MEAT & LIVESTOCK AUSTRALIA

OJD farm

Development of computer models to describe the epidemiology of Johne's disease in sheep

Project number OJD.027

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1. EXECUTIVE SUMMARY

The pathogenesis, epidemiology and options for control of Johne's disease in sheep were reviewed and mathematical models developed to simulate the spread of Johne's disease within infected flocks, and between flocks on a regional basis. The models also allow the evaluation and comparison of various control options at both flock and regional levels.

1.1 OJD Flock Model

This model simulates spread of OJD within an infected flock. Within the simulated flock, sheep progress between states of susceptible (SUS), Immune (IM), Latent (LT), Light shedding (LS), Heavy shedding (HS) and Clinical cases (CC) and age groups according to defined rules and the input values specified for the simulation. Animals may die or be culled according to the rules of the model.

The user can specify the numbers of sheep in each age-group and state at the beginning of the simulation, as well as values for the various parameters affecting transmission and progression of infection. The model simulates spread within the flock for a specified number of years. Each simulation can be run for a number of iterations, with Monte Carlo simulation used to provide random variation between iterations.

The effects of OJD on flock productivity can be simulated as effects on lambing percentage and on wool production and quality, in addition to the losses due to mortality/premature culling of affected sheep. Although these effects have still not been quantified, the model allows examination of 'what-if' scenarios to estimate the cost of OJD in infected flocks.

The model also allows simulation of various control strategies, including management control, vaccination and test & cull options. Simulations with and without the selected control options are run simultaneously and are compared within the model.

Results for each simulation are summarised as the mean, standard deviation and percentiles of the multiple iterations for each output variable of interest. Outputs from the model include the percentage of sheep in each state/age group at the end of each simulated year, the number and percentage of sheep dying from OJD each year, numbers of sheep tested or vaccinated each year and the amount of wool produced and return from wool sales each year.

1.2 OJD Regional Model

This model simulates spread of OJD between properties within a region. Properties are simulated on a grid of farms, and spread may occur by local spread between adjoining farms, or through the movement of replacement sheep between farms. Spread is simulated for a specified number of years, and the simulation is repeated for a number of iterations to provide a distribution of output values. Each iteration will produce different output values because of the use of probability distributions for some input values, and Monte Carlo simulation to randomise some model processes.

The model simulates spread within each infected flock once it becomes infected, as well as simulating the effect of vaccination on prevalence in the flock. Therefore, the longer a flock has been infected the higher the prevalence and the greater the risk of spread. Conversely, once a flock starts vaccinating, prevalence declines progressively, with a corresponding decline in the risk of spread to other flocks.

Control options that can be used include vaccination, surveillance and quarantine and movement controls on sale/purchase of sheep. The effect of various control strategies can be evaluated by comparing simulations with and without the strategy in place, or with variations of several proposed strategies.

Results for each simulation are summarised as the mean, standard deviation and percentiles of the multiple iterations for each output variable of interest. Outputs from the model include the percentage of flocks infected at the end of each simulated year, and the percentage of flocks tested, quarantined or vaccinated each year.

1.3 Validation and sensitivity analysis of models

Both models were validated by comparison of model outputs with existing data, and appear to provide realistic estimates of the spread of infection both within and between flocks, depending on the input values used. Because of the use of Monte Carlo methods, results varied considerably between iterations with the same input values, due to random chance. This was particularly apparent with the regional spread model, where the prevalence of infected flocks was closely linked to the simulated number of infected studs.

Model output also varied substantially between simulations depending on the input values used. For both models, the most important variables contributing to this variation where age and breed susceptibility, contact rate between susceptible sheep and potentially infected faeces and the probability of an infected sheep progressing from the latent (LT) state to become a light shedder (LS).

1.4 Conclusion

There is still inadequate data available to accurately estimate the true values for many of the parameters involved in spread and progression of OJD infection at both within-flock and regional levels. However, these models provide an opportunity to investigate the effects of assumed realistic values on the rate of spread of infection. In addition, the models allow estimation of the likely costs of disease, and the effectiveness and cost-benefit of proposed control strategies, particularly at the farm level.

As more precise estimates of the values of key parameters become available, the models will allow a rapid assessment of the likely impact of these values on our understanding of the disease.

2. INTRODUCTION

Johne's disease, or paratuberculosis, is a bacterial infection affecting mainly ruminants, and causing a chronic, granulomatous enteritis. Johne and Frothingham first described the condition as an atypical form of tuberculosis in cattle in 1895. It was subsequently re-named pseudo-tuberculosis, or Johne's disease in 1905 by Bang (Chiodini et al., 1984). The first case of Johne's disease in sheep was reported in 1911 (Stockman , 1911, cited by Manktelow and Hellstrom, 1979. The causal organism is very closely related to *Mycobacterium avium*, and although taxonomically it is now known as *Mycobacterium avium* subspecies *paratuberculosis* (Thorel et al., 1990), it is often still referred to as *Mycobacterium paratuberculosis* (Juste, 1997).

Johne's disease now has a virtually worldwide distribution in cattle, sheep and goats, and has also been diagnosed in many other species of domestic and wild ruminants, including deer and South American camelids. Natural infection of rabbits, foxes, stoats and other wildlife with *M. paratuberculosis* has also been recorded (Greig et al., 1997; Beard et al., 1999; Beard et al., 2001). A number of mono-gastric species have been infected experimentally, although infection did not usually result in occurrence of clinical disease or typical lesions of paratuberculosis (Chiodini et al., 1984; Sharp, 1997).

M. paratuberculosis has also been isolated from humans suffering from Crohn's disease, although there is still considerable debate about its role in the aetiology of Crohn's disease (Chiodini and Rossiter, 1996; Anonymous, 2000).

Ovine Johne's disease was first diagnosed in Australia in 1980, in the central tablelands region of New South Wales (Seaman et al., 1981). By the end of 2000, the disease had been confirmed in more than 800 flocks in all Australian States and Territories except for Queensland and the Northern Territory (Sergeant, 2001a), although the disease was still highly clustered geographically. In early 1999, agreement was reached for a 6-year national program for the control and evaluation of ovine Johne's disease in Australia, funded jointly by governments and industry. A key component of the national program is support for research into the epidemiology of Johne's disease in sheep in Australia, and the identification and evaluation of options for on-farm control or eradication.

Mathematical models can be used to solve real-world problems by translating them into mathematical descriptions that can then be analysed and solved using standard mathematical techniques (Murthy DNP et al., 1990). Although models provide only a very simplified representation of the real world, they can be very useful for comparing alternative interventions, or for identifying specific areas requiring further research, and quantifying the potential benefits of research. Disease modelling is particularly useful because it allows the investigation of a variety of disease control options that would often not be possible or practical to evaluate experimentally or in field trials.

Modelling of OJD may have a number of benefits. Outcomes may be able to be predicted at various time points in the progression of the disease, depending on the control measures instituted at those points. This will assist the development of policy options for disease control and the evaluation of disease and economic outcomes from a range of possible inputs on an industry basis. Individual farmers or groups of farmers may also benefit on a "micro" scale to identify, measure and weight risk factors to assist in the development of control strategies. It is arguable how much information is available for OJD to develop these models, although the information base is rapidly expanding. The benefit of modelling, particularly on a regional level, is greatest early in the progression of the disease but at the very time information is limited. Given these imperatives, models are often developed without all the information that is desirable. This may be overcome by providing a range of scenarios and choosing the "most likely" options.

3. OBJECTIVES

The objectives of this project were stated in the terms of reference and are repeated here:

3.1 A within-flock model for the spread of ovine Johne's disease

Develop a generic computer simulation model that describes the onset, progression and control of OJD within an infected flock and describes the change in infection and mortality rates, progression of infection and ability to detect disease as controls are applied. The model should include, but not be limited to the effect of:

a) Biology of the organism (survival) and host factors (infective dose, incubation period, age, time since infected, breed, expected mortality, production effects, stress factors such as internal parasitism, lactation)

b) Management factors that may affect the expression of the disease (grazing system, water supply, method and degree of hand feeding, stocking rate, risk of lateral spread from neighbours, age of culling, purchase of sheep and their "source")

c) Available diagnostic tests taking into account test performance depending on the age of the sheep and stages of disease.

d) Control options such as methods to reduce shedding of bacteria on grazed areas (vaccine, early culling), the production and manipulation of "safe" pastures for grazing by some classes of stock.

The model should determine what are the likely disease outcomes, including changes in disease prevalence and mortality rates, production losses and cost/ benefit, given a particular level of control input.

The model should be sufficiently generic so that it can be adapted to a range of management and production systems within Australia.

This model should be tested using data from a range of known infected farms over time.

3.2 A model for the regional spread of ovine Johne's disease

Develop a generic computer model that describes the progression of OJD between flocks in a region or area taking into account the available surveillance tools, rate of lateral spread, specific control options (such as movement restrictions and vaccination of infected and neighbour farms). The outcomes should be a description of the effect of varying levels of control activities on the rate of spread and prevalence of infected flocks, including the financial implications.

This model should be tested using data already known in regard to spread of infection in endemic areas.

4. EPIDEMIOLOGY, PATHOGENESIS AND CONTROL OF OJD

This review is intended to summarise the key issues in the epidemiology and pathogenesis of the disease, and provide a basis for the development of mathematical and simulation models of the spread and impact of paratuberculosis in sheep flocks in Australia. It draws on information from the published literature as well as on a number of other recent reviews on the subject, and incorporates new information not available to previous reviewers (Stehman, 1996; Sweeney, 1996; Sharp, 1996; Whittington and Sergeant, 2001; Manning and Collins, 2001).

4.1 Biology of *M a paratuberculosis*

M a paratuberculosis is a Gram-positive, acid-fast bacterium, closely related to *Mycobacterium avium*. It is defined as an obligate pathogen of animals and therefore is assumed to be unable to multiply in the environment (Thorel et al., 1990). It is dependent on mycobactin as an exogenous source of iron for growth and replication *in-vitro* (Chiodini et al., 1984). *M a paratuberculosis* can be differentiated from *M avium* based on cultural characteristics or by DNA analysis using the IS*900* insertion sequence that is specific to *M a paratuberculosis* (Collins et al., 1990; Vary et al., 1990; Whipple et al., 1990). However, mycobacteria other than *M a paratuberculosis* that were positive to IS*900* PCR have recently been identified, so that restriction endonuclease analysis of PCR product should be undertaken to confirm the identity of PCR-positive isolates (Cousins et al., 1999).

Detailed DNA analysis has shown that there are numerous strains of *M a paratuberculosis*, which can be broadly categorised into two groups, identified as C (cattle) and S (sheep) (Collins et al., 1990). Generally, C types have been isolated mainly from cattle, goats, deer and camelids, while S types have been isolated mainly from sheep and goats. Although there appears to be a host-preference for the different strain types, this preference is not absolute, and some crossover of infection has occurred (Whittington et al., 2001; Sergeant, 2001a). Johne's disease in sheep in Australia has been caused almost exclusively by S strains of *M a paratuberculosis* (Whittington et al., 2000; Sergeant, 2001a).

4.2 Transmission of infection

Spread of paratuberculosis is primarily via the faecal-oral route, with clinically affected animals excreting large numbers of organisms and causing significant environmental contamination. Young animals are exposed to faecal contamination of the udder, fodder and the environment, providing ample opportunity for exposure to an infectious dose of *M a paratuberculosis*.

Dissemination of infection to other tissues does occur, including to the uterus, supramammary lymph nodes, udder and sexual organs, and it may be excreted in milk and semen (Stehman, 1996; Sweeney, 1996; Eppleston and Whittington, 2001), and intra-uterine infection has been confirmed in cattle (Sweeney, 1996). The likelihood of foetal infection, and of excretion in milk, increases with the severity of infection, with most clinical cases likely to have disseminated infection. The level of foetal infection ranged from 8.6% of foetuses in asymptomatic cows to 20% – 40% in clinical cases (Sweeney, 1996). However, although some cattle may be infected *in utero* or directly from infected milk, this does not appear to be a major source of spread (Chiodini et al., 1984; Sweeney, 1996). The importance of vertical transmission in sheep is still unclear, but is probably relatively minor compared to horizontal transmission to lambs during and soon after lambing via faecal contamination of the udder and environment.

Although it has been suggested that cases infected *in utero* may progress more rapidly than post-natal infections, this has not been confirmed (Sweeney, 1996).

4.2.1 Infectious dose

The infective dose of *M a paratuberculosis* is not known, but appears to be fairly low (Chiodini et al., 1984). Infection may result from single or multiple oral exposures, and the infectious dose may be as low as 10^3 – 10^7 organisms (Brotherston et al., 1961; Reddacliff et al., 2001). The importance of ongoing

exposure is unknown, but may be important in establishing infection, particularly at low levels of contamination.

4.2.2 Susceptibility to infection

Susceptibility to infection with *M a paratuberculosis* is likely to be affected by many of the same factors that affect the progression of infection in infected animals (see below). Age and breed are the two specific factors most commonly regarded as affecting susceptibility to infection, although any stress resulting in reduced general immunity may also result in an increased susceptibility. Variations in susceptibility may be measured in terms of the number of organisms required to establish infection, or the probability that infection will establish for a specified dose of organisms.

In cattle, susceptibility to infection appears to be highest in young animals and declines progressively with age. Cattle are generally assumed to be highly resistant to infection by about 1 year of age, and infection of adult cattle may require much higher infective doses and result in longer incubation periods than is the case with neonatal infection (Whitlock and Buergelt, 1996). Sheep are also likely to have an increased level of resistance as adults, although this has not been confirmed. It is also unclear whether age-resistance is due to failure of infection to establish, or due to the greater ability of older animals to contain and reject the infection once it occurs.

An estimated cumulative dose of 10^3 organisms given over 10 weeks was sufficient to establish infection in 3-week-old British-breed lambs (Brotherston et al., 1961), compared to a single dose of $>10^7$ organisms required in merino weaners (>12 weeks of age) (Reddacliff et al., 2001). However, comparison of infectious doses between studies must be treated with some caution because of variations in culture and enumeration techniques. In fact, the estimated dose from Brotherston et al. (1961) might have underestimated the true dosage by 1 – 2 $\log_{10}$ (Reddacliff and Whittington, 2000). The infectious dose for adult sheep has not been determined. Overall, the probability of infection establishing in sheep is probably dependent on both the age of the animal and the cumulative dose of organisms to which it is exposed (Sweeney, 1996).

The greater apparent susceptibility of younger ruminants is possibly due to the relatively low proportion of T-lymphocytes in the ileal Peyer's patches favouring mycobacterial survival in this area. As involution of the ileal Peyer's patches occurs with age this susceptibility decreases (Miyasaka et al., 1983; Reynolds and Morris, 1983; Lugton, 1999; Clarke, 1997).

