancy between the two models, however, they have large standard errors and their confidence intervals include the boundary values of 0 and 1.

Heritability estimates obtained using the animal model (one sire and multiple/ missing sire models) are presented in Table 2.21. The heritability estimates from the two models are similar and close to those obtained from the dam model (Table 2.20). It should be noted, however, that the available information for the animal model analysis is the same as for the dam model analysis; hence the broad agreement of the results is not surprising.

Table 2.20. Trait dam effects (σ^2_D / σ^2_P) and estimated lamb heritabilities (h^2) [α]

	Dam effects ± s.e. (scaled outcome)	**Heritability ± s.e. (scaled outcome)**	**Dam effects ± s.e. (binary outcome)**	**Heritability ± s.e. (binary outcome)**
FR	0.02±0.02 (NS)	0.08±0.08 (NS)	0.15±0.12	0.60±0.48
ID	0.08±0.02	0.32±0.08	0.07±0.04	0.28±0.16
Loco	0.07±0.02	0.28±0.08	0.06±0.03	0.24±0.12

[α]FR = max. footrot lesion score. ID = maximum interdigital dermatitis score. Loco = maximum locomotion score. NS = not significantly different to zero – zero is included in the range of standard error values.

Table 2.21. Heritability estimates for lamb disease phenotypes using the animal model.

	h^2 lamb (all sires different/missing)	h^2 lamb (all sires the same)
FR	0.09±0.08	0.09±0.07
ID	0.30±0.09	0.28±0.08
Loco	0.28±0.09	0.26±0.08

[α]FR = max. footrot lesion score. ID = maximum interdigital dermatitis score. Loco = maximum locomotion score.

Heritability estimates were also obtained from a regression model. Using regression, heritability for footrot was not significantly different from zero, locomotion heritability was estimated at 0.28±0.04 and ID heritability was estimated to be 0.33±0.03. These match closely with our previous estimates of heritability using estimations based on maternal effects (Table 2.20) and the animal model (Table 2.21). The regression model uses very different methods of estimation from the dam effects and animal models, so agreement between models provides some confidence in the results.

2.4.4 - Litter effect

Using likelihood ratio tests it was determined that litter effect was not significant in the presentation of footrot lesions in lambs. For both ID lesions and locomotion scores the litter effect was statistically significant. When fitted, the dam variance component was no longer significant and thus most of the variation was accounted for in litter effects. An interpretation is that locomotion and ID are more subject to variation between years, possibly due to differing environmental factors, whilst the

dam genetic component of the footrot score is more stable across years, suggesting that it is more likely to reflect the genetic component. However, the lack of knowledge of sires makes partitioning of variances between additive genetic and litter effects difficult, so these conclusions are tentative.

2.4.5 - Combined Foot Scores

Heritability for the combined foot score phenotype was not estimable when data for all lambs over both years were analysed. In ewes the across-year repeatability of combined foot scores was 0.23 ± 0.04.

When using a shifting scale of positive disease classification in a binary analysis (Table 2.22), heritabilities were not estimable for combined foot scores of ≥7 and ≥8, or for ≥1. This may be due to the lack of cases where severe footrot lesion scores were observed (7 with CFS of 7 and 3 with a CFS of 8).

It should also be noted that although CFS is represented as a linear scale of zero to eight, the differences between the clinical outcomes of the scores are probably not the same so the relationship between the scoring system and the true trait is not linear. For example, the transition between scores zero and one represents the development of a symptomatic infection, which may be a large difference taking time for bacteria to multiply and progress from a subclinical colonisation to the appearance of visible signs. However, from score one to score two, which is a slight increase in inflammation, may be a much slighter difference and thus a smaller true gap between these scores is expected. This may affect the heritabilities estimated with different cut-off points as it may be that the difference between the two scores

either side of the cut-off is much greater in some cases than others. It may also result in scores being misclassified when the differences between them are unclear.

Heritability increased as the binary cut-off was moved towards higher scores, with the highest heritability seen for a combined score of 6 or greater, which is equivalent to a footrot lesion score of 2.

Table 2.22. Heritability (h^2) estimates for combined foot score when the threshold between positive and negative scores was shifted.

Values classed as 0 (-ve)	Values classed as 1 (+ve)	Heritability
0	≥1	Not estimable
≤1	≥2	0.06±0.05
≤2	≥3	0.08±0.05
≤3	≥4	0.13±0.06
≤4	≥5	0.15±0.12
≤5	≥6	0.26±0.14
≤6	≥7	Not estimable
≤7	8	Not estimable

2.4.6 - Repeated measures (lambs)

For the binary models, the permanent environmental effects converged to zero in all cases. This was also true for scaled models for both ID and FR. Locomotion scores showed an additional across-time effect (0.11). The convergence of permanent environmental effects to zero for all binary models and two of the scaled models suggests that repeated measures analysis of a time-dependent trait such as footrot may present difficulties in interpretation.

Heritability values were also calculated from the repeated measurements data (Table 2.23) using the animal model.

Table 2.23. Heritability estimates for lamb disease phenotypes using the animal model and taking the maximum scores for each of three consecutive months.

Disease Phenotype	Heritability (h^2)
FR	0.01 ± 0.01 (NS)
ID	0.06 ± 0.01
Loco	0.13 ± 0.05

* NS = not significantly different to zero – zero is included in the range of standard error values.

2.4.7 - Repeatability values (ewes)

Two models were used to provide estimates of across-year and within-year repeatability values. In the first, the basic ewe model, the between-year repeatability value for footrot lesions was 0.10±0.03, for interdigital dermatitis lesions was 0.20±0.03 and for locomotion score was 0.07±0.03.

Table 2.24. Repeatability estimates from repeated measures model run for single and combined years of the study.

	Repeatability			
	2005	**2006**	**05/06 (between years)**	**05/06 (within years)**
Max* FR	0.04±0.02	0.08±0.02	0	0.06±0.01
Max* ID	0	0.11±0.02	0.05±0.01	0
Max* loco	0.29±0.02	0.23±0.02	0.01±0.02	0.27±0.03
Bin* FR	0.08±0.11	0.21±0.11	0	0.15±0.08
Bin* ID	0	0.19±0.08	0.08±0.04	0
Bin* loco	0.21±0.03	0.25±0.04	0.04±0.04	0.19±0.04

*Max = maximum values used. Bin = binary values used (0 = no disease, 1 = disease).

The second model used repeated measurements to get within-year as well as between-year repeatability estimates (Table 2.24). Repeatability values range from 0.04 to 0.29.

For both versions of the model using all the data, there was no within-year repeatability for ID while footrot showed no between-year repeatability. There were both within-year and across-year repeatability in lameness although the within-year values were much higher. Repeatability values in 2006 were higher than 2005 for both footrot and ID lesions, but lower for locomotion scores when a scaled scoring system was used.

2.4.8 - Bivariate models

The genetic and phenotypic correlations are shown in Tables 2.25 and 2.26.

Table 2.25. Phenotypic and repeatability effect correlations between ewe traits ± s. e.[α]

Traits	FR	ID	Loco
FR	-	0.89±0.16	0.87±0.24
ID	0.34±0.02	-	1.00
Loco	0.28±0.02	0.41±0.02	-

[α] Repeatability effect and phenotypic correlations above and below the diagonal, respectively

Repeatability and dam effects correlations are assumed to approximate genetic correlations. Genetic correlations are all high, indicating a high degree of similarity in genetic control of the three phenotypes. They range from 0.87 to 1.00 (±s.e.) in ewes while in lambs they range from 0.57 to 1.00 (± s.e.). Phenotypic correlations in ewes range from 0.28 to 0.41 (±s.e.) and in lambs from 0.18 to 0.45 (±s.e.).

Table 2.26. Phenotypic and maternal effect correlations between lamb traits ± s.e.[α]

Traits	FR	ID	Loco
FR	-	1.00	0.57±0.40
ID	0.27±0.02	-	0.82±0.17
Loco	0.18±0.02	0.45±0.02	-

[α] Maternal effect and phenotypic correlations above and below the diagonal, respectively

2.5 - Discussion

A large longitudinal dataset was used to assess the relationship between foot health in ewes and their lambs. The principal objective was to estimate the proportion of variability that can be accounted for by the genetic relationship, by estimating heritability values for three distinct foot disease phenotypes – lameness, footrot lesions and interdigital dermatitis lesions.

The study detailed above provided extensive data on individual animals over time. For practical purposes this large quantity of data needed to be reduced in order to make the analysis and interpretation of results feasible. Without reduction the number of records to be analysed would be over 130,000 with the observations

differing in frequency of measurement for each animal, and constantly changing animal state across time. This would make interpretation of results difficult. Consequently, the maximum disease score achieved by each sheep in a specified time period was chosen for use in the main analysis because, given the available data, this most accurately reflects a sheep's underlying susceptibility to the disease phenotype in question.

Although these data are not normally distributed they were analysed using statistical methods where normality is assumed or, more specifically, normality of residuals. Due to the extreme numbers of non-positive disease scores the distributions are difficult to transform to a normally distributed variable. This is not an ideal way to analyse such skewed data, however it is difficult to avoid other than by categorising the data differently. Taking footrot in lambs as the most extreme example, the numbers in each category reduce greatly as the scores increase, with only 3 animals displaying a footrot lesion score of the highest severity (4). For this reason analyses were also run using binary outcomes which helps to address problems caused by the skewed nature of the data, grouping all affected animals together and compensating for the low numbers of sheep with extreme phenotypes.

The data are also limited due to the lack of available sire data for the lambs. This lack of pedigree data makes it difficult to gain accurate estimates for heritability, but the use of the animal model simulating a range of different numbers of sires, as well as parent-offspring regressions, attempted to address this issue.

Another point that should be taken into consideration when interpreting the results presented in this paper is the observation bias between different treatment groups. Intervention groups were observed much more frequently than control groups and

there was therefore greater opportunity to observe lesion and locomotion scores of all magnitudes. The fact that the highest average ID lesion scores were seen in the sheep that remained in the intervention group for the entire study is not a reflection on the efficacy of treatment but rather a consequence of observing the sheep more frequently. Details of the effects of treatments on prevalence and incidence of disease are presented in Wassink *et al.* (2010).

It should be noted that results obtained in this paper are not comparable to those presented in Wassink *et al.* (2010) because we have used different methods and outcomes which did not take into account any effects of treatment or improvements seen. Rather this study has focused on single maximum scores observed, regardless of which treatment a sheep was receiving or at which time point it was observed to have that score, in order to best estimate each sheep's true susceptibility to disease.

Results from this study give moderate heritability estimates in lambs for ID lesions and locomotion scores (0.14 -0.32), indicating that there is potential for improvement to be made using targeted breeding schemes. Criteria for breeding programmes would need to balance economically beneficial production traits and improvement in animal health to create a practical plan that would benefit both farmers and their flocks. Selection for footrot prevalence has already been used successfully in the case of Broomfield Corriedales (Skerman and Moorhouse, 1987), but whether it could be effective in the UK climate is unclear.

Environmental factors are significant in the patterns of footrot seen, so any programme for genetic improvement would also have to take account of the local and changing environment. It has long been known that footrot is greatly influenced by the climate as the bacteria are thought to only survive in the environment in warm,

wet pastures. In the UK in particular this is challenging as the climate is ideally suited to the survival of *Dichelobacter nodosus* in the environment (Whittington *et al.,* 1995 (in Green and George, 2008)) and we have no consistent periods of the year where temperature or rainfall levels (UK Met Office; Green and George, 2008) prohibit bacterial survival. This is an aspect of disease presentation which has not been considered in our study, however it is vital for future studies to include environmental data in order to get the most accurate information about disease patterns and the ways in which they could be better managed.

Footrot is a problem not only in the UK but globally, and a great deal of work on footrot has been done in Australia and New Zealand, where the sheep farming industry is larger than in the UK and the environment is very different. Both Skerman *et al.* (1988), and Raadsma *et al.* (1994) published heritability estimates for footrot based on studies in Oceania. Skerman *et al.*'s heritability estimates ranged from 0.12 to 0.28 based on 13 inspections of foot integrity between 1979 and 1983, while Raadsma *et al.* obtained estimates of between 0.06 and 0.28 in a challenge study. This latter study used repeated measurements (approx. 11 per sheep following induced and natural challenges with *D. nodosus*) so estimates for repeatability were also made, ranging from 0.18 to 0.70. For neither of these studies was lameness considered. Estimates of disease phenotype heritability in lambs from the Oxfordshire data presented in this paper range from 0.08 to 0.32 and as such correspond with previously published estimates, despite considerable differences in climatic conditions.

A more closely matched study both in terms of climate and methodology was that of Nieuwhof *et al.* (2008b), who published the results of a Scottish study examining foot lesion scores in different sheep breeds. Their heritability estimates ranged from

0 (in Scottish Blackface (SBF) lambs) to 0.26 (severe lesions in SBF ewes) which are within the standard errors calculated for our estimates.

Nieuwhof's study had the advantage of readily available pedigree data but only recorded one lesion score (combining footrot and interdigital dermatitis in a continuous scale, similar to the CFS used in this study) per sheep per year (two per year for mules), meaning that the most severe lesions an animal developed might have been missed if observations coincided with post-infection immune periods. This could hinder assessment of genetic susceptibility to disease and provide inaccurate representations of the true susceptibilities of individuals to disease. Infrequent scoring may result in artificially low heritability estimates which in turn may result in less accurate decisions being made about which sheep should be used for breeding purposes. The disadvantage of having incomplete pedigree information in the current data is offset by the frequent observations of disease including two distinct foot lesions and lameness, all three of which are closely linked, giving a more comprehensive view of the disease states of individual animals. Future studies will ideally include both repeated observations of disease and full pedigree data.

Our results showed that much lower levels of disease were seen in lambs than in ewes in both 2005 and 2006. It is hypothesised that this is because lambs' feet have not yet been exposed to damage or disease and so there are fewer opportunities for infections to become established. Older animals, whose feet are more worn and have had repeated infections in the past, are thought to be more susceptible to getting further infections. We have no information on disease history of the ewes prior to this study so we cannot examine this further.

One of the things remaining unclear from this study is how heritability of different disease phenotypes changes with age. We have been unable to gain estimates of heritability for adult ewes due to the lack of available pedigree data. There is also no information from lambs older than approximately six months as at this time the lambs were removed from the study (mostly sent to slaughter). Heritability may vary with age as the environment can have a larger or smaller effect on animals at different stages of their development. For example, if there was a shortage of food at a time when the lamb was at the peak of its growth phase this may adversely affect its development, while food shortages for a grown ewe may have less of an effect as they are already fully developed.

All repeatability values obtained from our study are quite low which suggests that in general there is high variation both between and within years. Between-year repeatabilities using multiple measurements are different to those obtained using a single measurement per year but for all three disease phenotypes the repeatability is reduced in the repeated measures model where maximum scores for each of three consecutive months were examined. This suggests that there is high variability within years but the overall highest score in a year is more stable. This corresponds with the idea of a maximum susceptibility which may be under partial genetic control, although environmental factors also play a role in the variation of these phenotypes. When repeated measures are included in the models, no consistent differences between binary and scaled outcomes are discernible. This makes it difficult to assess whether there is benefit in using a scaled scoring system rather than a simple binary diseased or healthy classification, which could impact greatly on the practicalities of repeated measurements in the field. In order for a scaled scoring system to be effective, farmers would need to be fully trained in the use of that

scoring system so that scores between farms and observers would be comparable. This would take time and manpower. It is much easier to say whether an animal has a lesion or not than to categorise that lesion on a 4 point scale, so if there is little information lost between the two systems then a binary (healthy/diseased, lesion/no lesion, lame/not lame) scoring system would be desirable as it is easier to implement.

Covariance and correlation also need consideration when analysing genetically linked phenotypes. They provide an estimate of how two traits may alter when only one of them is selected for by considering the associations between them. If selection on a more easily measured trait (e.g. locomotion score) may reduce other undesirable traits (e.g. lesion scores) then a selection programme will both have greater impact and have a greater chance of being properly implemented. Locomotion scores may be less time-consuming to observe (and thus cheaper in terms of labour) than inspecting in detail the feet of large numbers of sheep, so this would be the desirable way to assess disease phenotype. Temporal associations between foot lesions and locomotion score have been demonstrated (Kaler *et al.*, 2011) and this study aimed to further that work by considering the underlying genetic correlations along with the phenotypic correlations.

Our results show a large difference between genetic and phenotypic correlation values, probably due to uncertainties in the epidemic process and observation/scoring processes. Results presented here show high genetic correlations between the three disease phenotypes in lambs and ewes, despite lower phenotypic correlations. This suggests potential for the reduction of all three disease phenotypes by selection on only one. However, this would need to be considered in each flock in which selection was desired as in the data presented here the majority of lameness in the flock was caused by FR and ID, which may not be the case in all flocks.

While it is clear that there is some genetic component to the susceptibility or resistance to footrot it is equally clear that the environmental conditions to which animals are exposed are important. The analysis presented here considers only a small amount of information on non-disease factors and those only in the context of variables such as age, breed, sex and litter size. A number of these factors were significant in the outcome of clinical disease in lambs (Tables 2.17 and 2.19) and ewes (Tables 2.16 and 2.18). Though not all factors were measured in both lambs and ewes it is still clear that the factors which affect ewes do not always affect lambs and *vice versa*. This suggests that genetics and environment have changing roles to play as animals grow older and perhaps as environmental conditions around them change. As the predisposing factors do not remain the same throughout an animal's lifetime, different strategies may need to be employed for animals of different ages to achieve optimal results in a selection programme.

We have seen that footrot is a complex disease that is affected by a number of different factors which may change over the course of an animal's life. The three phenotypes observed are closely correlated genetically, although it is not always possible to see such close correlations in phenotypes due in part to the large effect environment has on disease presentation.

The moderate heritability levels calculated for this disease suggest that there is potential for a breeding programme selecting for resistant sheep to achieve progress in reducing overall incidence and prevalence over a number of years. However, such a breeding programme must be carefully designed to take account of the individual farm conditions as different environments will require different strategies.

The next step in exploring the possibilities afforded by genetic control of this disease is to create a large scale simulation model where different selection strategies and interventions may be tested in a range of environments to determine what may be a practical route forwards. The model developed to address this is presented in chapter 3, with a range of conventional interventions presented in chapter 4 and the potential for genetic selection explored in chapter 5. As additional data become available these should also be incorporated in order to give as accurate a model as possible. It is hoped that in the future, genetic selection may be a viable and worthwhile option to pursue in the ongoing battle against footrot.

Chapter 3: Development of a stochastic, individual-based simulation model of ovine footrot and sensitivity analysis of the completed model.

3.1 - Introduction

To fully understand endemic diseases such as footrot, and work towards long term solutions for control, genetics, epidemiology and their interaction must be considered in detail and simultaneously. Modelling has been used in a limited way to explore the potential for a reduction in footrot prevalence, particularly in the deterministic model of footrot produced by Nieuwhof *et al.* (2009). However, the complex nature of the disease has not yet been fully addressed in a simulation model.

In this study, a stochastic, individual-based, genetic-epidemiological model of footrot was developed that included sheep demography, individual host genetic effects and full flock life cycles with the following aims:

1) To evaluate the relative significance of different parameters (e.g. infection rate, bacterial survival time) on disease outcomes observed within a flock
2) To examine the effects of current control measures using different protocols
3) To determine the potential of genetic selection for resistance to footrot

In this chapter I present the structure of the model, along with some basic outcomes and a sensitivity analysis to determine how variations in parameters of unknown value affect disease outcomes. An individual-based model was chosen so that genetic variation between individuals could be explored and stochasticity was included because the flock sizes used are relatively small, meaning that rare events can be important.

The work presented in this chapter has been published in *Preventive Veterinary Medicine*, volume 108, pages 294-303.

3.2 - Materials and methods

The model description follows the ODD (Overview, Design concepts, Details) protocol for describing individual- and agent-based models as defined by Grimm *et al.* (2006, 2010).

3.2.1 - Purpose

The purpose of this model is to explore the interaction between host genetics and disease processes in footrot, by comparing the observable disease outcomes under a range of different conditions. It should allow comparisons of homogeneous and heterogeneous populations and include the effects of population structure on the outcomes of different treatment and selection strategies. Outcomes include the impact on short term disease prevalence or incidence and on the longer term population means for genetically controlled traits such as susceptibility.

3.2.2 - Entities, state variables and scale

3.2.2a - Population

The model population comprises sheep in three categories – ewes, lambs and rams. A base population of 200 ewes is simulated, with female lambs kept each year as replacements. The number of lambs born to each ewe is sampled from a Poisson distribution with mean 1.5 and a maximum number of lambs set at three. This does result in a more even spread of litter sizes between 0 and 3 than in field data (Figure 3.1) but is sufficient to approximate flock dynamics.

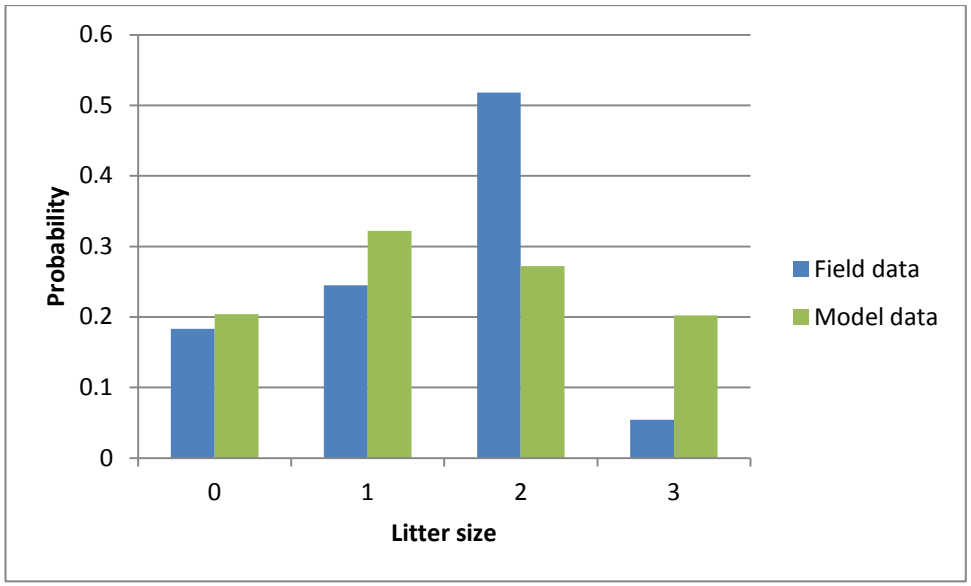

Figure 3.1. Litter sizes in field data (Wassink *et al.*, 2010) and generated in the simulation model.

Table 3.1. Sheep data determined at birth and remaining fixed for life.

Field name	Description
IDNum	Unique individual ID number
YearOfBirth	Year in which sheep was born
Dam	Dam ID number
Sire	Sire ID number
Sex	0/1 (male/female)
Susceptibility (Sus)	Applied susceptibility phenotype (≥0)
TrueSus	True susceptibility phenotype (may be <0)
GTSus	Genetic term for susceptibility
Recoverability (Rec)	Applied recoverability phenotype (≥0)
TrueRec	True recoverability phenotype (may be <0)
GTRec	Genetic term for recoverability
Revertability (Rev)	Applied revertability phenotype (≥0)
TrueRev	True revertability phenotype (may be <0)
GTRev	Genetic term for revertability

Data recorded for each ewe and lamb include genetic values which are set at birth and are dependent on parents' genotypes (Table 3.1), current status (e.g. disease state

and age) and disease history. Animal phenotype and genotype definitions are given below. Rams do not participate in any disease events and only identification numbers and genetic information used to calculate genetic values for their lambs are recorded.

3.2.2b - Host genetics

Within the population, sheep have unique genetic characteristics comprising three phenotypes - susceptibility, recoverability and revertability. Susceptibility governs the probability that a sheep will initially become infected, recoverability determines the length of time a sheep takes to recover from disease and revertability affects how quickly a sheep reverts to a susceptible state following a period of immunity.

In the records for each sheep a single genetic trait (i.e. susceptibility, recoverability and revertability) is represented by three parameters, the applied phenotype, the true phenotype and the genetic term (Table 3.1). The applied phenotype is a value ≥ 0 which is a term derived for the purposes of the model and based on the true phenotype value. It is set to a value ≥ 0 because the disease traits in this model cannot have negative values e.g. a negative susceptibility would indicate that a sheep had a negative probability of becoming infected which in real terms might equate to that sheep not only being resistant to infection but also providing protection for other sheep against infection, something that is not biologically possible in this situation.

The true phenotype is a value calculated based on a breeding value (from parents and a Mendelian sampling term), the population trait mean and a residual term as described below. This may result in a negative value. If the true phenotype is ≥ 0 it will be the same as the applied phenotype; if the true phenotype is <0 the applied phenotype is set to 0. The genetic term represents the genetic component of the true

phenotype and it is this value that contributes to the phenotypes of a sheep's offspring.

All traits with a genetic component are assumed to be polygenic, i.e. affected by variants in many genes, and under partial genetic control. Under this situation, we may assume the central limit theorem, and sample animal genotypes from a normal distribution, the variance of which is a function of the trait variance and heritability.

For each trait the phenotype, *P*, for each sheep, *i*, may be defined as comprising the following components:

$$P_i = \mu + g_i + e_i \qquad (1)$$

where μ is the trait mean in an unselected population, g_i is the genetic component (expressed as a deviation from 0) and e_i is the residual component (expressed as a deviation from 0), which is also assumed to be normally distributed.

The variance of P_i is the phenotypic variance of the input trait, denoted by σ^2P and the variance of g_i is $\sigma^2A=h^2\sigma^2P$, where h^2 is the trait heritability. Assuming that g_i and e_i, are uncorrelated, then the variance of the residuals is $\sigma^2e=(1-h^2)\sigma^2P$.

The simulation procedure was as follows. The population comprised founder animals, i.e. those without recorded or known parents and, in subsequent generations, progeny whose parents were known. Each founder animal had a genotype, or breeding value, g_i, for each genetically controlled input trait randomly sampled from a normal distribution, $N(0, \sigma^2A)$, where σ^2A is estimated as defined above. The breeding values for each trait for each progeny were constructed as $(gsire+gdam)/2$ plus a Mendelian sampling term (Falconer and Mackay, 1996). This term accounts for recombination events at meiosis and it was randomly sampled from a $N(0,$

$0.5\sigma^2 A)$ distribution (Falconer and Mackay, 1996). The residual for each trait for each animal was sampled from $N(0, \sigma^2 e)$. The phenotype for each animal was then calculated from Eq. 1 being simply the sum of the trait mean, the breeding value and the residual term. All phenotypes for the traits considered should be positive values; on the few occasions when a negative value was obtained, it was set to zero.

3.2.2c - Bacteria

Bacteria are transmitted between sheep via contaminated pasture and the model includes two parameters to account for this: ε determines the rate at which bacteria are lost from pasture as a result of bacterial death, and α is the rate of shedding of bacteria from a single infected sheep to the pasture per unit time. As values for shedding are unknown $\alpha = 1$, i.e. 1 unit is shed per sheep per day, and the number of currently infected sheep linearly determines the total rate of contamination per day. In the absence of new shedding, bacteria in the environment decay exponentially, with a mean survival time of one week assumed in the model.

3.2.2d - Time scale

Each model run represents 20 years of real time and is modelled in continuous time. The use of 20 years was decided because this represents a practical time period over which changes may be seen and in which farmers may be interested. Although further developments may be seen over longer time periods, it is more useful to be able to look at the benefits that may be seen during the working life of a farmer over a time period for which they may be willing to plan. A 20 year model run provides

short to medium term predictions for a genetic management plan, with the ability to look at it in conjunction with short term treatment and control methods.

All rates stated are per day unless otherwise specified.

3.2.3 - Process overview and scheduling

There are two categories of process in this model, fixed time and random time events.

3.2.3a - Fixed time events

This category comprises population processes and recording points. The model is assumed to start on the first day of the year, i.e. January 1st. On this date the year is updated and annual values, such as the number of infections per year, are recorded.

Lambing is modelled to occur on March 1st with all lambs being added to the model at this time. All fixed lamb values (Table 3.1) are calculated and recorded at this point, including the identity of parents and genetic values from parental genotypes. The age of the remaining ewes is also updated on this day.

On September 1st culling and slaughter occur. At this point all old ewes (aged 5) are culled. All male lambs are sent for slaughter. Enough female lambs are kept to maintain the base population of 200 ewes, with the remaining also sent for slaughter. Those sheep culled or sent for slaughter are removed from the model, although full data are retained for each removed individual for subsequent analysis.

3.2.3b - Random time events

Those events occurring at random times within the model are events representing disease processes. The footrot infection process is modelled as shown in Figure 3.2.

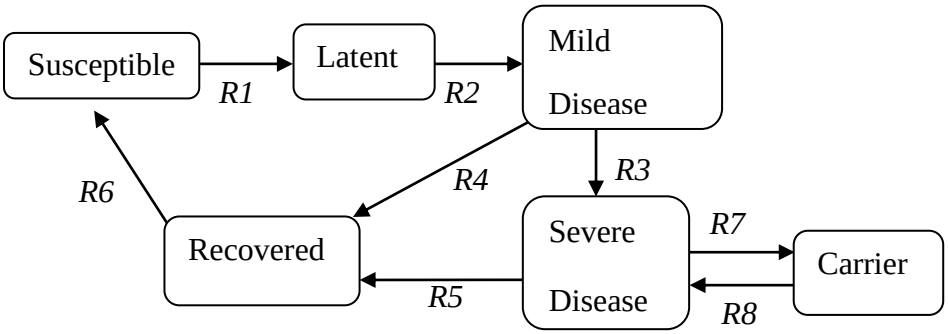

Figure 3.2. Footrot infection states. Full transition rates (R1 – R8) are given in Equations 2 – 9.

Only sheep in the states which may be classified as describing disease, i.e. those with visible clinical signs, are infectious and contribute to bacterial load in the environment. Mild and severe infection states may be considered to represent interdigital dermatitis and footrot respectively and sheep in both states contribute equally to bacterial contamination of the environment. Latently infected sheep represent the time between infection and the appearance of clinical signs.

Following extended periods with a flock completely free from disease, disease may still recur (Egerton *et al.*, 2002; Abbott & Egerton, 2008), suggesting a role for carrier sheep. Transitions between states are driven by the rates given in Table 3.2 and the resulting resting times in each state are exponentially distributed. Full rate equations determining transitions between states are given below (Equations 2 – 9). When sheep move from one state to another their disease status and disease history are updated and rate equations are recalculated to reflect the new situation.

Table 3.2. Parameter values used in the model. (FR = clinical signs of infection)

Parameter	Transition(s) affected (Figure 3.2)	Definition	Source and notes	Base value	Variation in sensitivity analysis
β	R1	Infection rate	Unknown – tested on sample model runs to determine values for base and variations.	5×10^{-5}	1×10^{-2}, 1×10^{-3}, 1×10^{-4}, 5×10^{-5}, 2.5×10^{-5}, 1×10^{-5}
ρ	R2	Rate of conversion from latent to FR (conversion rate)	Egerton, Roberts, Parsonson, 1969a, 1969b	0.14 (average duration 1 week)	constant
ψ	R3	Rate of progression from mild to severe FR (progression rate)	Beveridge 1941 (inferred: sheep recover from mild infection after ca. 2 weeks, so if not recovered by this point it is hypothesised that animals are likely to progress)	0.07 (average duration 2 weeks)	constant
γ	R4 R5	Rate of transition from FR to recovered (recovery rate)	Beveridge 1941 Roberts, Egerton, Parsonson 1969a, 1969b	0.033 (average duration 4 weeks)	constant
ζ	R6	Rate of reversion from recovered to susceptible (reversion rate)	Egerton, Roberts 1971	0.029 (average duration 5 weeks)	constant

Parameter	Transition(s) affected (Figure 3.2)	Definition	Source and notes	Base value	Variation in sensitivity analysis
ω	R7	Rate of transition from FR to carrier (carrier rate)	Treating this as the same as γ – sheep hypothesised to recover as normal, i.e. no longer show clinical signs, but harbour pockets of infection inside the hoof, becoming carriers instead of recovered sheep.	0.033 (average duration 4 weeks)	triangular distribution 0.01-0.07, peak at 0.03
φ	R8	Rate of conversion from carrier to FR (relapse rate)	Base value set to be equal to ζ, however there is no explicit measurement of this parameter.	0.029 (average duration 5 weeks)	triangular distribution 0.01-0.07, peak at 0.03
ε	R1 (indirectly due to affecting amount of bacteria in environment)	Death rate of bacteria in environment	Beveridge 1941 There is evidence that this rate varies by environment.	0.14 (average duration 1 week)	triangular distribution 0.05 – 0.5, peak at 0.14
α	R1 (indirectly due to affecting amount of bacteria in environment)	Rate of shedding of bacteria from infected sheep (shedding rate)	Unknown – using undefined units to include shedding processes (but not defining true bacterial load).	1	Constant

Parameter	Transition(s) affected (Figure 3.2)	Definition	Source and notes	Base value	Variation in sensitivity analysis
h^2	n/a	True heritability for genetically influenced traits	Unknown. The observed heritability for footrot occurrence is ca. 20%, suggesting that the true heritabilities of underlying traits are likely to be higher.	0.5	triangular distribution 0-1, peak at 0.5
σ^2	n/a	Variance of underlying genetically controlled traits	Unknown.	0.1	uniform distribution 0.01-0.50

3.2.4 - Design concepts

3.2.4a - Basic principles

Footrot is an infectious disease of sheep where bacteria are transmitted between animals via contaminated pasture. Homogenous mixing of the population of ewes and lambs is assumed, so that all sheep are equally likely to be exposed to contaminated pasture. Sheep are modelled as distinct individuals with their own unique genetic makeup that partially determines their susceptibility to and recovery from disease. These genetic traits are inherited from the sire and dam according to standard quantitative genetics principles, as described above. No specific age or sex effects are included.

3.2.4b - Stochasticity

The model is stochastic, with stochasticity incorporated into three areas of the model.

i) Genetic inheritance. A Mendelian sampling term is incorporated into the equations used to calculate a lamb's genetic trait values. This accounts for random recombination during meiosis and means that offspring with the same sire and dam have different genetic trait values.

ii) Disease events. The time between disease events (state transitions) is randomly drawn from an exponential distribution whose expected value is calculated based on the sum of the individual permissible event rates at that time point. The probability of specific events is based on the permissible state transition rates at that time point, with the precise event drawn using a random number. Finally, random numbers are also used to determine which sheep is affected by the event, based on its individual propensity for that transition.

iii) Population dynamics. The allocation of sires to lambs is determined at random, and the number of lambs born to each ewe is sampled from a Poisson distribution with mean 1.5, and a maximum number of lambs set at three.

3.2.4c - Observations

From the model we are able to make a number of observations. Full records of disease for each individual are kept and may be analysed to determine times spent in each state, and population level prevalence and incidence at any time point. The model also includes records of bacterial levels in the pasture and demographic information such as the age structure of the population over time.

3.2.4d - Explanations: heritability

Heritability is the proportion of variation in a trait that may be accounted for by additive genetics. Heritabilities are generally estimated from the outputs of variance component estimation techniques such as ANOVA or residual maximum likelihood. In this model, the heritability may be estimated for an output trait or phenotype such as the number of FR episodes. However, this phenotype may be the result of a combination of multiple underlying processes controlled by many genes, and the heritabilities of these processes are not readily measurable. The heritability of these underlying processes is referred to here as the true input heritability. In this model this may be considered as the heritability of the input traits, i.e. susceptibility, recoverability and revertability.

3.2.5 - Initialisation

Prior to running models for analysis a base population was generated. This is a population of 200 ewes that were present at the end of a 50 year simulation of the model with base parameter values. The use of this base data set minimises heterogeneity in initial conditions so that subsequent outputs may be more readily compared. Parameter values are set according to Table 3.2, as determined from published data and experimental values.

3.2.6 - Submodels

3.2.6a - Disease state transitions

The following equations give the transition probabilities for each state transition (R1 – R8, as shown in Figure 3.2).

R1: $$\beta\Sigma(S_i.Sus_i)E \quad (2)$$

where β is the infection rate, S_i is the susceptible state of sheep i (0/1), Sus_i is the susceptibility of sheep i and E is the degree of bacteria present in the environment.

R2: $$\rho\Sigma(L_i) \quad (3)$$

where ρ is the conversion rate and L_i is the latently infected state of sheep i (0/1).

R3: $$\psi\Sigma(Im_i) \quad (4)$$

where ψ is the progression rate and Im_i is the mildly diseased state of sheep i (0/1).

R4: $$\gamma\Sigma(Im_iRec_i) \quad (5)$$

where γ is the recovery rate, Im_i is the mildly diseased state of sheep i (0/1) and Rec_i is the recoverability of sheep i.

R5: $$\gamma\Sigma(Is_i.Rec_i) \quad (6)$$

where γ is the recovery rate, Is_i is the severely diseased state of sheep i (0/1) and Rec_i is the recoverability of sheep i.

R6: $$\zeta\Sigma(R_i.Rev_i) \quad (7)$$

where ζ is the reversion rate, R_i is the recovered state of sheep i (0/1) and Rev_i is the revertability of sheep i.

R7: $$\omega\Sigma(Is_i) \quad (8)$$

where ω is the carrier rate and Is_i is the severely diseased state of sheep i (0/1).

R8: $$\varphi\Sigma(C_i) \quad (9)$$

where φ is the relapse rate and C_i is the carrier state of sheep i (0/1).

b) Timesteps

The time until the next disease event occurs (timestep) is calculated as:

$$-log(RN) / \Sigma (R1:R8) \quad (10)$$

where RN is a random number sampled from a uniform distribution and $R1:R8$ are the rates calculated as above (Equations 2 – 9).

In certain circumstances these timesteps may be large and the expected time to the next disease event may result in there being fixed time events that need to happen earlier. In those cases, i.e. for lambing, culling and the start of each year, the timestep is altered so that it takes the model to the next time at which a fixed event is scheduled to occur. The model is then updated accordingly and a new timestep is calculated based on the new data.

3.2.6c - Bacterial processes

i) Addition of bacteria to the environment

$$\alpha\Sigma(Im_i + Is_i).timestep \quad (11)$$

where α is the shedding rate, Im_i is the mildly diseased state of sheep i (0/1), Is_i is the severely diseased state of sheep i (0/1) and *timestep* is the amount of time elapsed since the last event.

ii) Removal of bacteria from the environment (bacterial death)

$$\varepsilon E.timestep \quad (12)$$

where ε is the death rate of bacteria in the environment, E is the degree of bacteria present in the environment and *timestep* is the amount of time elapsed since the last event.

3.2.7 - Sensitivity analysis

The model was run for 50 years with base parameter values (Table 3.2) to obtain a population at equilibrium and this population was used as the start for each run of the model in the sensitivity analysis. The sensitivity analysis was performed using ANOVA (Saltelli *et al.*, 2008) to examine the contribution to variance of outcomes for the non-constant parameters in Table 3.2.

Four areas (represented by 6 parameters) have been identified where little or no experimental data are available and these were examined in the sensitivity analysis. These four areas are:

1. Survival time of (viable) bacteria in the environment - ε.

2. Carrier sheep properties – ω and φ determine the likelihood of sheep becoming carriers (no clinical signs and no bacterial shedding) and the rate at which they revert to an infectious state with clinical signs.

3. Host genetics - h^2 and σ^2 determine the proportion of phenotype determined by additive genetic effects and the variance of the trait of interest.

4. Infection rate - β determines the probability of a susceptible sheep becoming infected.

Distributions of parameter values (Table 3.2) were divided into five sections of equal probability and the mid-point value of each section was calculated. Using Latin hypercube sampling (LHS) these sections were sampled without replacement to give five combinations of five parameters (one LHS set) (Helton & Davis, 2003). This

was repeated four more times to give five LHS sets – a total of 25 parameter combinations. Each of these 25 parameter combinations was run with β values of 1 x 10^{-2}, 1 x 10^{-3}, 1 x10^{-4}, 5 x 10^{-5}, 2.5 x 10^{-5} and 1 x 10^{-5}, a total of 150 simulations. Each sensitivity analysis model was run with a simulated real time of 20 years, with the first ten years' data discarded to allow the system time to approach equilibrium following the change in parameters from base values.

ANOVA was used to analyse the resulting output data of the model: Disease outcomes, i.e. total number of new infections in year 20 (*numinf*), total number of lame days (mild or severe footrot) in year 20 (*tld*), and genetic outcomes, i.e. estimated heritability of number of episodes of lameness (*hepy*) in lambs (years 11-20) and estimated heritability of number of lame days (*hldpy*) in lambs (years 11-20). For disease outcomes (*numinf* and *tld*) data from the final year (year 20) were used and for genetic outcomes (*hepy* and *hldpy*) data from the final ten years were evaluated.

ANOVA models were of the following form:

$$Y = \omega + \varphi + h^2 + \sigma^2 + \varepsilon + \beta + e \qquad (13)$$

where Y is the observed outcome of interest e is the residual or error term and the other factors are input parameters as described in Table 3.2. Model fit and bias were checked.

Sire and dam effects within each individual simulation was also calculated using results from an ANOVA model of the following form:

$$Y = sire + dam + e \qquad (14)$$

where *sire* and *dam* are the two parents and *e* is the residual or error term.

Observed heritability was then calculated as:

$$Heritability = 2(Vsire + Vdam) / VP \qquad (15)$$

where *Vsire* and *Vdam* are the sire and dam variances from the ANOVA, and *VP* is the total observed (phenotypic) variance, i.e. *Vsire + Vdam + residual variance*.

All model simulations were programmed in MatLab R2008b Student and ANOVA models were performed using constrained (Type III) sums of squares.

3.3 - Results

To illustrate the types of outputs obtained and the variability between simulations, results from five model runs with base parameters (see Table 3.2), are shown in Table 3.3. Graphs are also included to show an overview of data from ten runs of the model with parameters set to base values. Figures 3.3 to 3.5 show mean values for the number of new infections per year, number of lame days per year and mean prevalence per year respectively, with error bars showing the 95% confidence intervals for each value.

A series of graphs was also plotted for Run 1 (Table 3.3), showing the number of lame days in the first year of life for sheep in the final population against the three genetically controlled traits – susceptibility, recoverability and revertability for cases where only a single trait was varied, with others fixed to 1 (Figure 3.6) or where all

traits varied simultaneously (Figure 3.7). In both cases, all three traits had highly significant ($p<0.001$) effects on the number of days sheep were lame.

Table 3.3. Outcomes from five runs of the model with parameters set to base values.

Outcome	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**
New infections in Yr20 (*numinf*)	756	810	800	909	803
New episodes in Yr20 (incl. carrier reversions)	1286	1408	1560	1375	1290
Mean episodes per infected sheep, Yr20	3.10	3.27	3.27	3.17	3.07
Median episodes per infected sheep, Yr 20	3	3	3	3	3
Inter-quartile range of episodes per infected sheep, Yr 20	2	2	2	2	2
Total lame days in Yr20 (*tld*)	27338	29367	32129	26506	28084
Mean lame days per infected sheep, Yr20	65.7	68.0	67.2	60.5	66.7
Median lame days per infected sheep, Yr 20	52.5	60	55	53.5	56
Inter-quartile range of lame days per infected sheep, Yr 20	61	57	55	51	57
Heritability of number of disease episodes in lambs (*hepy*)	0.25	0.21	0.21	0.19	0.18
Heritability of the number of lame days in lambs (*hldpy*)	0.25	0.22	0.23	0.18	0.20
Prevalence 1st January	0.20	0.16	0.22	0.20	0.22
Prevalence 1st April	0.22	0.21	0.23	0.20	0.19
Prevalence 1st July	0.26	0.30	0.23	0.29	0.26
Prevalence 1st October	0.22	0.19	0.21	0.15	0.22

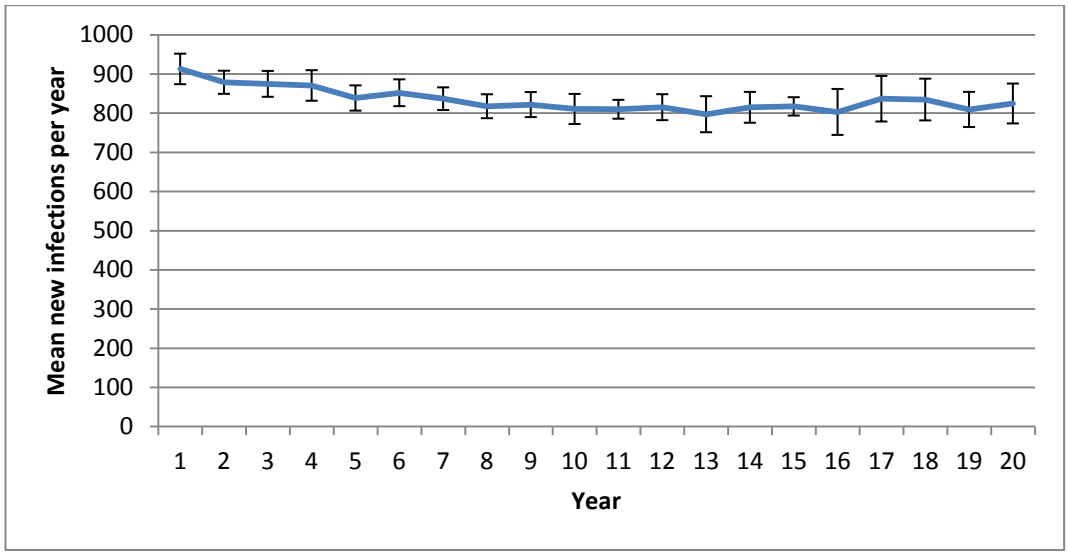

Figure 3.3. Mean new infections per year from ten base runs with 95% confidence intervals shown as error bars.

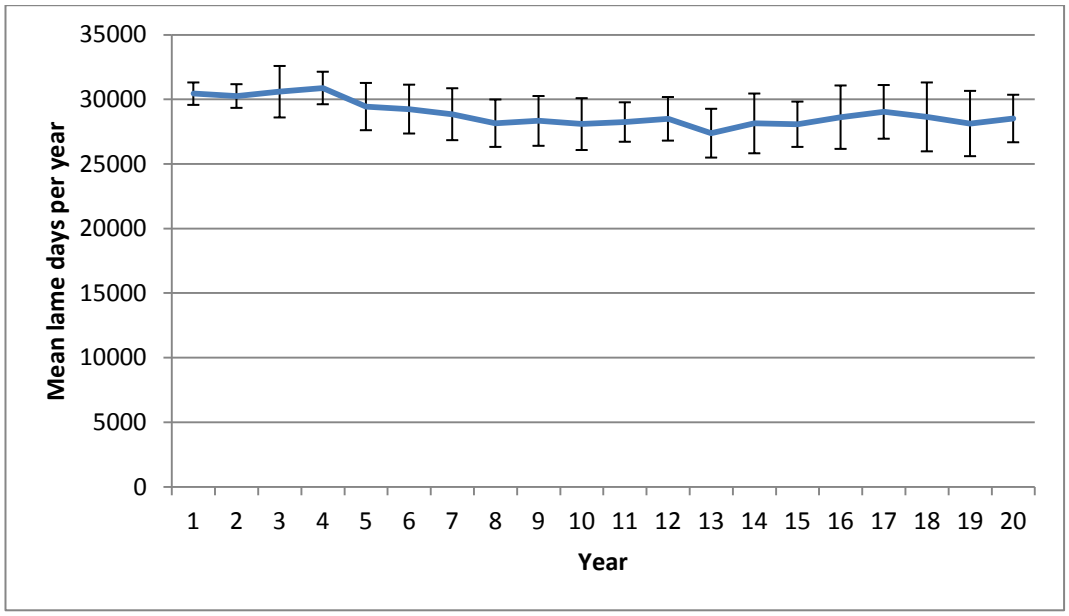

Figure 3.4. Mean lame days per year from ten base runs with 95% confidence intervals shown as error bars.

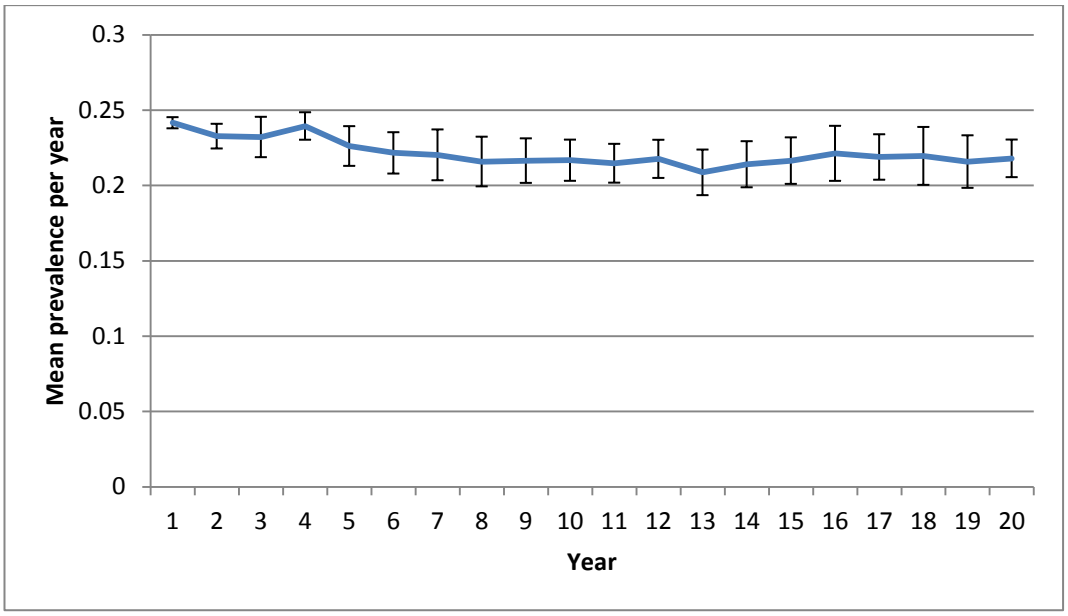

Figure 3.5. Mean prevalence per year from ten base runs, with 95% confidence intervals shown as error bars.

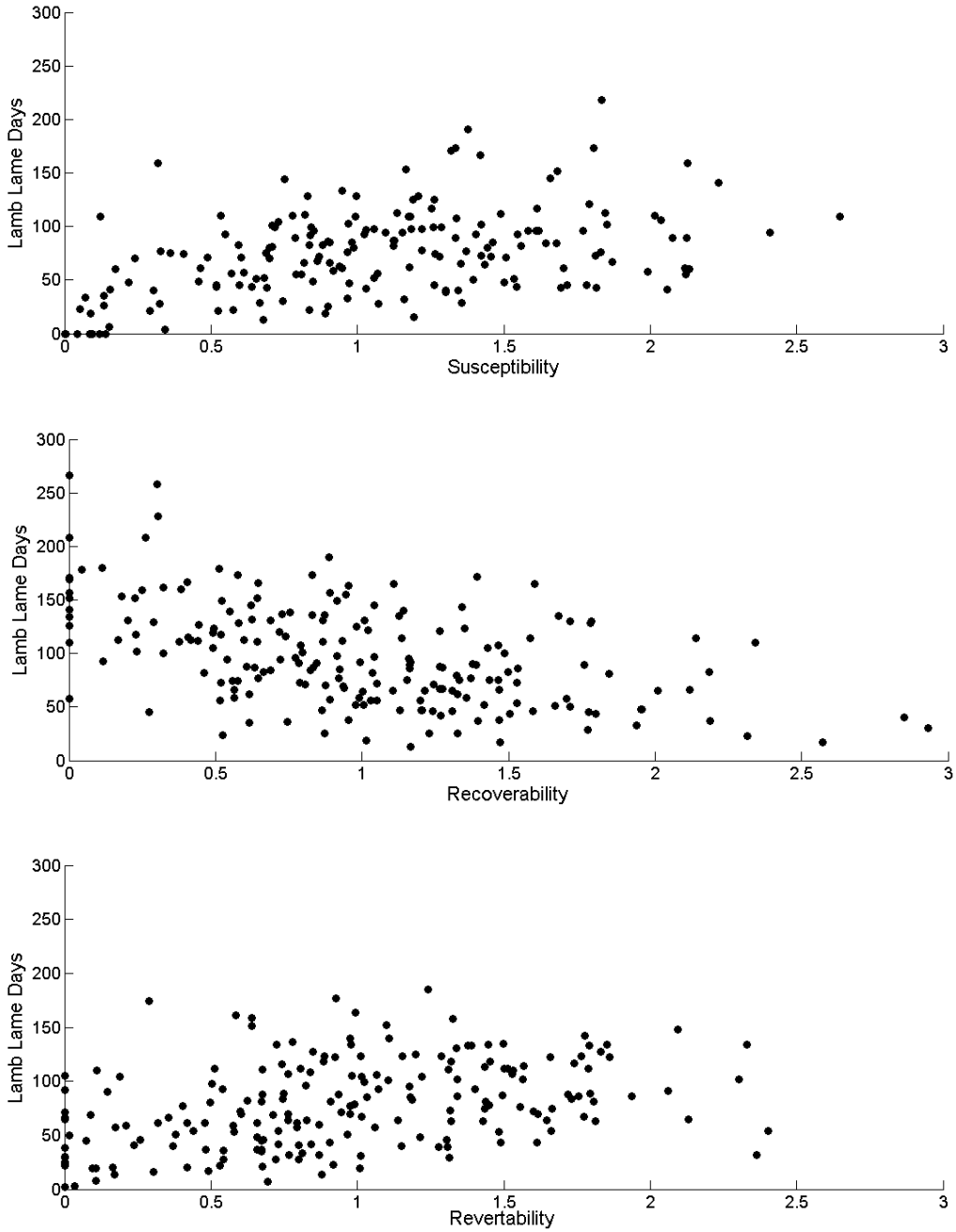

Figure 3.6. Genetically controlled traits and their effects on the number of lame days per sheep in their first year of life. Only data for sheep alive in the final population are plotted for clarity with the trait of interest varied and other traits fixed to 1.

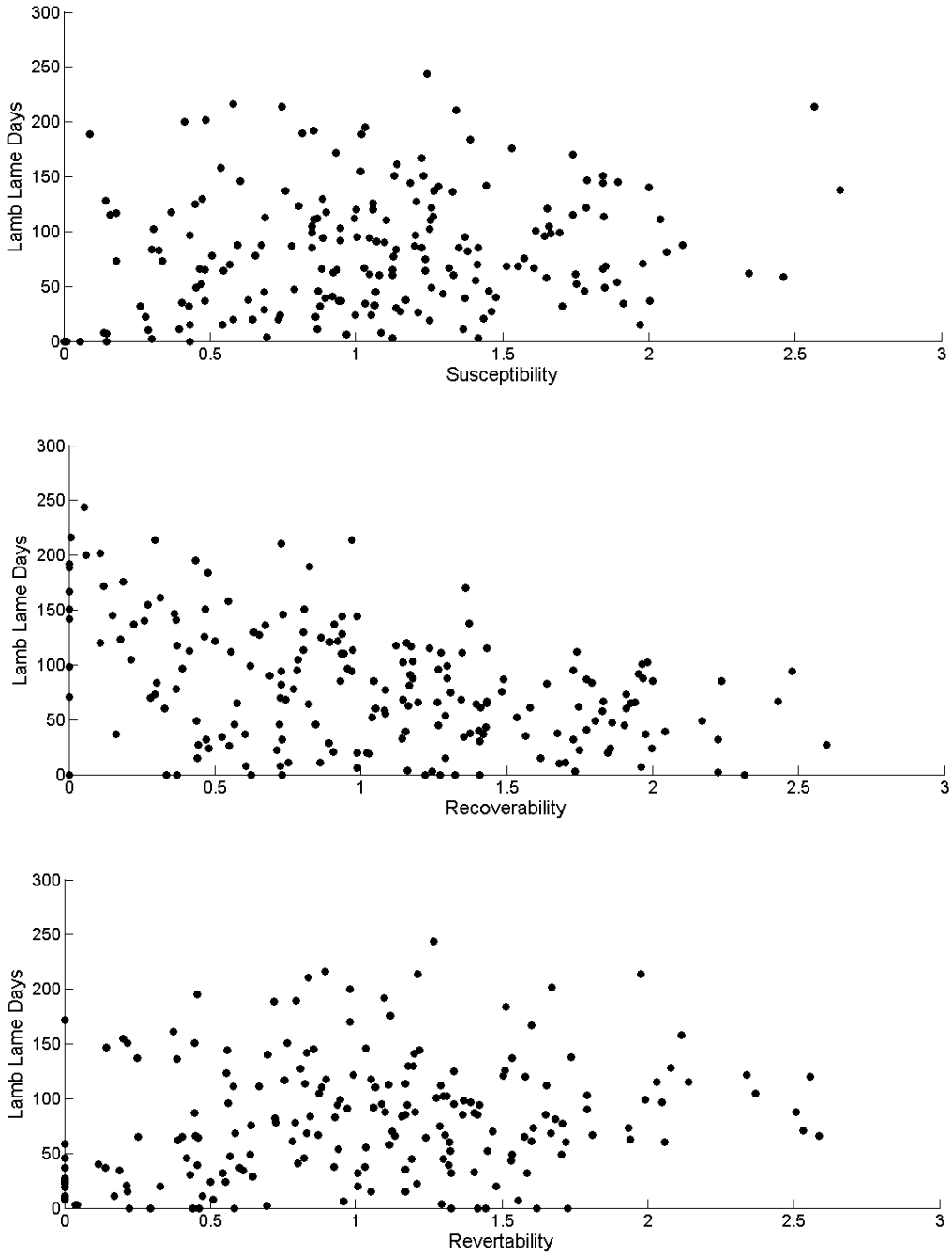

Figure 3.7. Genetically controlled traits and their effects on the number of lame days per sheep in their first year of life. Only data for sheep alive in the final population are plotted for clarity. All three traits were varied simultaneously.

In the sensitivity analysis, the ranges of the number of new infections in year 20 and the total number of lame days in year 20 are shown in Table 3.4.

Table 3.4. Ranges of number of new infections and total number of lame days per year in sensitivity analysis with differing values of infection rate β.

Infection rate β	Number of New Infections (range)	Total Number of Days Lame (range)
$1x10^{-2}$	1046 — 1635	33288 — 54871
$1x10^{-3}$	995 — 1444	32631 — 51390
$1x10^{-4}$	617 — 1133	24393 — 41675
$5x10^{-5}$	311 — 1057	9538 — 36583
$2.5x10^{-5}$	31 — 633	1027 — 28210
$1x10^{-5}$	0 — 254	0 — 10666

Note: There were 25 runs per infection rate, with different combinations of other parameters in each run.

Observed heritability for the number of lameness episodes per year ranged from 0.012 to 0.28 (mean 0.18), and for the number of days spent lame from 0.01 to 0.41 (mean 0.19).

In all ANOVA models, β was a significant factor ($p<0.01$). ε (bacterial death rate) was significant ($p<0.01$) for new infections and total number of lame days per year, and ω (carrier rate) was significant ($p<0.01$) for total lame days per year. No other factors were significant in any model. Variation in β made the greatest contribution to variation in all outcomes, with variation in ε making the second largest contribution, as shown by the magnitude of the F-values (Figure 3.8). Residuals were generally close to being distributed as expected and when residuals were plotted against fitted values no pattern of systematic bias was observed, although for heritability traits the variation in residuals tended to be larger for smaller values.

Full input and output data from the sensitivity analysis and ANOVA models are contained in Appendix A.

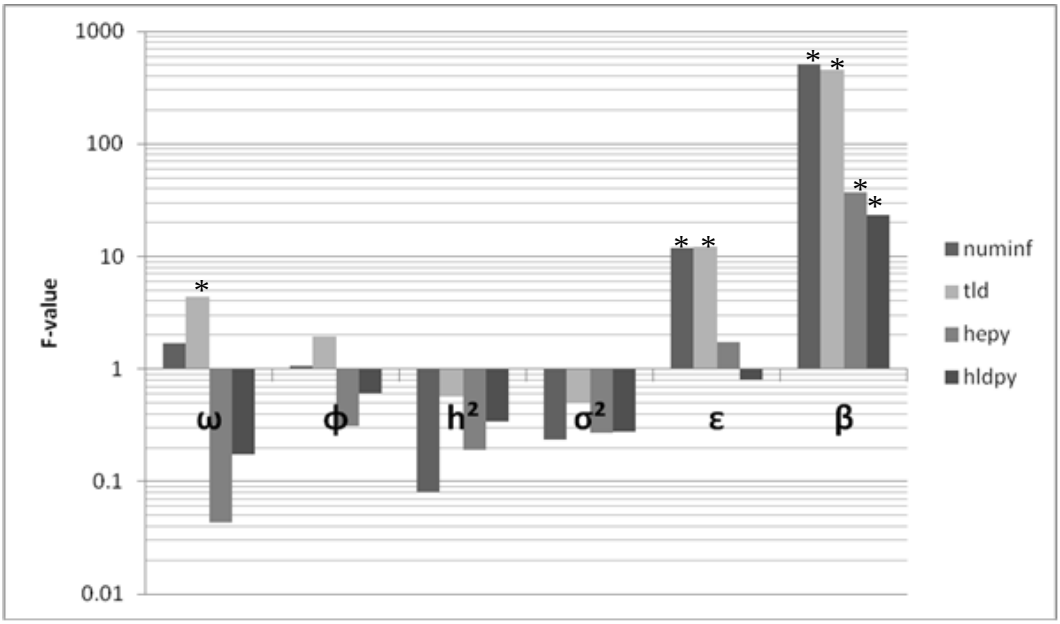

Figure 3.8. Influence of variation in input parameters on disease and host genetic outcomes, assessed by ANOVA F-values and plotted on a $\log_{10}$ scale with significant values ($p<0.01$) marked by *. *numinf* is the number of infections in the final year of simulation; *tld* is the total number of lame days in the final year of simulation; *hepy* is the heritability of the number of disease episodes in lambs; *hldpy* is the heritability of the number of lame days in lambs. Symbols are defined in Table 3.2.

3.4 - Discussion

A stochastic, individual-based model was constructed to simulate the epidemiology of footrot in a sheep flock which included genetic (heritable) processes relating to the host. The model includes four core areas that contribute to disease presentation and spread – population dynamics, host genetics, transmission of infection and bacterial dynamics in the environment. Footrot is a complex disease and there are still many

unknown variables that contribute to its epidemiology. The aims in producing this model were to investigate the interactions between host genetics and epidemiology in the presentation of footrot in a model sheep flock, and to determine the influence of parameters for which data are not available.

Prevalence seen in the model with β = 5x10-5 was about 25% (Table 3.3) which is higher than is generally seen in field data, but no treatment or control measures are currently included in the model. It is also expected that prevalence will be higher than in field data as the model has perfect recording of disease – no episodes, no matter how short the duration, are missed and all sheep are continuously observed. Finally, every episode of disease was assumed to cause lameness and this is not the situation from field data (Kaler *et al.*, 2012; Conington *et al.*, 2008). Peaks in prevalence of disease occur following the birth of lambs, which suggests that some of the seasonal dynamics seen in footrot (Wassink *et al.*, 2003; 2004) might be caused by increases in the susceptible population and density of sheep when lambs are born. The mean prevalence of disease increases asymptotically with increasing infection rate (from close to 0 at β = 1x10-5 to just over 0.3 at β = 0.1, Table 3.3), so that the lowest values used in the sensitivity analysis will create lower flock prevalences than the base values.

Figures 3.6 and 3.7 show the number of lame days in the first year of a sheep's life plotted against the three genetically influenced traits included in the model – susceptibility, recoverability and revertability. The general trends seen in these figures are that the number of lame days increase as susceptibility and revertability increase and decrease as recoverability increases. When all three traits are varied simultaneously (Figure 3.7) it should be noted that for both recoverability and revertability there are a number of points that lie on the axes because both of these

traits are dependent on a sheep becoming infected, i.e. having a susceptibility high enough to permit infection to occur, in order to be observable. Revertability is additionally dependent on recoverability because sheep need to be able to enter a recovered state before their revertability phenotypes become apparent. As recoverability and revertability are both dependent on susceptibility to be observable, it could be concluded that disease patterns are most strongly affected by susceptibility values. Figure 3.7 also illustrates the fact that even when there are clearly defined genetically influenced traits, as included in this model, outcomes are masked by individuals' experiences and thus it is difficult to accurately determine heritability because of the many layers of noise present.

During the development of this model four areas were identified where parameter values are not available (carrier sheep, survival time of viable bacteria in the environment, host genetics and infection rate). Infection rates were pre-allocated based on initial model testing to give a wide range of disease outputs. Distributions of probable values were assigned to the other parameters controlling these four areas and used to perform sensitivity analysis. There is a possibility, because only the most probable values are represented in the model, that the true values lie outwith those used in the sensitivity analysis. However, the values have been assigned using, where possible, data from published sources (Table 3.2) or estimates from field data (Wassink *et al.*, 2010), and it is likely that the true values lie within the ranges used.