Variations in susceptibility between breeds of cattle have also been suggested, although this may relate to abundance of the breed and management factors affecting transmission and progression of infection rather than true differences in susceptibility (Chiodini et al., 1984). Breed-differences in susceptibility in sheep have not been documented. However, there have been anecdotal reports of variations in the level of disease between breeds, with British breeds and their crosses regarded as more resistant than merinos. In one study, fine-wool merinos were found to be more prone to clinical disease than stronger-wool merinos, which were also more prone than British and cross-breeds (I Lugton, personal communication). A study in Dutch dairy cattle also found that there was evidence of genetic effects on susceptibility to infection with *M a paratuberculosis*, with a heritability estimate of 0.06 (Koets et al., 1999). It is not known whether any breed-effect on susceptibility acts through true resistance to infection, or whether animals become infected at a similar rate, but are less likely to progress to clinical disease.

4.2.3 Excretion rate

Generally, animals in the early stages of infection are faecal-culture negative, although they may excrete *M. a paratuberculosis* below the limits of detection for current culture techniques (Whitlock and Buergelt, 1996). However, compared to the levels of excretion from more advanced cases, early (latent) cases probably do not contribute significantly to the overall level of environmental contamination except in recently infected herds or flocks. As the infection progresses, the level of faecal excretion of *M. a paratuberculosis* also increases. Initially, excretion may be intermittent, becoming more constant as the disease progresses (Chaitaweesub et al., 1999; Whittington et al., 2000). The average excretion rate for five sub-clinically infected sheep in one study was 1×10^8 organisms per gram of faeces, or about 8×10^{10} organisms per sheep per day (Whittington et al., 2000). Excretion rates for earlier cases (intermittent and light shedders) have not been quantified.

4.2.4 Survival of M a paratuberculosis in environment

M a paratuberculosis is capable of surviving for extended periods in the environment, in faeces, soil or water. In bovine faeces, survival for periods of 8 – 11 months has been reported and in tap water for up to 14 months (pH = 5 and pH = 8.5) and 17 months (pH = 7) (Jorgensen, 1977; Vishnevski et al., 1940, cited by Wray, 1975; Lovell et al., 1944; Larsen et al., 1956). Under Australian conditions, sheep strains of *M a paratuberculosis* survived >12 months in faecal pellets in a shaded location, and for 48 weeks in the sediment of a water trough deliberately contaminated with infected faeces (Whittington, 2001).

The rate of survival is affected by environmental and climatic/seasonal factors, but the specific effects are not well understood. Unfavourable conditions of high exposure to sunlight and desiccation are likely to reduce survival, whereas survival will increase when conditions are cool, moist and protected. Even within a single paddock, some areas will harbour viable organisms for much longer than others, depending on the local micro-environment. This may be exacerbated by the movement of faecal pellets in run-off or by wind to low-lying sheltered areas that may favour survival and also be preferentially grazed.

Although soil acidity may have some effect on survival rates, studies have demonstrated prolonged survival (9 – 14 months) under a pH range from 5 – 8.5 in water (Lovell et al., 1944; Larsen et al., 1956). These findings suggest that any effect of soil pH on survival of *M a paratuberculosis* is unlikely to be important in the overall epidemiology of the disease. Exposure to direct sunlight is probably the main factor affecting the duration of survival of *M a paratuberculosis* in the environment, probably associated with temperature flux (Whittington, 2001). In these studies, moisture levels, lime application and exposure to UV light had no apparent effect on survival. However, survival for 4 – 9 weeks was recorded in unshaded locations in western NSW during early summer.

According to current knowledge, *M a paratuberculosis* is by definition an obligate parasite of animals, and therefore is unable to multiply in the environment, so that the level of contamination cannot increase other than through excretion of organisms from infected animals. Thus the duration and level of contamination on properties following de-stocking will generally depend on the initial level of contamination (determined by the number of infected sheep, their excretion rates and the duration of excretion) and the rate of decay (determined by factors affecting bacterial survival).

Studies undertaken to estimate decay rates for *M a paratuberculosis* found that decay appears to be bi-phasic, with an initial rapid decline over the first 8 – 10 weeks, followed by a much slower decline over succeeding months (Whittington, 2001). In some experiments there also appeared to be a period of dormancy of the organism, followed by an apparent increase in the number of viable organisms present above previous levels. The observed decay rates in these studies were about 0.2 – 0.4 $\log_{10}$ per week for the first 8 –10 weeks post-contamination, and 0.05 – 0.1 $\log_{10}$ per week thereafter, in a shaded environment. Decay rates under non-shaded conditions were not measured, but were inferred from the survival data for unshaded locations, with estimates ranging from 1 to 6 $\log_{10}$ per month (Whittington, 2001).

The possible occurrence of dormancy and subsequent increase in bacterial counts also cast some doubt on the assumption that *M a paratuberculosis* is unable to multiply in the environment.

4.3 Pathogenesis and progression of infection in infected sheep

Although the pathogenesis of paratuberculosis is still not well understood, it appears that it progresses through a number of stages, as described in Table 1. These hypothesised stages correspond to the changes that occur in histological lesions, and cellular and antibody mediated immune response as the disease progresses. Although the stages are described as distinct steps in the development of the disease, there is in fact no clear distinction between stages, but rather a gradual progression of the infection through successive stages. However, considering the pathogenesis as a progression through a series of distinct stages is useful for a better understanding of the epidemiology and diagnosis of paratuberculosis. These hypothesised stages correspond approximately with the stages of disease in cattle described by Whitlock and Buergelt (1996) and also with the range of histological lesions in sheep described by Perez et al. (1996) and Clarke (1997).

Following exposure, infected animals undergo a variable, but generally long latent period (Stehman, 1996; Chiodini et al., 1984). It appears that some animals with latent infections may eliminate the infection without ever progressing, while others may remain in a latent or incubatory state throughout their productive life, without ever exhibiting clinical signs. In many flocks with established infection, it is likely that the majority of animals become infected, but that many of them subsequently eliminate the infection and are probably resistant to re-infection (Gilmour et al., 1978; Perez et al., 1996, Chiodini, 1996; Clarke, 1997).

After a variable period of time, infection starts to progress in a proportion of latent cases. The trigger to start this progression is unknown, but is possibly associated with waning of the CMI dominated immune response. As lesions become more severe, animals will start to shed bacteria in their faeces, initially intermittently and eventually continuously, as the sheep progress through the various stages shown in Table 1. It may be possible for light or heavy shedders, or even clinical cases to recover and eliminate the infection, but this is probably a rare event (Gilmour et al., 1978; Hagan, 1938; Hagan and Zeissig, 1935). In sheep, the course of the disease is more rapid than in cattle, with clinical cases commonly seen in sheep 2-3 years of age, and sometimes at less than 12 months of age (Stehman, 1996; Denholm, 1996) and faecal shedding detected as early as 9 –12 months of age (Chaitaweesub et al., 1999; Eppleston et al., 2001).

The factors affecting the probability and rate of progression through these stages are not fully understood. However, progression of infection is likely to be affected by many of the factors that are important in initial establishment of infection. Specifically, the (cumulative) dose of *M a paratuberculosis* received by an animal and the age at infection are thought to be important factors in affecting progression of infection (Julian, 1975; Whitlock and Buergelt, 1996; Whittington and Sergeant, 2001; Stehman, 1996).

Soil type and pH have also been suggested as possibly affecting the prevalence of disease, although the mechanism of action for any effect is unknown (Johnson-Ifearulundu and Kaneene, 1997; Reviriego et al., 2000). Similarly, breed and genetic susceptibility might also affect the rate of progression of infection in addition to or instead of any direct effect on susceptibility to infection (Manning and Collins, 2001; Koets et al., 1999).

The ongoing level of exposure to *M. a paratuberculosis* appears to be an important factor in the pathogenesis of paratuberculosis, with the rate at which the disease progresses in individual animals affected by the initial dose rate, and probably also by the level of ongoing exposure. The level of exposure may also be one factor involved in progression of latent cases as continuous exposure to *M. a paratuberculosis* organisms eventually overcomes the ability of CMI to contain the infection (Whittington and Sergeant, 2001; Clarke, 1997).

Finally, because CMI appears to have a key role in suppressing infection, any factors affecting the ability of an animal to maintain an effective CMI response are likely to affect progression of disease (Chiodini, 1996; Juste, 1997; Clarke, 1997; Manning and Collins, 2001). Such factors may include herd/flock management, nutritional stress, pregnancy and lactation, occurrence of other diseases such as internal parasites and pregnancy toxaemia, and other stresses (Seaman and Thompson, 1984; Julian, 1975; Stehman, 1996; Lacetera et al., 2001).

Given the long incubation period and chronic nature of the disease, only a very small proportion of the infected sheep in an infected flock will show signs of clinical disease at any one time. The majority of infected sheep will have either latent or sub-clinical infection, as described for cattle (Whitlock and Buergelt, 1996). Many sheep may also have recovered from the infection and be immune, while some may be excreting *M a paratuberculosis* but still be seronegative.

4.3.1 Effect of soil type and pH on expression of Johne's disease

The effect of soil type and pH on the occurrence of Johne's disease is still unclear, despite a number of reports suggesting an association. This is further complicated because the likely mechanism of action is also unclear.

It has been hypothesised that high pH and low availability of iron in the soil may affect survival and multiplication in the environment (Johnson-Ifearulundu and Kaneene, 1997). However, this appears unlikely because pH appears to have only a moderate effect on organism survival in water and application of lime did not affect survival under experimental conditions. Also, because *M a paratuberculosis* is defined as an obligate pathogen of animals, it is assumed not to undergo multiplication in the environment (see above).

An alternative hypothesis is that high soil pH acts to reduce the level of available iron in the animal, limiting the ability of the organism to multiply and cause disease in infected animals (Richards, 1989b). As early as 1935, an association between low soil pH and the occurrence of clinical Johne's disease in cattle was reported in England, with subsequent reports from France, the Netherlands, United States of America and South Africa also supporting this hypothesis (Smythe, 1935; Gasse, 1962, cited by Kopecky, 1977; Jansen, 1948; Kopecky, 1977; Richards, 1989a; Michel and Bastianello, 1999; Reviriego et al., 2000). The application of lime to pastures has also been suggested as being protective against Johnes's disease. Clinical Johne's disease is reported to have disappeared from the island of Jersey after the adoption of widespread liming of pastures in the early 1900's (Spicer, 1936), and liming of pastures was associated with a reduction in the likelihood of being sero-positive for Johne's disease in US dairy herds (Johnson-Ifearulundu and Kaneene, 1998).

However, some of these reports are anecdotal in nature, and are not supported by detailed data to support the hypothesis (Smythe, 1935; Gasse, 1962; Spicer, 1936; Richards, 1989a). Other reports present more detailed analyses, but do not effectively demonstrate that the observed differences between regions or herds were not due to other causes, particularly the underlying distribution of the infection due to historical or industry-related factors or to other measures implemented concurrently with liming.

In Australia, there is little doubt that the occurrence of Johne's disease in sheep is strongly correlated with soil pH. However, the main sheep producing areas of eastern Australia generally have acid soils because of pasture-improvement practices over many years. Therefore, because the disease was originally introduced into an area of acid soils and has since spread to surrounding areas in more recent years, it is impossible to distinguish any effect of soil type from the underlying geographic and temporal pattern of infection. Similarly, Johne's disease is more common in dairy cattle than in beef breeds in Australia, with dairies predominantly found in areas of high pasture improvement and acid soils. Again, it is difficult to demonstrate any clear effect of soil type because of the underlying pattern of infection in the industry.

Regardless of any possible association between acid soils and the occurrence of Johne's disease, there are other agronomic and production reasons for controlling soil acidity. Any additional control of Johne's disease achieved through liming of soils would be an added benefit.

Table 1: Stages of infection due to paratuberculosis

Classification	Duration[a,b]	Histological lesions[c]	Lesion type[c]	Stage[b]	% of herd/flock[b]	Gross lesions & clinical signs[c]	Serological tests[a]	Tests for CMI[d]	Faecal shedding[a]
Latent	Months – Years	Mild focal or multi-focal lesions, no visible afb's, some animals tissue culture positive.	Type 1	Stage 1	60 – 70%	Nil	Ineffective, sensitivity close to zero (<10%)	Not well characterised –potentially moderate-high	Not detectable
Light shedders	Months	Spreading focal lesions, some with a few afb's visible	Type 2	Stage 2	20 – 30%	Nil	Generally poor sensitivity (10-40%)	Sensitivity decreasing	Intermittent culture positive, low numbers of organisms
Heavy shedders	Weeks – Months	Progressive lesions, afb's common	Type 3a	Stage 2		early gross lesions may be observed, no clinical signs	Moderate sensitivity (40-70%)	Poor sensitivity	Usually culture positive, increased concentration of organisms
Clinical	Weeks – Months	Advanced multibacillary or paucibacillary lesions	Types 3b, 3c	Stage 3 Stage 4	5 – 10% 1 – 2%	Obvious gross lesions and clinical signs	High sensitivity (>70%)	Poor sensitivity	Heavy, constant faecal shedding, very high concentration of organisms.

[a] Whittington and Sergeant, 2001; [b] Whitlock and Buergelt, 1996; [c] Perez et al., 1996;
[d] Reliable estimates for sensitivity of CMI tests are not available for sheep (Collins, 1996; Hietala, 1992)

4.4 Infection in other species

Although strains of *M a paratuberculosis* have been grouped generally into sheep and cattle strains, which are genetically and epidemiologically quite different, this host-preference is not absolute, and some cross-over of infection between species does occur. In Australia, sheep strains have been identified in cattle, and cattle strains have been isolated from sheep, both in small numbers of cases (Sergeant, 2001a). In addition, both strains are capable of infecting goats, and goats are capable of transmitting the sheep strain back to sheep (Whittington and Taragel, 2000).

Infection with a sheep strain of *M a paratuberculosis* has also been confirmed in macropods on Kangaroo Island in South Australia (P Cleland, personal communication). The significance of this finding for the sheep industry generally is still unclear, as the level of co-grazing of sheep and macropods on Kangaroo Island is unique. Testing of 300 kangaroos from 10 OJD-infected properties in New South Wales failed to find any evidence of macropod infection under conditions of less intensive contact (Abbott, 2000).

Infection of other wildlife, including rabbits, foxes and other species may be possible under conditions of high contamination (Greig et al., 1997; Beard et al., 1999; Beard et al., 2001). However, there has been no evidence of this occurring in Australia, despite testing of >600 rabbits from 13 farms in New South Wales and Victoria — two farms infected with bovine Johne's disease and 11 with ovine Johne's (Abbott, 2000; Kluver et al., 2000).

To date, other species (domestic or wildlife) have not been implicated as a significant source of spread of OJD in Australia.

4.5 Spread within flocks

Spread of infection within an infected flock is dependent on exposure of susceptible animals to infected faeces, either through mixing of infected and susceptible sheep, or by grazing susceptible sheep in areas previously contaminated by infected sheep. Vertical transmission from an infected ewe to its lamb is also likely to occur, but probably only makes a limited contribution to the overall spread of infection through the flock.

In a recently infected flock, infection will often occur initially in a small number of sheep which are either carrying the infection when introduced or have become infected following exposure to infected faeces from another flock in the area. In many cases these initial cases will be in the early stages of infection and may take months to years before they start shedding significant numbers of organisms and even longer before they show clinical signs. The rate of spread from these initial cases will depend on the number of cases and their stage of infection, the management of the flock and other factors affecting susceptibility and progression of infection as described above.

The degree of mixing of sheep is likely to have an important role in the rate of establishment and spread in a flock. In flocks where all age groups are run together and/or paddocks are rotationally grazed (or cell grazed) on a regular basis, there may be regular exposure of susceptible sheep (particularly lambs and weaners) to contaminated faeces. This will increase the rate of spread of infection, and also the rate of progression in infected sheep.

However, in many situations, such as where sheep are set-stocked in age-groups, exposure may only occur sporadically, such as at mustering for shearing, lamb marking and for other management activities, or through periodic changes of paddock. Under these circumstances, infection may be limited to one or a small number of sub-populations within the flock for a long time, before spreading more generally throughout the flock. For example only a small number of lambs present at the time the initial infected sheep are excreting may become infected initially, and it is not until these sheep are adults several years later that the infection spreads further (Whittington and Sergeant, 2001). Thus, following introduction, infection may be highly clustered in age-cohorts or other sub-populations of the flock and may take many years to spread throughout the flock.

4.6 Diagnosis

Diagnosis of paratuberculosis poses a difficult challenge. During the early stages of infection, when the infection is dormant or just starting to progress, cell-mediated immunity (CMI) dominates the animal's response to infection and these animals will be negative to serological tests (Clarke, 1997). Some early cases may be excreting low levels of *M a paratuberculosis* in their faeces, often intermittently, and so may also be difficult to detect even by faecal culture. As the infection progresses excretion of *M a paratuberculosis* increases, but animals may be faecal culture positive for many months before becoming seropositive or showing signs of disease (Chaitaweesub et al., 1999; Whittington and Sergeant, 2001). The presence of seronegative animals intermittently excreting *M a paratuberculosis* and providing a source for further spread of infection provides a major challenge for the early detection of infection, and for prevention of spread within and between flocks.

Detection of infection is particularly difficult in low prevalence flocks, and in flocks that have only recently been exposed. In recently infected flocks, where the initial level of exposure tends to be quite low, there are consequently low numbers of clinical and pre-clinical cases until sufficient time has elapsed for the disease to progress in individual animals and spread in the flock to a detectable level. This process may take some years. During this period, the majority of infected animals are likely to be latent or sub-clinical cases, and infection will be difficult to detect (Whittington and Sergeant, 2001). In contrast, after many years of build up of infection, some flocks report substantial losses each year, and detection of infection is relatively easy (Eppleston and Simpson, 1999; Eppleston et al., 2000; Lugton, 2001).

Because of the nature of the disease, and the production systems involved, diagnosis of paratuberculosis in sheep is usually made on a flock basis, rather than in individual animals. Generally, diagnosis relies either on investigation of suspect clinical cases, or the screening of a (large) sample of animals from the flock using a screening test such as serology or pooled faecal culture (Anonymous, 2001).

The characteristics of currently available tests for the detection of paratuberculosis have been summarised by Whittington and Sergeant (2001). Briefly, all tests suffer from imperfect sensitivity and/or specificity. However, tests for CMI, such as the gamma interferon test, provide the best hope for early detection of infection in live animals, before they have had an opportunity to spread the infection. Pooled faecal culture also appears to be a highly sensitive test and is capable of detecting flock-infection very early. However, because this is a culture-based test, infected animals will be excreting significant numbers of organisms before detection, providing opportunities for further spread of the disease. Serological tests such as the agar-gel immuno-diffusion test have very poor sensitivity until quite late in the course of disease, and therefore are better suited to identification of animals for postmortem examination and rapid confirmation of infection in flocks with well-established infection.

Guidelines for the use of screening tests under the national program have been established to provide a high level of flock-sensitivity except in recently infected and/or low-prevalence flocks. Pooled faecal culture is now the preferred test for market-assurance and surveillance testing, except where a rapid result is required and/or the disease is likely to be well established in the flock, when serology may be used as an alternative (Anonymous, 2001). The recommended sample sizes for flock screening are 350 head using pooled faecal culture and 875 using serology.

4.7 Losses due to disease

Johne's disease causes progressive wasting and eventual death in clinically affected animals, and additional production losses in sub-clinical cases. Although the effects of the disease are generally obvious, direct disease-related losses from Johne's disease are traditionally difficult to estimate because of the prolonged sub-clinical period, and the difficulty in accurately attributing the cause of death in many cases. In sheep, the direct losses are mainly associated with increased mortality rates due to the occurrence of clinical cases and decreased wool production and fertility in clinical and pre-clinical cases. Additional indirect costs may occur due to having fewer excess sheep for sale and lost trading opportunities because of restrictions on movements from known or suspected infected flocks, and from high-prevalence regions.

4.7.1 Mortality rates

Estimates of mortality rates due to ovine Johne's disease vary considerably, and are mainly anecdotal, based on farmer reports. Estimation is further complicated by the need to distinguish mortalities due to Johne's disease from those that die with Johne's disease and those that are unrelated to Johne's disease (McGregor et al., 2001). Under Australian conditions, mortalities generally increase over time, and commonly reach 5% – 15% per annum and occasionally higher (Eppleston and Simpson, 1999; Eppleston et al., 2000, M Evers, personal communication). Detailed investigations and estimation of mortality rates associated with Johne's disease and other causes have been undertaken in one New South Wales flock (McGregor et al., 2001). In this flock, all adult sheep dying during four one-week periods at about three-monthly intervals were postmortemed to determine the cause of death, and overall and Johne's-disease-specific mortality rates were estimated. The annual mortality rate in sheep over 12 months of age for this flock was 21.5% and the estimated annual mortality rate due to Johne's disease was 14.6%.

4.7.2 Production losses

In cattle, sub-clinical Johne's disease is known to cause decreased milk production in the lactation(s) prior to onset of clinical signs, as well as reduced fertility and increased incidence of mastitis. Reductions in milk production of 6% – 16% have been recorded in cows prior to onset of clinical signs (Kennedy and Benedictus, 2001).

In sheep in Spain, ELISA positive ewes produced about 10% less milk than ELISA negative cohorts (Aduriz et al., 1994). In Australia, ELISA positive sheep gained weight at about 7 grams/day less than ELISA negative sheep (Chaitaweesub et al., 1999). In the same study there was no difference in wool production between ELISA positive and negative groups. Some producers have reported reduced fertility and decreased wool production and wool quality in heavily infected flocks, although investigations were not undertaken to confirm the cause or magnitude of these losses (T Hayes, personal communication). Thus, although reductions in wool production, wool quality and lambing percentage in sub-clinically affected sheep are likely, the magnitude of these effects is unknown.

4.8 Control of infection on-farm

Effective control of infection on-farm depends on reducing exposure of susceptible sheep (particularly lambs and weaners) to infected faeces, and slowing the progression of infection in animals that do become infected. In dairy cattle this is possible through calf-management programs to isolate susceptible calves from adult faeces (Collins, 1994; Kennedy and Benedictus, 2001). However, such an approach is not feasible for sheep, and alternative methods must be used. Unfortunately, there has been little research in this area, so there are few well-tested recommendations that are known to be effective.

The main strategies that have been proposed for control of ovine Johne's disease in Australia are (adapted from Anonymous, 2001):

1) Vaccination – A killed vaccine is currently being evaluated on a number of properties in New South Wales, and is showing considerable promise as a control measure (see below). Despite the apparent effectiveness of vaccination, it should not be relied on as a sole method of control, but rather as one of a number of strategies that can be used in combination to provide effective control.
2) Grazing management – Grazing management strategies can be used to provide low-risk pastures and water supplies for lambing and weaning using 'low-risk' adult sheep, cattle or crop rotations, in a similar manner to creation of low-risk paddocks for internal parasites. Grazing management should also be directed at ensuring adequate nutrition and minimising the effect of internal parasites, nutritional stress and other diseases on the flock. Grazing management may also be used strategically to reduce the level of contamination on parts of a property by heavy grazing to reduce vegetation cover followed by short-term de-stocking (3 – 4 months), particularly during summer, to facilitate decay of the organism in the environment.
3) Selective culling – Early culling of known or suspected infected animals and their progeny/cohorts will reduce the overall level of pasture contamination.

4) Segregation – Separation of the flock into high- and low-risk groups and segregation of these groups onto different parts of the property may allow progressive reduction in disease levels and selective culling of high-risk groups.
5) Breeding strategies – Various strategies can be used to isolate lambs at or soon after birth, to reduce their exposure and therefore the level of infection. Strategies include snatching lambs at birth and various artificial breeding techniques. These strategies have still not been fully evaluated, however if only animals that are faecal-culture negative at the time of lambing or semen/embryo collection are used and lambs are not exposed to infection from other sources (e.g. colostrum, milk, pasture, recipient sheep) they should be effective in preventing transmission.
6) Purchase replacements – In some circumstances, purchasing of replacement sheep from a low-risk flock (SheepMAP status ≥ MN1) rather than keeping home-bred replacements may be a feasible and effective way to reduce the level of infection in the flock.
7) Liming soils – There is some evidence to suggest that reducing soil acidity by liming of soils may reduce the level of clinical Johne's disease (see above). However, the evidence to date is not conclusive, and survival of *M a paratuberculosis* in alkaline water for >9 months suggests that any effect of liming on survival of the organism may be limited. An indirect effect of soil acidity on progression of infection in infected animals may be possible, but has not been adequately investigated to date.

4.8.1 Vaccination

Vaccination currently provides the best prospects for effective on-farm control of ovine Johne's disease. An imported, killed vaccine (Gudair™ CSL Ltd) is currently being trialed on three properties in New South Wales, and has recently been registered for use in sheep in Australia. The vaccine is also being evaluated in adult sheep on one heavily infected property.

Overseas reports suggest that vaccination of lambs will reduce the levels of both faecal shedding of *M a paratuberculosis* and of clinical disease in infected flocks (Cranwell, 1993; Juste et al., 1994; Sigurdsson, 1960; Juste, 1997; Chiodini et al., 1984). Preliminary results from the trial flocks are very encouraging, with both OJD-mortalities and faecal shedding of *M a paratuberculosis* delayed and reduced in vaccinated sheep, compared to unvaccinated cohorts (Eppleston et al., 2002). Based on these results, and on anecdotal reports from producers using the vaccine, vaccination is likely to be a very useful tool in assisting to control ovine Johne's disease in Australia.

Vaccination should not be regarded as a panacea, as it is not always effective in reducing the level of infection, with prevalence of faecal shedding in cattle in 25 long-term vaccinated herds not statistically different from that in 29 unvaccinated herds (Kalis et al., 2001). For maximum effectiveness, vaccine should be used as part of an overall management strategy to control Johne's disease in the infected flock. Management practises to minimise exposure of young sheep to *M a paratuberculosis* contamination are essential to maximise the effectiveness of the vaccine and sheep should be vaccinated as lambs, generally at lamb marking, to provide maximum protection. Adult vaccination may be effective in reducing subsequent losses due to disease (Corpa et al., 2000; Crowther et al., 1976). However, the effectiveness of vaccination at older ages is uncertain, and adult vaccination may not always be effective (McGregor et al., 2002).

There are also a number of drawbacks to use of the vaccine, including the occurrence of persistent injection-site lesions that can result in downgrading of carcases, severe injection-site reactions in humans following accidental injection and interference with immunological tests for the presence of humoral or cellular immunity to *M a paratuberculosis* (Eppleston et al., 2001; MacDiarmid, 1987; Juste et al., 1994; Gwozdz et al., 2000).

Although vaccination is not 100% effective in preventing infection, it apparently does substantially reduce infection and excretion rates, and over time will significantly reduce overall levels of contamination on infected properties. Given the ongoing reduction in contamination resulting from an effective vaccination program, it may be possible in the longer term to eradicate infection from infected properties using a combination of vaccination and grazing management. This hypothesis has never been adequately tested, although clinical disease has been eliminated from parts of Iceland following a long-term vaccination program (Siguroarson and Gunnarsson, 1983; Fridriksdottir et al., 1999). Simulation modelling has also

suggested that eradication is a possible outcome from long-term vaccination (Juste and Casal, 1993; Sergeant, 2002). If this was the case it may make local and regional eradication of the disease possible in the long term, without the necessity for widespread de-stocking programs.

In addition, use of vaccine in flocks which have been exposed to infection, but in which it has not been confirmed may be an effective means of preventing establishment and further spread of infection. While this may prove an effective method of control, it makes the subsequent determination of the status of the flock very difficult, because of the substantial reduction in infection and excretion rates following vaccination. This also applies to infected flocks that have been vaccinating for many years. In these flocks, it may be necessary to vaccinate for up to 2 – 3 generations to ensure maximum suppression of infection, and then to cease vaccination if a return to an infection-free status is required. To demonstrate that infection has been prevented or eliminated it would be necessary to cease vaccinating for several years and then test unvaccinated sheep as adults using pooled faecal culture to determine if the infection has persisted.

4.9 Spread between flocks

Johne's disease is usually introduced into previously clean herds or flocks through the introduction of an infected animal. The main mechanism of spread between farms, particularly for the introduction of infection into previously uninfected areas, is therefore through the movement of infected sheep. Analysis of surveillance data for New South Wales showed that up to 42% of forward traces from infected flocks in the Residual Zone resulted in infection, as did about 23% for traces from the Control Zone (Sergeant, 2001b).

Direct farm to farm environmental spread, such as by water, or spread by fomites is possible but has never been confirmed (Sweeney, 1996). However, experience in NSW has shown that local spread of infection between neighbouring properties is very common, and that the risk of a flock being infected increases with the number of infected neighbouring flocks. Analysis of surveillance data for New South Wales showed that up to 75% of neighbours to infected flocks in the Residual Zone may also be infected, compared to about 40% in the Control Zone, suggesting that the risk from neighbours is greater in areas that have been infected for longer (Sergeant, 2001b). Although the mechanism of spread is unknown in most cases, straying sheep, common use of land or facilities, or movement of infected faeces in run-off water or wind are the most likely explanations.

Infected wildlife provide another possible means of local spread, although this is probably a minor factor, except perhaps on Kangaroo Island, and similar environments where macropod populations are large and concentrated. Macropods are more likely to contribute indirectly to spread through damage to boundary fences allowing continuing straying of sheep. Other, less likely, mechanisms for local spread include spread of contamination by blowflies (Fischer et al., 2001) or nematode larvae (Whittington et al., 2001;Lloyd et al., 2001).