The model is stochastic so, in principle, it is desirable to obtain many replications of the model with a wide range of parameter values to account for both fluctuations between runs and the wide range of possible values for unknown parameters. ANOVA was used to compensate for the limited range of values used and also to allow easy investigation of scenarios where several parameters varied

simultaneously, and in this approach the model stochasticity is contained in the residual variance. This approach reduces the need for multiple runs because effects of all parameters are considered at the same time instead of individually, whilst still accounting for variation in all parameters (Saltelli *et al.*, 2008).

Disease outcomes are more sensitive than estimated heritabilities to variation in input parameters, and estimated heritability values are affected more by infection rate ($p<0.01$) and bacterial death rate (not significant) than by variation in true heritability and genetic variance. This suggests that the infection rate and the death rate of the bacteria not only combine to drive the system but they also effectively mask the genetic components that we wish to measure. The dominance of infection parameters in determining outcomes means that it may be difficult to use observed outcomes from field data in single epidemiological scenarios to infer accurately the strength of genetic control of underlying traits describing the infection process, other than to infer that they must be heritable. This difficulty is compounded by the scenarios modelled in which there are multiple underlying traits which are genetically controlled.

One of the aims in constructing this model was to investigate the use of selection strategies to reduce the incidence and prevalence of footrot. However, currently the precondition for embarking on such a selection scheme is the value of the observed heritability (e.g. Nieuwhof *et al.*, 2008b; Conington *et al.*, 2008) and our current model results suggest this to be largely inaccurate in representing the true heritability.

Under certain model conditions heritabilities of nearly 0.3 were observed, which would suggest that in the right conditions effective selection could be achieved.

However, under other conditions heritability was very low or not possible to estimate. With field data it may be hard to infer the true strength of genetic effects because heritability estimates can vary greatly with disease prevalence, but it may still be possible to determine the sheep that show the optimal reaction to bacterial exposure, i.e. those with a high resistance phenotype. If correlations between ranked estimated breeding values calculated under different infection pressures are high then highly resistant sheep may be identified. However, if animals' ranks are very different with different infection rates it would suggest that different genotypes perform better in different environments, i.e. there isn't a single 'best' genotype to cover all environments. In such cases these genotype by environment interactions would need to be considered, making efficient selection more difficult.

Infection rate and death rate of the bacteria in the environment are clearly important values because they control a large proportion of the variance in disease outcomes. However, these are both difficult to estimate from field data, and will likely vary significantly over time and space. With respect to the bacterial death rate, soil must be examined for live bacteria and both survival and viability must be considered. In order to get values for this parameter it would be necessary to conduct infection trials to determine for how long the bacteria remained capable of causing new infections when transmitted in a natural way through contact with infected pasture, continuing the work by Whittington (1995). Infection rate is also difficult to determine experimentally because bacteria are not transmitted directly between sheep but via contaminated pasture. This raises further questions, including whether all sheep have been equally exposed to bacteria, what area of the pasture is contaminated, what the infectious dose is and which sheep are susceptible at any one time, all of which would require highly controlled conditions to answer with any accuracy.

These results indicate that the infection rate and the rate at which bacteria die in the environment are the most significant factors in the incidence and prevalence of footrot seen in sheep flocks. They also suggest that for footrot, and perhaps for other similar persistent, infections with environmental and host reservoirs, the observed/estimated heritability is not a reliable measure of the extent of genetic control of the underlying resistance traits. In chapter 5 this model is utilised to address the potential for genetic selection under these circumstances and how this potential can be assessed. However, first the model will be used to assess the effectiveness of a number of more traditional control measures to compare different protocols for currently available treatment and control strategies (Chapter 4).

Chapter 4: Modelling the impacts of epidemiological control methods on the incidence and prevalence of footrot.

4.1 - Introduction

Footrot is an infectious disease of sheep caused by the bacterium *Dichelobacter nodosus.* Due to its infectious nature, interventions targeted at reducing disease in individuals also reduce the risks for other members of the flock due to lowered exposure to the pathogen. A range of control and treatment methods is commonly used in the field to manage and reduce the prevalence of footrot in sheep flocks, including pasture rotation, foot trimming, footbathing, antibiotic treatments (topical and parenteral), selective culling and vaccination (Wassink and Green, 2001; Kaler and Green, 2009). This study simulates the impact of four of these methods - pasture rotation, selective culling, antibiotic treatment and vaccination - on footrot in a UK sheep flock.

This study focuses on the effects of individual control measures and different protocols for their use and quantifies their impact on the whole flock, including those not directly affected by the intervention. The aims were to quantify the impact of each control method and to assess the impacts of different protocols that might be used to implement each of these methods. Impacts are assessed both in terms of mean flock performance over time, in terms of footrot severity or prevalence, and also in terms of the risk, i.e. the probability of achieving certain outcomes. The latter is possible because of the stochastic nature of the model. This study does not consider interactions between measures.

4.2 - Methods

4.2.1 - The base model

All models were simulated using the base model described in Chapter 3, which was extended to include control and treatment options. To briefly recap, the model represents a flock of 200 ewes in which footrot is present, with lambing and finishing/culling events each year. Each sheep may be in one of six disease states - susceptible, latently infected, presenting mild clinical signs, presenting severe clinical signs, recovered (immune) or carrier (no clinical signs). Sheep with clinical signs shed bacteria into the environment where they survive for a mean of seven days. The model is stochastic, individual-based and run in continuous time. It also includes host genetics where three resistance traits (susceptibility, recoverability and revertability) are passed on from parents to offspring using quantitative genetics calculations to determine lamb values at birth.

For each control measure, three disease outcomes were used to measure the impact made on disease levels. These are: (1) the number of new infections per year, (2) the total number of lame days seen in the flock per year and (3) the mean prevalence per year.

Each model scenario was run ten times (five times for vaccination experiments) to obtain a range of values to be used in the analysis of the above outcomes. Ten runs with no control measures applied were also performed and used as a comparison to determine the magnitude of the effects for each control measure.

4.2.2 - Pasture Rotation

The assumption was made that all new pastures to which sheep are transferred are completely free from *D. nodosus*. Therefore, the model code specified that the variable *Environment*, representing the number of *D. nodosus* on the pasture at any given time, was set to zero at fixed time points to mimic the effects of moving sheep to new pasture free from contamination. Time intervals used for rotation were: six months (at lambing and culling), one month, fifteen days and seven days Although it is recognised that 15d and 7d are unlikely to be achievable on a real farm, these are included to aid understanding and interpretation.

4.2.3 - Selective Culling

At the annual cull in September further options were added to allow selective rather than random culling. Three criteria to score sheep with respect to footrot were explored to determine the relative effects of using different ranking methods, with animals having the highest scores being preferentially culled:

1. The number of episodes each sheep had in the last 12 months (culling by episodes)
2. The total number of days each sheep spent lame in the last 12 months (culling by lame days)
3. The number of times a sheep was seen to be diseased in the last 12 months when the whole flock was checked only on the first day of every month (culling by monthly observation)

When it was time to cull sheep (1st September) all ram lambs were first removed. Following this, the desired criterion was used to determine the worst N percent (where N is between 5 and 25) of female sheep based on their performance over the past 12 months (6 months for lambs), where N is the percentage of female sheep to be culled based on performance. This culling protocol favours the culling of ewes as they have 12 months of observations instead of the 6 available for lambs, but also ensures that any female lambs that are particularly bad are also removed from the flock. Finally, further female lambs were culled at random to reduce the population to the base value of 200 ewes if necessary.

Five different cull percentages were used to examine the effects of the above criteria, viz. 5, 10, 15, 20 and 25% of female sheep, again recognising that these might not be feasible in the field.

4.2.4 - Antibiotic treatment

It is assumed that antibiotic treatment kills the *D. nodosus* present in the hoof, reduces host inflammation promotes healing and stops a sheep from shedding infectious bacteria. Antibiotic treatment may not always be successful, but for purposes of the model we assume it halts shedding and removes infectious bacteria from the surface of the hoof, although pockets of infection may remain internally. In terms of the model structure, treated sheep are moved from an infectious to a non-infectious state and more specifically from diseased to either a recovered state or a carrier state if the infection is not fully cleared. The state to which a sheep transitions is dependent on its own *recoverability* value, as it moves to the state to which it would have naturally progressed following infection. In other words, we assume that

administration of antibiotic immediately curtails the current infectious period, but does not alter the subsequent immunological state.

Two treatment protocols were tested, either to (1) treat all diseased sheep, with both mild and severe clinical signs or (2) treat only sheep with severe clinical signs. The number of doses administered over time was recorded.

With each of these protocols it was assumed that there was a detection rate of 100% so all diseased or severely diseased sheep were treated each time the flock was observed. Different frequencies of observation were also tested, resulting in treatments being administered every day, every three days, once a week, once every two weeks and once a month. A minimum time of ten days between treatments for an individual sheep was applied to allow time for the antibiotics to act and the sheep to recover. It is recognised that some of these protocols are of theoretical interest only.

4.2.5 - Vaccination

Theoretical vaccines were administered once per year to all ewes in the flock, at the start of the year, and were considered to have one of eight different effects on each sheep's response to infection, dependent on the protocol modelled. Vaccination of lambs was not considered. The eight models were signified by the letters A to H and are described in further detail below. For each model, five runs were performed with each of four effect/effect length combinations, i.e. 90% or 50% efficacy combined with 6 or 12 month length of effect. Mean outputs from the five runs performed with a single set of parameters were used to compare results from different protocols and against base values.

The vaccine effect was considered to be directly related to the sheep's original base values for genetic traits, i.e. the vaccine efficacy is determined by the underlying host genotype. A 90% effect improved the traits by 90% of their original values. For example, a sheep with a revertability of 1 would have its peak vaccine effect at a revertability of 0.1, a 90% reduction or improvement in the base value. Similarly, a sheep with a base recoverability of 1 would have its peak vaccine effect at a recoverability of 1.9, a 90% increase or improvement in the base value. The impact of 50% or 90% effectiveness on actual transition times (in days) is shown in Table 4.1.

Table 4.1. Mean transition times between states for sheep with base values of 1 for all three traits under 50% and 90% vaccine effects.

Transition	**Mean transition times (days)**		
	Base	**50% vaccine (peak)**	**90% vaccine (peak)**
Susceptible to latent	200*	400*	2000*
Diseased to recovered	30	20	16
Recovered to susceptible	35	69	345

*These values assume 100 infectious units of bacteria in the pasture, the values vary greatly with different contamination levels.

A summary of the eight vaccination models is given in Table 4.2, and each model is described below.

Model A: it was assumed that a vaccine improved recoverability and revertability, i.e. sheep recovered more quickly and remained immune for longer. The values for recoverability and revertability were improved over 14 days following vaccination until they reached the maximum vaccine effect. After this point the values gradually reverted each day in a linear fashion towards the sheep's base genetic values for the remainder of the vaccine effect length.

Table 4.2. Vaccine assumptions used.

	Direct Effect		Maternal Effect			
Model	Recov. & Revert.	Suscept.	Recov. & Revert.	Suscept.	Sustained effect	Residual effect
A	X					
B	X	X				
C	X		X			
D	X	X	X	X		
E	X				X	
F	X	X			X	
G	X					X
H	X	X				X

Model B: the same protocol as Model A except that susceptibility was also affected. This means that in addition to recovering more quickly and remaining immune for longer, the sheep were also less likely to become infected. Susceptibility follows the same pattern as revertability, decreasing to a peak vaccine effect for the first 14 days and then reverting gradually back to base values for the remainder of the vaccine effect length.

Model C: the same protocol as Model A but with the addition of a maternal immunity effect on recoverability and revertability for the first two months (60 days) of a lamb's life, passed on from vaccinated dams. Maternal effects were coded using the same pattern of effects as vaccination but over a shorter duration. Recoverability and revertability values were increased for the first two days of a lamb's life, reaching a peak that is the same as the vaccine effect (i.e. 50% or 90% improvements on base values). After this time, the values reverted gradually to base values in a linear fashion for the remainder of the 60 day effect.

Model D: the same protocol as Model B but with the addition of maternal effects which were implemented as described in Model C. Maternal effects were assumed to occur for all three trait values, recoverability, revertability and susceptibility.

Model E: the same protocol as Model A except that the full vaccine effect was sustained until the end of the vaccine effect period i.e. there is no gradual reversion phase. This is to examine the impact of vaccination on disease if a high level of immunity can be maintained, for example with repeated booster doses of the vaccine. As the vaccine cannot be administered immediately prior to or following lambing (MSD Animal Health), the time when footrot is at its peak prevalence, this also allows us to investigate whether having a full vaccine effect at peak prevalence would reduce disease. Base values of recoverability and revertability were restored after the vaccine effect length was reached.

Model F: the same protocol as Model B except that the full vaccine effect was sustained until the end of the vaccine effect length as in Model E above. Base values of recoverability, revertability and susceptibility were restored after the vaccine effect period was concluded.

Model G: the same protocol as Model A except that a residual effect of 50% or 25% of the vaccine effect remained after the vaccine effect had worn off. This means that during the second phase of vaccine effect where the effect is wearing off, the effects gradually revert to a point that is better than the sheep's own base value. For example if a 90% vaccine effect was used with a 50% residual effect the effects would revert to a value 45% better than the sheep's base value. Similarly using a 50% vaccine effect with a 25% residual effect would result in a reversion to a trait value that was

12.5% better than the sheep's own base value i.e. the sheep were permanently more protected.

Model H: the same protocol as Model B, except that residual effects remain after the primary vaccine effect has worn off. This is described in the Model G protocol.

4.2.6 - Outcomes

Model results were evaluated using mean disease outcomes per year. These were used to compare the effects of different strategies within control methods, e.g. pasture rotation every week versus every fortnight, and also to compare the effects of different methods, e.g. vaccination versus selective culling. Additionally outcomes from each of the ten repeated simulations per method were compared with each of ten base run outcomes in a pairwise manner. The results from this were used to give probabilities of different magnitudes of benefit using the different strategies investigated in this study.

4.3 - Results

4.3.1 - Pasture rotation

There was a decrease in new infections (Figure 4.1), total lame days and prevalence of lame sheep as the rate of pasture rotation increased, with patterns seen being very similar. Weekly pasture rotation resulted in a mean decrease of 31.6 - 52.7% in new infections, 30.8 - 49.9% in the total number of lame days and 28.4 - 47.6% in mean annual prevalence when compared with a base run with no control measures applied. Rotating pasture every 15 days gave slightly smaller improvements in comparison to

the base values, with mean decreases of 11.8 - 27.9% in new infections, 12.9 - 23.4% in lame days and 13.1 - 22.7% in mean yearly prevalence. A small improvement was seen with monthly rotation, with mean decreases of up to 17.1% in new infections, up to 12.4% in total lame days and up to 11.8% in mean prevalence. No clear effects were observed when six monthly pasture rotation was applied. Effects remained approximately constant over time, meaning this method does not have a cumulative effect. Variability between simulations (flocks) was not changed by the use of pasture rotation.

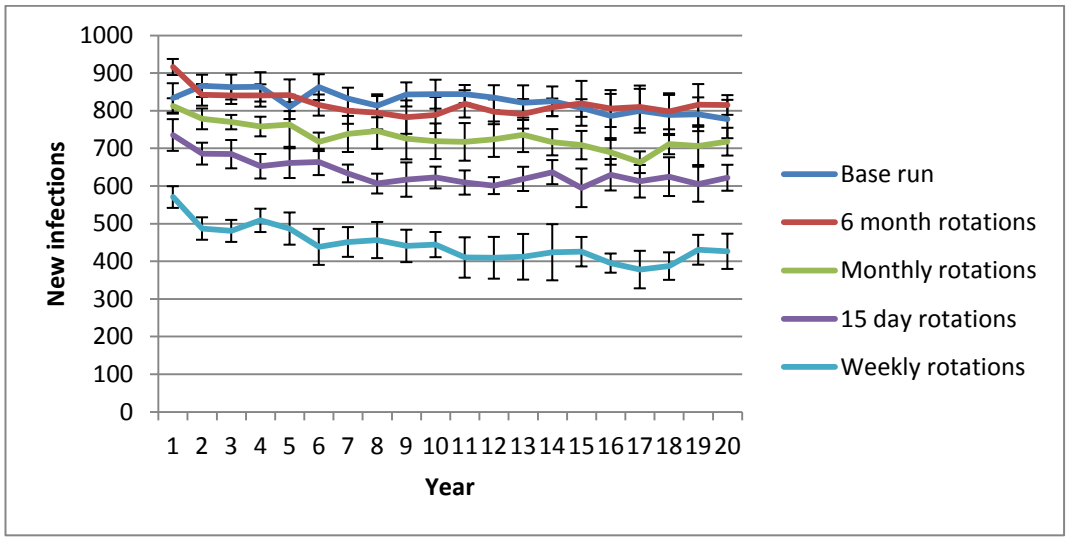

Figure 4.1. Mean number of new infections per year by frequency of pasture rotation, obtained from ten model runs per scenario. Error bars show 95% confidence intervals.

To give a better idea of variation between runs, the number of new infections in year ten, from ten runs of each pasture rotation model was also plotted (Figure 4.2)

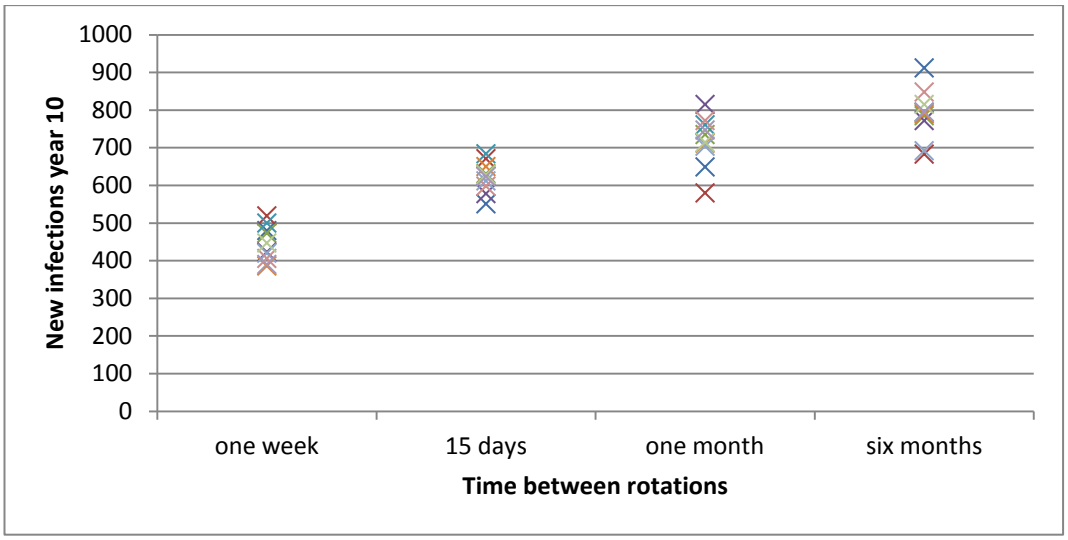

Figure 4.2. New infections in year ten from ten runs of each pasture rotation model.

4.3.2 - Selective culling

The numbers of female sheep culled per year for the 5-25% culling protocols are shown in Table 4.3, which includes variation between runs (flocks) and between years in the same run. Variation in numbers of female sheep culled is due to the variability of number of female lambs born per year.

Table 4.3. Number of female sheep selectively culled for each culling percentage used.

Cull percentage	Number of female sheep selectively culled
5	13-17
10	28-34
15	41-52
20	53-70
25	68-88

Reductions in disease following selective culling were gradual, continually decreasing for a number of years before eventually reaching a relatively stable value, as shown in Figure 4.3.

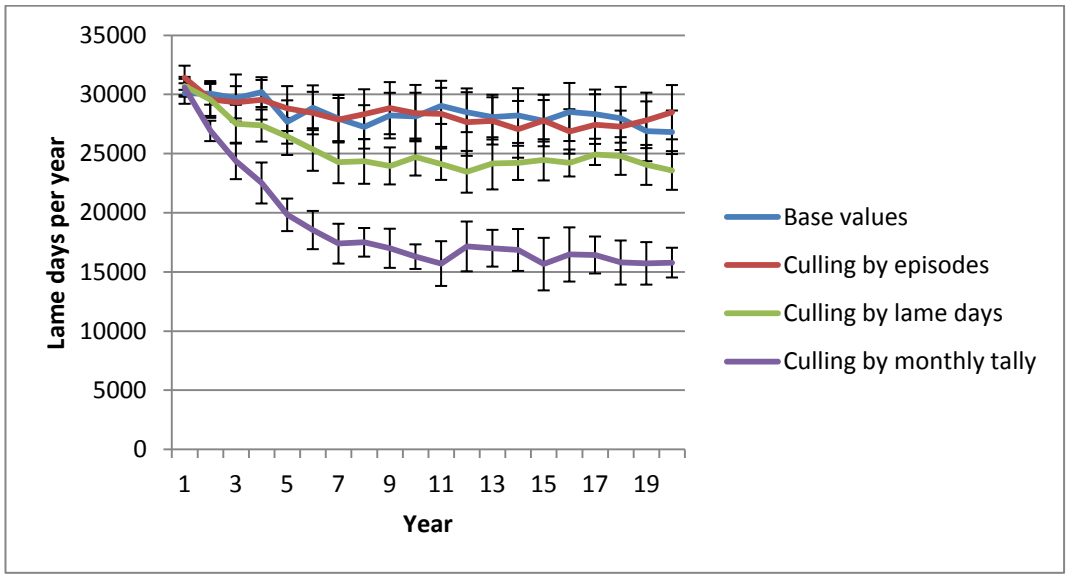

Figure 4.3. Mean total lame days per year when culling 15% of female sheep using three different selection criteria. Error bars represent 95% confidence intervals.

When using the same selection criterion, greater reductions in disease were seen when larger proportions of female sheep were culled. For example, when culling by monthly tally each increase of five percent in the culling amount resulted in a 10-16% improvement in mean annual prevalence when compared with values from the base mean with no control measures (Figure 4.4).

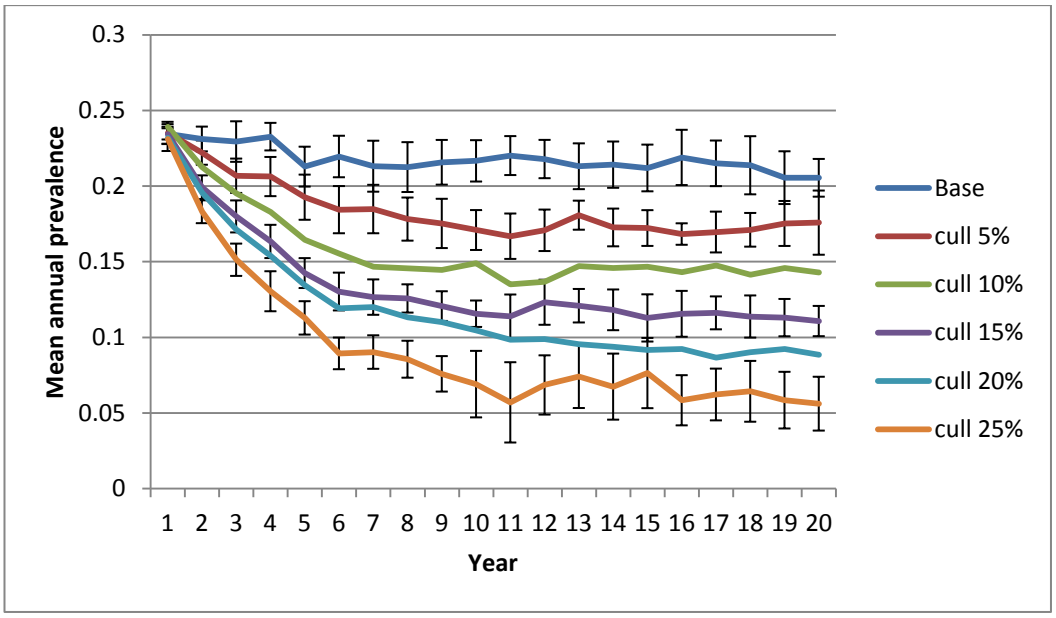

Figure 4.4. Mean prevalence of footrot over time when culling different percentages of female sheep using monthly observation data. Error bars show 95% confidence intervals; these are shown only on a selection of data for clarity.

The different culling protocols resulted in different outcomes with respect to the number of lame days and the number of new infections. Table 4.4 shows a sample of these using data from years five and ten, taken from ten repeated model runs.

At year five, progress was still being made, while by year ten disease prevalence and incidence had approximately levelled out. The numbers of lame days and new infections shown in Table 4.4 highlights the fact that although mean values decreased steadily with increasing cull percentages, there was considerable overlap in actual values given the variation between repeated runs.

Table 4.4. Range of disease outcomes in years 5 and 10 with different culling strategies (ten model runs per strategy).

Cull criterion	Percentage culled	New infections Yr 5 (mean)	New infections Yr 10 (mean)	Lame days Yr 5 (mean)	Lame days Yr 10 (mean)
No culling (base)	0	741-900 (839)	721-925 (811)	26503-34877 (29445)	23963-33131 (28089)
Number of footrot episodes	5	687-871 (782)	687-850 (756)	28846-35495 (31159)	25176-34457 (30193)
	10	657-796 (704)	581-696 (653)	24357-32578 (29329)	24913-30069 (27268)
	15	556-781 (690)	522-709 (606)	25109-33264 (28809)	22972-32082 (28443)
	20	547-691 (611)	480-577 (528)	25260-34634 (28509)	23559-30793 (26704)
	25	542-666 (583)	396-554 (461)	24795-30980 (27954)	21700-36825 (25782)
Number of lame days	5	712-848 (797)	674-830 (732)	25867-30216 (28217)	23839-30481 (26000)
	10	710-883 (790)	665-841 (735)	24087-30573 (27069)	22535-31139 (26210)
	15	671-843 (748)	574-800 (709)	22845-28985 (26450)	19614-27314 (24711)
	20	634-793 (712)	570-713 (626)	21380-29312 (25016)	20342-26263 (22397)
	25	524-783 (665)	459-629 (572)	16121-30392 (24600)	13739-25421 (20329)
Monthly observation score	5	652-910 (815)	696-833 (755)	21639-28846 (25520)	20842-27222 (23280)
	10	691-936 (794)	607-850 (732)	19885-25981 (22643)	16422-25815 (20381)
	15	605-855 (738)	531-696 (635)	17580-22628 (19826)	13159-18317 (16293)
	20	528-810 (694)	516-684 (597)	14375-24488 (18842)	12337-16630 (15059)
	25	465-713 (630)	10-636 (408)	11349-19560 (16582)	226-15875 (10106)

To address the issue of overlap and to determine the probability of benefit over base values using each run, complete pair-wise analysis was used, comparing each base run value with each run using selective culling. Table 4.5 shows the probability of benefit (i.e. of achieving a reduction in lame days or new infections in comparison with base values) in years five and ten, along with the probability (in brackets) of achieving a reduction in new infections or lame days of over 25% in comparison with base values.

It should be noted that the outcomes seen with this method, comparing ten intervention runs with each of ten base runs, would not give the same outcome as sampling 100 different outcomes from interventions and comparing these against 100 different base run outcomes. The resulting probability distribution from the 10x10 method would be flatter and wider than with 100 different outcomes, so care must be taken when using these results. However, with the limited amount of data available this method is preferable to simply having ten individual comparisons of randomly selected pairs of outcomes.

Culling based on monthly observations had the greatest impact on disease outcomes, with maximum values of more than double those of the second best results, seen with culling by lame days, for both total lame days and mean annual prevalence. A possible explanation for this result is that using monthly observation data all animals are measured for disease under the same conditions. This removes environmental variations, for example in infection pressure so all sheep are compared under equal probability of infection.

Table 4.5. Probability of reduction in new infections and lame days in years 5 and 10 using different culling strategies, along with probabilities that the reduction is greater than 25% of base values.

Cull criterion	Percentage culled	Probability of reduction in new infections Yr 5 (>25% reduction)	Probability of reduction in new infections Yr 10 (>25% reduction)	Probability of reduction in lame days Yr 5 (>25% reduction)	Probability of reduction in lame days Yr 10 (>25% reduction)
Number of footrot episodes	5	0.79 (0)	0.82 (0.01)	0.3 (0)	0.27 (0)
	10	0.98 (0.04)	1.0 (0.21)	0.45 (0.02)	0.6 (0)
	15	0.97 (0.20)	1.0 (0.51)	0.57 (0.02)	0.44 (0.03)
	20	1.0 (0.63)	1.0 (0.97)	0.63 (0.02)	0.61 (0.03)
	25	1.0 (0.79)	1.0 (0.99)*[1]	0.67 (0.01)	0.76 (0.12)
Number of lame days	5	0.74 (0)	0.91 (0.03)	0.64 (0)	0.74 (0.05)
	10	0.74 (0)	0.83 (0.02)	0.76 (0.04)	0.68 (0.06)
	15	0.9 (0.01)	0.92 (0.09)	0.82 (0.07)	0.84 (0.09)
	20	0.96 (0.08)	1.0 (0.32)	0.91 (0.18)	0.92 (0.38)
	25	0.99 (0.25)	1.0 (0.68) *[2]	0.88 (0.2)*[3]	0.99 (0.53)*[4]
Monthly observation score	5	0.57 (0.02)	0.83 (0)	0.85 (0.17)	0.88 (0.26)
	10	0.68 (0)	0.82 (0.07)	1.0 (0.44)	0.94 (0.59)*[8]
	15	0.91 (0.08)	1.0 (0.29)	1.0 (0.8)	1.0 (0.98)*[9]
	20	0.97 (0.22)	1.0 (0.56)	1.0 (0.84)*[6]	1.0 (1.0)*[10]
	25	1.0 (0.38)	1.0 (0.92)*[5]	1.0 (1.0)*[7]	1.0 (1.0)*[11]

Note: starred values also had non-zero probabilities of achieving a reduction of more than 50% compared with base values. Probabilities were: *[1] - 0.19; *[2] - 0.01; *[3] - 0.01; *[4] - 0.07; *[5] - 0.45 (P>75% reduction - 0.10); *[6] - 0.08; *[7] - 0.19; *[8] - 0.01; *[9] - 0.10; *[10] - 0.26; *[11] - 0.78 (P>75% reduction - 0.12).

4.3.3 - Antibiotic treatment

The impact of antibiotic treatment varied greatly according to the protocol used, and for some protocols it almost entirely eliminated the disease. When all diseased sheep were treated once a week or more frequently, the number of new infections was

reduced to below 50 new infections per year (Figure 4.5) and the total number of lame days per year was reduced to less than 200 (Figure 4.6).

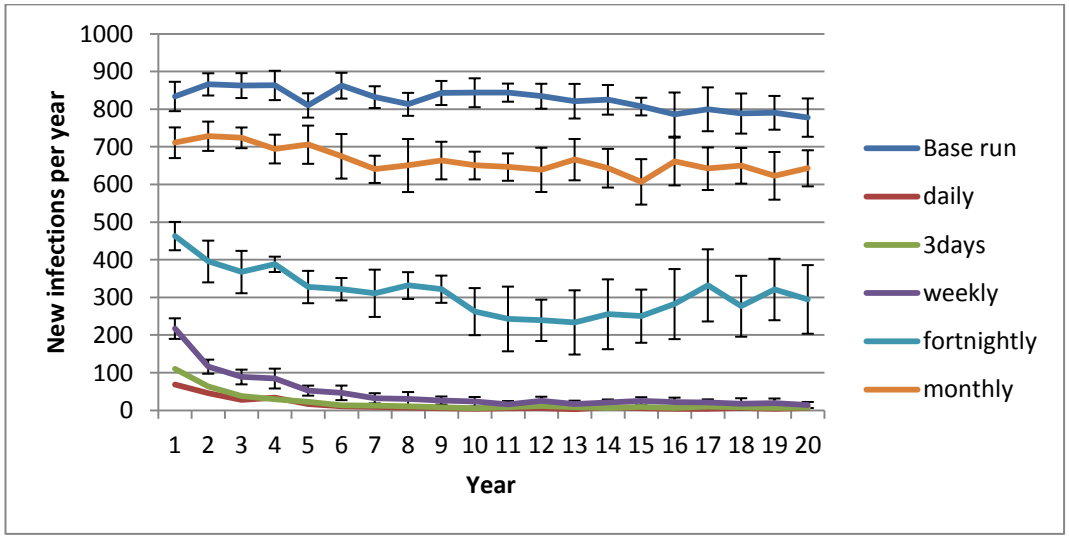

Figure 4.5. New infections per year when treating all diseased sheep with antibiotics at different intervals. Error bars represent 95% confidence intervals (not shown on daily or 3days lines for clarity).

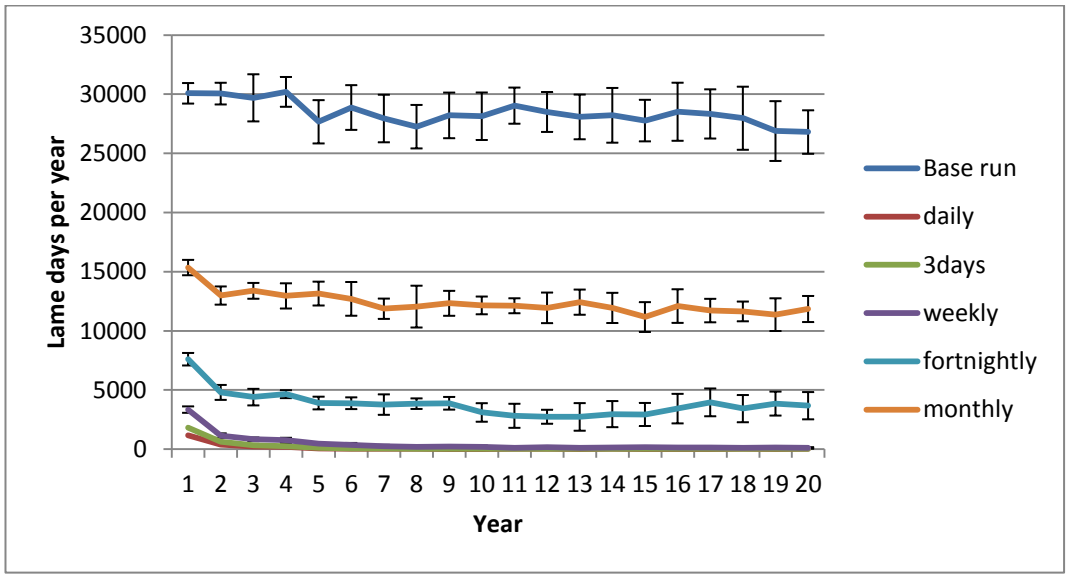

Figure 4.6. Lame days per year when treating all diseased sheep with antibiotics at different intervals. Error bars represent 95% confidence intervals (not shown on daily or 3days lines for clarity).

In contrast, if only severely diseased sheep were treated, even when treating daily, the number of new infections did not fall below 300 (Figure 4.7) and lame days remained greater than 4000 per year (Figure 4.8).

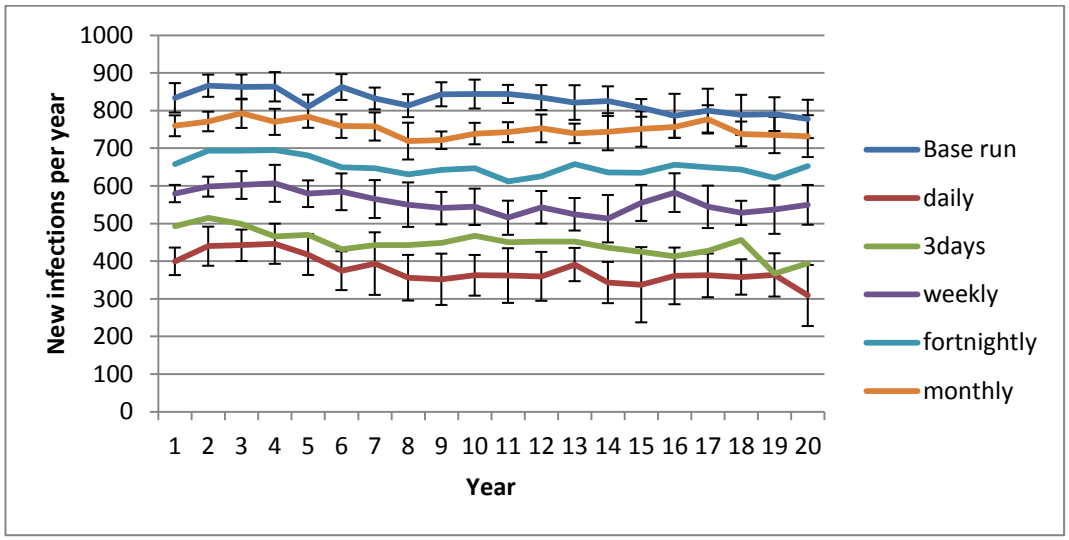

Figure 4.7. New infections per year when treating severely diseased sheep with antibiotics at different intervals. Error bars represent 95% confidence intervals (not shown on all data for clarity).

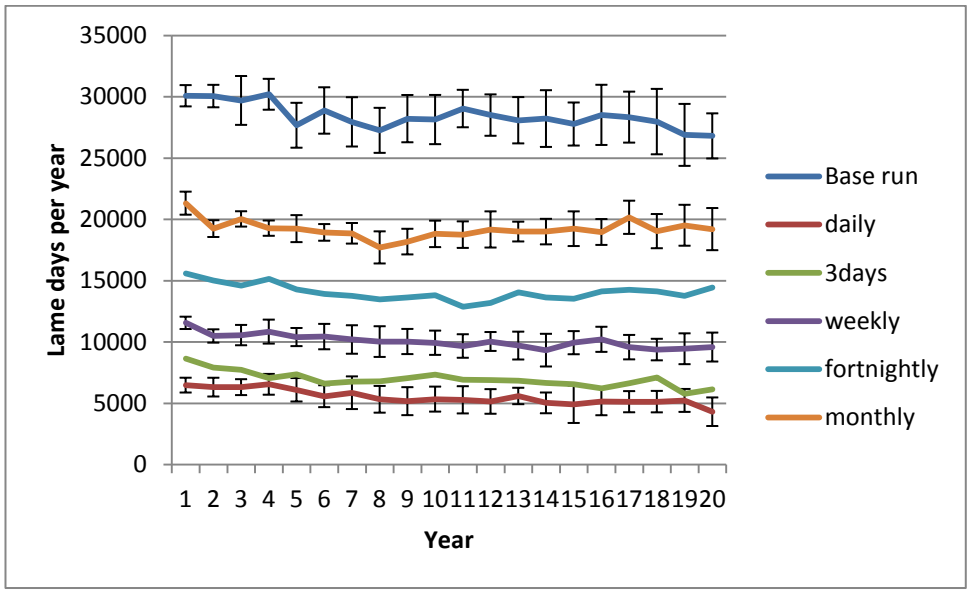

Figure 4.8. Lame days per year when treating severely diseased sheep with antibiotics at different intervals. Error bars represent 95% confidence intervals (not shown on all data for clarity).

There was a consistent reduction to a mean annual prevalence of < 0.05 with treatment of all sheep affected with footrot fortnightly or more frequently. When only treating severely diseased sheep daily treatment was required to consistently reduce prevalence to 0.05, although on occasion treatments of severely diseased sheep every three days also gave a mean annual prevalence of below 0.05.

It is also interesting to consider the mean doses of antibiotic administered per year when treating all diseased sheep (Figure 4.9) and treating only severely diseased sheep (Figure 4.10).

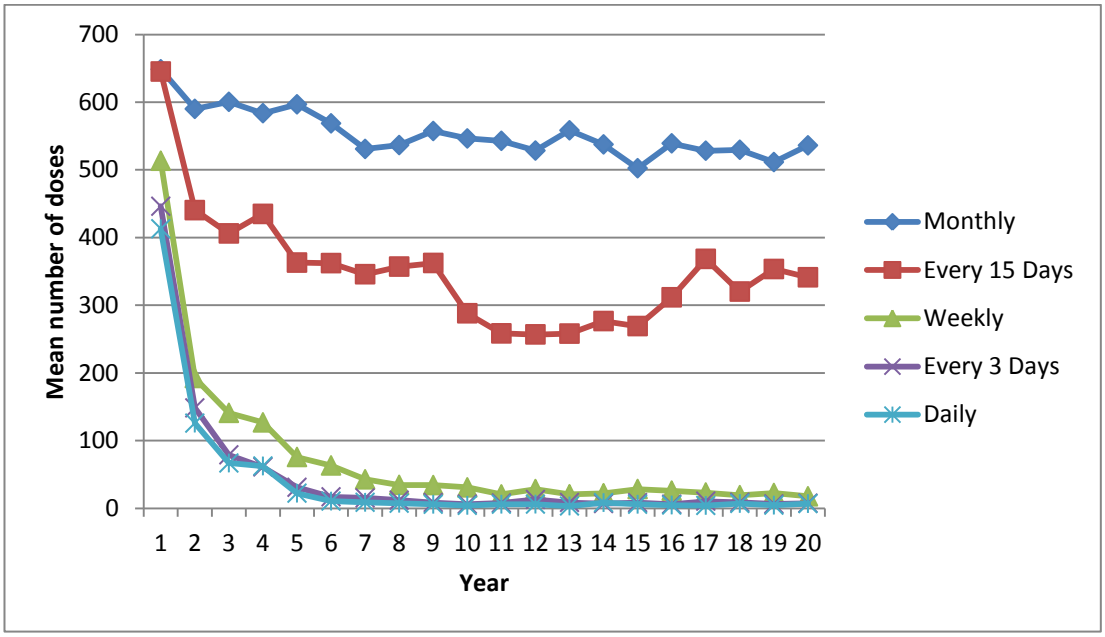

Figure 4.9. Mean doses of antibiotic administered per year when treating all diseased sheep at different intervals.

When treating all diseased sheep at least once a week the number of doses of antibiotic needed rapidly reduced to less than 50 doses per year. When only severely diseased sheep were treated with antibiotics the number of doses remained high, with

all treatment intervals requiring, on average, more than 400 doses per year. Treating all sheep at more frequent intervals resulted in a much lower total number of treatments over time when a twenty year period for each treatment protocol was simulated (Table 4.6).

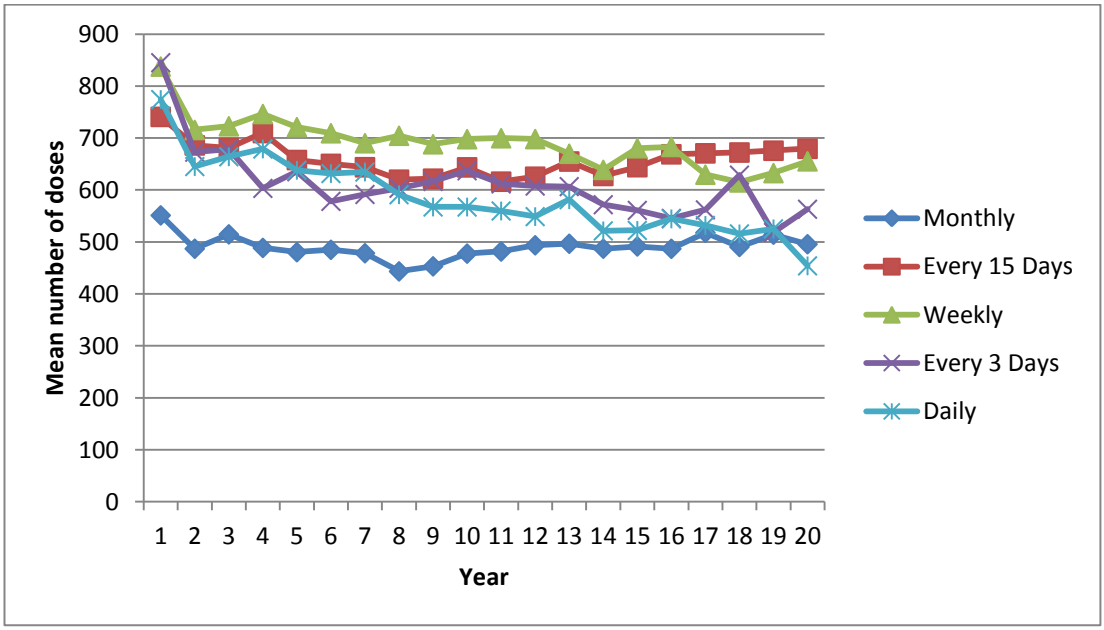

Figure 4.10. Mean doses of antibiotic administered per year when treating severely diseased sheep at different intervals.

Table 4.6. Mean total doses of antibiotic administered over 20 years with different treatment protocols.

	Mean total doses administered over 20 years	
Treatment interval	**Treat all diseased sheep**	**Treat severely diseased sheep**
Daily	783	11700
Every 3 days	907	12240
Weekly	1483	13837
Every 15 days	7018	13191
Monthly	11072	9817

4.3.4 - Vaccination

4.3.4a - Model A

Vaccination using protocol A had low impact on disease outcomes even when using vaccines with 90% effect and 12 month effect length. Mean prevalence following vaccination according to Model A is shown in Figure 4.11. The pattern of new infections and total lame days was similar. It is believed that the small effects of vaccination are due to the high density of susceptible lambs post-lambing, coupled with waning vaccine effects in ewes. If even a small amount of contamination remained on the field at lambing then new lambs quickly become infected and the contamination, and prevalence of footrot, rises. Further vaccine models were explored (below) to examine this.

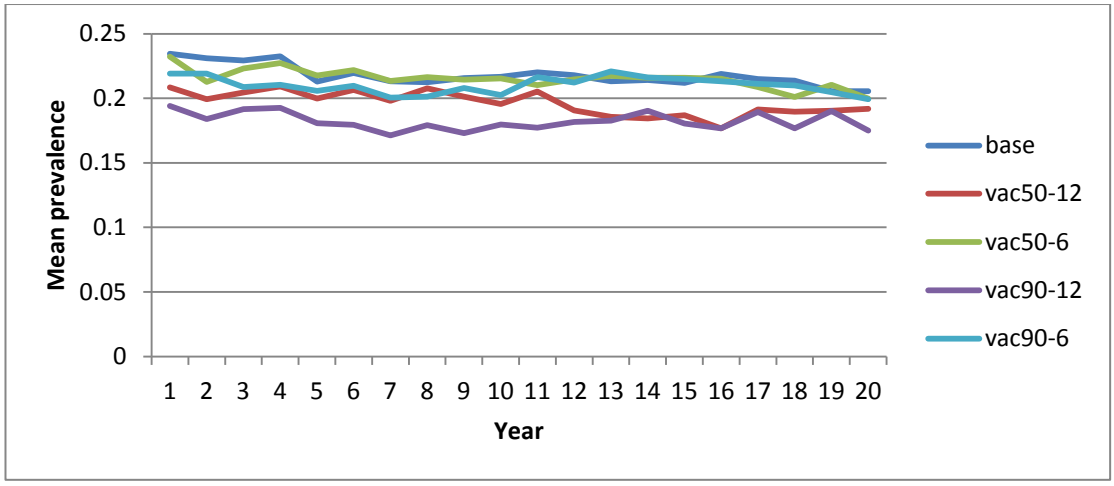

Figure 4.11. Mean prevalence following vaccination with different strengths of vaccine according to Model A protocol. base: no vaccination; vac50-12: vaccine effect 50%, vaccine effect length 12 months; vac50-6: vaccine effect 50%, vaccine effect length 6 months; vac90-12: vaccine effect 90%, vaccine effect length 12 months; vac90-6: vaccine effect 90%, vaccine effect length 6 months.

4.3.4b - Model B

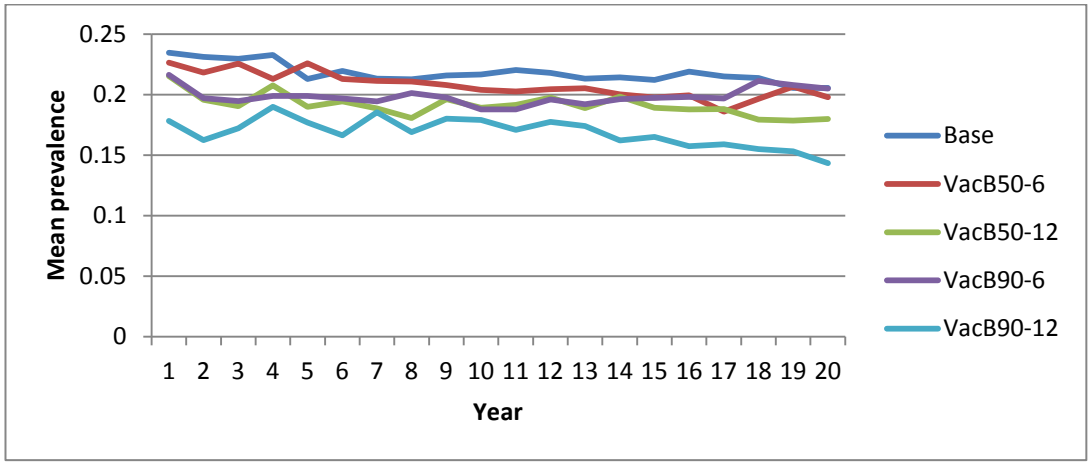

Figure 4.12. Mean prevalence following vaccination with different strengths of vaccine according to Model B protocol. Base: no vaccination; VacB50-6: 50% vaccine effect, 6 month vaccine effect length; VacB50-12: 50% vaccine effect, 12 month vaccine effect length; VacB90-6: 90% vaccine effect, 6 month vaccine effect length; VacB90-12: 90% vaccine effect, 12 month vaccine effect length.

When susceptibility was included in the vaccine effect the impact on disease outcomes was slightly improved but only when using the 90% 12 month vaccine. Mean prevalence following vaccination according to Model B protocol is shown in Figure 4.12. The patterns of new infections and total lame days were similar.

4.3.4c - Model C

The addition of a two month maternal effect to the Model A protocol had no significant effect, with outcomes very close to those seen using the Model A protocol.

4.3.4d - Model D

The addition of a two month maternal effects period to the Model B protocol improved the vaccine impact slightly resulting in a more marked difference between the base values and the vaccinated values except for the 50% 6 month vaccine which still shows no effect. The 90% 12 month vaccine showed the greatest impact on disease prevalence, reducing the mean prevalence to ~0.14.

4.3.4e - Model E

In Model E the effects of the vaccine were maintained at full strength for the duration of the vaccine effect length. The impact on disease outcomes were more pronounced than in previous models, with the prevalence reduced to approximately 0.12-0.13 from the base of approximately 0.22.

The number of new infections was reduced to just over 500 (base just over 800) and the total lame days per year were reduced from ~28000 to ~17500.

4.3.4f - Model F

With the Model F approach, where again full effects were sustained, using a vaccine with a 90% effect and 12 month effect length, mean prevalence was reduced to ~0.1 (Figure 4.13), new infections were reduced to ~400 and lame days per year were reduced to ~15000.

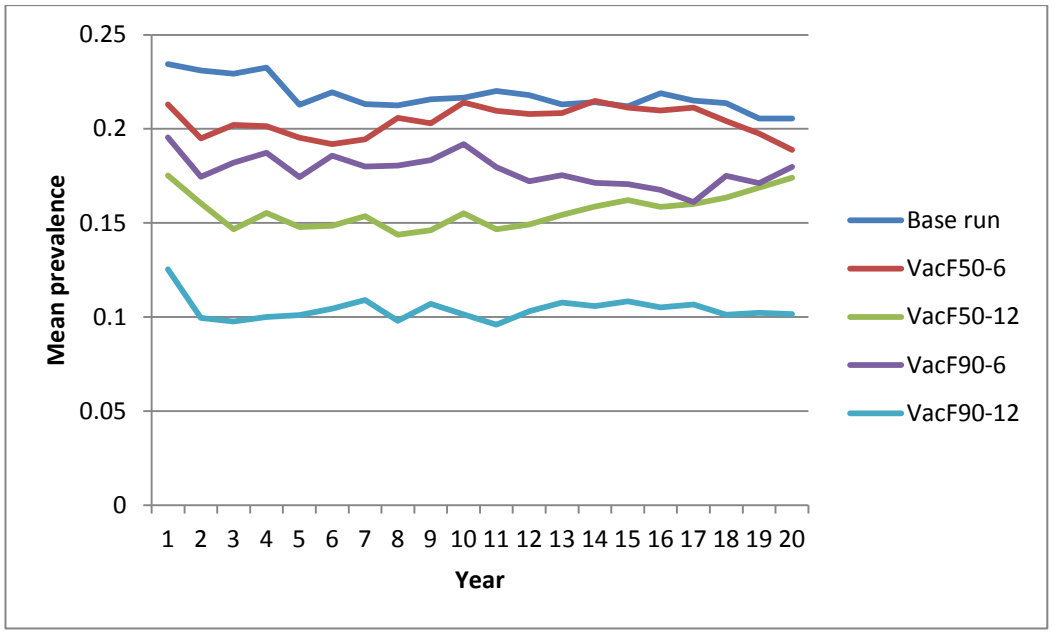

Figure 4.13. Mean prevalence following vaccination with different strengths of vaccine according to Model F protocol. Base run: no vaccination; VacF50-6: 50% vaccine effect, 6 month vaccine effect length; VacF50-12: 50% vaccine effect, 12 month vaccine effect length; VacF90-6: 90% vaccine effect, 6 month vaccine effect length; VacF90-12: 90% vaccine effect, 12 month vaccine effect length.

These are the largest effects seen with any vaccine protocol for all three disease outcomes measured and constitute an improvement of approximately 50% on base values where no vaccination is administered. In this model the vaccine effect is sustained at peak value so there is no waning immunity at lambing, thus helping to keep infections in ewes at a lower level.

4.3.4g - Model G

Vaccination using Model G protocol with residual vaccine effects of 50% and 25% gave lower improvements than those seen with Model E protocol but better than those with Model C protocol. In these models some immunity remains even after the

vaccine effect has worn off i.e. the immune system receives a small permanent boost which contributes to the added reduction in disease prevalence over the Model C protocol. Prevalence is reduced to ~0.13 in 12 month models with both 25% and 50% residual effects and for other models the prevalence remains between ~0.16 and ~0.2.

4.3.4h - Model H

Mean prevalence when vaccinating according to Model H protocol was reduced to just over 0.1 with a 90% vaccine with a 25% residual effect and a 12 month effect length (Figure 4.14), which was the best outcome observed in models where waning immunity was included.

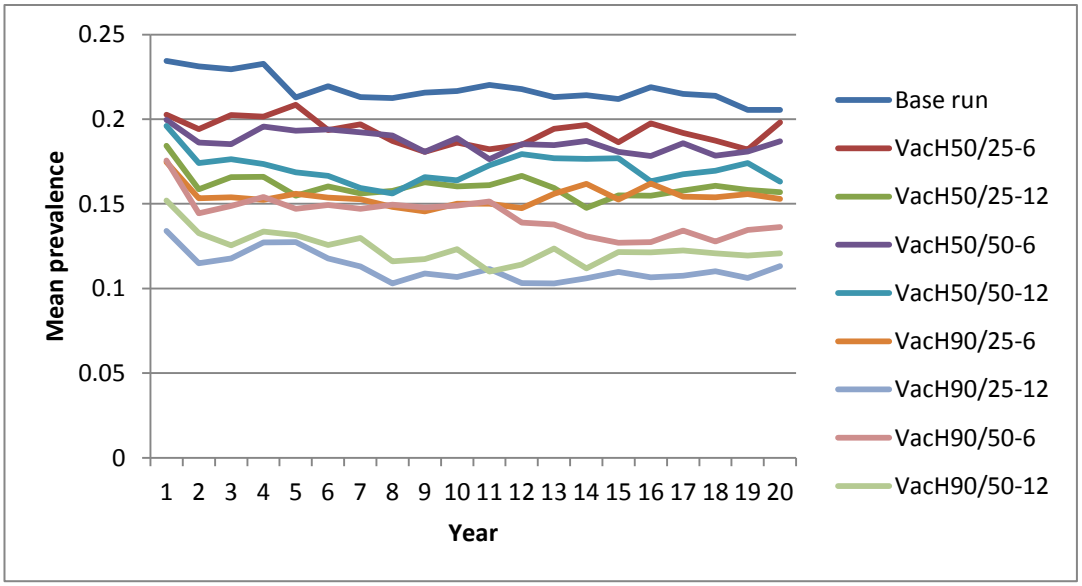

Figure 4.14. Mean prevalence following vaccination with different strengths of vaccine according to Model H protocol. Base run: no vaccination; VacHx/y-w indicates x% vaccine effect, y% residual effect and w months effect duration

4.3.4i - Comparison of different vaccine models

Figures 4.15 and 4.16 present a comparison of mean prevalence outcomes following vaccination according to different model protocols, using the 90% vaccine with a 12 month effect length and the 50% vaccine with 12 month effect length respectively. These outcomes are from models where recoverability and revertability parameters are affected by vaccination. Figures 4.17 and 4.18 show the same results for models where recoverability, revertability and susceptibility parameters are altered following vaccination. Models assuming a 6 month effect length showed similar patterns but with much smaller effects.

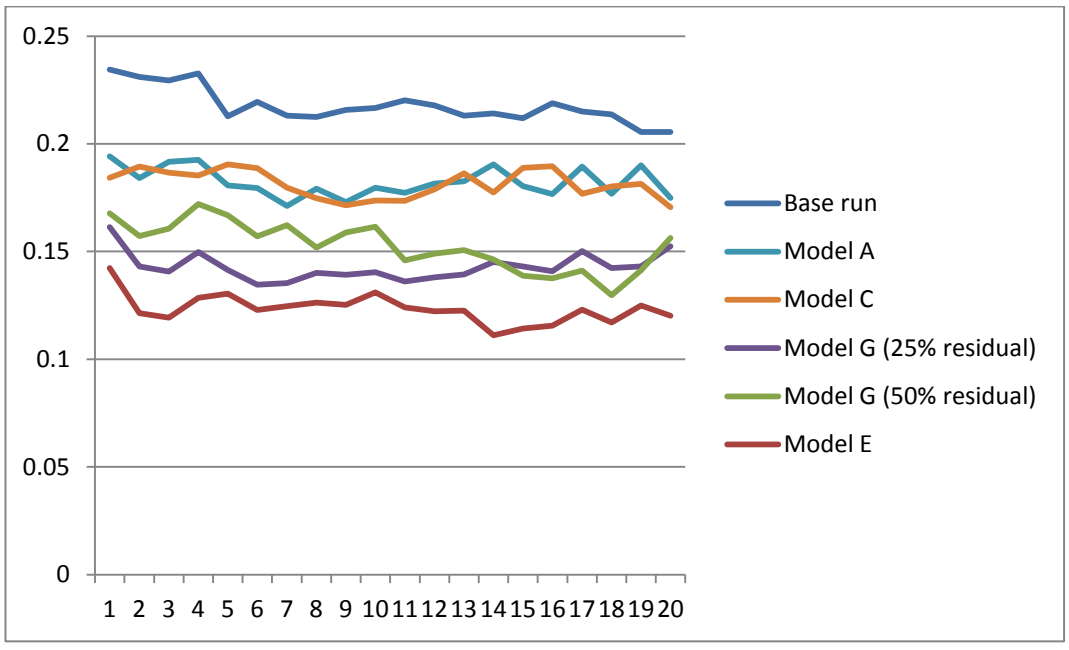

Figure 4.15. Mean prevalence following vaccination with a 90% vaccine effect and a 12 month vaccine effect length, using different model protocols.

It can be seen that Models A and C produce very similar effects with the 90% 12 month vaccine (Figure 4.15), reducing mean prevalence to ~0.18. Model G gives slightly improved effects with both 25% and 50% residual vaccine effects, which

become indistinguishable by around year 13 at a mean prevalence of ~0.14-0.15. Model E, where the full vaccine effect continues for 12 months gives the greatest reduction in mean prevalence, to ~0.12-0.13.

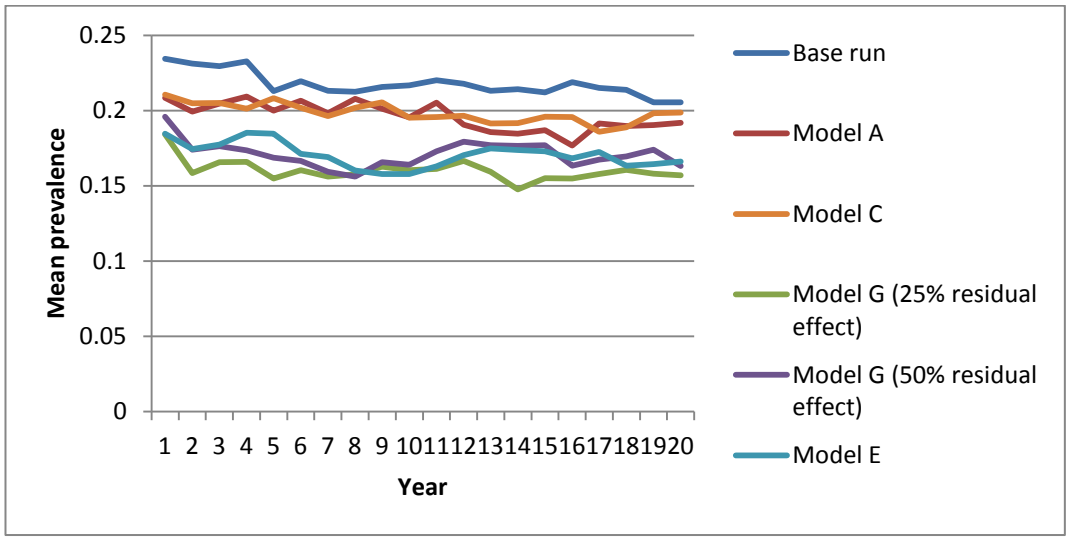

Figure 4.16. Mean prevalence following vaccination with a 50% vaccine with a 12 month vaccine effect length using different model protocols.

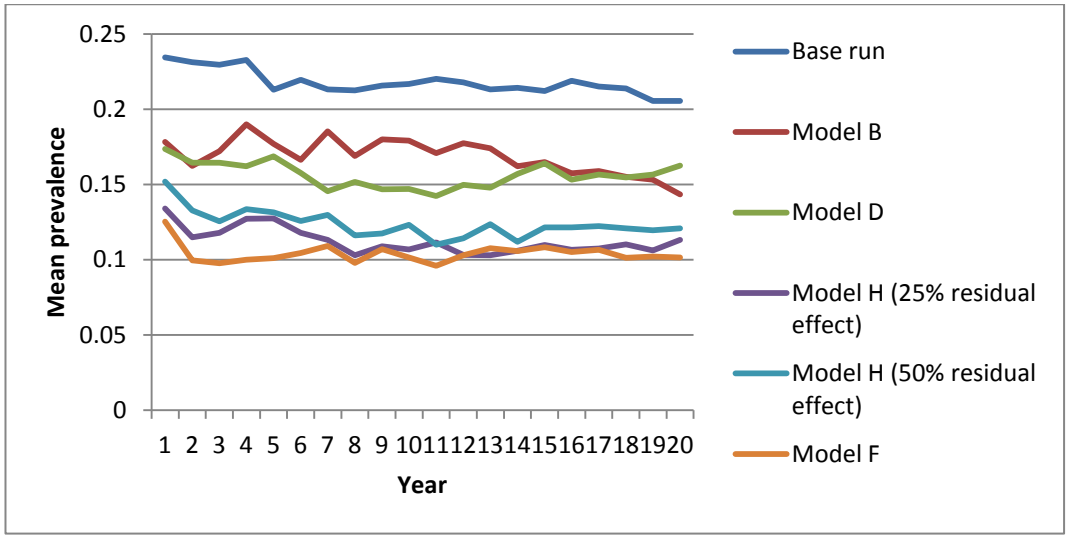

Figure 4.17. Mean prevalence following vaccination with a 90% vaccine with a 12 month vaccine effect length using different model protocols.

When using a 50% vaccine effect with a 12 month effect length (Figure 4.16) the impacts on disease prevalence are reduced as might be expected. Models A and C again give similar results, reducing prevalence to ~0.19-0.20. Models G and E also give similar results, reducing prevalence to ~0.16-0.17.

When susceptibility is also altered by vaccination the reduction in prevalence is greater. Using a 90% vaccine with a 12 month effect (Figure 4.17), mean prevalence was reduced to between ~0.1 (Model F) and ~0.16 (Models B and D). When using a 50% vaccine with a 12 month effect (Figure 4.18) the impact on prevalence was less than seen with the 90% vaccine and was very similar to the results seen using models where only recoverability and revertability are affected by vaccination.