4.10 Control of local, regional & inter-regional spread

Control of inter-farm and inter-regional spread of Johne's disease in Australia relies on a combination of voluntary restrictions on the movement of sheep between regions (zones) of different status and regulatory controls placed on properties known or suspected to be infected (Sergeant, 2001a). During 2001, restrictions on infected flocks have been eased to allow limited trading of low-risk sheep (including vaccinated sheep) subject to certain conditions. Movements of sheep between zones of different status require specified testing of the source flock and a declaration of status by the owner of the flock of origin (Anonymous, 2001).

Although these measures are likely to reduce the spread of disease somewhat, their effectiveness is limited by:

- Movements that have already occurred in the past with the potential to initiate new (unidentified) foci of infection in otherwise low-risk regions;
- Inability of current tests and strategies to detect infection before there has been the potential for further spread of disease.
- Non-compliance of some producers; and

- Lack of measures to prevent local spread between neighbouring farms.

Thus, while strict compliance with zoning and movement controls may prevent future introductions of infection into a region, additional measures are required to minimise spread from infected flocks (whether identified as infected or not) within the local area/region.

Possible measures to help limit spread of infection between farms and regions include (adapted from Sergeant, 2001b):

- Continued restrictions on the movement of sheep between zones, based on a risk assessment approach and negative flock status.
- A risk-based approach to trading for infected/suspect flocks to encourage compliance.
- Prompt and thorough investigation of flocks identified by tracing in low prevalence districts to determine status.
- Pre-emptive vaccination or depopulation of infected and high-risk suspect flocks in low prevalence districts to minimise the risk of further spread.
- Ongoing surveillance programs in all zones for detection of infected flocks.
- Intensive surveillance of neighbours of infected flocks in low prevalence districts to ensure early detection and implementation of control measures.
- Prevalence reduction on infected farms through management changes and vaccination.
- Vaccination (compulsory or voluntary) of flocks neighbouring known infected flocks.
- Vaccination (compulsory or voluntary) of all flocks in a district/zone.
- Use of vendor declarations when selling/purchasing sheep within a zone.
- Development of group strategies to work together to control and eliminate the disease on an area basis.

4.11 Conclusion

Additional research is still required to fully understand the epidemiology and pathogenesis of Johne's disease in sheep. An improved understanding of factors affecting the establishment and progression of infection are essential for the development of effective strategies for the control and eventual eradication of this infection on infected farms. However, even with current knowledge and tools, there are a number of options that can be tried.

At present, vaccination appears to be the key to successful control of ovine Johne's disease in Australia. However, vaccine trials are still incomplete, and vaccine should not be regarded as a panacea. It is essential that other methods for reducing excretion and survival of the organism are explored and utilised to support vaccination and provide effective control.

5. SYSTEM CHARACTERISATION

5.1 Flock dynamics

A conceptual model of the structure and dynamics of a sheep flock is shown in Figure 1 and Table 2. In this characterisation the term 'year' is used to describe a 12-month period, matching the production cycle within the flock. It does not necessarily equate to a calendar year.

Lambs (0 years old) enter the flock at the beginning of each production year, at a rate that varies according to the breed, age of adults at joining, nutritional and seasonal factors, the Johne's disease status of individual sheep and the occurrence of other diseases.

One-year-old sheep may be either retained from the previous year's lambs, or purchased from an external source, depending on the type of flock and management policy. A sufficient number of one-year-olds are retained or purchased to replace culls and deaths during the previous year, so that the number of adult sheep in the flock at the beginning of each year remains approximately constant. Any lambs not retained in the flock are culled.

Rams are also introduced as 1 – 2-year-olds, and are normally retained for a fixed period unless they die or are culled earlier. Sufficient new rams are purchased or retained each year to maintain the required ram percentage. Rams are generally purchased but may be retained from lambs, depending on the type of flock and management policy.

During each year a proportion of each age-group die or are culled at a rate determined by their age, Johne's disease status and occurrence of other diseases. The balance of the age-group progress to the next age-group at the end of the year. Animals in the oldest age group (age=m yrs) are all culled at the end of each year.

Figure 1: Conceptual model of the age structure and dynamics of a sheep flock

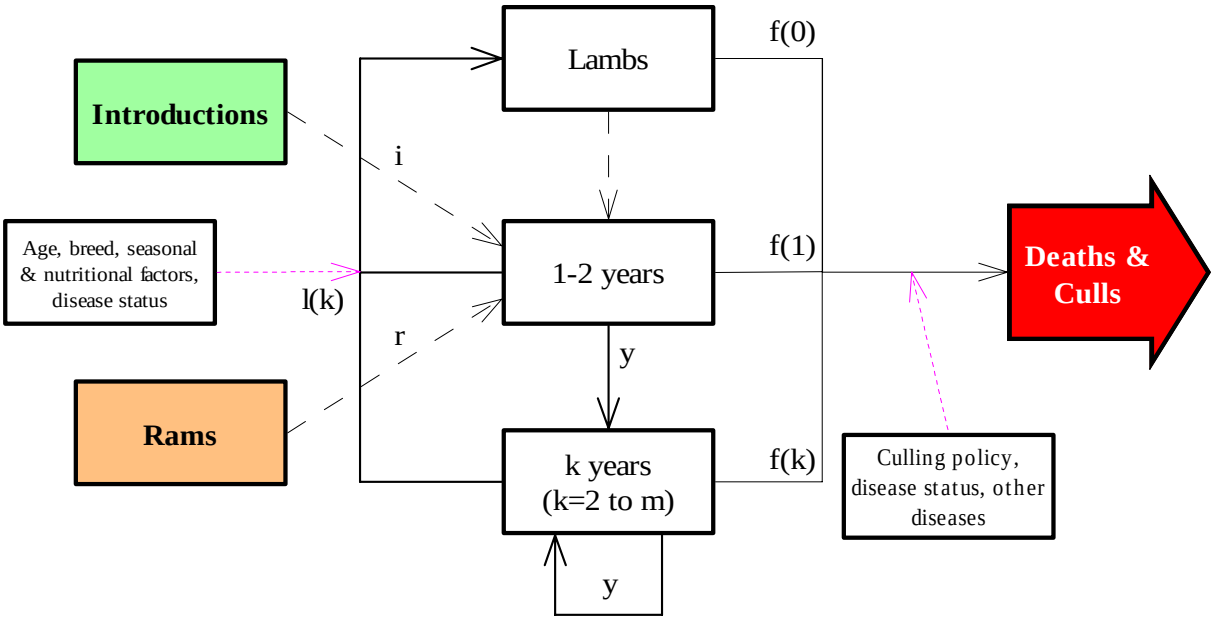


Table 2: Parameters and variables for a model of flock dynamics and age structure

Parameter/Variable	Description
y	Time in years
k	Age of sheep in years
l(k)	Age specific lambing rate (%) – for k=1 to m
f(k)	Age-specific culling and mortality rate (%) – for k=0 to m
i	Number of introductions (replacements)
r	Number of ram replacements
m	Maximum age of flock – f(m) = 100%

5.2 Spread of infection within an infected flock

In an infected flock, animals may exist in any of the states described in Table 3. Animals enter the flock as Births or Purchases and move between States, die or are culled at various rates as shown in Figure 2 and Table 4.

Table 3: Description of disease states for a conceptual model of the spread of ovine Johne's disease in an infected flock

State	Abbreviation	Description
Susceptible	SUS	Susceptible to infection if exposed
Immune (Resistant)	IM	Immune/resistant to infection (or re-infection), assumed to be life-long
Latent	LT	Incubating infection but not infectious
Light Shedder	LS	Actively infected and shedding *M a paratuberculosis* at low levels and/or intermittently
Heavy Shedder	HS	Actively infected and shedding high levels of *M a paratuberculosis* continuously
Clinical	CC	Infected and exhibiting clinical signs of disease

5.2.1 Transition from SUS to LT (a)

The rate of transition of sheep from SUS to LT states (a) is equivalent to the probability of a sheep becoming infected during a time period, and is determined by the probability of exposure to infected faeces during that time period, the cumulative dose of organisms ingested and the probability that infection will establish if exposure occurs. These probabilities are in turn affected by a range of factors as shown in Figure 3 and Table 5.

5.2.2 Transition from LT to LS (b)

The transition rate for sheep from LT to LS (b) is equivalent to the probability of progressing between the states during a time period. A range of factors may affect this probability, as shown in Figure 4 and Table 6. Generally, progression from LT to LS is suppressed by an effective CMI response. Therefore any factors reducing CMI capability are likely to increase the probability of progression, as are other factors, including ongoing challenge, cumulative dose and possibly breed.

5.2.3 Transition from LS to HS, HS to CC and CC to death

The transition rates for sheep from LS to HS (c), HS to CC (d) and CC to death (e) may be affected by the same factors affecting progression from LT to LS (Figure 4 and Table 6). However, once progression of infection starts in an individual, the CMI response has already been compromised and conditions favouring progression are already established. Therefore, any additional effect of these factors on further progression is likely to be relatively minor, and is assumed to be negligible compared to their effect on (b).

Figure 2: Conceptual model for the spread of Johne's disease within an infected flock

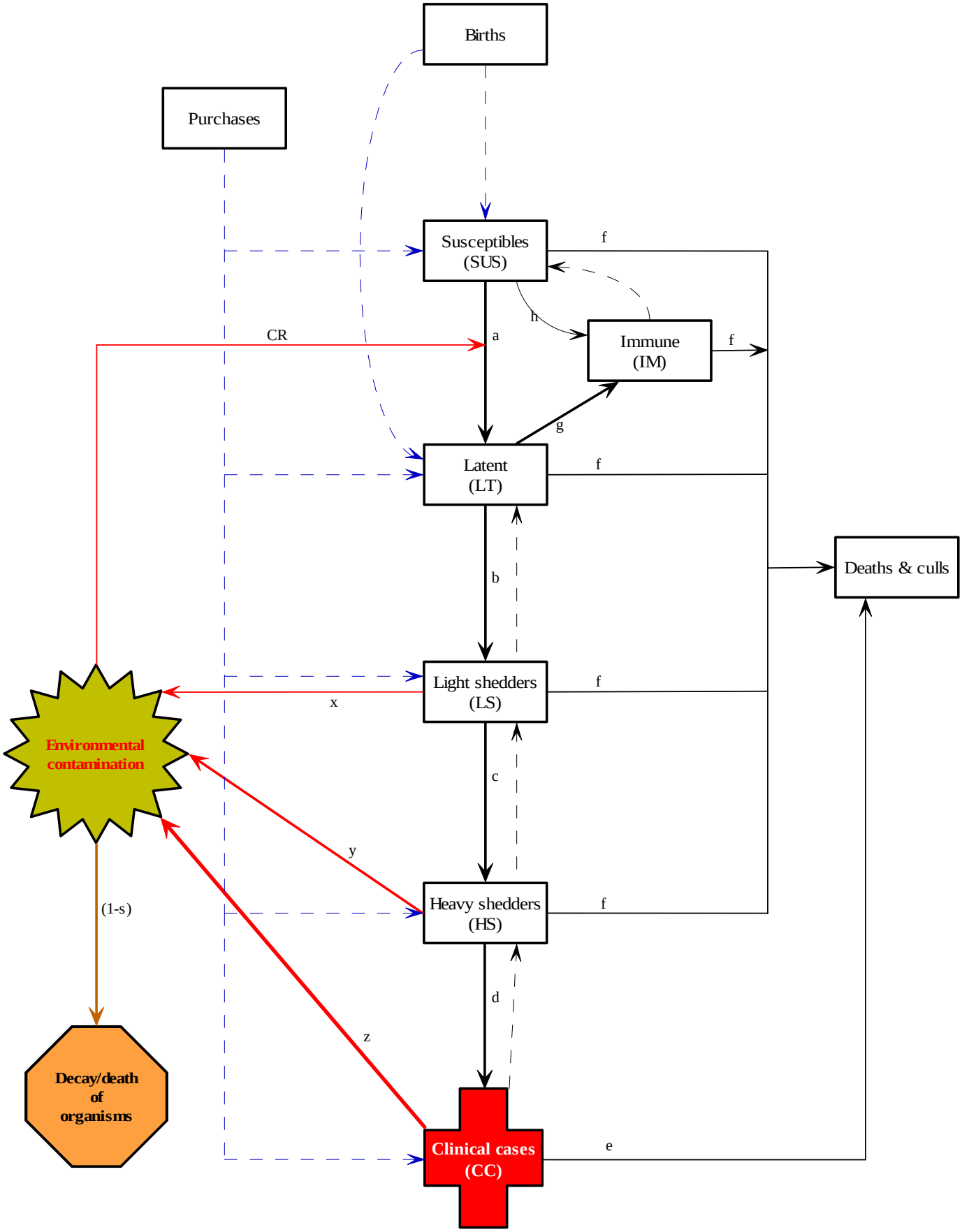


Table 4: Transition parameters and variables for a model of the spread of ovine Johne's disease within an infected flock

Variable/ parameter	Description
a	Probability of infection
b	Transition rate from LT to LS
c	Transition rate from LS to HS
d	Transition rate from HS to CC
e	Transition rate from CC to Dead (= Clinical case mortality rate)
f	Culling/death rate of sheep other than clinical cases
g	Transition rate from LT to IM
h	Transition rate from SUS to IM (= vaccine efficacy)
S	Survival rate of *M a paratuberculosis*
x	Excretion rate for LS animals, as a proportion of CC
y	Excretion rate for HS animals, as a proportion of CC
z	Excretion rate for CC (= 1)
CR	Contact rate parameter (average number of effective contacts with sheep faeces per sheep per time period)
M	Mycobacterial contamination level

* Transition rates are the probability of the transition occurring per time period (1 month) = 1/(average time to transition in months)

Figure 3: Factors affecting the probability of exposure to infection, and the probability of infection establishing in a susceptible sheep

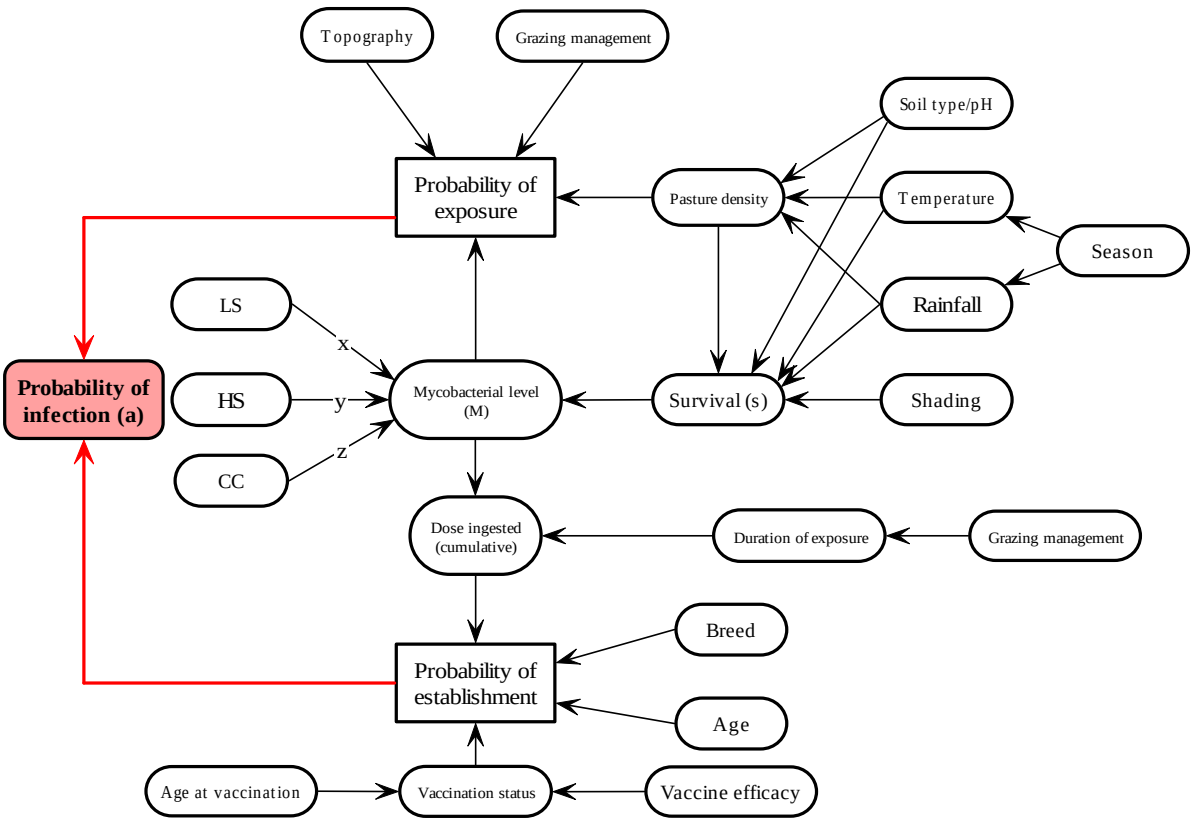


Table 5: Factors affecting the rate of transition from SUS to LT states.

Factor	Effect
Probability of exposure	
Topography	Collection of faeces in run-off areas preferentially grazed by sheep increases probability of exposure
Grazing management	Set-stocking reduces probability of exposure in uninfected mobs compared to rotational or cell-grazing.
Level of mycobacterial contamination	The probability of exposure increases with increased numbers of *M a paratuberculosis* in the environment, which is affected by: ➢ Numbers of cases in each of LS, HS and CC states and their excretion rates ➢ The survival rate of mycobacteria over time ➢ Shading – increased shading increases mycobacterial survival
Pasture density	Increased pasture density reduces the probability of exposure by reducing ingestion of soil/litter but increases mycobacterial survival, due to shading and protection of organisms. Pasture density is affected by: ➢ Temperature ➢ Rainfall ➢ Soil type ➢ Season – primarily through effects on rainfall and temperature These factors have also been postulated as having a direct effect on mycobacterial survival, with survival increasing with lower temperatures, higher rainfall and acid soils.
Status of dam (mother)	Lambs born to infected ewes may be infected prior to or soon after birth directly from their mother. The probability of exposure of unborn lambs increases progressively with the stage of disease (state) in the dam from LS through to CC.
Probability of establishment	
Dose ingested	The probability of infection establishing increases with increasing (cumulative) dose of organisms ingested, which is affected by: ➢ Level of mycobacterial contamination, as discussed above ➢ Duration of exposure – longer exposure results in increased doses of mycobacteria ➢ Grazing management – affects the duration of exposure depending on the length of time sheep remain in contaminated paddocks
Age	The probability of infection establishing for a given dose is thought to decrease with increasing age, although the specific relationship is unclear
Breed	British breeds and cross-breeds are postulated to be more resistant to infection than merinos
Vaccination status	Vaccination prior to exposure reduces the probability of infection establishing, depending on: ➢ Age at vaccination – vaccination as lambs is likely to be more effective than at a later age ➢ Vaccine efficacy – the vaccine is not 100% effective, so a proportion of sheep will remain unprotected

5.2.4 Reversion to previous states

Although reversion of light and heavy shedders and clinical cases to the previous state is possible, this is probably a rare occurrence, and the rates indicated above are net rates after allowing for any reversion.

5.2.5 Culling/death rate (f)

The culling/death rate (f) for states other than CC is the probability of an animal dying or being culled during a time period. This rate is determined by the age of the animal, the age-specific mortality rates for the flock and the culling policy of the flock owner. Generally, all sheep are culled once they reach a pre-determined age, according to culling policy. Prior to that, losses each year are mainly low (3-5%) and are due to death or premature culling because of other diseases. The culling/death rate is unaffected by state, except for clinical cases, which are dealt with separately.

5.2.6 Transition rate from LT to IM (g)

The transition rate for sheep from LT to IM (g) is equivalent to the probability of a latently infected sheep eliminating infection and becoming immune during a time period. This probability is affected by the same factors affecting the probability of progression between states, except that the effects are reversed. Therefore, as the probability of progressing from LT to LS increases, the probability of progressing to IM decreases.

Table 6: Factors affecting the rate of transition between states LT-LS.

Factor	Effect
Probability of progression	
Dose ingested	The probability of infection progressing increases with increasing (cumulative) dose of organisms ingested, which is affected by: Level of mycobacterial contamination Duration of exposure Grazing management as shown in Table 4.
Vaccination status	Vaccination reduces the probability of infection progressing, depending on: Age at vaccination – vaccination as lambs is likely to be more effective than at a later age Vaccine efficacy – the vaccine is not 100% effective, so a proportion of sheep will remain unprotected
Age at exposure	The probability of infection progressing is thought to decrease with increasing age at exposure
Animal age	The probability of infection progressing is thought to increase with the age of the animal, at least until adulthood is reached
Breed	Proposed variations in breed-susceptibility to Johne's disease may be due to British breeds and cross-breeds being better-able to suppress infection, and reduce the rate of progression in infected animals
Factors affecting CMI response	Any factor that affects the animals ability to maintain an effective CMI response is likely to increase the probability of progression, including: Pregnancy and lactation Other diseases (internal parasites, pregnancy toxaemia, others) Nutritional stress
Soil type	Soil type has been postulated as affecting the prevalence of disease in a herd/flock/region, and may affect progression of infection, possibly through the availability of micro-nutrients essential for multiplication of *M a paratuberculosis*.

5.2.7 Transition rate from SUS to IM (h)

The transition rate for sheep from SUS to IM (h) is equivalent to the probability of a susceptible sheep becoming immune/resistant during a time period and is determined by vaccination status and vaccine efficacy.

The IM state (either from recovery or vaccination) is generally assumed to be life-long, although reversion to susceptible may be possible, depending on environmental, nutritional and physiological stresses.

5.2.8 Mycobacterial survival rate (s)

The mycobacterial survival rate (s) is the proportion of mycobacteria surviving between time periods. Thus, the level of mycobacterial contamination in the environment declines at a rate of (1-s) per time period. Mycobacterial survival may be affected by the following seasonal and environmental factors (see Figure 3 and Table 4):

- Shading
- Temperature
- Rainfall
- Soil type and
- Season

5.2.9 Mycobacterial excretion rates (x, y, and z)

Animals in LS, HS and CC states are assumed to contribute increasing amounts to the level of environmental contamination with *M. a paratuberculosis*. Excretion rates for LS, HS and CC states are x, y and z respectively. The overall level of environmental contamination at any time is made up of current contamination from existing cases, plus any residual contamination surviving from previous time periods. If the level of contamination introduced from new and existing cases exceeds the losses due to death of organisms the overall level of contamination increases.

5.2.10 Births and Purchases

At the time of birth, most lambs will be in the susceptible (SUS) state. However, a proportion of the lambs born to infected ewes may be latently infected at birth, with the proportion affected increasing with the severity of disease. If lambs are retained, the one-year-old sheep start the year with the same proportion of sheep in each state as existed in the lambs at the end of the previous year. Purchased sheep may be in any state when they enter the flock, depending on the purchasing policy of the owner.

5.2.11 Production effects

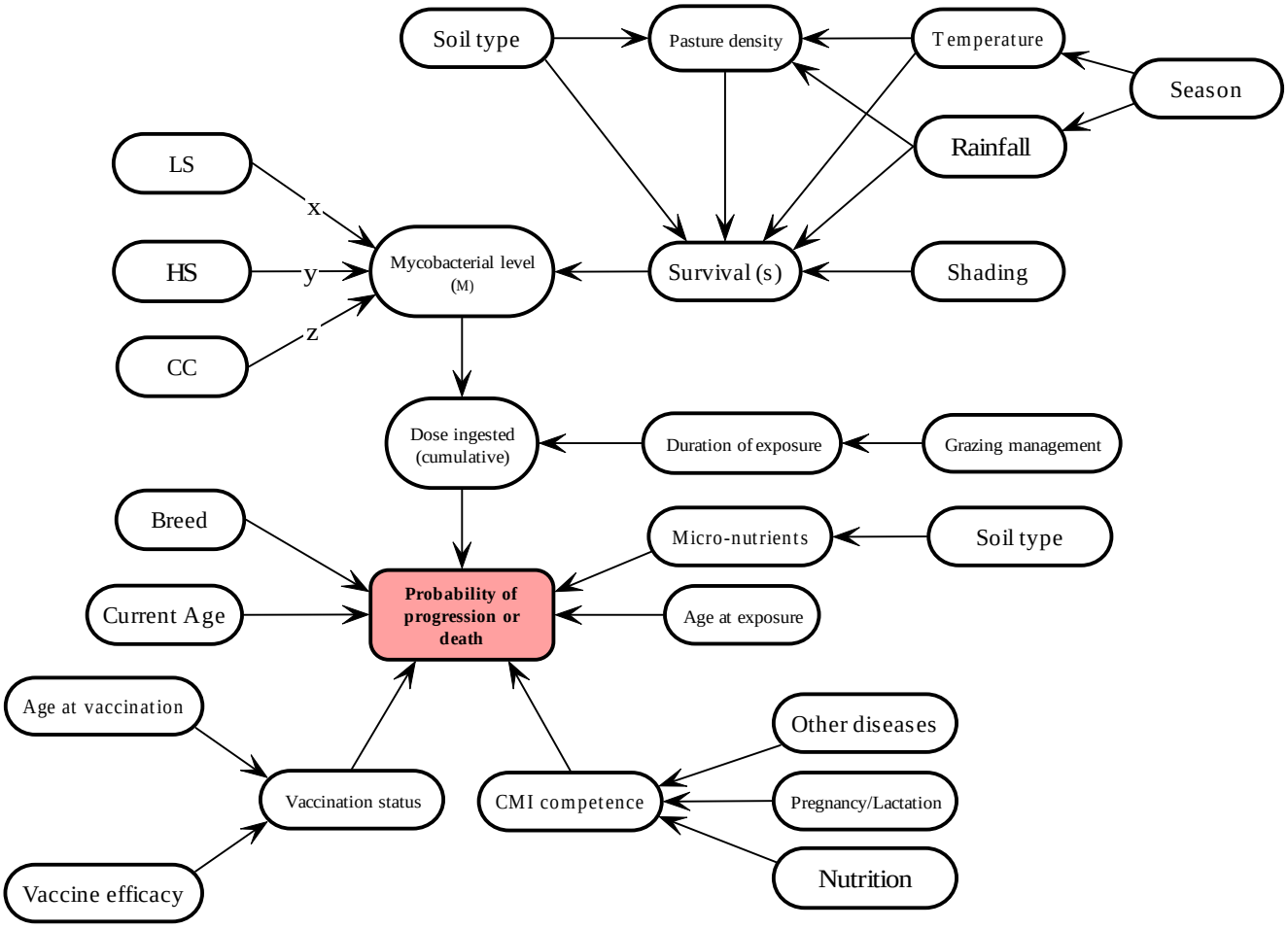
In addition to the premature death or culling of clinical cases, infection is assumed to affect flock productivity, through reduced pregnancy/lambing rates and reduced wool production and quality for affected sheep. Generally, these effects are assumed to increase with stage of disease in affected sheep.

5.2.12 Control of infection

Infection in infected flocks may be controlled by:

- Early culling of clinical cases;
- Purchase of replacement sheep instead of breeding replacements;
- Management changes to reduce exposure, progression or survival of the organism (for example rotation of sheep with other enterprises, improved nutrition and disease control, etc);
- Vaccination (including possibly adult vaccination); and
- Test & cull – while theoretically possible this is not a practical or feasible option in most sheep flocks.

Figure 4: Factors affecting the probability of Latent infection progressing



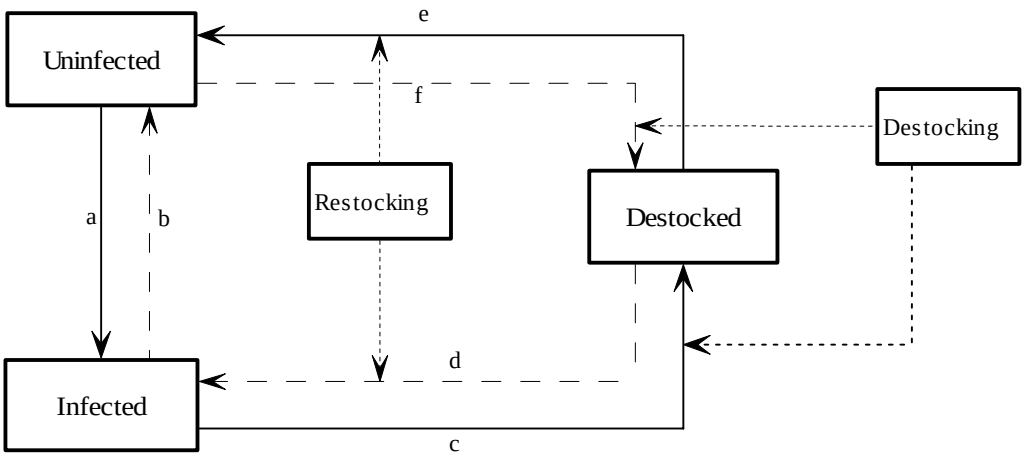
5.3 Spread of infection between flocks

Regional spread of OJD is simply an extension of spread within a farm, with spread being dependent on either movement of infected sheep between farms as sales/purchases, or exposure of susceptible sheep to infected sheep or faeces from farms in close proximity (for example common use of land, straying sheep or environmental transfer of faeces). Farms may be either infected, uninfected or destocked, as shown in Figure 5, with movements between these states affected by a range of factors as described in Table 7.

Uninfected flocks may become Infected by purchasing infected replacement sheep, or by local spread from neighbouring or nearby infected properties. The rate of transition to Infected is therefore dependent on factors affecting both these forms of contact. Once Infected, the risk of further spread from a flock is dependent on prevalence within the flock, and measures taken to prevent further spread, including quarantine and vaccination.

Destocked flocks normally remain destocked for at least 1.5 – 2 years, after which they may restock at any time. When flocks restock they revert to either Uninfected or Infected, depending on whether the restocker sheep purchased are infected or not. Alternatively, destocked flocks may remain destocked indefinitely.