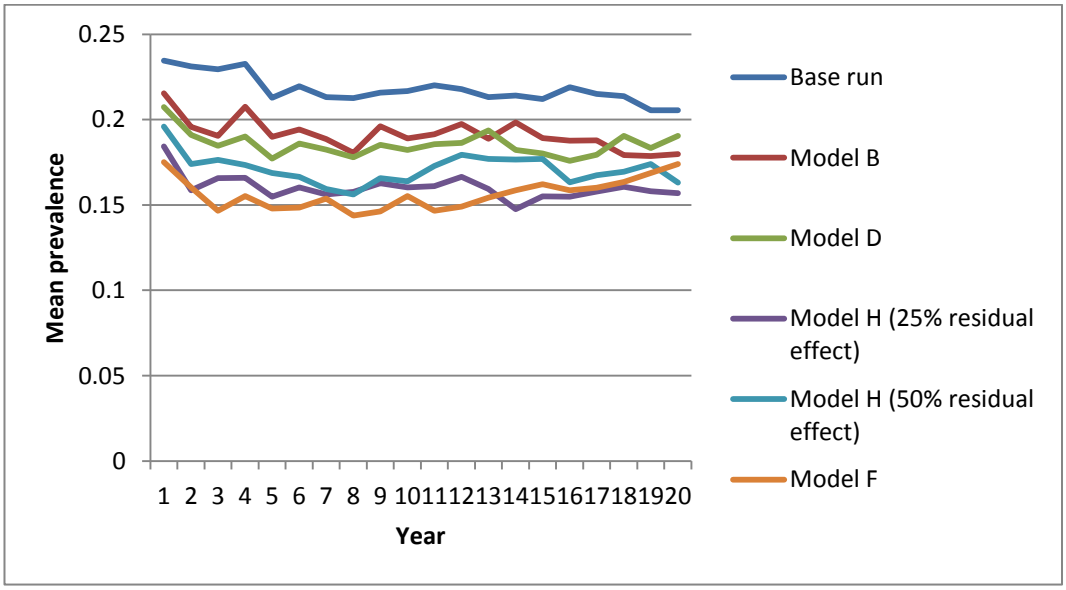

Figure 4.18. Mean prevalence following vaccination with a 50% vaccine with a 12 month effect using different model protocols.

It is also possible to look at the probability of achieving improvements over base values using pair-wise comparison as considered in the culling data above. When applying a 90% vaccine with a 12 month effect length all protocols A-H resulted in a

probability of at least 0.9 that the number of lame days and number of infections in years 5 and 10 (taken as examples as in the culling data above) was reduced below that of base levels. However, the probabilities are more varied when a 25% reduction in base values is desired, ranging from 0 to 1 (Figure 4.19). Only Model F gave a consistent probability of 1 in the reduction of lame days and new infections by at least 25% across both years. Model F also had a non-zero probability of reducing the number of infections and number of lame days by 50% in both years (0.48-0.5 for new infections; 0.52-0.7 for lame days). Maximum reductions of 57% and 63% in new infections, and 63% and 65% in lame days were seen in years 5 and 10 respectively using Model F. No other model showed probabilities above 0.1 for reductions of greater than 50% in new infections or lame days in either year.

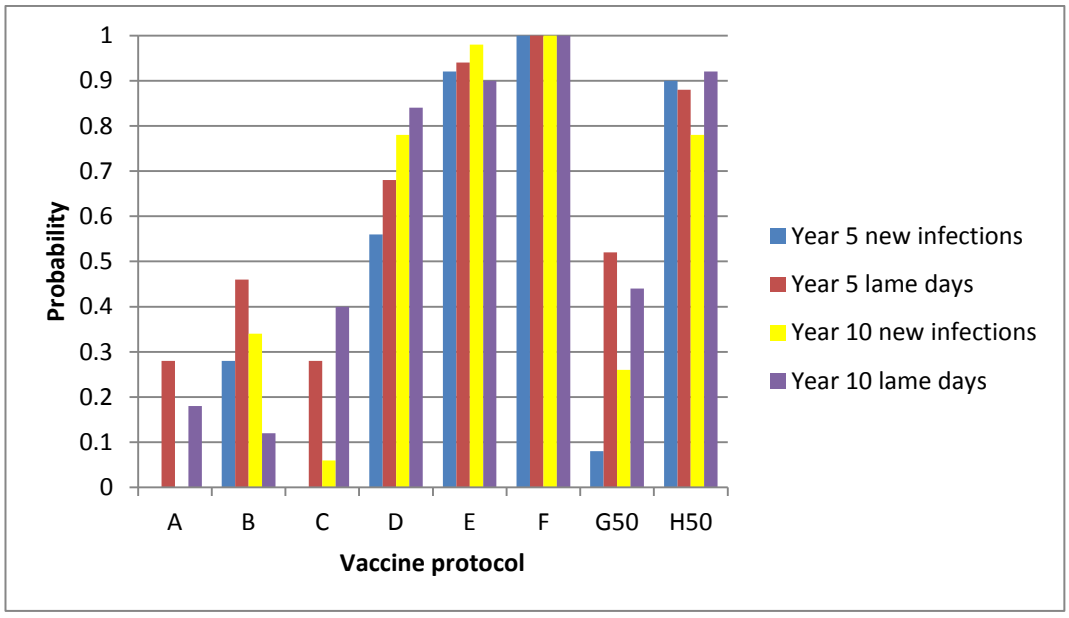

Figure 4.19. Probability of achieving a 25% reduction in lame days and new infections in years 5 and 10 when compared with base values using different vaccine models. Models G and H are shown with the 50% residual effect applied.

4.4 - Discussion

This study has explored the effects of four different treatment and control methods used to reduce the incidence and prevalence of footrot in sheep flocks. All four methods reduced footrot incidence and prevalence, but the extent of these effects varied greatly. Antibiotics were the most effective at reducing disease levels, particularly if all diseased sheep and not only those with severe clinical signs were treated. Pasture rotation showed moderate effects, as did selective culling, although the latter did not reach peak effect until some years had passed, while the effects of pasture rotation were more immediate. Vaccination was less beneficial than other methods and given the effectiveness of other controls it would seem to be a less favourable option, particularly in the long term as the duration of immunity is so short. It should be noted that some of these methods would not be practical in a field setting but are explored to gain further information about the biology of the disease and the theoretical impacts that may be achieved.

The differences between disease outcomes when using pasture rotation with different intervals are large. In the simulations undertaken for this study, the smallest benefit observed with weekly rotation was still better than the largest effect seen when pasture rotation is only carried out every 15 days. This is because *D. nodosus* from infected sheep quickly contaminate the fresh pasture. When rotating sheep every seven days peak contamination levels have not yet been reached but with 15 day rotations a much higher level of environmental *D. nodosus* is able to accumulate, causing higher exposures and more infections. Improvements of up to approximately 50% in the number of new infections and total number of lame days per year were seen. When compared with the base rate this shows that frequent pasture rotation can

have a big impact on the incidence and prevalence of disease. Such large differences are promising but there are a few caveats to note.

This analysis was based on the assumption that new pastures were completely free from all causative bacteria, which should be the case if ten days have elapsed since affected sheep were last on that pasture. A survival length of 7 days was suggested by Beveridge (1941) and this is the mean value used in this model, allowing for some bacteria to survive longer than this. However, it is difficult to experimentally determine the survival time of viable *D. nodosus* as it is an anaerobic species that is difficult to culture. PCR analysis can be used to identify *D. nodosus* (Calvo-Bado *et al.*, 2011) but this does not distinguish between viable and non-viable bacteria. As the survival time of bacteria is a key determinant of disease transmission and the effects of pasture rotation, the data generated here, although based on the best data available, must be used cautiously until we can more accurately determine these values. Further modelling may also be used to explore the effects of different bacterial survival times on disease outcomes.

It is likely that pasture rotation would be recommended for use in combination with other control measures and future work could look at how combinations of these control measures may be best used to most efficiently control footrot in UK sheep flocks, something for which no data are currently available. This was not done in this study as it aimed to explore the individual effects of control methods and to see how variation in strategies used for each of these controls affected the disease outcomes observed. The next step would be to explore different combinations of control methods because when planning disease control strategies it would be useful to know if the different interventions act in synergy to boost effects, or work against each other to reduce the effects.

Selective culling could be an effective way to reduce footrot in a flock over a period of time. The improvements seen in the model were incremental over a number of years before eventually stabilising to a reduced incidence and prevalence of footrot. However, the perceived success of a selective culling programme is dependent on a number of factors.

Firstly there is the consideration of what criterion to use when selecting the 'worst' sheep. In this study we have explored three different criteria and the results show that the differences can be large even when culling the same number of sheep. For instance, as can be seen in Figure 4.3, culling 15% of sheep (41-52 sheep) based on monthly observations reduced the total number of lame days by almost half while culling based on the number of episodes of disease a sheep had experienced had very little impact at all.

The number of episodes a sheep has experienced does not reduce the number of lame days because it is only indirectly linked to the number of lame days a sheep experiences; they are controlled by different underlying traits. The number of episodes is largely determined by the sheep's susceptibility while the number of lame days is controlled by the joint effects of the sheep's susceptibility and recoverability. A sheep may have a very high susceptibility and thus have a high number of episodes, but if its recoverability is also very high then the time spent lame may be very short. Conversely a sheep with low susceptibility and low recoverability may be infected very rarely but spend a long time affected during each episode. Culling based on the number of episodes does not capture this variation in time spent lame and so has only a small effect on total lame days, although its effect on new infections is greater.

There is also a question of whether these measures may be confounded by other variables. For example, culling based on the amount of time a sheep is lame per year seems to give better results than culling based on the number of times a sheep becomes lame. However, if antibiotic treatment is used, the number of days a sheep spends lame will be artificially reduced and so may no longer provide a clear indicator of which truly are the worst sheep for culling purposes.

Perhaps surprisingly, culling based on monthly observations of all sheep, made on the first day of every month, gives the best results. This is despite the fact that both total lame days and total episodes are measures that use all available data, with every episode and lame day accurately recorded no matter how short the duration of that episode. Yet monthly observations use far less data - only one day per month is taken into consideration and the wealth of other data available is discarded. It is thought that the increased impact using this criterion is due to the fact that comparing all sheep on the same day each month reduces environmental variation between measurements and thus gives a more accurate representation of the best and worst sheep. When using all data it is difficult to tell which sheep are the first to become affected or which are affected at periods of low prevalence, which might be a better indicator of highly susceptible sheep. This is partially accounted for when all sheep are compared on the same day as the flock disease conditions at that time are consistent between sheep. If measurements are taken at low prevalence only those sheep that become affected when infection pressure is low will be tallied and because there are only 12 measurement points per year a difference of one or two will have a greater impact on selection.

In this study, a fixed percentage of sheep were culled each year, which did not reduce over time. An alternative approach would be to cull sheep that had total disease

episodes or lame days greater than a threshold amount e.g. all sheep with more than three episodes. This would result in reduced numbers of sheep being culled as the prevalence within the flock was lowered through disease control methods and thus may be more desirable to farmers. This method was attempted using the model but due to the high numbers of episodes and lame days per sheep (a result of the perfect recording of all episodes no matter how short the duration) the numbers of sheep remained large over time unless high thresholds were used.

Selective culling reduces footrot prevalence because it removes diseased sheep that are likely to be the highest shedders of *D. nodosus*, which in turn reduces the amount of *D. nodosus* on the field. This explains why culling based on the number of lame days gives better results than culling based on the number of episodes - those sheep that spend longer in a diseased state contribute bacteria into the environment for a greater period of time than those that get infected many times but recover rapidly from each episode. The selection models presented here are based on a partially closed flock where no new ewes are brought in from outside and where rams do not contribute to disease cycles. However, there are so few rams and they are present only briefly within the flock that their contribution to infection cycles would be negligible. The efficacy of culling would be greatly reduced if sheep with very low recoverability were brought in from external flocks because on becoming infected these would add to the contamination of the pasture for long periods of time and thus reduce the benefits of culling the greatest shedders from the flock.

Selective culling of diseased sheep was also hypothesised to reduce susceptibility by removing sheep with the highest susceptibility values through culling. However, the effects on genetic traits were extremely small. Whilst there is a correlation between lameness and susceptibility, it is not perfect (see Chapter 3, Figures 3.2 and 3.3), so

any selection method will have less than perfect accuracy. An alternative approach is to use genetic selection which, instead of removing the worst sheep, selects only the best to breed from; this approach is explored in Chapter 5.

In these models it is assumed that all diseased sheep shed equally but there are no data available on variation in shedding between sheep. If some sheep are greater shedders than others this could greatly alter the disease dynamics and selection methods would have to take into account shedding levels when selecting the sheep to be culled. This would also apply if there are sheep that have asymptomatic infections but still shed bacteria as it would not be possible to determine these by visual inspection and other selection methods would need to be considered. Although the model includes carrier sheep it does not allow for these to be shedders as current evidence suggests that carrier sheep maintain internal pockets of bacteria not exposed to the pasture, for example in microvesicles (Beveridge, 1941; Roberts and Egerton, 1969). If these do shed then other ways of identifying sheep to be culled may need to be utilised, for example foot integrity has been closely linked with lameness (Beveridge, 1941; Kaler *et al.*, 2010) and this may be an alternative approach.

It is also possible that the amount and duration of shedding may be in part due to the dose of *D. nodosus* at infection, i.e. a potential dose effect. This is to some extent random but is also linked to the degree of contamination in the pasture at the time of infection. At times of high contamination the probability of a large dose at infection would increase whilst at times of low contamination a smaller dose might be more probable, although this effect was not included in the model. If there were a dose effect then reducing contamination on the pasture would greatly assist in reducing shedding as more infected sheep would be shedding at a lower rate and thus the contamination of pasture would remain lower.

In this model, and from data collected during field studies, antibiotic treatment is very effective at reducing the prevalence of lameness if administered to all diseased sheep within a few days of developing clinical signs (i.e. lameness and/or lesions) not just those whose clinical signs have progressed to a severe state (Wassink *et al.*, 2010). Treating all diseased sheep results in much greater improvements to the disease burden observed within the flock than treating only those with severe clinical signs; also observed by Green *et al.* (2007). This is probably because even mildly affected sheep carry *D. nodosus* on their feet (Moore *et al.*, 2005; Calvo-Bado *et al.*, 2011) and are likely to be contributing bacteria to the soil. Thus they help maintain a level of contamination that allows many more sheep to subsequently become infected. However, as mentioned above, the similarity (or difference) in shedding rates between infected individuals is not clear from field data. The homogeneous shedding from diseased sheep assumed in this model may affect the outcomes of the antibiotic treatment protocols. If only severely diseased sheep shed bacteria, or shed substantially more than sheep with only mild clinical signs, then we might expect to see some more improvements when only severely affected sheep were treated, although mildly affected sheep will continue to propagate the infection within the flock.

Not only does treating all diseased sheep at frequent intervals give the greatest reduction in disease prevalence, but also results in fewer treatments required over time compared with treating only sheep with severe clinical signs. This makes it cost effective in the long run and also gives a much greater benefit to the health and welfare of the sheep. The mean total doses for treating all diseased sheep daily for a period of 20 years was 783. The equivalent mean over 20 years for treating only severely affected sheep once a week was 13837, which is close to 20-fold more

doses. This may be also be considered as a reduction of the risk of lameness by nearly 20 fold, which is considerable and suggests great benefit to sheep health in flocks adopting the strategy of treating all lame sheep as soon as clinical signs become evident. The reduction in costs that may be achieved with an optimal treatment strategy is also considerable, with each dose of antibiotic costing approximately £1 (Wassink *et al.*, 2010), and this is prior to calculation of any increased incomes from healthier sheep with better body condition scores and greater live weight at time of sales which should also be seen if footrot incidence and prevalence were reduced (Wassink *et al.*, 2010). This reduced use of antibiotics may also be beneficial because there is lower pressure on pathogens to evolve resistance traits.

In vaccine models, all ewes were vaccinated at the start of every year. It was decided not to vaccinate lambs because it is not common practice in flocks. However, this means that during the period between lambing and culling less than half the population is protected by the vaccine (lambs are born at a mean rate of 1.5 per ewe). If there is any contamination left on the pasture following lambing then disease may easily be propagated when such a low proportion of the flock have been vaccinated. This will affect the amount of impact vaccination can have on disease prevalence when used in isolation.

Our study gives vaccine benefits of up to ~65% over non-vaccinated models while published estimates of efficacy have been between 46% and 100% (Hindmarsh *et al.*, 1989; Liardet *et al.*, 1989; Duncan *et al.*, 2012) so our best vaccine strategy falls in the lower range of estimates from field studies. However, the range of outcomes from field studies is large and estimates from those studies where vaccination had a strong positive effect were made in the presence of other control measures and so

may be biased. For instance, a recent study by Duncan *et al.* (2012) reported a vaccine efficacy of 62%, based on reduced risk of new disease with footrot after vaccination compared with incidence rate in unvaccinated sheep. However, all sheep, from both groups, with footrot were treated with long-acting amoxicillin at the start of the study and again if they were affected at monthly follow-up visits. Although a control group was used where antibiotics but no vaccine were administered, it is difficult to determine what the individual propensity for disease may have been for sheep in each group. It is also possible that vaccination and antibiotic treatment combine to give benefits that are greater than the sum of their individual effects, which may be the case as they approach from different stages of the infection process – vaccination reduces new infections while antibiotics reduce the length of infections.

The specifics of the genetic basis for resistance are unclear and in our model we have represented the genetic effects simply as three heritable traits controlling probability of becoming infected, time taken to recover from disease and length of immune period following recovery. However, how this translates into a vaccine response is not clear, hence the use of multiple model strategies to explore possible vaccine effects. With field vaccine results varying so greatly it is therefore difficult to assess how accurate our model is at simulating vaccine effects. Further data on the length of vaccine effects with current vaccines and the genetic processes controlled by vaccination would be desirable to help validate the model outcomes for this control strategy, particularly in the case of vaccination without the use of further control methods.

There are a few key outcomes from this experiment that highlight important things to consider when planning a footrot control programme; with the proviso that these

come from a theoretical model with certain assumptions. The first is that curtailing infection is a highly effective approach to reducing disease because it reduces the contamination of the pasture, resulting in fewer future infections and thus improving the welfare of the sheep. This can be seen in both culling and antibiotics results. Culling sheep based on the number of days they had spent lame reduced the total number of lame days in the flock by a greater amount than culling based on the number of episodes. Antibiotics administered to sheep early in the disease process, i.e. when they were experiencing only mild clinical signs, reduced disease significantly more than waiting until sheep showed severe clinical signs before administering treatments. Treating all sheep with clinical signs not only reduced disease prevalence very quickly, but also reduced the number of future treatments required so that over a twenty year period it resulted in nearly 20 times fewer doses being administered. This is because even mildly affected sheep shed bacteria onto the soil and the faster an infection is curtailed, the lower the contamination of the pasture will be.

The second key observation is that effective selection for culling purposes can be made using limited numbers of observations on sheep, provided that the sheep are observed on the same date. This study used monthly observations to try and mimic a realistic observation protocol in the field, and this resulted in very effective outcomes following culling based on these observations. It is believed that the process of examining sheep on the same day reduces environmental variation and thus the worst sheep may be accurately determined even if all sheep are only examined once a month. However, the model set up led to sheep with many short episodes of lameness and others with longer episodes, this has not been validated with field data. It also assumed a 100% detection rate of lameness and infection, and it is likely to be

lower than this in the field which would reduce the effectiveness of this selection protocol.

Chapter 5: The potential for genetic selection to reduce footrot in the UK sheep population.

5.1 – Introduction

It has been established in a number of studies from around the world that resistance to footrot is partly under genetic control, with an estimated heritability of between 0.1 and 0.3 (Skerman *et al.*, 1988; Raadsma *et al.*, 1994; Niewhof *et al.*, 2008). In the UK, control rather than elimination is currently more feasible (Green and George, 2008) because the climate facilitates transmission of the causative bacterium throughout the year. This removes the opportunity to eliminate in a period of no transmission, which is the approach used in many parts of Australia. In addition, over 90% of flocks have footrot (Wassink *et al.*, 2003) and so elimination would leave a flock highly susceptible to re-infection from other infected flocks, arising from either poor biosecurity or purchase of infected stock. The host does not mount a strong immune response to *D. nodosus* and so reinfection and repeated disease events occur in sheep (Beveridge, 1941). In this situation genetic selection to improve the underlying resistance of a sheep flock may be desirable.

Evidence that it is possible to breed for footrot resistance has been available for several decades. In the 1970s and 1980s a flock of sheep in New Zealand was selectively bred for footrot resistance over a period of approximately 15 years. These sheep originated from the Corriedale breed and following the intense selection programme for footrot resistance were subsequently known as the Broomfield Corriedale line. When later challenged with *D. nodosus*, both experimentally and naturally, significantly fewer Broomfield Corriedales were affected and to a lesser

severity than other Corriedale sheep with which they were compared. A lower prevalence of footrot was also observed in the offspring of Broomfield Corriedale sires mated with unselected ewes than from the offspring of comparable sires and unselected ewes (Skerman and Moorhouse, 1987).

This success suggests that breeding for footrot resistance is possible but in this Corriedale flock certain measures were taken that would not be practical in most flocks. For example, for a period of nine years approximately 85% of all females bred were culled with an initial cull rate of approximately 75% applied to ewe hoggets (Skerman and Moorhouse, 1987) to achieve the resistance traits desired. These high culling rates would be prohibitive for many flocks, but it is possible that a selection program based on selecting superior ram lambs to become sires would also increase flock resistance to footrot.

Genetic selection could be used with conventional control methods such as treatment and selective culling to provide longer term benefits of disease reduction and resistance. However, treating diseased sheep could mask the differences between individuals in genetic susceptibility and thus make selection more difficult. In addition, it is known that the use of antibiotic treatment reduces flock prevalence (Wassink *et al.*, 2010) and at low prevalence estimated heritabilities of resistance traits are lower (Niewhof *et al.*, 2008; Bishop and Woolliams, 2010). This may be partly due to incomplete exposure to infection; those sheep that are not exposed to *D. nodosus* will not be able to express their resistance phenotype.

This chapter explores the potential for genetic selection for footrot resistance in a simulated UK flock. It examines the effects of selective breeding in which sire breeding values are estimated from performance records measured on all animals in a

flock. The effect of selective breeding is investigated both in isolation and in conjunction with other control methods (pasture rotation, selective culling of ewes and antibiotic treatment) to analyse the interaction between long and short term control methods. All analyses are carried out using the simulation model presented in Chapters 3 and 4.

Several hypotheses are tested:

1. Genetic selection of breeding rams based on disease observations:
    a. improves resistance traits for footrot
    b. reduces the prevalence of footrot in a sheep flock
2. Genetic selection used in combination with conventional control measures gives greater benefit than either genetic selection or epidemiological controls in isolation.
3. Antibiotic treatment affects the identification of the most resistant rams, by masking the disease phenotype.
4. Improvements seen from genetic selection are the result of a combination of improvements in resistance traits (direct effect) and reduced environmental contamination (indirect effects).

5.2 – Methods

For the purposes of this set of simulations, two flocks are used. The first is a pedigree flock; this is the flock from which rams are selected based on their estimated breeding values (EBVs). The second flock is a standard commercial flock, which is the flock in which the selected rams are used for breeding. The effects of selection for footrot resistance are evaluated in the commercial flock where no selection is

carried out but selected rams are used. In some scenarios particularly susceptible ewes are culled. In the pedigree flock, from which rams are selected, several treatment control strategies are employed specifically to investigate how clinical management affects identification of the genetically most resistant animals.

5.2.1 – Selection of rams for breeding

The pedigree flock model was run for 20 years, initially with no control and random selection of sires with regard to footrot resistance, i.e. they were assumed to be selected for other uncorrelated traits. Data on the first six months of life of ram lambs were extracted - after this time ram lambs were removed from the flock. Three disease traits – number of footrot episodes, number of lame days and monthly observation score (see Chapter 3) – were recorded together with lamb, sire and dam identification and year of birth. These data were used to create input files for ASReml (Gilmour *et al.*, 1996), which was used to calculate EBVs for ram lambs for each of the three disease traits. The mixed effects model used was of the following form:

$$\text{outcome } Y = \text{mu} + \text{year} + \text{animal} + e$$

Where outcome Y is the trait of interest, mu is the population mean, year is a fixed effect term for year of birth, animal is the individual animal genotype included as a random effect and e is an error term. Covariances between animals were assumed to arise from additive genetic relationships, as described by the numerator relationship matrix **A**. **A,** and its inverse $\mathbf{A}^{-1}$, were calculated directly in the ASReml package from pedigree relationships between animals, i.e. from knowledge of the sire and dam of each animal. This is a software package designed to fit linear mixed models

to large data sets in order to estimate variance components, particularly for cases where data structures are complex and unbalanced. Its calculations are based on a restricted maximum likelihood method and the algorithms it uses have been optimised specifically for estimation of genetic parameters such as heritabilities and genetic correlations.

Outputs from the mixed effects model include an estimated breeding value (EBV), which is calculated independently for each trait of interest. The EBV is simply the solution for each individual in the random animal term. The five ram lambs with the best EBVs for each footrot trait from each year of the simulation were selected (from a total of approximately 150 ram lambs per year) as the breeding sires for a simulation in the commercial flock.

This process was repeated ten times for each disease trait to give ten different sets of sires per trait, which were then used as inputs for ten iterations of the breeder to finishing flock model using each selection criterion.

This study also attempted to determine the effects of antibiotic treatment, which reduces disease prevalence, on the ability to select the best rams for breeding. Two further sets of rams were identified using the above process, this time from pedigree flocks using one of two sets of antibiotic treatment protocols. These protocols were to treat all diseased sheep once a week or to treat only severely diseased sheep once a week.

The success of genetic selection may be assessed in terms of two outcomes in the commercial flock. The first is its effect on disease levels within a flock, which may be determined by looking at the change in incidence and prevalence over time when genetic selection is applied. The second outcome is the effect genetic selection has

on the underlying genetic traits controlling disease resistance in individuals, as measured by the change in the population mean.

The observed changes resulting from genetic selection may be split into direct effects arising from the change in the mean values for each genetically determined trait, along with indirect effects arising as a consequence of decreased bacterial burden in the environment. To disentangle these two effects a series of simulations was run where the environmental load of *D. nodosus* was kept constant throughout the genetic selection programme, at a level equivalent to the median load in simulations where no control or selection took place. The outcomes from these models are then compared with the results seen using genetic selection in a flock where the bacterial load is allowed to vary. These outputs are then used to estimate the proportion of improvements from genetic selection that are the consequence of direct genetic versus indirect environmental benefits.

5.2.2 – Simulation models

Selective breeding was considered both in isolation and in combination with a range of treatment protocols. The models used were as follows:

1. Genetic selection alone, using each of the three disease traits as selection criteria.
    a. Rams selected from a pedigree flock where no control or selection is implemented.
    b. Rams selected from a pedigree flock using antibiotic treatment to treat either all diseased sheep or only severely diseased sheep.

c. Rams selected from a pedigree flock where no control or selection is implemented. Selection halted after ten years.
d. Rams selected from a pedigree flock where no control or selection is implemented. Environmental levels of *D. nodosus* kept constant at the median level seen in base runs with no control or selection, in order to disentangle the direct effects of selection from the indirect effects arising from decreased *D. nodosus* in the environment.

2. Genetic selection plus pasture rotation at weekly, fortnightly and monthly time intervals.
3. Genetic selection plus selective culling of 5%, 15% and 25% of female sheep according to each selection criteria defined in Chapter 4.
4. Genetic selection plus antibiotic treatment of all diseased sheep or only severely diseased sheep at daily, weekly and monthly intervals.

Table 5.1 gives an overview of all simulation protocols. Each protocol was run ten times with the same parameters to give a range of results. Numbers of new infections and lame days, mean prevalence and mean population values for the three genetic traits (susceptibility, recoverability and revertability) were extracted for analysis. Mean outcomes were compared to give overviews of the disease patterns seen with each protocol, and the means of genetic traits in lambs born each year were used to determine progress in the underlying resistance traits. Pair-wise comparisons of model data, comparing ten runs with each control or selection protocol with each of ten base runs with no control or selection (a total of 100 comparisons per model protocol), were used to give probabilities of achieving benefit under a range of model conditions. As mentioned in Chapter 4 it should be noted that the outcomes seen with this method, comparing ten intervention runs with each of ten base runs, would not

give the same outcome as sampling 100 different outcomes from interventions and comparing these against 100 different base run outcomes, so care must be taken when using these results.

Table 5.1. Simulation protocols used in this study.

Model	Pedigree flock		Commercial flock				General	
	No selection or control	Antibiotic treatment	Breeding from selected rams	Pasture rotation	Selective culling	Antibiotic treatment	Selection halted after ten years	Environmental contamination kept constant
1a	x		x					
1b		x	x					
1c	x		x				x	
1d	x		x					x
2	x		x	x				
3	x		x		x			
4	x		x			x		

Female lambs were retained in the commercial flock each year to maintain a breeding population of 200 ewes, as described in Chapter 3. This means that genetic progress should be continued as offspring of selected sires pass on their improved genotypes to offspring in the next generation.

All models use extended versions of the base model as described in Chapter 3, with control measures implemented as in Chapter 4.

5.3 - Results

5.3.1 - Genetic selection in isolation

When using genetic selection in isolation, there are mean reductions in new infections (Figure 5.1), total lame days (Figure 5.2) and mean prevalence (Figure

5.3) when compared with a base situation of no control methods. These reductions are initially rapid then stabilise after approximately 10 years.

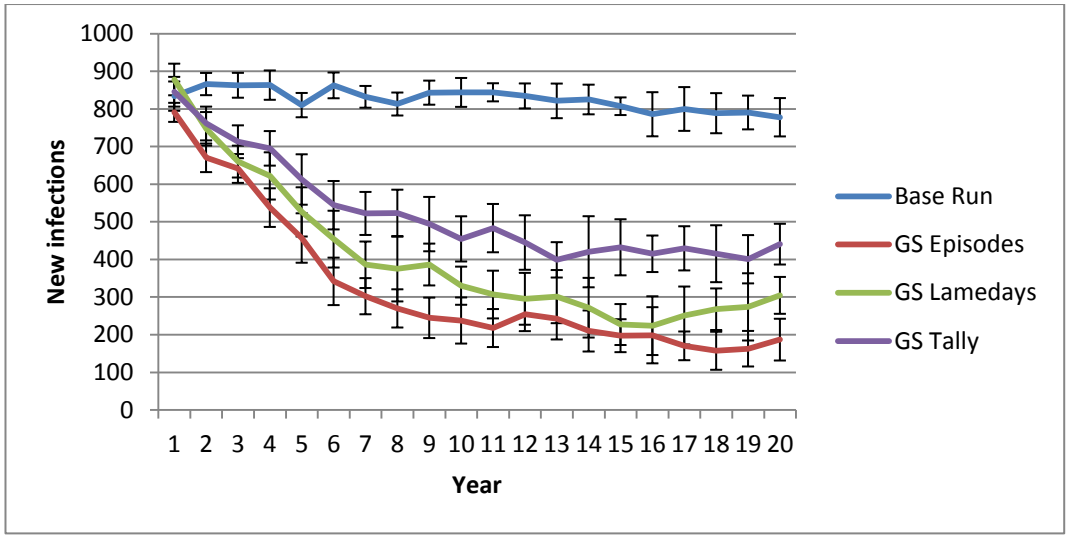

Figure 5.1. Mean effect of genetic selection on the number of new infections observed annually. Base Run - no selection applied; GS Episodes - selection based on number of episodes; GS Lamedays - selection based on number of lame days; GS Tally - selection based on monthly observations. Error bars indicate 95% confidence intervals.