Figure 5: Conceptual model for spread of OJD between flocks



5.3.1 Control options

Control of regional spread of OJD may be by:

- Surveillance and quarantine of known or suspected infected flocks;
- Vaccination of known infected flocks, neighbouring flocks or other flocks of unknown status;
- Destocking of infected flocks; or
- Restrictions on movement of sheep, based on either testing or vaccination requirements.

Table 7: Factors affecting spread of OJD between farms

Transition	Rate	Affected by
Uninfected to Infected	a	▪ numbers of sheep purchased; ▪ prevalence of infection in the source flocks (if infected); ▪ effectiveness of measures to prevent the purchase of infected sheep; ▪ numbers of neighbouring/local properties that are infected; ▪ prevalence of infection in neighbouring flocks (if infected); ▪ likelihood and level of contact with sheep/faeces from infected properties; ▪ effectiveness of measures to prevent introduction from neighbouring properties; ▪ effectiveness of measures taken to prevent establishment of infection if introduced; and ▪ vaccination coverage of flocks.
Infected to Uninfected	b	Failure of infection to establish or maintain on the property because of: ▪ Vaccination or management preventing continued transmission *of M a paratuberculosis* on the property; ▪ Lack of transmission from few infected introductions or early cases; and ▪ Extinction of infection because of lack of sufficient susceptible animals to maintain and spread (eg purchasing all replacements).
Infected to Destocked	c	▪ National/State policy; ▪ State or Zone in which property is located; ▪ the perceived costs/benefits; ▪ the likelihood of successful eradication; and ▪ may be progressive destocking of individual flocks.
Destocked to Infected	d	▪ Likelihood of restocking ▪ The numbers of replacement sheep purchased;
Destocked to Susceptible	e	▪ the prevalence of infection in the source flocks (if infected); and ▪ the effectiveness of measures to prevent the purchase of infected sheep.
Uninfected to Destocked	f	▪ Changes in ownership/management; and ▪ Enterprise profitability

6. MODEL SPECIFICATION AND ANALYSIS

6.1 Model 1 – a simple mass-action model of within-flock spread (OJD Spread Model v1.0)

This model is a simple mass-action model of spread within an infected flock. Main assumptions for this model are:

- There is random mixing and exposure to *M a paratuberculosis* of sheep in the flock;
- Sheep exist in the following states: Susceptible (SUS), Immune (IM), Latent (LT), Light Shedders (LS), Heavy Shedders (HS) and Clinical (CC) (see Figure 2);
- Transitions between states occur as shown in Figure 2 and Table 4, except that sheep enter the system as births or purchases in the SUS state only, at a rate to maintain a constant population;
- The rate of new infections (a) is proportional to the proportion of SUS and the accumulated level of infection in the flock at the end of the previous time period;
- Once infected, sheep progress through the various states at predetermined rates, which are effectively net rates of progression – reversion to a previous state is not specifically included in the model;
- Sheep are culled or die at a fixed rate (f) for all states, except CC, which are removed at a different rate (e) to all other states;
- Transitions from SUS to IM (h) occur because of age or due to vaccination,;
- The level of contamination in any time period is measured in terms of 'CC-equivalents', and is calculated as the number of CC animals, plus specified proportions of the numbers of HS (y) and LS (x) animals;
- A specified proportion of organisms (s) survive in the environment between successive time periods

Transition rates and other variables for the model are described in Table 8:

Table 8: Important variables for a model of the spread of ovine Johne's disease in an infected flock

Variable/ parameter name	Description
CR	Contact rate parameter (average number of effective contacts with sheep faeces per sheep per time period)
M_t	Mycobacterial contamination level at time = t
SUS_t	The number of SUS animals at time = t
IM_t	The number of IM animals at time = t
LT_t	The number of LT animals at time = t
LS_t	The number of LS animals at time = t
HS_t	The number of HS animals at time = t
CC_t	The number of CC animals at time = t
N	Population (total flock) size

The model is specified by the following equations:

$a = CR \times M_t/N$

$M_{t+1} = xLS_t + yHS_t + zCC_t + sM_t$

$LT_{t+1} = (1\text{-}f)LT_t + a(1 – h)SUS_t – (b + g)(1\text{-}f)LT_t$

$LS_{t+1} = (1\text{-}f)LS_t + b(1 – f)LT_t – c(1\text{-}f)LS_t$

$HS_{t+1} = (1\text{-}f)HS_t + c(1 – f)LS_t – d(1\text{-}f)HS_t$

$CC_{t+1} = (1\text{-}g)CC_t + d(1 – f)HS_t$

$IM_{t+1} = (1\text{-}f)IM_t + h(1 – f)SUS_t + g(1\text{-}f)LT_t$

$SUS_{t+1} = N – (LT_t + LS_t + HS_t + CC_t + IM_t)$

6.1.1 Implementation

Deterministic and stochastic versions of this model were implemented using Excel (Microsoft Corporation) and @Risk (Pallisade Corporation).

For the deterministic model, fixed-value estimates of the various contact and transition rate parameters were used for all input parameters, while outputs were the percentage of animals in each state at the end of each time period. Monthly transition rates were estimated as the inverse of the mean estimated time to transition (in months) for each state.

For the stochastic model, fixed estimates were also used for input parameters. However, for each iteration of the model, the actual number of animals changing between states at the end of each time period was estimated using the RiskBinomial function of @Risk. This estimated the actual number of animals for each transition from the number of animals in the previous state that were available and the transition rate for that transition. For this model, culls and deaths were assumed to be removed first, followed by any animals that became immune. The remaining animals were then available to progress to the next State. (There was a precedence established in the transition probabilities – Culls > Immune > others).

By adjusting the input values the model could be adapted to suit time periods of t = 1 month through to t = 12 months.

Outputs from the stochastic model also included the percentage of animals in each state at the end of each time period for each iteration. The model was run for 1,000 iterations to provide a distribution of possible results associated with chance variations in the transition process. The mean percentages in each state at each time period from the stochastic model were very similar to the corresponding values from the deterministic model.

The stochastic model was also implemented using Visual Basic to create a stand-alone program. The Visual Basic version was similar except that the probability of replacement sheep being in any state was specified by the user.

6.2 Model 2 – OJD Spread Model v2.0

This model is a more complicated model allowing for a more realistic simulation of flock age structure, disease dynamics and control options. The structure and assumptions of Model 2 are similar to that of Model 1 and Figure 2, except as described below:

6.2.1 Model Design

6.2.1.1 Spread of infection

Transition rates and other variables for Model 2 are described in Table 9. Assumptions and calculations for this model are:

- Sheep are assumed to exist in the same states as for Model 1: Susceptible (SUS), Immune (IM), Latent (LT), Light Shedders (LS), Heavy Shedders (HS) and Clinical (CC) (see Figure 2);
- The simulated flock comprises age categories of lambs/weaners (< 1 year old), hoggets (1 – 2 years old) and up to 5 age-cohorts of adults (≥2 years old);
- The internal time period for the model is 3 months, with output summarised on an annual basis.
- The probability of new infection (a) at each time step depends on the level of environmental contamination, age and breed susceptibility of animals and management actions to reduce exposure, and is calculated as:

$$a = CR \times M_t/N_{total} \times Susceptibility_{age} \times BreedSusceptibility \times (1 - ExposureReduction)$$

- The probability of progression from LT to LS (b) depends on age, breed, vaccine and management effects and the ongoing level of ecposure, and is calculated as:

$$b = Progress_{age} \times (1 + CR \times M_t/N_{total}) \times BreedProgression \times (1 - VaccineProgression) \times (1 - ManagementProgression)$$

- The probability of recovery from LT>IM (g) is calculated from the average time (months) to transition, adjusted for effects of breed, management and vaccination as:

$$g = [1 - (1 - 1 / TimeToTransition_{state})^l] / [BreedProgression \times (1 - VaccineProgression) \times (1 - ManagementProgression)]$$

- The probability of progression for other transitions (c=LS>HS, d=HS>CC, e=CC>Death) are calculated from the average time (months) to transition as:

$$Probability(c, d, e) = 1 - (1 - 1 / TimeToTransition_{state})^l$$

- To allow for random variation in infection and progression between states, the numbers of animals in each age group changing state at each time step are calculated using a binomial function as:

$$NewVacc_{age,t+1} = Binomial(SUS_{age,t}, h)$$
$$NewRecovered_{age,t+1} = Binomial(LT_{age,t}, g)$$
$$New_{LT,age,t+1} = Binomial((SUS_{age,t} - NewVacc_{age,t}), a)$$
$$New_{LS,age,t+1} = Binomial((LT_{age,t} - NewRecovered_{age,t}), b_{age})$$
$$New_{HS,age,t+1} = Binomial(LS_{age,t}), c)$$
$$New_{CC,age,t+1} = Binomial(HS_{age,t}), d)$$
$$OJDDeaths_{age,t+1} = Binomial(CC_{age,t}, e)$$

- The new total numbers of animals in each age group and state at each time step are calculated as:

$$IM_{age,t+1} = IM_{age,t} + NewVacc_{age,t+1} + NewRecovered_{age,t+1}$$
$$LT_{age,t+1} = LT_{age,t} + New_{LT,age,t+1} - New_{LS,age,t+1} - NewRecovered_{age,t+1}$$
$$LS_{age,t+1} = LS_{age,t} + New_{LS,age,t+1} - New_{HS,age,t+1}$$

$$HS_{age,t+1} = HS_{age,t} + New_{HS,age,t+1} - New_{CC,age,t+1}$$
$$CC_{age,t+1} = CC_{age,t} + New_{CC,age,t+1} - OJDDeaths_{age,t+1}$$

Table 9: Input parameters and variables for a model of the spread of ovine Johne's disease in an infected flock

Variable/parameter name	Description
age	Age of sheep
l	Length of time period = 3 (months)
b_{age}	Age-specific probability of transition LT $\rightarrow$ LS per time step
$SUS_{age,t}$	The number of SUS animals by age at time = t
$IM_{age,t}$	The number of IM animals by age at time = t
$LT_{age,t}$	The number of LT animals by age at time = t
$LS_{age,t}$	The number of LS animals at by age time = t
$HS_{age,t}$	The number of HS animals by age at time = t
$CC_{age,t}$	The number of CC by age at time = t
$N_{state,age,t}$	Population (flock) size by state and age at time = t
$Susceptibility_{age}$	Relative age susceptibility, compared to young lambs
$Deaths_{age}$	Age-specific death rate due to causes other than OJD
BreedSusceptibility	Relative susceptibility due to breed, compared to a fully susceptible breed
$NewVacc_{age,t}$	Number of new IM cases due to vaccination (SUS>IM) during one time-step
$NewRecovered_{age,t}$	Number of new IM cases due to recovery from infection (LT>IM) during one time-step
$New_{state,age,t}$	Number of new cases in LT, LS, HS or CC states due to progression from previous state during one time-step
$OJDDeaths_{age,t}$	Number of deaths due to OJD during one time-step
LambingPercent	Average lambing percentage for simulated flock
$LambingEffect_{state}$	Effect of infection state (LS, HS or CC) at lambing on lambing percentage
BreedProgression	Relative probability of progressing from LT>LS due to breed-effect, compared to a fully susceptible breed.
VaccineProgression	Relative reduction in probability of progressing from LT>LS due to vaccination, compared to an unvaccinated animal.
ExposureReduction	Relative reduction in exposure due to management changes in a control program
ManagementProgression	Relative reduction in probability of progressing from LT>LS due to management changes as part of a control program.
$TimeToTransition_{state}$	Average time to transition from one state to the next in months

- New lambs are born at the beginning of each year, and all lambs are assumed to be SUS at birth. Lambing percentage is adjusted for any assumed effect of OJD, and because lambing percentage may exceed 100%, the number of lambs is calculated as:

$$N_{SUS,lambs,t+1} = Binomial(N_{adults,t}, (LambingPercent \times (1 - LambEffect_{state}) / 2) + Binomial(N_{adults,t}, (LambingPercent \times (1 - LambEffect_{state}) / 2)$$

- At the end of each time period, the number of deaths due to causes other than OJD are calculated and the revised total numbers of animals in each age group and state for the start of the next time period are calculated as:

$$Deaths_{state,age,t} = Binomial(N_{state,age,t}), f)$$
$$N_{state,age,t+1} = N_{state,age,t} - Deaths_{state,age,t}$$

- At the end of each year, the remaining lambs progress to become the following years hoggets and adults progress in age by one year. All adults in the oldest age group are culled and replaced by sufficient young adults (2-year-old), recruited either from the previous year's hoggets or as purchases, to maintain constant numbers of adults at the start of each year. If hoggets are

retained as replacements, the proportion of new adults in each state is the same as in the hoggets at the end of the year, or if replacements are purchased, the proportions in each state are as specified by the user.

- Progression from SUS to IM (h) only occurs if vaccination is included as part of a control program;

6.2.1.2 Flock productivity

- Infection is assumed to affect wool production (kg/head), wool quality (price received) and lambing marking percentage, depending on the state of the sheep at shearing and lambing.

6.2.1.3 Control options

Available control options include: early culling of clinical cases; purchase of all replacements; implementation of measures to reduce exposure to infection, survival of *M a paratuberculosis* or progression of clinical cases; vaccination or test and cull.

- Vaccination (if used) is assumed to have two separate effects. These effects are:
  -Vaccine efficacy, which is the proportion of SUS animals becoming IM following vaccination; and
  -Vaccine effect on progression, which is the percentage reduction in the probability that a vaccinated animal will progress from LT to LS in any time period, compared to an unvaccinated animal of the same age.

- If a control program is in place, the probability of infection (a) is reduced by the percentage specified by the user for management to reduce exposure;

- If a control program is in place, the probabilities of progression LT>LS and LT>IM are adjusted for management and vaccination effects;

- If a control program includes purchase of replacements, all replacement sheep are assumed to be purchased after the start of the program, regardless of the source of replacements previously;

- If early removal of CC animals is included as a control options, sheep are culled at the end of the first time period in which they became CC (i.e. e = 1);

- If test and cull is chosen as a control option, each sheep in the selected age group (except cast-for-age ewes) is subjected to a Bernoulli trial to determine its test result. For SUS and IM sheep, the probability of a positive result (p) is the assumed test specificity for that state, and for other states p is the test sensitivity for that state; and

- Testing is undertaken at the end of each year, and test positive animals are all culled and replaced, in addition to normal cast-for age ewes.

6.2.2 Implementation

The model was implemented as a stochastic model in Visual Basic 6, as a stand-alone program. Key features of the model implementation are:

1) Inclusion of production parameters and the effect of disease on production;
2) Allowance for implementation of controls from a specified year after the model commencement;
3) Incorporation of a wide range of control options;
4) Direct comparison of with and without control options in a single run of the model
5) Comprehensive on-screen summary output in tabular and graphic form;
6) Detailed output to text files for further analysis;
7) Suitability for subsequent economic analysis using model output; and
8) Outputs presented as mean and percentiles of results from multiple iterations of the model.