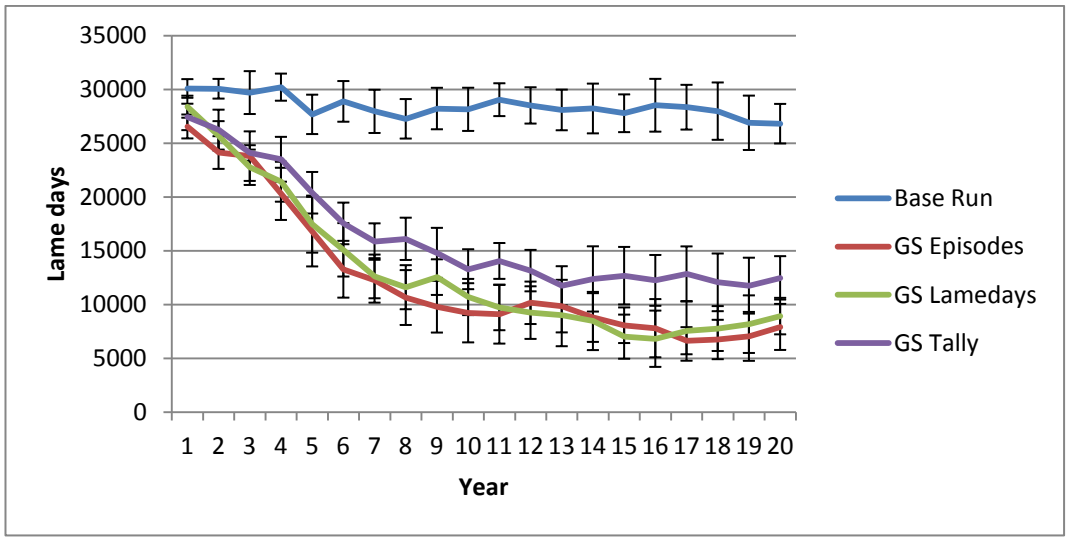

Figure 5.2. Mean effect of genetic selection on the total number of lame days observed annually. Base Run - no selection applied; GS Episodes - selection based on number of episodes; GS Lamedays - selection based on number of lame days; GS Tally - selection based on monthly observations. Error bars indicate 95% confidence intervals.

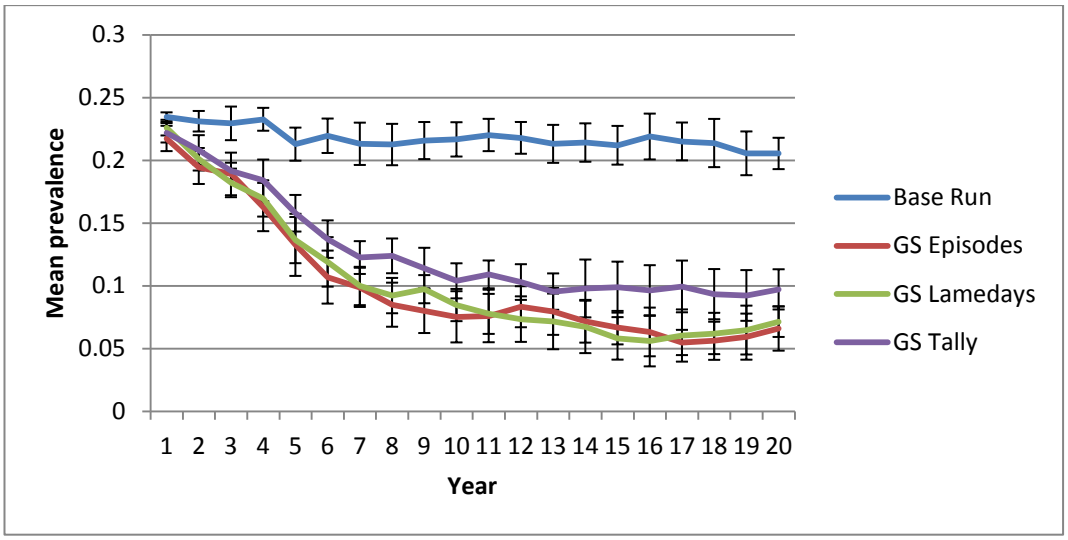

Figure 5.3. Mean effect of genetic selection on mean annual prevalence. Base Run - no selection applied; GS Episodes - selection based on number of episodes; GS Lamedays - selection based on number of lame days; GS Tally - selection based on monthly observations. Error bars indicate 95% confidence intervals.

All selection methods gave a probability of 0.95 or greater that any improvements over base values would be seen at both year 5 and 10, with probabilities of at least 0.85 that the improvements would be greater than 25% of base values. The probabilities of achieving a reduction of 50% over base values for lame days and new infections were more varied (0-0.98) as shown in Figure 5.4. In year 10, selection based on episodes also gave a probability of 0.41 that a 75% reduction in lame days and new infections would be achieved, with selection by lame days giving a probability of 0.02 of achieving the same magnitude of reduction.

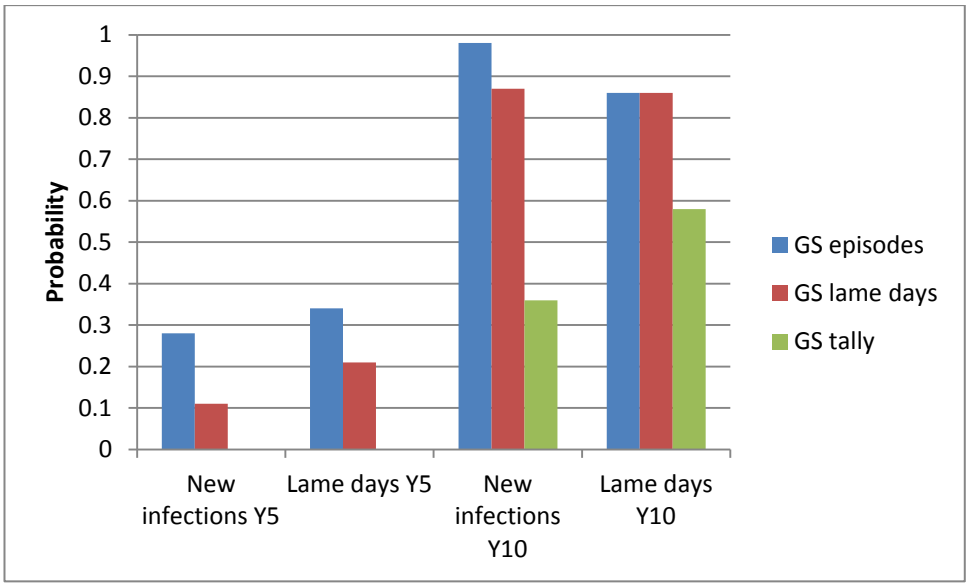

Figure 5.4. Probability of achieving a 50% reduction in the numbers of new infections and lame days in years 5 and 10, in comparison with base values without selection.

Selection based on the number of footrot episodes a ram lamb experienced resulted in a marked decrease in susceptibility of up to 70% in comparison with the base model but had little or no effect on recoverability or revertability. Selection based on lame days and monthly observations also resulted in a decrease in susceptibility, of up to 55% and 35% respectively. These two methods also gave improvements to recoverability (up to 29% and 32% respectively) and, to a smaller extent, revertability (up to 19% for both methods). A comparison of effects of the three selection methods on susceptibility, recoverability and revertability in the commercial flock is shown in Figures 5.5 to 5.7, with the values in the pedigree flock represented by the base run.

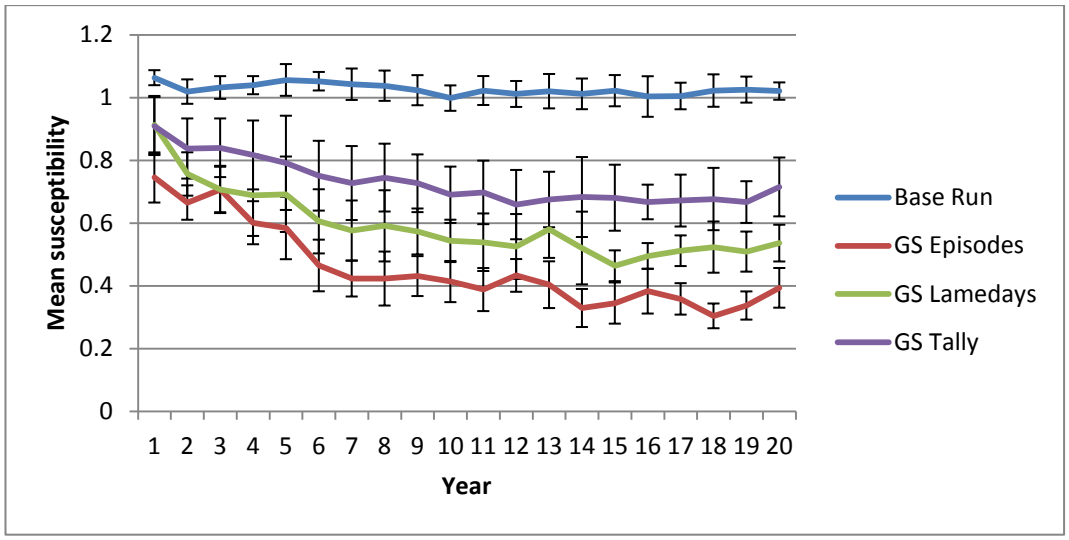

Figure 5.5. Mean effect of genetic selection on susceptibility in the breeder to finishing flock. Base Run - no selection applied; GS Episodes - selection based on number of episodes; GS Lamedays - selection based on number of lame days; GS Tally - selection based on monthly observations. Error bars indicate 95% confidence intervals

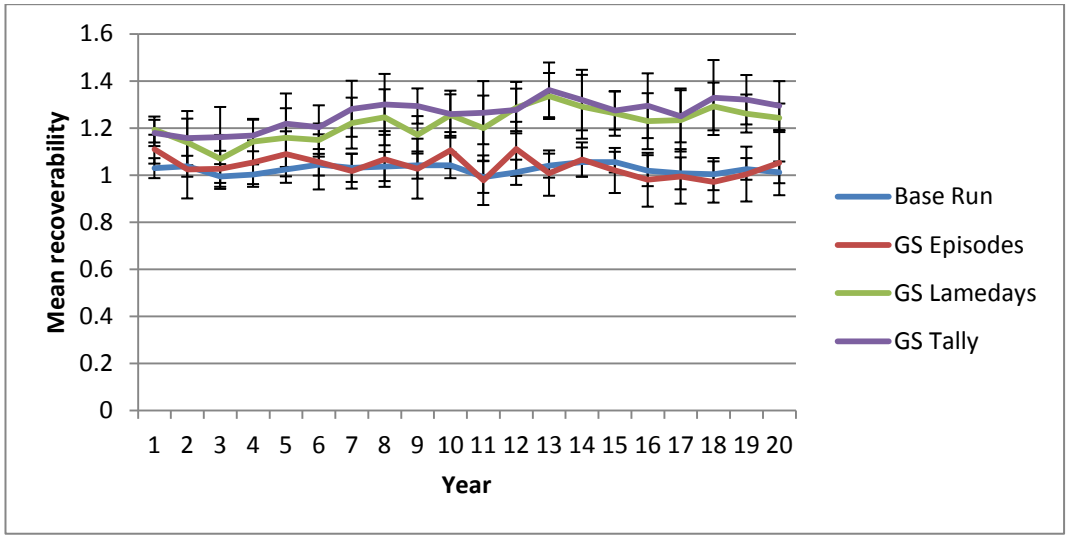

Figure 5.6. Mean effect of genetic selection on recoverability. Base Run - no selection applied; GS Episodes - selection based on number of episodes; GS Lamedays - selection based on number of lame days; GS Tally - selection based on monthly observations. Error bars represent 95% confidence intervals.

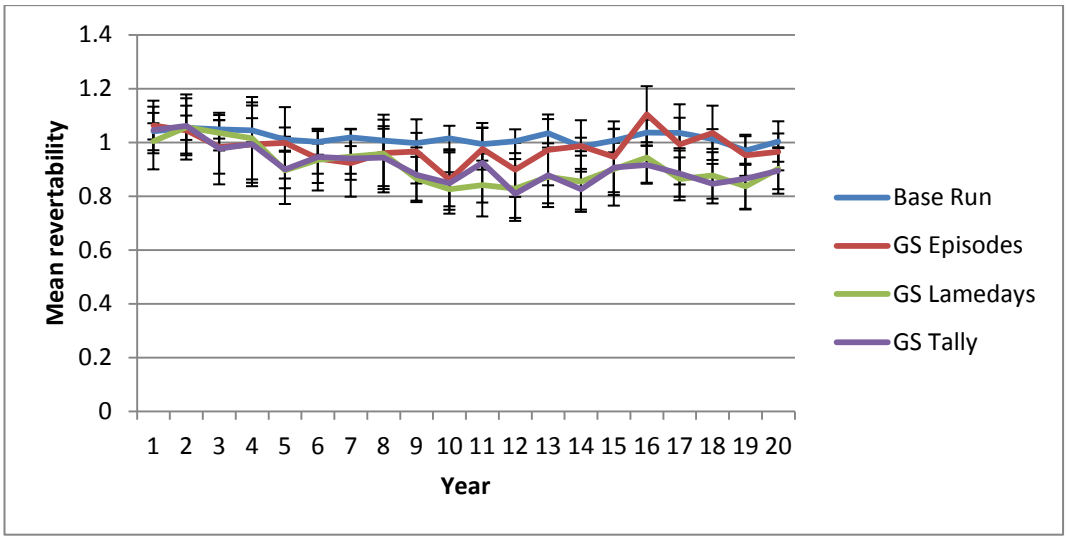

Figure 5.7. Mean effect of genetic selection on revertability. Base Run - no selection applied; GS Episodes - selection based on number of episodes; GS Lamedays - selection based on number of lame days; GS Tally - selection based on monthly observations. Error bars represent 95% confidence intervals.

When genetic selection was halted after ten years, reverting to random selection of rams, i.e. with genetic values calculated as in the base model, there was an increase in the number of new cases (Figure 5.8) back to approximately the same level as prior to selection. This result was the same for all methods of selection. All progress in genetic values, i.e. susceptibility, recoverability and revertability, was also lost when selection was halted after ten years, with values reverting to those seen prior to the start of selection, as shown in Figure 5.9. This is because once selection is halted and breeding rams are randomly chosen with regards to footrot resistance (it is assumed they are chosen for other breeding goals) it effectively means that the flock is now breeding for increased susceptibility because the breeding rams are likely to have higher susceptibilities than the sheep in the improved commercial flock.

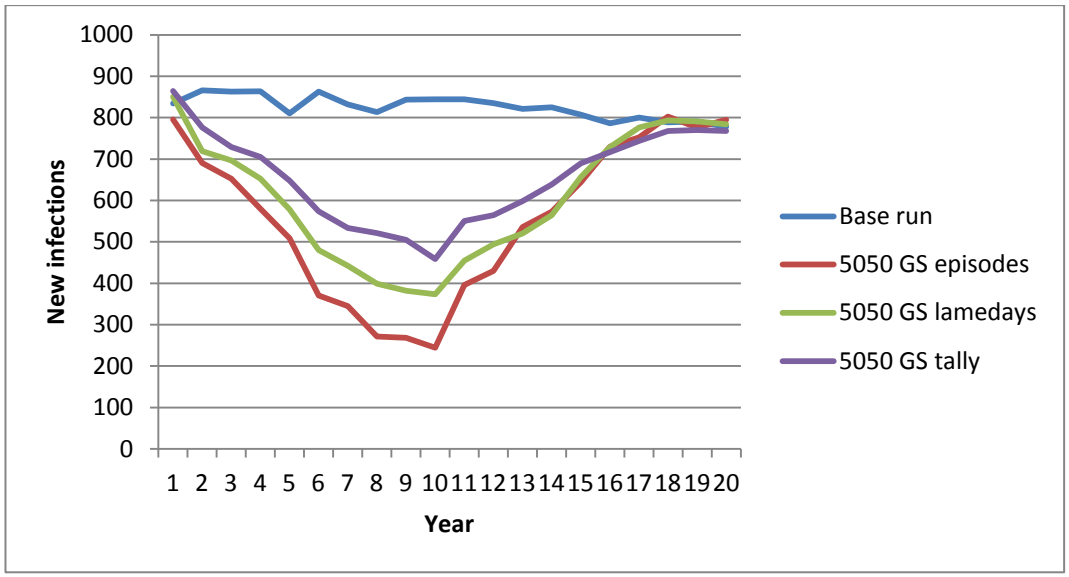

Figure 5.8. Mean new infections per year with genetic selection applied for ten years and then reverting to no selection of rams. Base run - no selection applied; 5050 GS episodes - selection based on number of episodes years 1-10; 5050 GS lamedays - selection based on number of lame days years 1-10; 5050 GS tally - selection based on monthly observations years 1-10.

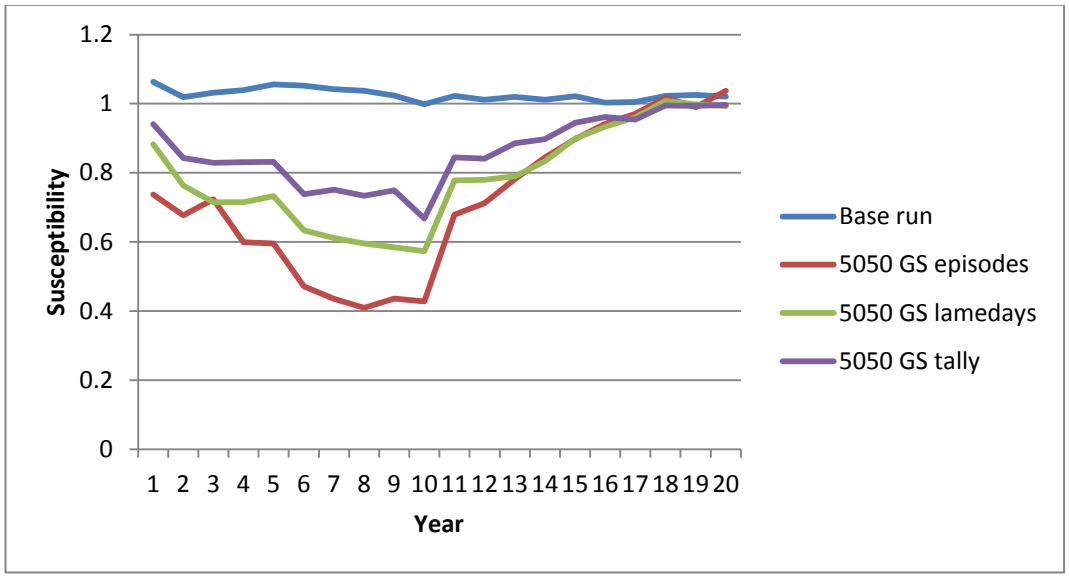

Figure 5.9. Mean susceptibility in the population with genetic selection applied for ten years and then reverting to no selection of rams. Base run - no selection applied; 5050 GS episodes - selection based on number of episodes years 1-10; 5050 GS lamedays - selection based on number of lame days years 1-10; 5050 GS tally - selection based on monthly observations years 1-10.

To separate the direct and indirect effects of genetic selection, i.e. the reductions in disease outcomes from improvements in resistance traits and those resulting from a lower load of *D. nodosus* in the environment, models were also run where the load of *D. nodosus* was kept constant at 750 (infectious) units. This is the median value in base simulations where no controls or selection were applied in the flock. Keeping the bacterial load constant has no effect on genetic traits but it does change the number of new infections and lame days within the flock (Figures 5.10 and 5.11).

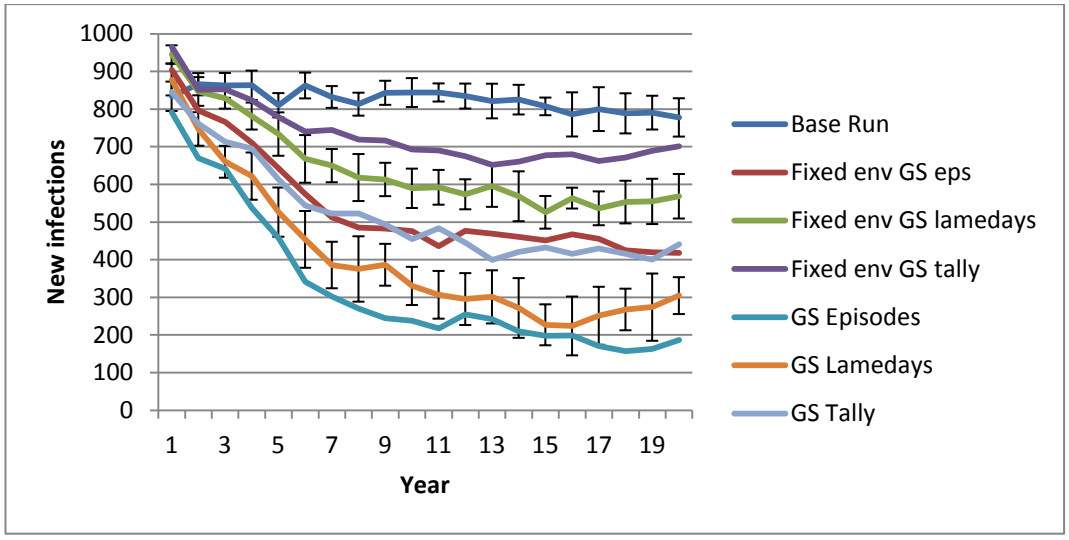

Figure 5.10. Number of new infections in flocks using different genetic selection criteria (GS episodes, GS lamedays and GS tally) in environments where the number of *D. nodosus* is fixed (Fixed env) or variable. Base values are also shown from a flock with variable bacterial levels and no selection or control. Error bars represent 95% confidence intervals and are shown only on base run and lame day data for clarity.

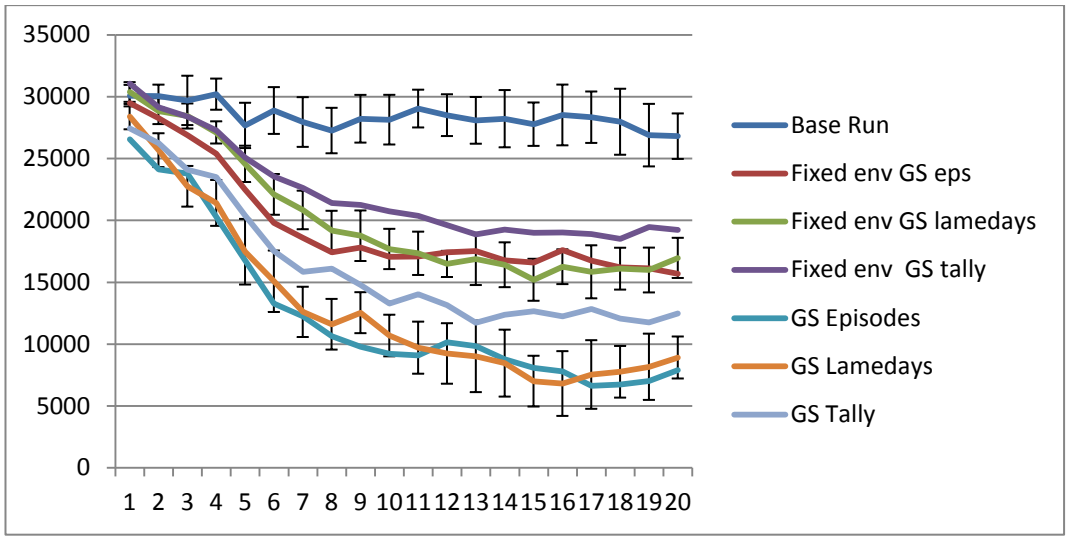

Figure 5.11. Number of lame days seen in flocks using different genetic selection criteria (GS episodes, GS lamedays and GS tally) in environments where the number of *D. nodosus* is fixed (Fixed env) or variable. Base values are also shown from a flock with variable bacterial levels and no selection or control. Error bars represent 95% confidence intervals and are shown only on base run and lame day data for clarity.

The proportions of reductions in new infections and lame days due to genetic effects and environmental effects are given for years 5 and 10 in Table 5.2.

Table 5.2. Proportions of reductions in lame days and new infections in years 5 and 10 (compared with base levels) attributable to direct genetic effects and indirect environment effects (i.e. reduction in levels of *D. nodosus* on the pasture). Data were rounded to two decimal places.

		GS episodes	GS lamedays	GS tally
New infections Y5	Reduction due to genetics	0.21	0.09	0.04
	Reduction due to environment	0.23	0.26	0.21
	Total reduction	0.44	0.35	0.24
Lame days Y5	Reduction due to genetics	0.19	0.11	0.10
	Reduction due to environment	0.21	0.26	0.17
	Total reduction	0.39	0.37	0.26
New infections Y10	Reduction due to genetics	0.44	0.30	0.18
	Reduction due to environment	0.28	0.31	0.28
	Total reduction	0.72	0.61	0.46
Lame days Y10	Reduction due to genetics	0.39	0.37	0.26
	Reduction due to environment	0.28	0.25	0.27
	Total reduction	0.67	0.62	0.53

5.3.2 - Genetic selection in combination with conventional control methods

Genetic selection in isolation reduces disease incidence and prevalence over time but treatment of diseased sheep is necessary to prevent pain and suffering in sheep with footrot, keeping disease levels low while genetic progress is underway. In combination with genetic selection, the benefits from conventional control methods may be enhanced. In this study, the combined effects of genetic selection and each of pasture rotation, selective culling of ewes and antibiotic treatment were examined. Treatment strategies in the commercial flock had no impact on their average genetic values for the three genetically controlled traits, so only disease outcomes are presented here.

Table 5.3. Disease outcomes in year ten when using pasture rotation alone and in combination with genetic selection.

	Control/selection methods	**Weekly rotation**	**Fortnightly rotation**	**Monthly rotation**
Mean number of infections at Yr 10 (range)	Rotation alone	444 (386-519)	623 (551-684)	719 (580-815)
	Rotation + episode selection	2 (0-17)	57 (0-402)	103 (0-296)
	Rotation + lame days selection	0 (0-1)	17.4 (0-121)	143 (0-308)
	Rotation + tally selection	0 (0-3)	101.3 (0-331)	342 (3-520)
Mean number of lame days at Yr 10 (range)	Rotation alone	16511 (13429-20062)	22854 (19225-28382)	25713 (20509-30859)
	Rotation + episode selection	197 (0-1693)	3043 (0-18223)	4424 (318-10414)
	Rotation + lame days selection	40 (0-295)	831 (0-5409)	4919 (156-11045)
	Rotation + tally selection	95.2 (0-671)	3575 (0-11216)	10405 (435-14548)

When pasture rotation was combined with genetic selection additional improvements of up to 100% (total elimination) occurred in the number of episodes per year and improvements over 99% were seen in total lame days per year (Table 5.3).

In Table 5.4 the combination of selective culling with genetic selection is considered, using 15% culling level and outcomes in year 10 to compare combined strategies.

Table 5.4. Disease outcomes in year ten when using a 15% culling strategy alone and in combination with genetic selection.

	Control and selection methods	Cull 15% by episodes	Cull 15% by lame days	Cull 15% by monthly observations
Mean number of infections at Yr 10 (range)	Culling alone	606 (522-709)	709 (574-800)	635 (524-696)
	Cull + episode selection	30 (0-126)	40 (1-189)	11 (0-81)
	Cull + lame days selection	61 (25-177)	67.5 (8-162)	49 (0-106)
	Cull + monthly observations selection	159 (31-292)	231.4 (106-341)	169 (15-339)
Mean number of lame days at Yr 10 (range)	Culling alone	28423 (22972-32082)	24711 (19614-27314)	16293 (13159-18317)
	Cull + episode selection	1515 (0-5761)	1969 (80-9276)	331 (0-2088)
	Cull + lame days selection	2183 (919-6315)	2076 (179-5269)	1222 (0-3044)
	Cull + monthly observations selection	5489 (531-11451)	6422 (2584-8951)	4003 (301-7925)

With all combinations large improvements are seen in the number of new infections and total lame days compared with culling alone. Genetic selection based on number of episodes gives consistently better outcomes than the other selection criteria, irrespective of which culling strategy is used. The most effective combination is selective culling based on monthly observations coupled with genetic selection based on the number of episodes which gives the lowest values for both disease outcomes. The second best combination is using the number of episodes as the criterion for both the selective culling and genetic selection.

Selective culling of ewes based on disease observations may be considered to be a form of genetic selection of ewes because selective culling removes the phenotypically most susceptible sheep. Figures 5.12 - 5.14 show the changes in susceptibility, recoverability and revertability when combining genetic selection based on episodes with selectively culling 15% of ewes by each selection protocol, along with the changes achieved with genetic selection alone.

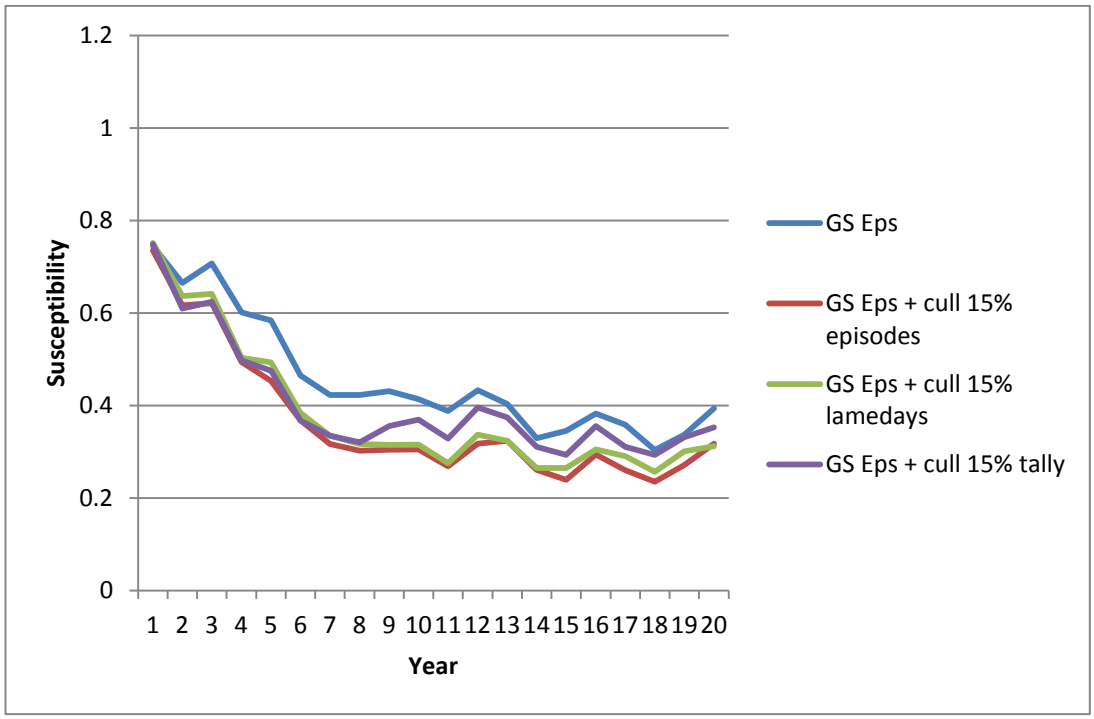

Figure 5.12. Effects on susceptibility when combining genetic selection based on episodes (GS eps) with selectively culling 15% of female sheep using different selection criteria (episodes, lame days and monthly observations/tally).

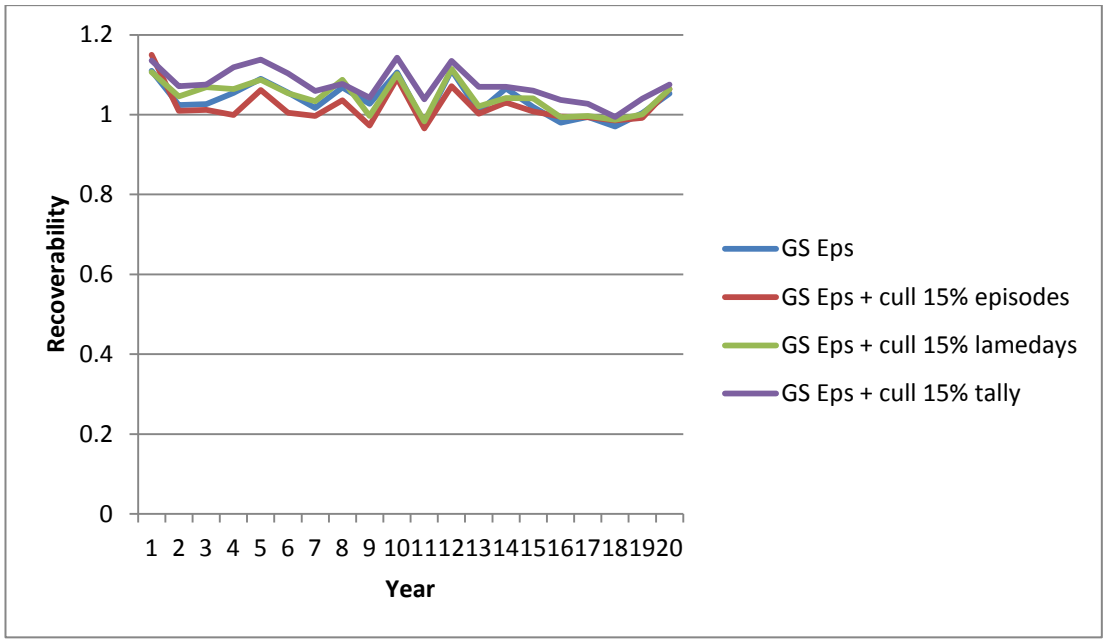

Figure 5.13. Effects on recoverability when combining genetic selection based on episodes (GS eps) with selective culling of 15% of female sheep using different selection criteria (episodes, lame days and monthly observations/tally).