The effects of control programs were calculated by running two duplicate models in parallel, one with controls implemented, and the other without. Because of random variations between the models, the two models produce slightly different outputs, even in the absence of a control program. However, the mean output values over multiple (5 – 10) iterations are very similar between the two models.

6.2.3 Model testing and validation

6.2.3.1 Estimation of model parameters

Suggested ranges and initial values for key model parameters were estimated as follows:

Parameter	Estimation and range of suggested values
Program control	
Number of years	The number of years to be simulated. Suggested range is 20 – 100 years, default value = 30 years
Number of iterations	The number of iterations (repetitions) to be done. Suggested range is 5 – 100, default value = 10
Lower and Upper Percentiles	Percentiles for summarising output. Suggested values 2.5/97.5%, 5/95% or 25/75%.Default values = 5% & 95%
Use random seed	Flag to use a random seed for the random number generator. Checked = random seed, unchecked = fixed seed, default = unchecked
Set random number seed	Seed value for the random number generator if “Use random seed” is unchecked. Inactive if “Use random seed” is checked. Default = 1
Purchase replacements?	Check to purchase all replacements, uncheck to self-replace. Default = unchecked
Years adults kept	The number of years that adults are kept for. Suggested range is 4 – 6, default = 5 years.
Initial numbers by state and age	The initial numbers of sheep in each state/age combination. Suggested range for total adults = 1000 – 5000, Default total = 2000.
State of purchased replacements	The probability that purchased replacement sheep are in each state. Must total 100%, default is 100% SUS, 0% for other states.
Disease parameters	
Transmission parameters	
Probability of progressing from LT to LS, by age	The probability of progressing is assumed to increase with age, because relatively few infected animals are shedding at detectable levels before about 1.5 – 2 years of age. The suggested range of values, based on beast-guess estimates, is: <5%/quarter for lambs (default=1%), 5 – 20%/quarter for hoggets (default=10%) and 20 – 50%/quarter for adults (default=40%).
Average time to transition between states	The time to transition between states is likely to vary with nutrition, physiological state, ongoing exposure to infection, occurrence of other diseases and other factors. Generally, as the disease progresses, the rate of progression is also likely to increase. Suggested ranges and default values for each transition are shown below, based on beast-guess estimates.
LS to HS	Suggested range: 6 – 12 months, default = 9 months
HS to CC	Suggested range: 3 – 9 months, default = 6 months
CC to Death	Suggested range: 1 – 6 months, default = 3 months
LT to IM	Suggested range: 3– 24 months, default = 12 months
Contact Rate	Contact is the average number of contacts (per year) of a sheep with potentially infected faeces. The suggested range is 25 – 150, equivalent to one contact per fortnight to three per week, and the default value is 100, equivalent to two contacts per week
Relative age susceptibility	Susceptibility is assumed to decrease with age, and susceptibility of hoggets and adults is estimated relative to a fully susceptible lamb. Although the degree of age resistance has not been quantified, suggested ranges and default values are: 5 –

	50% for adults (default=10%), 20 – 80% for hoggets (default=50%) and 100% (fixed) for lambs.
Bacterial characteristics	
Mycobacterial excretion rates	The rate of excretion of *M a paratuberculosis* by infected sheep, relative to clinical cases (CC).
CC (z)	Fixed as the reference state at 100% – other states are input as a percentage of CC
HS (y)	Heavy-shedding sheep are assumed to be excreting *M a paratuberculosis* in their faeces at relatively high levels, of the order of 1 – 2 logs less than CC. The suggested range is 1 – 10%, and the default value is set at 10% (equivalent to 1 $\log_{10}$ less than CC).
LS (x)	Light-shedding sheep are assumed to be excreting *M a paratuberculosis* in their faeces at much lower levels, of the order of 2 – 4 logs less than CC. The suggested range is <1%, and the default value is set at 1% (equivalent to 2 logs_{10} less than CC).
Mycobacterial survival (s)	The observed decay rate under favourable survival conditions ranged from 0.2 – 1.7 logs_{10}/month, equivalent to 0.6 – 5 logs_{10}/quarter (Whittington, 2001). Based on these results, the suggested range is 0.01 – 30%, and the default value is 10% (equivalent to a 1 $\log_{10}$ decline).
Breed effects	
Relative susceptibility due to breed	Variations in susceptibility due to breed have been suggested but not quantified. Suggested values are 80 – 100%, with a default value of 100% (fully susceptible)
Relative rate of progression due to breed	Variations in progression of LT to LS due to breed are also possible but have not been quantified. Suggested values are 80 – 100%, with a default value of 100% (fully susceptible)
Flock parameters	
Average wool cut	From NSW Agriculture sheep gross margins. Suggested range: 4 – 6 kg/head, default value = 5 kg/head
Standard deviation of wool cut	Suggested value 10% of average cut, default value = 0.5 kg/head
Average price for wool	From 2002 market quotations. Suggested range: 600 – 800 cents/kg, default value = 600 cents/kg (greasy).
Lambing %	From NSW Agriculture sheep gross margins. Suggested range: 80 – 120%, default value = 100%
Annual mortality rates	From NSW Agriculture sheep gross margins. Suggested ranges: 3 – 5% for adults (default = 3%), 3 – 5% for hoggets (default = 5%) and 5 – 10% for lambs (default = 7%).
Effect of disease on production	
Reduction in wool production	Any reduction in wool production due to infection is assumed to increase as the disease progresses, but has not been quantified. Suggested ranges and default values are: 0 – 5% for LS (default=0%), 5 – 20% for HS (default=5%) and 10 – 20% for CC (default=10%)
Reduction in wool price received	Any reduction in wool quality due to infection is also assumed to increase as the disease progresses, but has not been quantified. Any reduction in quality is measured as a reduction in price received for affected wool. Suggested ranges and default values are: 0 – 5% for LS (default=0%), 5 – 20% for HS (default=5%) and 10 – 20% for CC (default=10%)
Reduction in lambing percentage	Any reduction in lamb marking percentage due to infection is also assumed to increase as the disease progresses, but has not been quantified. Suggested ranges and default values are: 0 – 5% for LS (default=0%), 5 – 20% for HS (default=5%) and 10 – 20% for CC (default=10%)
Disease control Program	
Year control	The year of the simulation in which selected controls are first implemented. If the

program starts	year is greater than the number of years being simulated the controls are never implemented. Suggested range is 15 – 20 years, to allow disease to stabilise, or 5 – 10 years for early control. Default value = 999 years (no control program)
Management	Management options to reduce disease impact
Remove clinical cases	If checked, all CC animals are culled at the end of the time period in which they become CC. Default is unchecked.
Purchase replacements	If checked, all replacements are purchased, regardless of previous selection. State of replacements is as listed under the Model Parameters tab. Default is unchecked.
Reduction in exposure	This includes any measures taken by the farmer to reduce exposure of susceptible animals to infection through grazing management, etc. Suggested range is 0 –30%, default = 0%
Reduction in progression	This includes any measures taken by the farmer to reduce progression of LT animals to LS, for example through internal parasite control, improved nutrition, liming, etc. Suggested range is 0 –30%, default = 0%
Reduction in survival	This includes any measures taken by the farmer to reduce the survival of *M a paratuberculosis*, for example through grazing/pasture management, liming, etc. Suggested range is 0 –100%, default = 0%
Vaccination	
Use vaccination	If checked, lambs are vaccinated at about 3 months of age. Default is unchecked
Vaccine efficacy	Vaccine efficacy is the probability that a susceptible (SUS) sheep that is vaccinated will become immune to infection (IM), and assumed to be >50% – i.e >50% of susceptible lambs become IM if vaccinated. Results of ongoing vaccination trials should help quantify this effect. Suggested range is 50 – 95%, default value = 80%.
Effect of vaccine on progression	Vaccination of previously infected animals is assumed to reduce the probability that a vaccinated animal will progress from LT to LS in any time period, compared to an unvaccinated animal of the same age. Results of ongoing vaccination trials should help quantify this effect. Suggested range is 20 – 80%, default value is 50%.
Adult vaccination	If checked, all sheep are vaccinated initially regardless of age, with only lambs vaccinated thereafter. Default is unchecked
Test & Cull	
Test adults	If checked, all adults are tested at the end of each year, with any test-positive animals culled. Default is unchecked
Test hoggets	If checked, all hoggets are tested at the end of each year, with any test-positive animals culled. Default is unchecked
Test sensitivity, by State	Sensitivity of the surveillance test used will vary according to stage of disease and the test used. Suggested ranges and defaults (for serology) are: 0 – 5% for LT (default = 0%), 5 – 30% for LS (default = 20%), 20 – 70% for HS (default = 50%) and 50 – 95% for CC (default = 5%). Suggested ranges for PFC are: 0 – 5% for LT, 5 – 50% for LS, 50 – 90% for HS and 90 – 100% for CC.
Test specificity	Specificity of the test will also vary, mainly according to the test used. Suggested ranges and default values are: >99.5% for serology in SUS and IM, default = 99.9% and 100% for PFC.

6.2.3.2 Data for validation of the model

In order to verify the suitability of the model as a predictor of spread of OJD within infected flocks, model output was compared to available data from known infected flocks. Only limited data was available for comparison, with most of this relating to either prevalence of infection (estimated using histology/tissue culture or from the results of pooled faecal culture) or annual mortality rates reported by farmers. Suitable longitudinal data on changes in disease prevalence or mortalities over time was not available for comparison with the model, although anecdotal reports support the models findings that it may take >10 years for the mortality rate in infected flocks to peak. The data that was available for validation of the model is summarised in Table 10.

Crude mortality rates are likely to overestimate the true OJD-mortality rate by varying degrees, depending on the mortality rate due to other causes on each farm. In addition, farmer-estimates of OJD-mortality

also may be biased, depending on how the estimates were derived and the farmers' assumptions about the proportion of deaths that were due to OJD. Estimates will also vary depending on whether they were calculated as a percentage of the whole flock, of adults only or of affected mobs/age groups only. Based on the data in Table 10, the mortality rate due to OJD in flocks with established infection is likely to vary from about 5 – 15% when measured as a percentage of all adult sheep, but may be as high as 20 – 30% in individual mobs or age-cohorts.

Estimates of prevalence of infection based on histological or cultural examination of large numbers of sheep provide a more objective measure of prevalence but are also likely to substantially underestimate the true prevalence of infection (particularly early cases) unless adjusted for the (often unknown) sensitivity of the testing procedure used. From the data in Table 10, the prevalence of infected animals in flocks with established infection could range from about 10 – 40%, and possible higher, while the average percentage of adults that are actually shedding is probably >12%.

In one recently infected flock, the estimated prevalence of shedders (based on pooled faecal culture) in two cohorts of 5-year-old ewes was 2.5% before adjusting for the sensitivity of the test, equating to a true prevalence of probably 4 – 6%. These ewes were lambs at the time infection was first introduced into the property in a mob of purchased sheep.

Prevalence and mortality rates will also vary depending on local factors that may encourage or hinder progression of infection on each farm, and the length of time since the farm became infected. Therefore, any comparison of model output with real estimates must be treated with some caution.

The available data did not allow any validation of production effects or control activities, other than limited evaluation of vaccination in sheep up to 2 years post-vaccination.

Table 10: Estimates of prevalence in infected flocks used for validation of an OJD spread model.

Parameter	Group	Average (%)	Range (%)	Comments and Source
Crude mortality rate	2 yo 3 yo 4 yo	13 13.5 13	9 – 17 12 – 15 12 – 14	Based on shearing/crutching counts, higher values for 9 months only (Eppleston and Simpson, 1999).
Crude mortality rate	3 yo wethers 4 yo wethers Purchased 3 yo wethers	8 18 8		14 infected flocks in central & southern tablelands and south-west slopes of NSW (M Evers personal communication).
	Whole flock Ewes Wethers Crossbreds 2 – 4 yr old Purchased ewes	9 27 15 2 13 10	7 – 12+ 21 – 34 10 – 19	6 flocks (7, 7, 8, 10, 10, 12+) 2 flocks (21 & 34) 3 flocks (10, 15 & 19)
OJD mortality rate	155 flocks 0-2 yrs pd 3-5 yrs pd 6-9 yrs pd ≥ 10 yrs pd	3.7 2.4 4.3 5.4 5.9	0 – 20 0 – 13 0 – 20 0 – 18 1 – 20	Owner-estimated OJD-mortality rate by time (years) since infection or diagnosis (Eppleston et al., 2000).
OJD mortality rate	unvaccinated hoggets vaccinated hoggets	3.1 0.2		Three flocks in the OJD vaccination trial (Eppleston et al., 2002).
Histological prevalence by lesion type	Mixed ages	17 5 12	9 – 23	Six infected flocks from four farms (Sergeant et al., submitted). Mild paucibacillary lesions (score 1) Moderate-severe lesions (score ≥ 2)
Prevalence of infection by lesion type	Histology and/or tissue culture +ve Histo score 1 Histo score ≥ 2	37 6 16		145 x 3-yr-old sheep tested by histology and tissue culture (C Lambeth, personal communication) Mild paucibacillary lesions (score 1) Moderate-severe lesions (score ≥ 2)
Prevalence of faecal shedders	5 yo ewes	1.4 – 2.5		Recently infected flock, 5-6 years post-exposure (L Rast, personal communication).
Prevalence of faecal shedders	18 – 21 mths unvaccinated 18 – 21 mths vaccinated	8.8 0.9	3.4 – 17.3 0.5 – 1.1	Three flocks in the OJD vaccination trial. Estimates not adjusted for test sensitivity (P Windsor, personal communication).

6.2.3.3 Comparison of data with model output

The model was run for 100 iterations using the default values. The median and 90% interval for OJD mortality rates and prevalence of infection for the scenario at the end of 30 years are shown in Table 11.