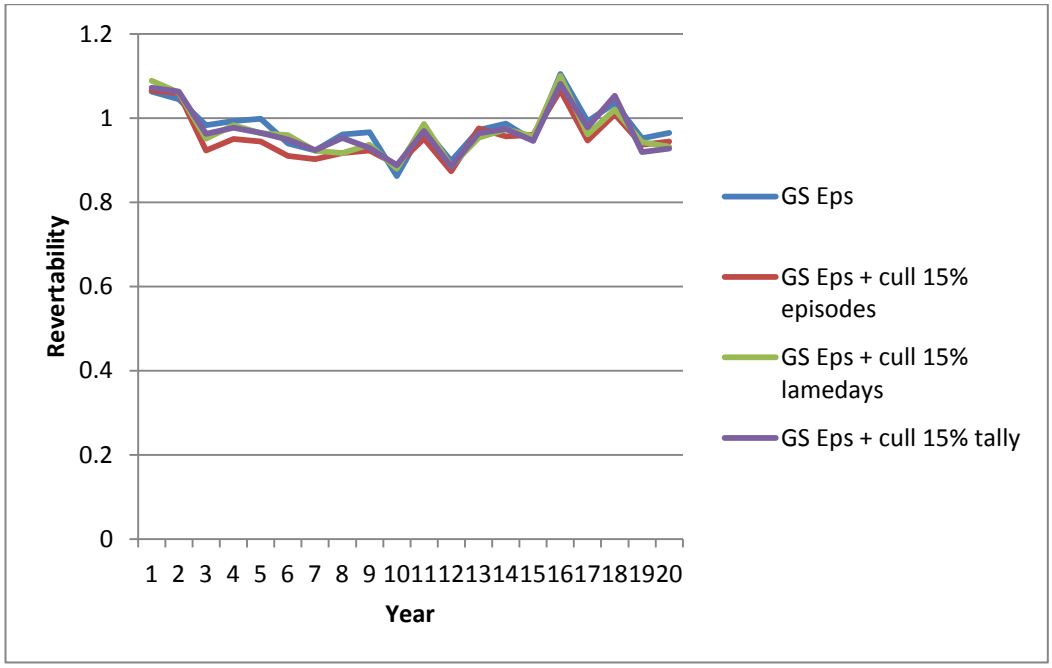

Figure 5.14. Effects on revertability when combining genetic selection based on episodes (GS eps) with selective culling of 15% of female sheep using different selection criteria (episodes, lame days and monthly observations/tally).

Similar magnitudes of effect were seen when using selective culling with genetic selection based on lame days and monthly observations. All of these models use the same set of sires, which accounts for the distinct patterns seen across outcomes, particularly the spikes seen in recoverability. There was a small additional reduction (~0.1) in susceptibility when genetic selection and selective culling were combined, with no clear effect on recoverability and revertability.

Table 5.5. Disease outcomes in year ten when using antibiotic treatment alone and in combination with genetic selection.

	Control/selection methods	**Treat all diseased sheep weekly**	**Treat severely diseased sheep weekly**
Mean number of infections Yr 10 (range)	Treatment alone	23 (2-59)	545 (444-693)
	Treatment + episode selection	2 (0-8)	54 (20-196)
	Treatment + lame days selection	3 (0-6)	77 (11-157)
	Treatment + monthly observations selection	3 (0-11)	129 (29-324)
Mean number of lame days Yr 10 (range)	Treatment alone	184 (19-619)	9937 (7878-12307)
	Treatment + episode selection	8 (0-43)	1257 (630-4113)
	Treatment + lame days selection	19 (0-44)	1398 (255-2419)
	Treatment + monthly observations selection	24 (0-105)	2223 (547-5555)

Table 5.5 shows the added benefits that result from combining genetic selection with antibiotic treatment of either all diseased sheep or severely diseased sheep, in

comparison with treatment alone. Genetic selection based on number of episodes reduced the mean number of new infections and number of lame days per year by more than 90% compared with treatment alone. There were mean improvements of more than 70% with all combinations, with the greatest improvement when combining genetic selection with treatment of all infected sheep.

In nearly all strategies combining genetic selection with conventional treatment methods, non-zero probabilities of achieving elimination by year 20 were observed. Figures 5.15 to 5.17 show the probabilities of elimination when using genetic selection combined with pasture rotation, selective culling and antibiotic treatment respectively. For the purposes of these data, elimination was defined as zero new infections and zero lame days in year 20 of the simulation.

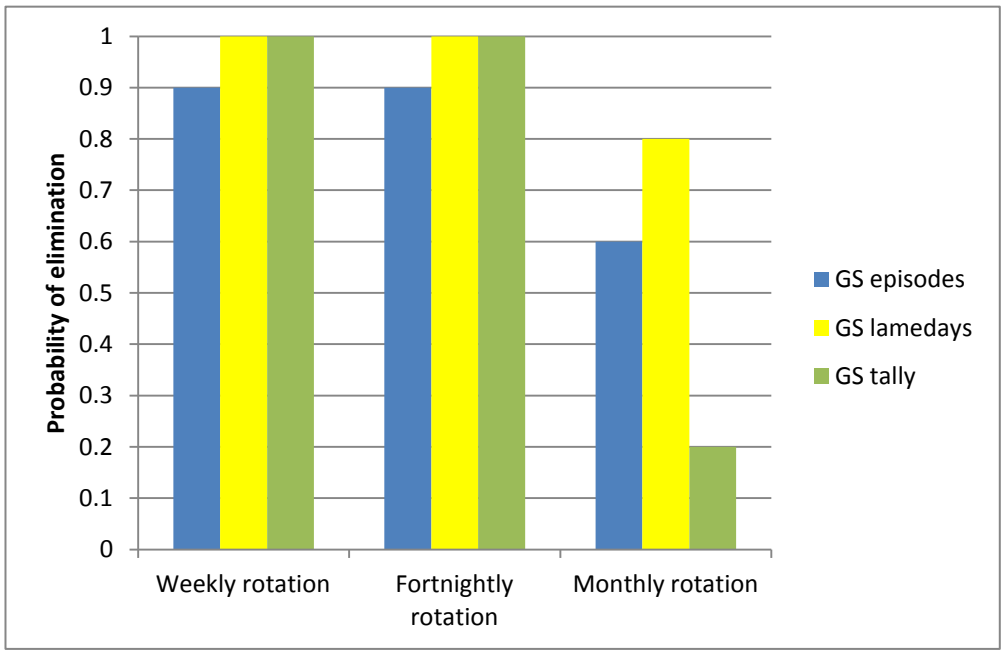

Figure 5.15. Probability of footrot elimination by year 20 using genetic selection and pasture rotation at different time intervals.

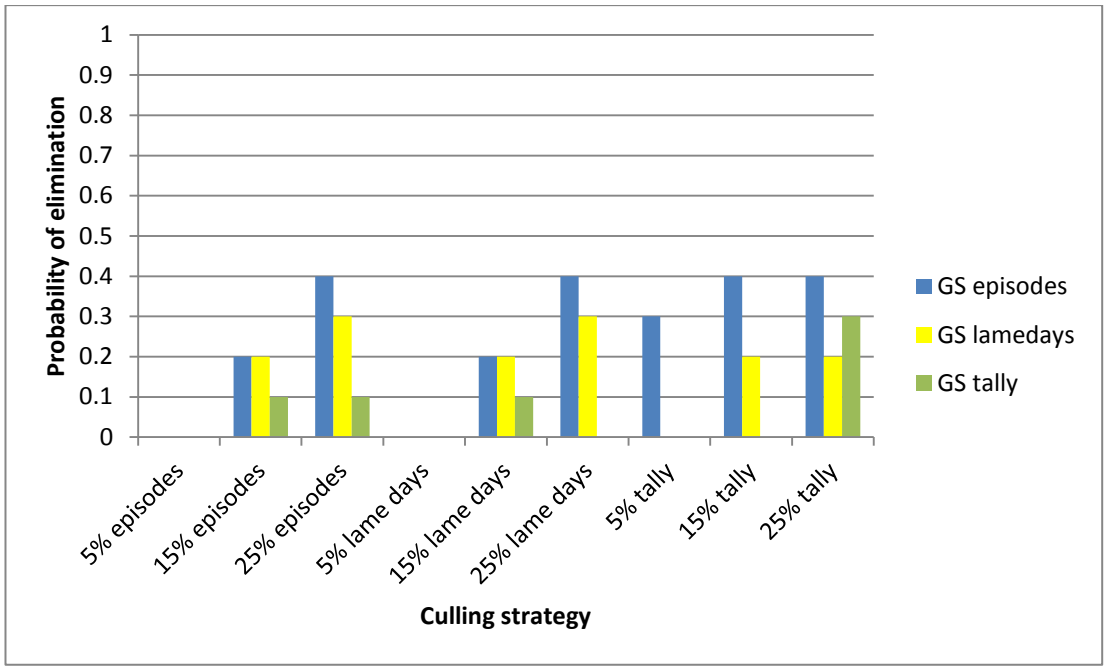

Figure 5.16. Probability of footrot elimination after 20 years when using genetic selection and selective culling of 5, 10 and 15% of female sheep based on number of episodes, number of lame days or monthly observations (tally).

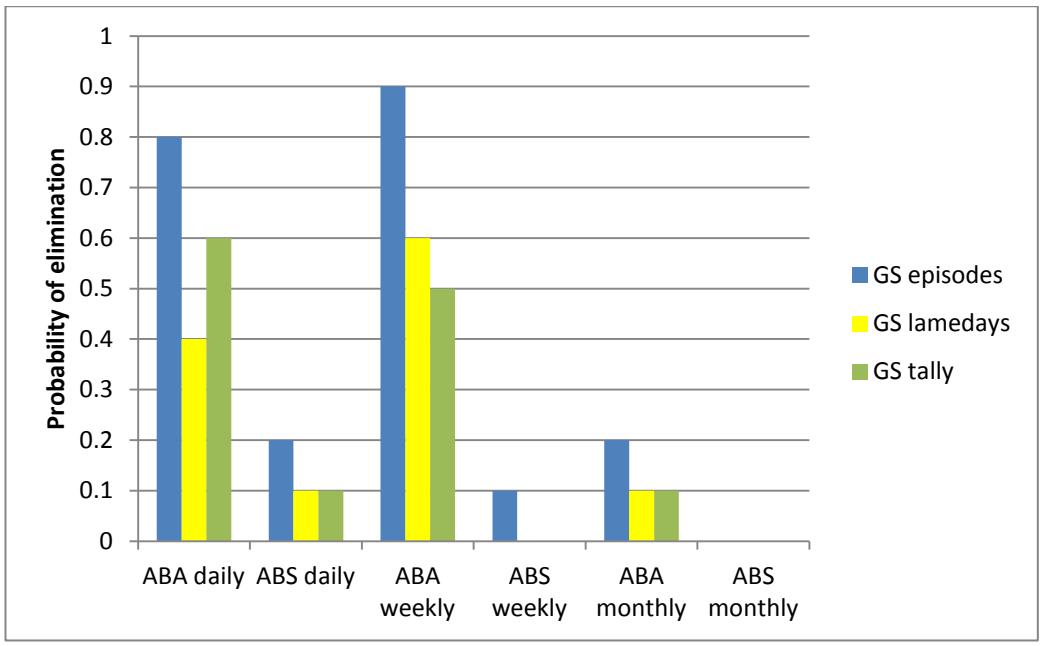

Figure 5.17. Probability of footrot elimination by year 20 when using genetic selection and antibiotic treatment of all diseased sheep (ABA) or severely diseased sheep (ABS) at different time intervals.

Pasture rotation plus genetic selection gave the highest probabilities of elimination, with antibiotic treatment of all diseased sheep weekly or daily also giving high probabilities of elimination. Selective culling never gave a probability of elimination > 0.4 regardless of the culling strategy and the highest probability of eradication with antibiotic treatment of only severely diseased sheep was 0.2.

5.3.3 - EBVs calculated from a flock using antibiotic treatments

It was hypothesised that the use of antibiotics to treat lame sheep would mask the differences between individuals, making the calculation of accurate EBVs more difficult because the covariance of true and estimated breeding values is reduced. This results in a lower rate of progress than when using EBVs calculated in a flock where no interventions were used. EBVs based on lame days and number of episodes were calculated under two antibiotic treatment situations, treating all diseased sheep once a week or treating only severely diseased sheep once a week. Rams selected from each of these treatment flocks were used as breeding rams in the finishing flock to assess the progress that may be made using rams selected from flocks with different treatment protocols and thus different disease prevalence.

The results show a distinct difference between the results from rams selected using the treat all diseased sheep strategy (ABA strategy) and those selected using the treat severely diseased sheep strategy (ABS strategy). Good progress was still made using the ABS strategy but very low impact on disease was seen with rams selected from a flock using the ABA strategy because heritability values in the ABA flock diminished greatly (Table 5.6) due to the low prevalence of disease.

Table 5.6. Heritability values calculated for number of episodes and number of lame days in flocks using different treatment protocols.

Selection flock and its treatment protocols	Mean heritability of number of episodes (min-max)	Mean heritability of number of lame days (min-max)
Base flock (no treatment)	0.09 (0.03-0.13)	0.09 (0.05-0.13)
ABA flock (treat all sheep with clinical signs weekly)	0.01 (0.00-0.04)	0.01 (0.00-0.03)
ABS flock (treat sheep with severe clinical signs weekly)	0.07 (0.04-0.09)	0.05 (0.00-0.10)

The resulting reduction in progress is presented in Figures 5.18 and 5.19, where the number of new infections over time and population mean susceptibility over time when using breeding rams from flocks using the ABA or ABS strategies is presented. ABA strategies significantly reduce the amount of progress made, with up to approximately 10% reduction in new infections and no clear benefit to genetic traits. ABS strategies still give good results, reducing both the number of infections and the mean susceptibility by approximately 40-50%.

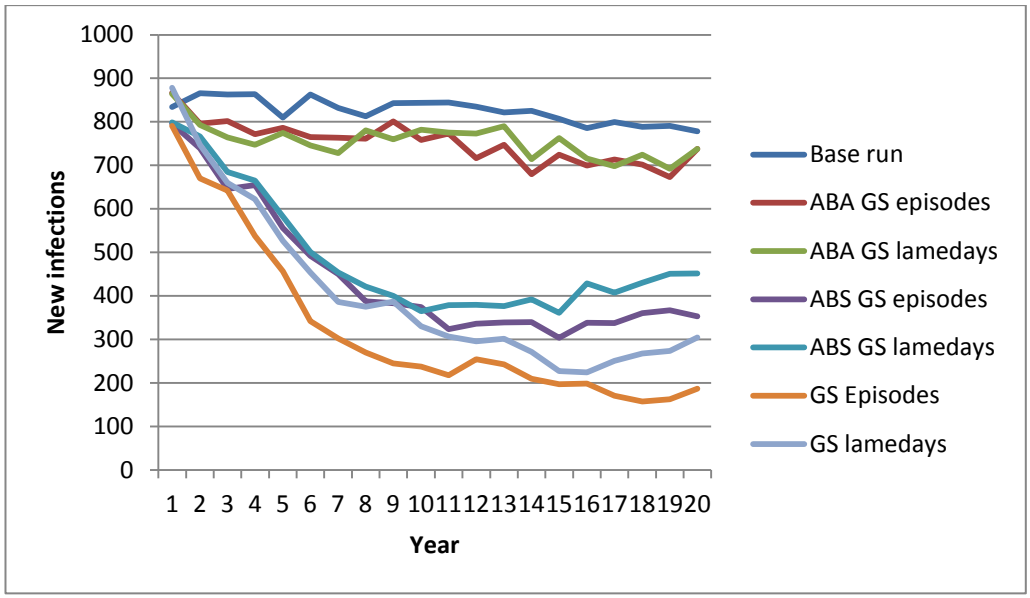

Figure 5.18. Mean new infections per year in flocks using genetic selection where selection took place in flocks employing different antibiotic treatment protocols. Base run - no selection applied; ABA - antibiotic treatment of all diseased sheep in the pedigree flock; ABS - antibiotic treatment of severely diseased sheep in the pedigree flock; GS episodes - selection based on the number of episodes; GS lamedays - selection based on the number of lame days.

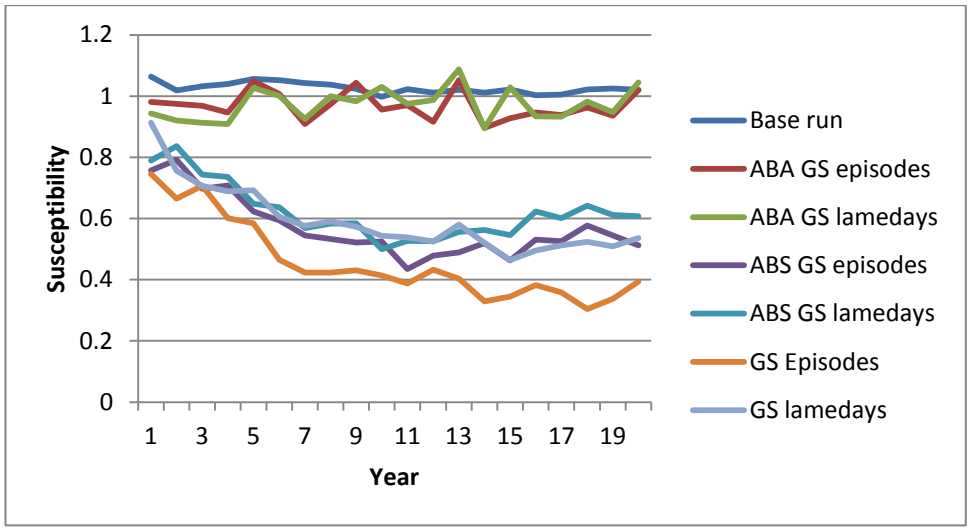

Figure 5.19. Mean susceptibility in the population in flocks using genetic selection where selection took place in flocks employing different antibiotic treatment protocols. Base run - no selection applied; ABA - antibiotic treatment of all diseased sheep in the pedigree flock; ABS - antibiotic treatment of severely diseased sheep in the pedigree flock; GS episodes - selection based on the number of episodes; GS lamedays - selection based on the number of lame days.

5.4 - Discussion

The results from this study show that genetic selection has the potential to reduce the prevalence and incidence of footrot within a sheep flock in the UK, with large reductions seen by year 10. This reflects results seen in the Broomfield Corriedale flock (Skerman and Moorhouse, 1988) and in models developed by Nieuwhof *et al.* (2009).

Nieuwhof *et al.* (2009) used a deterministic model with a homogeneous population to analyse the potential for genetic selection to reduce footrot (Nieuwhof *et al.*, 2009). However, the model developed for the current study has a number of advantages. Firstly it is a stochastic model that enables us to consider variation in outcomes and thus allows the quantification of risk associated with different management methods. Secondly, it is possible to simulate multiple interventions simultaneously to quantify the combined effects, for example using genetic selection in conjunction with antibiotic treatments. Thirdly, the outputs from the model are given over time, meaning that temporal aspects of disease patterns and the methods used to control them may be examined.

The Nieuwhof *et al.* (2009) model showed improvements in prevalence within a few generations, and a reduction in footrot to a prevalence of between 0.03 and 0.10 within 20 years, dependent on the model type used. The current model resulted in a prevalence of 0.05 to 0.1 dependent on the selection criterion used, which is close to those values predicted by Nieuwhof. Both models also demonstrated an effect greater than that due to genetic improvement alone, which is accounted for by the reduction in pathogen burden in the environment that results from the reduced disease prevalence.

Standard quantitative genetic theory only predicts effects that are the direct result of genetic improvement following selection and does not take into account additional improvements that may be the result of reduced pathogen burden. The added benefit from reduced pathogen burden was first discussed by Barger *et al.* (1989) in relation to breeding for resistance to parasite disease, yet it is still not routinely included in models predicting the effects of genetic selection.

It is possible to estimate the proportion of reductions in disease levels that are due to direct genetic effects and the indirect environmental effects caused by the reduction in pasture contamination seen with reduced levels of disease (Table 5.2). The results varied with selection method and over time but in general a higher proportion of disease reduction was due to lower environmental load in year 5 than in year 10. This is because the population mean for genetic traits continues to reduce over time and following the cumulative effects of multiple years of selection, the direct benefits increase further than the environmental effects. The data presented in Table 5.2, quantifying the direct and indirect effects of the selection process, show that the environmental effects are substantial and thus genetic selection results in much greater impact than may be expected from genetic improvements alone.

It is also probable that the models in this study underestimate the direct effects of selection. Selection in these models was based on the use of rams in a static flock, i.e. the rams were not in a flock that was itself undergoing selection. If high resistance to footrot was a desirable criterion for the use of rams in breeding programmes then it is likely that breeders supplying rams to other flocks would use this as a criterion for selection within their own flocks to ensure they had were competitive in the market. This would result in the selected rams having genetic values for resistance that were better than those used in the models, where there was

no selection in the flock from which the rams were obtained. This would in turn lead to a greater effect of selection which would be due to an increase in direct effects.

Good progress in disease reduction was made using rams selected on the number of lameness episodes, with smaller, but still positive, progress using selection based on number of lame days or monthly lameness observations. Selection based on the number of episodes also resulted in the largest decrease in the population's susceptibility values. This decrease in the population's susceptibility would contribute to reducing prevalence in two ways. Firstly, it reduces the probability of initial infections occurring so that a high disease prevalence is never reached. Secondly, when disease is already present in the flock, it reduces the probability of further infections and re-infections occurring and thus propagating the disease further. These two effects combine to slow the infection cycle and thus maintain disease at low levels.

Time spent lame and time until immunity is lost are both also affected by susceptibility as a sheep must be susceptible in order for recoverability and revertability phenotypes to be expressed. Susceptibility may be seen as the key parameter controlling disease presentation in individuals because the other two traits are only important once a sheep has become infected. Reducing susceptibility therefore also has an effect on the total lame days which contributes to the high impact with selection based on the number of episodes.

In the current model rams are selected from a static flock i.e. in the pedigree flock itself no selection for resistance is taking place. The positive results seen here may be further improved by selecting rams from a flock that was itself undergoing selection for footrot resistance. The trait variance is maintained in subsequent generations but

as the mean is reduced through selection the most resistant sheep in each generation should be less susceptible than in the previous generation. These rams would have higher EBVs, in terms of footrot resistance, and thus lead to further progress than seen when continually using rams from a flock with the same population mean for susceptibility year after year.

However, as Figures 5.8 and 5.9 show, selection must be maintained to keep lower prevalence and reduced susceptibility because when use of rams with favourable breeding values for resistance stops, and population average rams are once again used, values revert to those seen prior to the start of a selection programme. It is possible that stopping genetic selection on the commercial farm but continuing with selective culling would slow this reversion because it would continue to remove the worst sheep from the flock and those ewes with the least desirable resistance values would not be used for breeding. However, the effect of dams on the next generation is small; due to the large numbers of ewes used per generation and the low number of offspring per ewe, that this would most likely have only a very small effect. Sires have a much more immediate effect on the population genotypes because only a small number are used (approx. 1 ram per 40 ewes) and thus their genetic information is passed on to many offspring.

The cessation of selection for footrot might be due to breeding values for footrot no longer being available from the pedigree flock, which may occur if the farmer stopped recording footrot data on rams in the pedigree flock. Reversion to base values occurs within 7-8 years, which is undesirable and is equivalent to breeding for increased susceptibility. One approach the commercial farmer may consider is the use of ram lambs from within his own flock for breeding purposes i.e. using a closed flock approach to stop his genetic advantage being diluted by susceptible breeding

rams. After ten years of selection for footrot resistance these ram lambs would be sufficiently resistant to maintain a flock with a low expected prevalence of footrot. However, this would require a strong shift in management for the commercial farmer and would require careful consideration. There would be the added complications of inbreeding to consider if this shift in strategy were to be attempted. In addition, because antibiotic treatment affects identification of resistant rams, the management protocols used in the commercial farm might make accurate selection difficult.

The use of antibiotic treatments in the pedigree flock was very effective at reducing disease prevalence. This also affected the observable heritability (Table 5.6) because variation between individuals and hence the accuracy of the rams' EBVs was low, leading to reduced efficacy of selection. When sheep were only treated once they had shown severe clinical signs the loss of progress in the commercial flock was small because variation between individuals was still observable. However, treatment of sheep in the pedigree flock as soon as they became lame resulted in significant reduction in prevalence (to below 0.01) and so progress when using rams selected from that flock as breeding rams in the commercial flock gave poor results in both disease reduction (Figure 5.18) and underlying susceptibility (Figure 5.19). This is explained by the difference in observable heritability at high and low disease prevalence.

Heritability is a key determinant in the amount of progress that may be made using EBVs, but observable heritability varies with prevalence of disease. In general, higher prevalence results in higher heritability values which Nieuhof *et al.* .(2008b) hypothesise may be due to the fact that not all genes involved in resistance are expressed when infection prevalence is low. The variation between individuals is less distinct when disease prevalence is low, and so the full resistance phenotype is not

observable in individuals (Bishop and Woolliams, 2010). The optimum prevalence to observe variation in binary traits (e.g. infected versus non-infected) is 0.5 because as prevalence tends towards 0 or 1 from 0.5 less variation occurs between individuals (Bishop and Woolliams, 2010). Consequently it is more difficult to identify individuals that perform better, for example those that are more resistant to infection. Additionally, at low prevalence it is possible that not all sheep are exposed and so they are not able to express their resistance phenotype.

However, keeping prevalence high in order to gain maximum EBVs and thus make good genetic progress is highly unethical, and illegal in the UK, as this would adversely impact welfare and would cause sheep pain and discomfort. Some compromises must be made to ensure good welfare as well as possibilities for genetic progress. When treating only severely diseased sheep a much lower impact was seen on the estimation of EBVs. In practice farmers do not always treat on the first day a sheep is observed to be lame (Wassink *et al.*, 2010) so there may still be sufficient variation between individuals in a well-treated flock to enable effective ram selection. Further modelling could be used to quantify the interactions between welfare outcomes (e.g. lame days in pedigree flock) and selection outcomes (e.g. lame days saved in finishing flock).

Selection in this chapter is based on observable disease phenotypes, which are comprised of genetic effects and environmental effects, which means that accurate estimation of genetic effects is complex. A marker test has been developed and marketed in New Zealand based on allelic variation in the DQA2 gene (Hickford, 2000), a component of the MHC system, which is widely used in the New Zealand sheep industry. On Merino farms using the footrot gene marker test, reductions of up to 50% in control and prevention costs have been seen, with up to 70% reductions in

these costs on mid-micron farms (Greer, 2004). However, Conington *et al.* (2008) showed that there were no correlations between the outcomes of that test and footrot in UK sheep, which may be partly explicable by the differing profile of alleles at this gene between the sheep populations of the two countries (Genever, 2009). This suggests that while a marker test may be possible, the commercially available test is not currently useful in the UK setting. Currently, selection for increased footrot resistance would have to be based on observable disease phenotypes, an approach that several studies have suggested may be viable (Skerman and Moorhouse, 1988; Nieuwhof *et al.*, 2008b; Raadsma *et al.*, 1994).

Selection based on phenotype may not be as accurate as having clearly identifiable genetic markers partly because of variation between human observations. Kaler and Green (2008) showed that 83% of farmers could correctly identify interdigital dermatitis lesions and 85% for footrot lesions. However, footrot was also the most common misdiagnosis of lesions, with 47% of incorrectly identified lesions being classified as footrot. This may cause problems in selection because sheep susceptible to other foot problems may be incorrectly identified as being the most susceptible to footrot and thus the most footrot resistant sheep may not be used for breeding. However, if these sheep also suffered severely from other foot problems it may not be desirable to use them as breeding stock. Within the models developed here, it has been assumed that all footrot episodes are correctly identified and this may lead to over-estimation of effects.

The use of trained observers and the training of farmers may help to limit this problem. In a separate study, Kaler *et al.* (2009) examined the inter- and intra-observer reliability when assessing lameness in sheep. This study assessed observers who were asked to score lame sheep on a scale of 0 (not lame) to 6 (will not stand or

move) and found that exact agreement between observers was 68% while individual observers scored sheep the same in 76% of cases. They also found that the majority of differences between observers were one score. This may not cause problems if the point difference was between positive scores because lame sheep would still be identified and sheep with severe lameness would also be correctly identified. However, the greatest disagreements were seen between scores 0 and 1, which may have an impact on identifying mildly lame sheep. These sheep could still be shedding *D. nodosus* onto the soil and the inability to correctly identify these may cause problems in genetic selection programmes, when trying to identify the most resistant animals. Sheep that are consistently only mildly affected by footrot are not resistant but may be tolerant of the disease if no severe clinical signs develop. The model developed for the current study has not considered tolerance but it is an alternative approach to disease control. Future modelling could be used to explore the effects of breeding sheep that were tolerant to *D. nodosus* infection, although this would not lead to the indirect environmental effects that could make breeding for reduced susceptibility so effective. It would also require the model to separate infection and disease states, a process for which there is currently insufficient data.

In the commercial flock, imported rams with better EBVs may be used for selective breeding and when this is combined with traditional treatment and control methods both short term disease reduction and long term susceptibility can be reduced which is the optimal result. Elimination of footrot was seen in a number of simulations combining genetic selection with more traditional control methods (Figures 5.15-5.17). The most effective strategy for elimination of footrot was to combine genetic selection with pasture rotation or antibiotic treatment of all sheep displaying clinical signs of footrot. This is because these approaches reduce the flock exposure to *D.*

nodosus by either moving sheep to clean pasture or reducing the prevalence to very low levels with antibiotic treatment. When combined with the reduction in susceptibility achieved through genetic selection this strategy maintains low levels of pathogen in the environment through good management and reduces the chance of new infections occurring to re-contaminate the pasture.

These simulations consider selection on footrot alone. In practice selection would be based on a combination of predominantly production traits such as growth rate, live weight, muscle depth and fat depth, with health traits such as footrot resistance as secondary considerations. However, there is a strong drive towards functional selection (Conington *et al.*, 2004) including traits such as disease resistance, even if this slows down the rate of selection for other traits. It is still likely that EBVs for footrot would be considered unfavourably if they were adversely correlated with other profitable traits. An EBLEX study (Conington *et al.*, 2009) determined that footrot usually showed either positive correlations or no correlations (Genever, 2009), which makes it a practical possibility for inclusion in future sire selection schemes. To allow farmers to make informed decisions about how to use EBVs for footrot they would need to be included in a breeding index, with the footrot EBVs weighted according to relative worth. The use of footrot selection as part of breeding schemes for Texel sheep is under discussion (Raadsma and Conington, 2011) and it is believed to be a highly marketable trait.

In conclusion, these results are promising for the future use of footrot resistance traits in sire selection schemes. However, consideration must be given to the fact that this is a simulated model and results would need to be tested in a field situation. In practice, selection would not be based on footrot alone, but would be in combination with other traits, which would dilute the effects seen in footrot reduction. Selection

methods used would need to be carefully considered because the effects seen with different selection criteria may vary. The approach taken in flocks with low prevalence of disease would also need to be investigated because in these flocks selection may not yield such positive results due to the masking of differences between individuals.

Chapter 6. Discussion

6.1 - Introduction

In this thesis a model of ovine footrot was developed (Chapter 3) to examine the impacts of current control methods and determine the potential for genetic selection to reduce the prevalence of footrot in UK flocks. The model included host genetics controlling resistance traits, bacterial population dynamics, epidemiology and sheep population dynamics, four key areas controlling disease patterns that are rarely studied together. It was used to combine epidemiological and genetic data so that both short term (Chapter 4) and long term (Chapter 5) control methods could be simulated. Data analysis was also performed using a longitudinal data set collected by Wassink *et al.* (2010) to determine heritability estimates for three footrot traits (lameness, footrot lesions and interdigital dermatitis lesions) and to examine the associations between these traits (Chapter 2).

Results showed that genetic selection could be effective and in combination with short term control methods, such as antibiotic treatment and pasture rotation, elimination of the disease from an individual flock was possible (Chapter 5). However, it also highlighted issues that require further investigation. The accurate identification of susceptible and resistant individuals was dependent on prevalence and suggests that accurate selection would be difficult in flocks with low footrot prevalence (Chapter 5). The selection criteria used to identify the best performing sheep had different effects on disease outcomes and different criteria were required to give optimal results from selective culling and genetic selection programmes (Chapters 4 and 5). Prompt antibiotic treatment of all diseased individuals resulted in very low prevalence of disease and used far lower numbers of doses than when

waiting until clinical signs were more severe before treating (Chapter 4). Heritability estimates were within the range of those seen in other field studies both in the UK and globally, with high genetic correlation between the three traits suggesting they are closely linked (Chapter 2).

These outcomes show that there is potential for genetic selection to be used as a tool to reduce footrot prevalence but there are still areas that require further consideration before such programmes are implemented. The main findings of this study and their implications are discussed in the following sections.

6.2 - Modelling

Nieuwhof *et al.* (2009) previously developed a model of footrot and genetic selection to improve resistance but it was an extremely simple model comprised of a series of differential equations and thus lacking between-animal variability and sheep demography. This prevented the model from being used to explore intervention strategies such as antibiotic treatment and vaccination. The model developed in the current study (Chapters 3-5) expands on Nieuwhof's model in a number of ways, including unique genetic parameters controlling individuals' resistance to disease, population dynamics and full infection and disease cycles. It is also stochastic.

With stochastic models there is variation seen in outcomes due to randomised sampling at various points during each model run. It is desirable to obtain as many repetitions of a single model set up as is practical so that a large proportion of the possible variation is captured. In this study, due to the time available and model detail, the number of model runs used is very limited. This is a drawback of the study and further iterations of the model would have been ideal. However, it does serve to

illustrate the general principles in showing how different strategies compare and even with limited iterations the variation between outcomes can be seen. These outcomes can be used as guides to develop future studies in the field, which would be necessary before outcomes from modelling data are recommended as management strategies in the field. Future work could then include the use of this model to conduct high numbers of iterations and thus make the results more robust.

Variation between animals is a core component of the model developed in this study and one of the main deficiencies in the model presented by Nieuwhof *et al.* (2009). Variation has been addressed on two levels. Firstly, there is a basic assumption that not all animals are the same. This could be modelled using different categories of sheep, for example resistant and susceptible or good vaccine responders and poor vaccine responders. However, for footrot it is known from heritability studies (e.g. Skerman *et al.*, 1988; Raadsma *et al.*, 1994; Nieuwhof *et al.*, 2008b) that resistance to footrot is partly controlled by genotype and so individual genetic variation was included. This allows for a spectrum of responses to infection and treatments and makes analysis of genetic selection through breeding schemes possible.

The individual-based approach is necessary for modelling genetic selection for resistance to diseases where resistance phenotype falls on a continuous spectrum rather than simply resistant or susceptible. It enables researchers to consider the effects of individuals on the disease patterns observed within the flock and provides a framework for targeting interventions towards the least resistant individuals. Consideration of this type of approach may be possible with simple compartmental models, but the number of compartments and the processing power required would be extremely large, making it an undesirable way to approach the problem. It is also of limited use to use deterministic models because the stochastic processes are key to

the outcomes seen. Stochasticity is also necessary to allow a range of outcomes to be seen, which is important in assessing the level of risk when choosing new management strategies for reducing disease prevalence.

The model has been used to examine the effects of not only genetic selection, but also the combination of genetic selection with current conventional control methods because these would need to be used in conjunction to manage disease in short- and long-term situations. These improvements result in a model which provides more detailed and informative outcomes. Heritability estimates from the model were within the range of those seen in field studies (Nieuwhof *et al.,* 2008; Raadsma *et al.,* 1994; Skerman *et al.,* 1988), the use of antibiotic treatment in the model gave outcomes similar to those seen in field trials (Wassink *et al.,* 2010; Green *et al.,* 2007), and the combined direct and indirect effects of genetic selection were apparent as hypothesised in other field and theoretical studies (Nieuwhof *et al.,* 2008b; Bishop and Woolliams, 2010). These outcomes, reproducing patterns seen in field data from a number of sources, provide us with a number of potential approaches to disease control that may be tested in future field studies.

Although the model is able to reproduce field disease patterns there are a few areas that it may be useful to expand in future modelling work to gain greater understanding of the biology of the disease and its behaviour in sheep flocks.

The first of these is age-related susceptibility or expression of resistance phenotype. Nieuwhof *et al.* (2008b) showed heritability of zero in Scottish Blackface lambs and older sheep might have damaged feet, due to previous infections or general wear, that could predispose the feet to infection. It is hypothesised that as lambs grow older they become more susceptible to footrot because their feet become damaged and thus

D. nodosus can more easily gain access to the hoof. Kaler *et al.* (2010) showed associations between foot conformation and lameness in sheep which supports this hypothesis. The role of age-related changes requires further consideration and the individual-based nature of this model means that it could easily be included. However, although future modelling work may benefit from the inclusion of age-related factors there is currently insufficient data to effectively parameterise these effects.

The second factor that may enhance future models is the inclusion of environmental factors such as temperature and rainfall. In Australia, *D. nodosus* has been estimated to survive on pasture for approximately seven days and this length of time is diminished in extreme temperatures and arid conditions (Beveridge, 1941). We do not know the survival time of *D. nodosus* in the UK. In addition, the climate is such that temperatures and moisture levels are almost constantly in the range suggested to be favourable to *D. nodosus* and so usually have little effect on pathogen transmission. However, if the model were to be applied to other countries or used to investigate the effects of climate change on footrot, then temperature and moisture would be important factors to include.

Data on temperature and rainfall could be collected during field studies and are often available for past dates from organisations, such as the Met Office in the UK, who routinely collect weather and climate data. Whether it would be possible to apply this to footrot data to generate 'footrot forecasts' detailing risks is uncertain. This strategy has been used for other diseases that have an environmental compartment, for example helminths and vector borne diseases, and used to generate 'parasite forecasts'. In the UK, the National Animal Disease Information Service (NADIS) publishes a disease profile highlighting risks of parasites in particular regions of the

UK, based on meteorological data (e.g. Wilson *et al.*, 2012). The climate in the UK is almost always in the range thought to be conducive to *D. nodosus* survival and transmission so it is likely that a footrot forecast would not be of great help to UK farmers. However, it is possible that other countries may benefit from this type of forecast. To enable this type of model to be developed a consistent weather recording system would need to be in place, and further studies would need to be done to accurately determine the range of conditions under which viable *D. nodosus* could survive.

The third element that may be considered in future models is the fact that there are multiple strains of *D. nodosus* (Claxton *et al.*, 1983) and individual flocks have more than one strain present at any one time (Moore *et al.*, 2005). It may be useful to consider the different strains present within a flock because they can have different clinical outcomes (Stewart *et al.*, 1984). There is also the effect of *Fusobacterium necrophorum* to consider. It is believed that *F. necrophorum* plays a role in aiding the progression of footrot lesions (Beveridge, 1941; Witcomb, 2012) and may be another target for interventions. It is also unclear precisely how *F. necrophorum* and *D. nodosus* interact to cause the clinical signs of footrot and models may be used to explore the possible mechanisms underlying this process.

Further bacterial details do not seem to be critical in reproducing observed disease patterns because the current model is able to do this without the inclusion of multiple strains or species of bacteria. However, it is of interest in determining the specific biology of infection and the nature of interactions between strains and species to cause the clinical signs seen in footrot. It might also become important if different strains perform better in different climate conditions because this could cause a change in disease patterns seen following climate change e.g. hotter temperatures,

shorter rainy seasons etc. Understanding different strain behaviour might also be important if strain-specific vaccines were used to target disease within a flock. If different strains resulted in widely differing clinical outcomes (suggested by Stewart *et al.,* 1984) then the most virulent strains could be targeted using vaccination to reduce severity of disease. It would be important to understand the dynamics of the bacterial population of the hoof in this situation to minimise the risks of promoting an environment that allowed other virulent strains to flourish.

It is also possible that individual sheep have different levels of resistance or tolerance to different bacterial strains and species. This could greatly impact the way in which genetic selection programmes were designed because it would be desirable to improve resistance to a multiple strains rather than just one. The genetic basis for resistance to footrot is unclear at the present time and may be in part determined by the resistance to different strains or species that result in different clinical outcomes. For example, *Fusobacterium necrophorum* is thought to aid progression of footrot in sheep (Witcomb, 2012) and resistance to this may result in lowered severity of footrot, thus appearing to be reduced susceptibility to footrot. Improved knowledge of bacterial dynamics within the foot of sheep with footrot may therefore be useful in designing optimal genetic selection programmes.

While the models developed in this study are specific to footrot, they could easily be adapted to investigate other infectious endemic diseases of sheep and other livestock populations. For example *Corynebacterium pseudotuberculosis* infection, causing caseous lymphadenitis (CLA) and modelled by O'Reilly *et al.* (2008, 2010), is a chronic disease of sheep with a range of treatment options available that may be modelled using a similar framework.

6.3 - Epidemiological controls and genetic selection

The model was used to investigate different approaches to the management of footrot including conventional control methods (Chapter 4) and genetic selection (Chapter 5). These may be considered as short term and long term control methods that in conjunction may be more effective at reducing disease prevalence than if used on their own. Wassink *et al.* (2010) showed that prompt treatment of lame sheep with antibiotic was effective at reducing prevalence of footrot to 1 - 2%. The model was able to replicate these results, which indicates that it is providing a reasonable representation of the real world situation. It also showed that prompt treatment resulted in fewer doses being administered over time (even in the first year) than waiting until clinical signs were more severe before treating. This is something that could be tested in field trials.

Antibiotic resistance has not yet been seen in the case of footrot but if selection pressures on the pathogens change then this could become a problem. In principle, reduced use of antibiotics should help to reduce the probability of resistance developing because when fewer doses are used, bacteria are under less selection pressure to develop resistance. However, in this case the number of infections is also reduced so that although lower numbers of total doses are used, a higher proportion of the remaining pathogen is targeted by treatments. Thus, although the frequency with which *D.nodosus* are exposed to antibiotic is reduced, they may still be under high pressure to develop resistance because nearly all of the remaining population are exposed to it. This requires further consideration, but it raises the question of whether antibiotic treatment should routinely be used for all cases of footrot or targeted at specific severe cases to reduce the probability of resistance occurring.

The model showed vaccination to be less successful than other treatment and control options, although there are few robust papers on vaccine efficacy to determine if these outcomes are an accurate representation of true effects. The smaller effects were seen because vaccines were given to ewes and then a susceptible population of lambs was born, sufficient to spread disease. Low efficacy in the model may also be due to the way in which vaccine effects were characterised as having a proportional effect on genetic traits, which results in a disproportionate effect on transition times between disease states. This method was chosen for two reasons. Firstly, it is known that the effectiveness of vaccination in individuals is directly related to their underlying ability to mount an immune response. Immune effects were not modelled in this study so modification of resistance traits that may be partly responsible for immunity mechanisms was used as a proxy for these. Secondly, there are insufficient data to include more detailed immune response effects. The addition of immune response data is a possible approach that could be used to improve the representation of vaccination in future models, when sufficient data become available.

The efficacy of vaccination does vary quite widely in field trials (Hindmarsh *et al.*, 1989; Liardet *et al.*, 1989; Duncan *et al.*, 2012) and its effects have been estimated in conjunction with the use of antibiotic treatments. Because vaccines are not tested in isolation (often due to welfare considerations) individual effects of vaccines are not clear. It is possible that antibiotic treatment and vaccination interact to give higher benefits than might be seen alone. This is a likely outcome because although vaccination prevents new infections, the reduction in pathogen shedding following antibiotic treatment of diseased animals would lead to reduced exposure and thus also reduces the incidence of new infections. However, this lack of data makes it difficult to assess the accuracy of vaccination effects as given by the model.

Culling strategies and genetic selection were based on the same selection criteria and the modelling was used to determine which strategy for selection gave the optimal results. Selection based on a single monthly observation gave the best results for selective culling, while selection based on episodes gave the best results for genetic selection. The single monthly observation was equivalent to standardising the environmental variation between individuals because all sheep were observed on the same days. This allowed accurate identification of sheep that were consistently more diseased than other sheep because they were compared at times of equal infection pressure and under the same environmental conditions. It is those sheep that are diseased for long periods of time that contribute the most bacteria to the pasture and thus propagate infection within the flock. Genetic selection showed the best results when using the number of episodes as the selection criterion which is probably because this is the trait that is directly controlled by susceptibility. If a sheep has a very low susceptibility then it will never have an episode of footrot and expression of the other resistance phenotypes (high recoverability and low revertability) are dependent on this event. Thus if very low genetic values for susceptibility can be propagated through the flock it reduces the number of initial infections and thus diminishes the impact of the other resistance traits.

Footrot is a disease that has been present in UK sheep flocks for over a hundred years (Beveridge, 1941). The current situation is the result of not only human management and interventions, but also more than a hundred years of co-evolution between sheep and *D. nodosus* (and possibly other bacteria including *Fusobacterium necrophorum*). It is therefore probable that an equilibrium has been reached in terms of resistance and virulence. Altering this balance by selecting for footrot resistance may lead to increased selection pressure on the bacteria to evolve to overcome the

resistance mechanisms, leading to new strains with different mechanisms of action. The fact that resistance to *D. nodosus* has not already occurred in sheep may also be the result of resistance being linked to other traits with negative consequences, although as diseased sheep may still reproduce it is more probable that the selection pressures have not been high enough to force resistance to develop.

It is clear from the results that selective culling and genetic selection require different selection criteria to achieve optimal results. This is because the two approaches require different disease traits to be targeted and it highlights the key difference between short and long term control strategies. In the long term the desired outcome is to reduce the number of infections that occur, or eliminate the disease altogether, hence the optimal target is susceptibility which directly affects the probability of new infections occurring. In the short term, we want to quickly reduce disease prevalence and prevent suffering in individual animals - the best way of doing this is to curtail infections. This is one of the key outcomes of these models - for short term control it is best to curtail infections while for long term control it is desirable to prevent them from occurring in the first place - and this may be used to aid the design of control and selection programmes.

One aspect that has not been fully explored in this study is the economics surrounding the use of different treatment and control strategies and the costs and benefits surrounding different footrot management strategies. Wassink *et al.* (2010) demonstrated that prompt treatment of footrot with antibiotics resulted in an increase in gross margin of £6.30 per mated ewe. This demonstrated that even considering the costs of treatment, a financial benefit could be obtained by reducing the levels of footrot. For the genetic selection programmes considered in this thesis there is no economic analysis available. However, the reductions in footrot were considerable.

To date, footrot resistance has also showed either slightly positive or negligible effects on other profitable traits, so improving footrot resistance would not be at the cost of reducing other desirable features. This would suggest that genetic selection may also bring financial rewards due to the reduction of footrot, which, although not as rapid as with antibiotic treatment, would eventually be higher as there is no treatment cost associated. However, it is unclear how much the implementation of genetic management programmes would be and it is possible that rams with good footrot resistance traits would cost more than other rams if that became a desirable trait. A full economic analysis of the costs and benefits associated with genetic selection, along with the likelihood of it being adopted by industry, would be desirable before any large scale programmes are implemented.

The results obtained from this study of footrot may also be applied to other diseases of a similar nature. They have shown that infectious diseases where variation in susceptibility is partly under genetic control may be improved by genetic selection. Other (endemic and epidemic) diseases of livestock such as mastitis (sheep and cattle), infectious pancreatic necrosis (salmon), *E. coli* infection (pigs and poultry), PRRS (pigs) *Salmonella* spp. (poultry) and Marek's disease (poultry) have been identified as diseases where there is genetic variation in outcomes, economic impact and industry concern (Davies *et al.*, 2009). The investigation and development of genetic selection programmes to improve resistance for these diseases is underway in some cases (e.g. IPN: Storset *et al.*, 2007; Houston *et al.*, 2008) and may have potential to reduce prevalence of other diseases.

6.4 - Heritability and factors affecting disease presentation

This study is the first time that a large longitudinal data set from a UK setting has been used to comprehensively consider heritability for footrot resistance traits (Chapter 2). Previous studies have used limited observations per individual (Nieuwhof *et al.*, 2008b; Skerman *et al.*, 1988) or did have repeated observations but were conducted in Australia (Raadsma *et al.*, 1994), and resulted in heritability estimates of 0 to 0.29 on a range of footrot traits. The field data collected by Wassink *et al.* (2010) were used in the current study and allowed calculation of heritabilities for footrot lesions (0.09 - 0.60), interdigital dermatitis lesions (0.28 - 0.32) and lameness in lambs (0.24-0.28), but not in ewes because no pedigree information was available.

The results for interdigital dermatitis lesions and lameness were reasonably consistent with the results seen in other studies, but there were large variations in heritabilities for footrot lesions, with high standard errors. This may be explained in part by the fact that few lambs developed footrot during the time before they were removed from the flock so they were unable to express their full resistance phenotype (Wassink *et al.*, 2010; Nieuwhof *et al.*, 2008b; Bishop and Woolliams, 2010). Large standard errors may also be explained by the fact that, in terms of genetic studies, the available data set (Wassink *et al.*, 2010) was relatively small. The study by Nieuwhof *et al.* (2008b) in Scotland estimated a heritability of zero for footrot resistance in lambs, although positive results were seen in ewes from the same flock and breed. The low heritabilities seen for footrot in lambs, and the low prevalence of footrot lesions in lambs suggests an age effect that is not yet fully explored.

Age was a significant factor affecting presentation of lameness in ewes when analysed using mixed effects models but it did not significantly affect lesions (footrot or interdigital dermatitis). On average, older sheep showed increasingly higher locomotion scores, apart from the oldest sheep (those where broken mouth was observed) and these may have lasted in the flock to old age because of good performance, for example showing little or no footrot. This age effect seen in the data supports the hypothesis of Kaler *et al.* (2011) that as sheep get older they become more susceptible to footrot, perhaps because of damage incurred to the feet over time. The increasing severity with age suggests a possible treatment strategy focusing on minimising lameness, and thus damage to foot integrity, in lambs to prevent further infections later in life, although this requires further investigation. Day of birth, which affects the age of the lamb, was also significant in the presentation of lameness but not lesions in lambs which suggests that age-related effects may become apparent early in life.

It is unclear whether the lack of significance of age on presentation of lesion traits, while it is significant for lameness, is a true biological difference or an issue of data collection and analysis. Lameness and lesions have been seen to be closely linked in field studies (Kaler *et al.*, 2011) but in the mixed models analysis presented in Chapter 2 they showed only moderate phenotypic correlation (0.28 and 0.18 for footrot and 0.41 and 0.45 for interdigital dermatitis lesions in ewes and lambs respectively). However, genetic correlations were much higher (0.87 and 0.57 for footrot and 1.0 and 0.82 for interdigital dermatitis lesions in ewes and lambs respectively). This suggests that the two traits are controlled by similar responses in sheep but that there is noise in the data that reduces the correlation seen in observable phenotype.

The lack of pedigree data for ewes in this study restricted the analysis that was possible, for example, although the amount of disease observation data was good, no heritabilities for ewes could be calculated. Thus comparison of heritability to footrot traits over time could not be investigated. Future studies would benefit from having data available for both repeated longitudinal disease observations and full pedigree information so that disease in families over multiple years and at different ages could be more thoroughly investigated.

Breed was also significant in the presentation of disease, affecting lameness and both footrot and interdigital dermatitis lesions. Differences between breeds have been documented previously (e.g. Emery *et al.,* 1984) but this result, from multiple breeds in the same field environment, further supports the idea that genotype affects presentation of footrot. Little work has been done on analysing the differences between many different sheep breeds exposed to the same environment, and to different environmental effects. Flocks tend to be comprised of a single breed so differences between breeds may be exaggerated due to the infectious nature of footrot because the direct genetic effects lead to indirect environmental effects by reducing pathogen, thus making the breed appear even less (or more) susceptible.

There is still scope for work to be done to determine the most and least resistant breeds of sheep but resources may be better devoted to exploring the underlying genetics that result in different resistance phenotypes. The identification of genetic markers would be useful in developing an accurate test for footrot resistance. Although there is a commercially available test based on the DQA2 gene of the MHC (Hickford, 2000), it has shown little correlation with disease outcomes in the UK (Genever, 2009). A much larger data set of genetic information coupled with longitudinal disease observations would be needed from UK sheep in order to

explore associations between potential genetic markers of resistance and disease phenotypes. Studies in a range of different environments might also be needed to further explore the genotype by environment interactions that combine to produce observed footrot phenotypes.

There also remain questions regarding the appropriate measure of disease phenotypes to determine resistance and how these should be collected. The modelling in this study used three criteria, length of time spent lame, number of episodes of disease and monthly observations where each sheep was classified as healthy or diseased. Meanwhile, in the data collected by Wassink *et al.* (2010), observations of lameness, interdigital dermatitis lesions and footrot lesions were available. It has been seen from the modelling that different selection criteria result in different disease outcomes within a flock (Chapter 5) and so desired outcomes need to be carefully matched to selection criteria that target those specific outcomes. Determining accurate ways in which to measure these will be helpful in maximising the potential benefit. It would also be useful to develop further ways of calculating EBVs for footrot, perhaps using combinations of different disease phenotypes, e.g. a function of number of disease episodes and total time spent lame, to generate the most effective footrot resistance score.

6.5 - General conclusions

This study has shown that an individual-based model is able to reproduce observed patterns from a range of different disease and treatment outcomes observed in field studies of ovine footrot. Further field studies are needed to explore the outcomes from this model before recommendations about improved footrot control strategies

can be made to farmers. These could be targeted towards investigating combinations of control strategies and genetic selection. It would also be useful for future work to have a more comprehensive set of data which included longitudinal observations of different disease phenotypes, along with information on bacterial strains present on the hoof and full pedigree information. This would require input from scientists from the disciplines of quantitative genetics, epidemiology and microbiology in order to maximise the amount of useful data obtained. It is this type of collaborative study crossing multiple disciplines that will allow further developments to be made in our knowledge of the biology of diseases like footrot. Future modelling studies may also be useful to address issues such as climate change, which are long term effects that would be difficult to investigate in a field setting.

Outcomes from modelling need to be thoroughly tested in a field setting before they can be successfully used in practice. It is hoped that the results obtained from this study may be used to aid the design of field trials and ultimately promote good management practices that will lead to a reduction of footrot in the UK.

Chapter 7 - References

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Appendix A: Data from sensitivity analysis

Table A1. Parameter input values for sensitivity analysis

	1.1	**1.2**	**1.3**	**1.4**	**1.5**
ω	0.043	0.055	0.035	0.021	0.029
φ	0.035	0.029	0.055	0.021	0.043
h^2	0.279	0.59	0.41	0.5	0.721
σ^2	0.353	0.059	0.157	0.451	0.255
ε	0.114	0.28	0.215	0.373	0.163
	2.1	**2.2**	**2.3**	**2.4**	**2.5**
ω	0.043	0.021	0.055	0.035	0.029
φ	0.055	0.043	0.029	0.021	0.035
h^2	0.41	0.721	0.279	0.5	0.59
σ^2	0.451	0.059	0.255	0.157	0.353
ε	0.163	0.373	0.28	0.114	0.215
	3.1	**3.2**	**3.3**	**3.4**	**3.5**
ω	0.029	0.035	0.021	0.055	0.043
φ	0.055	0.021	0.035	0.043	0.029
h^2	0.41	0.721	0.279	0.5	0.59
σ^2	0.353	0.255	0.059	0.451	0.157
ε	0.114	0.373	0.163	0.28	0.215
	4.1	**4.2**	**4.3**	**4.4**	**4.5**
ω	0.043	0.029	0.021	0.055	0.035
φ	0.021	0.055	0.029	0.035	0.043
h^2	0.59	0.721	0.41	0.279	0.5
σ^2	0.157	0.451	0.255	0.353	0.059
ε	0.163	0.373	0.114	0.215	0.28
	5.1	**5.2**	**5.3**	**5.4**	**5.5**
ω	0.055	0.021	0.035	0.029	0.043
φ	0.043	0.021	0.029	0.055	0.035
h^2	0.721	0.5	0.279	0.59	0.41
σ^2	0.157	0.451	0.353	0.255	0.059
ε	0.163	0.215	0.114	0.28	0.373

All parameter sets were then run with each of the following infection rates (β): a) 0.01; b) 0.001; c) 0.0001; d) 0.00005; e) 0.000025; f) 0.00001. Outputs are referenced as parameter set number followed by infection rate letter, e.g. 1.1a.

Table A2. Sensitivity analysis outputs: new infections in year 20.

	1.1	**1.2**	**1.3**	**1.4**	**1.5**
a	1118	1118	1345	1283	1342
b	1134	995	1386	1155	1286
c	1079	744	922	767	1110
d	875	493	811	391	851
e	603	114	456	66	383
f	132	0	50	0	0
	2.1	**2.2**	**2.3**	**2.4**	**2.5**
a	1457	1246	1061	1143	1346
b	1346	1346	1064	1120	1242
c	987	807	726	1018	876
d	809	538	517	905	764
e	540	121	187	506	301
f	66	0	0	178	0
	3.1	**3.2**	**3.3**	**3.4**	**3.5**
a	1265	1179	1357	1119	1232
b	1295	1016	1444	1128	1102
c	1097	617	1133	805	1023
d	1057	311	970	543	639
e	591	119	630	182	239
f	134	0	24	0	0
	4.1	**4.2**	**4.3**	**4.4**	**4.5**
a	1046	1431	1413	1085	1335
b	1114	1285	1305	1262	1219
c	840	766	1115	837	786
d	760	492	1018	623	625
e	354	159	633	233	211
f	34	0	208	0	0
	5.1	**5.2**	**5.3**	**5.4**	**5.5**
a	1276	1434	1378	1635	1282
b	1138	1273	1276	1298	1166
c	948	982	1116	961	744
d	768	737	940	654	355
e	388	427	629	215	31
f	70	0	254	0	0

Table A3. Sensitivity analysis outputs: total lame days year 20

	1.1	1.2	1.3	1.4	1.5
a	39670	38330	48417	40438	46327
b	39334	34004	47751	39717	42623
c	36234	25519	32534	28713	39427
d	34891	16364	31323	14358	29908
e	20836	4736	17605	3237	12250
f	5017	0	2380	0	0
	2.1	**2.2**	**2.3**	**2.4**	**2.5**
a	48187	46031	33549	36954	45857
b	44644	49601	36019	38279	45054
c	36840	26834	24393	32363	31364
d	32148	24216	17661	28173	31589
e	24168	6341	7114	17493	11722
f	3352	0	0	6336	0
	3.1	**3.2**	**3.3**	**3.4**	**3.5**
a	54871	36049	44973	39346	36087
b	51390	32631	45652	40883	38323
c	37595	22889	41675	27933	29996
d	36583	9538	33207	17980	23541
e	22784	4544	28210	6678	8042
f	6119	0	872	0	61
	4.1	**4.2**	**4.3**	**4.4**	**4.5**
a	33288	46703	48596	33995	45493
b	35389	46423	47928	39826	43314
c	27486	30030	40891	27270	28989
d	25864	17986	36166	20088	24821
e	11880	9904	22670	8078	8249
f	1513	0	9592	0	0
	5.1	**5.2**	**5.3**	**5.4**	**5.5**
a	42817	38032	42700	48550	41766
b	38228	40168	40797	43840	38777
c	33494	30252	34890	36635	26415
d	26149	25529	28517	26474	12114
e	15205	17165	24568	8981	1027
f	3960	0	10666	0	0

Table A4. Sensitivity analysis outputs: heritability of number of episodes in lambs

	1.1	1.2	1.3	1.4	1.5
a	0.2286	0.1903	0.2422	0.1805	0.2809
b	0.2229	0.2349	0.1878	0.18	0.2326
c	0.2301	0.2284	0.227	0.2088	0.2174
d	0.205	0.1759	0.2178	0.1848	0.2439
e	0.2042	0.1226	0.1916	0.1625	0.1692
f	0.0774	0.0928	0.0483	0.035	0.1303
	2.1	**2.2**	**2.3**	**2.4**	**2.5**
a	0.2342	0.2285	0.2013	0.1819	0.2154
b	0.2066	0.2748	0.2338	0.2465	0.2187
c	0.1843	0.219	0.217	0.2451	0.2103
d	0.1619	0.162	0.1468	0.1955	0.2176
e	0.2083	0.1446	0.0903	0.2357	0.1614
f	0.0562	0.207	0.1132	0.1927	0.1882
	3.1	**3.2**	**3.3**	**3.4**	**3.5**
a	0.1831	0.1817	0.2017	0.261	0.2525
b	0.2172	0.1823	0.231	0.225	0.214
c	0.2215	0.2324	0.2236	0.1949	0.1999
d	0.2187	0.141	0.1945	0.2047	0.2034
e	0.1974	0.0275	0.1925	0.093	0.1523
f	0.1356	0.1935	0.0477	0.0128	0.0709
	4.1	**4.2**	**4.3**	**4.4**	**4.5**
a	0.1836	0.2223	0.2196	0.2522	0.2705
b	0.2244	0.1953	0.1905	0.2271	0.2326
c	0.2391	0.1804	0.2073	0.2236	0.2462
d	0.2135	0.1834	0.221	0.1858	0.2149
e	0.145	0.0342	0.2242	0.1199	0.1442
f	0.0796	0.0568	0.1408	0.0778	0.0288
	5.1	**5.2**	**5.3**	**5.4**	**5.5**
a	0.1882	0.2019	0.2616	0.2201	0.2403
b	0.2312	0.2026	0.1941	0.253	0.2775
c	0.2664	0.1836	0.2241	0.2097	0.1881
d	0.2293	0.2216	0.253	0.1476	0.1486
e	0.1748	0.1584	0.2044	0.1413	0.0545
f	0.0867	0.2321	0.1055	0.1397	0.0168

Table A5. Sensitivity analysis output: heritability of number of lame days in lambs

	1.1	1.2	1.3	1.4	1.5
a	0.2326	0.1629	0.2879	0.1802	0.2637
b	0.1804	0.2229	0.2331	0.2195	0.2615
c	0.2326	0.175	0.2732	0.1884	0.249
d	0.2209	0.1689	0.1973	0.1886	0.2691
e	0.2185	0.111	0.159	0.1608	0.1415
f	0.0918	0.1106	0.0333	0.0081	0.1559
	2.1	**2.2**	**2.3**	**2.4**	**2.5**
a	0.2724	0.2977	0.2155	0.1719	0.261
b	0.2119	0.2505	0.1517	0.2078	0.2511
c	0.2481	0.2246	0.2083	0.2044	0.231
d	0.2262	0.1587	0.1695	0.1862	0.2141
e	0.2009	0.1261	0.0847	0.2041	0.1521
f	0.0707	0.4054	0.1585	0.1709	0.1299
	3.1	**3.2**	**3.3**	**3.4**	**3.5**
a	0.1855	0.1356	0.2163	0.2225	0.2279
b	0.2585	0.2107	0.2193	0.2634	0.2406
c	0.2354	0.2245	0.2185	0.193	0.181
d	0.2227	0.1484	0.2625	0.195	0.1753
e	0.239	0.0273	0.1777	0.1112	0.113
f	0.1361	0.229	0.0411	0.0199	0.0327
	4.1	**4.2**	**4.3**	**4.4**	**4.5**
a	0.1782	0.282	0.2122	0.2463	0.2858
b	0.2046	0.2909	0.2344	2397	0.2509
c	0.1565	0.1865	0.217	0.2444	0.2768
d	0.2003	0.2055	0.229	0.1964	0.2379
e	0.1083	0.0379	0.2477	0.1324	0.129
f	0.1238	0.1609	0.1022	0.1098	0.0304
	5.1	**5.2**	**5.3**	**5.4**	**5.5**
a	0.2012	0.2134	0.2409	0.2242	0.2098
b	0.2405	0.1978	0.1817	0.2812	0.2983
c	0.2526	0.1839	0.2093	0.203	0.1896
d	0.2386	0.2087	0.2081	0.2058	0.1336
e	0.1673	0.145	0.191	0.129	0.0443
f	0.087	0.2023	0.1222	0.1574	0.0221