Table 11: Summary results after 30 years for 100 iterations of an OJD spread model using default input values

Parameter	Age	Median	5 – 95% interval
Annual OJD mortality rate	Whole flock	4.7	4.1 – 5.3
	Adults	12.3	10.8 – 13.6
	6+ yo	2.3	0.7 – 4.6
	5-6 yo	7.6	4.7 – 10.2
	4-5 yo	16.6	12.5 – 21.2
	3-4 yo	20.4	16.7 – 25.4
	2-3 yo	8.6	5.9 – 11.2
	Hoggets	0.9	0.5 – 1.3
	Lambs	0	0
	Vaccinated hoggets	0.3	0.1 – 0.5
Prevalence of infected animals	Whole flock	53.5	51.5 – 55.5
	Adults	32.3	29.2 – 34.3
	6+ yo	3.1	1.4 – 5.6
	5-6 yo	10.6	7.5 – 13.9
	4-5 yo	25.5	21.6 – 29.4
	3-4 yo	41.3	36.6 – 46.1
	2-3 yo	54.3	49.9 – 58.8
	Hoggets	54.7	52.0 – 57.0
	Lambs	75.8	73.2 – 78.8
Prevalence of shedders (LS+HS+CC)	Hoggets	16.9	14.0 – 19.6
	3-4 yo	37.3	30.0 – 45.8
	4-5 yo (5 yrs post exposure)	0.5	0 – 2.1
	4-5 yo (high exposure)	5.2	2.3 – 10.7
	Adults	20.6	17.2 – 23.7
	Vaccinated hoggets	5.1	2.4 – 6.9

6.2.3.4 OJD mortality rates

Median annual OJD mortality rates estimated by the model ranged from 0 to about 14% of adults, depending on time since infection and random variation in the model. Rates stabilised after about 20 years and averaged 12.3% (90% interval 10.8 – 13.6%) at 30 years. The median annual mortality rate calculated across the whole flock after 30 years was 4.7% (90% interval 4.1 – 5.3%), and ranged from 1 – 25% for different adult age groups. Some individual simulations peaked as high as 29 – 30% after about 20 years, before dropping back to about 25% in later years. These rates were comparable to the estimated range of values from the available data, particular considering the variability and generally subjective nature of the data.

Three flocks involved in the vaccination trial provided reasonably reliable data on mortality rates due to OJD in unvaccinated hoggets (~ 2 years old) in heavily infected flocks. Deaths in these flocks due to OJD averaged 3.1%, compared to a predicted rate of 0.9% from the model. The higher than predicted mortality rate in these flocks is probably due to the very high level of infection in these flocks, and the deliberate challenging of these sheep by co-grazing with clinical cases. In addition, although the model only predicted 0.9% mortalities for the year, there were an additional 1.4 – 2.3% of hoggets that were clinical cases at the end of the year, and would be expected to die soon after.

Some individual flocks reported mortality rates substantially higher (or lower) than those predicted by the model. Higher than expected mortality rates could be due to:

- highly favourable conditions for spread and progression of infection;
- high mortality rates due to other diseases blamed on OJD; or

- high mortality rates in individual age-cohorts.

Lower than expected rates could be due to:

- situations unfavourable to spread and progression of infection;
- calculating mortalities across the whole flock instead of just adults; or
- relatively recently infected flocks in which prevalence has not yet peaked.

6.2.3.5 Prevalence of infection

The simulated median prevalence of infected animals after 30 years was about 54% across the whole flock, ranging from 76% in lambs to 3% in aged ewes, and generally decreased with age, as infected sheep either died or recovered from infection. Median prevalence for all adults was about 32% (90% interval 29.2 – 34.3%), well within the estimated range of 10 – 40%. The estimated prevalence for 3 year-old sheep (41.3%, 36.6 – 46.1) was similar to the observed prevalence of histological or tissue culture positive sheep for this age-cohort in one flock (37%).

6.2.3.6 Prevalence of shedding animals

The model estimated that after 30 years about 20.6% (17.2 – 23.7%) of adults and 16.9% (14.0 – 19.6%) of hoggets would be shedding *M a paratuberculosis* in faeces. The simulated estimate for adults was consistent with the estimated range of >12%, allowing for the fact that some light or intermittent shedders may have remained undetected. However, the simulated estimate for hoggets was considerably higher than the observed value for hoggets from the vaccination trial. Much of this difference could be due to the fact that many of the shedding hoggets (>50%) are likely to be light shedders and that the sensitivity of pooled faecal culture for detection of these sheep is likely to be correspondingly poor. Therefore, assuming a sensitivity of about 50%, the average prevalence of shedders in these hoggets would be about 18%, within the predicted range from the model.

In one flock, estimated prevalence based on pooled faecal culture in 5-year-old ewes was 2.5% and 1.4% 5 and 6 years respectively after introduction of infection. Assuming a sensitivity of pooled faecal culture of about 50%, the true prevalence in these ewes is likely to be about 3 – 5%, which is greater than the range predicted by the model (0 – 2.1%). However, this simulation was based on the original introduction of only four infected sheep, rather than a larger number as was probably the case with this flock. Repeating the simulation with higher initial exposure (34 infected sheep), consistent with purchasing a large mob of infected ewes resulted in a predicted prevalence of 2.3 – 10.7%.

6.2.3.7 Effect of vaccination

Simulated mortality rates and prevalence of shedders in vaccinated hoggets at the commencement of a vaccination program were 0.1 – 0.5% and 2.4 – 6.9% respectively, slightly higher than the observed values of 0.2% and 0.9% in the vaccination trial flocks. Some of the difference in prevalence of shedders is likely to be due to underestimation of the true prevalence of shedders in the vaccinating flocks. However, the lower than expected mortality rate, and the lower prevalence, even after adjusting for the sensitivity of pooled faecal culture, suggest that the vaccine is probably more efficacious than the 80% assumed in the model.

6.2.3.8 Sensitivity analysis

A sensitivity analysis was undertaken to evaluate the importance of possible errors in input values. For this analysis, the value of each variable was increased or decreased by a variable amount, depending on the default value and the assumed likely range of realistic values for the parameter. Each simulation was run for 10 iterations and all other variables were held constant at the default values.

Because the main observable effect of OJD on-farm is the death of affected animals, this analysis used the annual mortality rate in adults as the outcome of interest. Variations in the time taken to reach the peak mortality and the median and range of adult mortality rates at 30 years were the outputs compared between simulations.

The results of the sensitivity analysis are summarised in Table 12, with detailed results shown in Table 13. Many effects were relatively small, or required a substantial change in the input value to cause any change in the annual mortality rate. In addition, many variables affected mainly the rate of spread within the flock, with little effect on the final mortality rates observed. The main variables affecting the overall mortality rate were those affecting the rate of progression in individual sheep

Table 12: Summary of an analysis of the effect of variations in input values on model output

Effect	Input variables	Comments
No effect or very minor effect	Background (non-OJD) mortality rates	
Effect on time to peak mortality only	Initial numbers of infected sheep	Increased numbers = earlier peak.
	Lambing percentage	Decreased lambing % = earlier peak, increased lambing % = later peak
	Age susceptibility (hoggets and adults)	Increased susceptibility = earlier peak, Decreased susceptibility = later peak.
	Breed susceptibility	Decreased susceptibility = later peak. Very low susceptibility (50%) resulted in major delay in peak mortality to ~ 90 years, and a lower peak.
Effect on mortality rate at 30 years only	Years kept	Decreased years kept = increased mortality rate
Effect on both time to peak mortality and mortality rate at 30 years	Purchase replacements	Infection died out if replacements were purchased instead of bred
	Excretion rates for HS & LS states	Increased excretion rates = earlier peak and higher mortalities and vice versa
	Survival of *M a paratuberculosis*	Increased survival = earlier peak and higher mortalities
	Probability of progression LT>LS (all ages)	Increased probability = earlier peak and higher mortalities and vice versa
	Time to change between states LS>HS, HS>CC	Decreased time = earlier peak and higher mortalities and vice versa
	Time to change between states CC>Dead	Increased time = earlier peak and higher mortalities and vice versa
	Time to change between states LT>IM	Increased time = earlier peak and higher mortalities and vice versa
	Contact rate	Increased rate = earlier peak and higher mortalities and vice versa
	Breed effect on relative rate of progression	Decreased relative rate = later peak and lower mortalities

Specific effects worth noting included:

- If adult or adult and hogget susceptibility were assumed to be zero, the resulting epidemic was significantly delayed (peak at 33 years for adult susceptibility=0, 50 years for adult susceptibility =hogget susceptibility =0), and mortality rates at 30 years were reduced.
- Assuming both adults and hoggets were 100% susceptible resulted in a very early peak of mortality (22% at 9 years) with a subsequent drop back to levels similar to the default values
- Changes to the probability of progression and the time between states also resulted in changes in the distribution of mortality rates between age groups of adults
- Assumed low values for breed susceptibility and relative rate of progression resulted in major delays to the peak mortality and reduced mortality rates. For example, an assumed breed susceptibility of 50% resulted in a peak mortality of 9.7% (range 4.2 – 11.6%) at 90 years.

Variables that had a major effect on annual mortality rates, and therefore warrant further investigation included:

- Age susceptibility - infection died out if adults were assumed to be resistant to infection and replacement sheep were all purchased;

- Breed effects on susceptibility and rate of progression – decreased breed susceptibility resulted in a significant delay and reduction in mortality rates;
- Probability of progression from LT to LS for adults, hoggets and lambs – variations in these rates had a substantial effect on mortality rates.
- Contact rate – variations in contact rate also had a major effect on mortality rates.

Pre-natal or peri-natal infection of lambs from their dam was initially included in the model, but was found to have no effect on the resulting mortality rate and was therefore removed from the model.

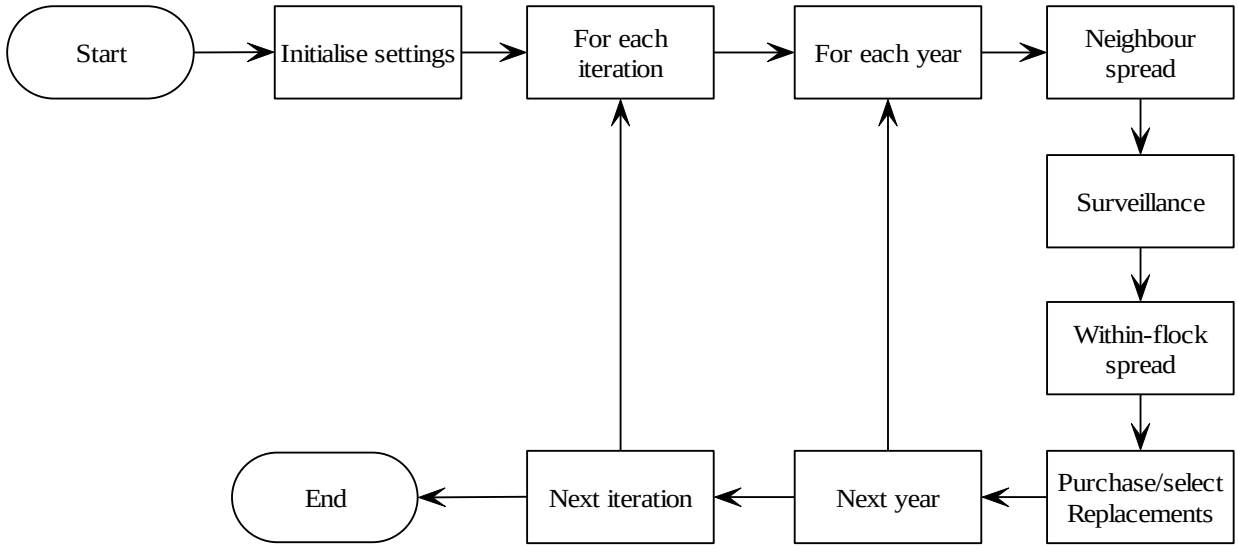
Table 13: Results of a sensitivity analysis for a simulation model of the spread of OJD in an infected flock

OJD spread model #2	Years to peak mortality	Mortality at 30 years		Comment
Parameter change	Median	Median	Range	
Default	21	11.7	10.4-13.3	
Purchase replacements	11	0		(time to extinction)
Keep 4 years only	20	14.6	11.9-16.1	
HS=1%	26	11.1	2.4-11.8	
HS=20%	17	12.3	11.7-14.5	
LS=0.1%	21	11.3	10.0-13.2	
LS=5%	18	13.5	11.2-13.9	
survival =0	28	11.3	10.4-12.1	
survival=20	20	12.8	11.5-13.5	
survival=50	13	14.9	14.1-16.6	
Adults progress 15%	28	9.4	6.3-10.8	
Adults progress 35%	16	13	11.2-14.7	
Hoggets progress 1%	29	9.2	7.5-10.3	
Hoggets progress 10%	16	17	14.5-17.9	
Lambs progress 0%	22	11.8	10.5-13.0	
Lambs progress 5%	18	17.7	16.2-20.0	
LS>HS=6 months	19	12.5	10.9-14.2	
LS>HS=12 months	26	11.3	10.6-12.3	
HS>CC=3 months	19	12.8	11.1-14.7	
HS>CC=9 months	24	11.5	10.8-12.8	
CC>D=1 month	27	9.9	7.5-11.7	
CC>D=6 months	17	13.9	13.4-15.7	
LT>IM=12 months	23	10	7.8-12.0	
LT>IM=24 months	18	16.9	15.8-18.8	
CR=80 contacts/year	30	9.7	0-11.0	
CR=120 contacts/year	18	13.1	11.7-14.3	
Adult susceptibility =0%	>30	9.5	5.8-11.5	
	33	12	10.8-13.2	
Adult susceptibility =20%	17	12.1	10.6-13.1	
Hogget susceptibility = 20%	28	12.1	8.7-13.1	
Hogget susceptibility = 80%	16	12.5	10.1-13.6	
Breed susceptibility = 80%	22	12.1	10.5-14.0	
Breed susceptibility = 50%	90	9.7	4.2-11.6	
Breed progression = 80%	19	10.5	9.2-11.8	
Breed progression = 50%	35	6.1	5.3-7.4	
Breed sus=progress=80%	26	10.6	8.7-11.5	
Number LS at start = 50	13	12	11.1-13.3	
Number HS at start=20	15	12.1	10.5-13.6	
Lambing % = 80	18	12.8	11.6-14.4	
Lambing % = 120	24	11.3	10.1-13.2	
Adult Mortality = 1%	22	12.1	9.7-13.1	
Adult Mortality = 5%	23	12.2	10.4-14	
Hogget Mortality = 3%	19	11.8	10.6-13.4	
Hogget Mortality = 7%	21	12	10.9-13.9	
Lamb Mortality = 5%	21	12.1	10.5-13.0	
Lamb Mortality = 10%	21	12.4	10.6-13.6	

6.3 Model 3 – Regional Spread Model v1.0

The OJD regional spread model is based on the interactions between a large number of individual flock models, to simulate the spread of OJD between flocks, either through local spread between adjoining flocks, or through movements of replacement sheep. A simplified structure of the model is shown in Figure 6, and additional input parameters are listed in Table 13.

Figure 6: Structure of a regional spread model for OJD



The main features of this model are:

6.3.1 Set-up and general structure

- Spread of infection within each flock in the regional model is simulated using a simplified version of the individual flock model (Model 2);
- A single randomly-selected flock is assumed to be infected at the start of the simulation, with the number of sheep in each state specified by the user;
- The total number of farms in the region being simulated and the proportions that do not run sheep, are studs or that purchase all replacements can be specified by the user;
- Disease transmission parameters for infected flocks can be set by the user in a similar manner to the individual-flock model;
- Flock sizes, lambing percentages, relative flock susceptibility and probability of contact between neighbouring flocks vary randomly between flocks according to triangular distributions specified by the user, or can be set explicitly by the user;
- The proportion of flocks purchasing from outside the specified region, and the probability of sheep purchased from outside being from an infected flock and the proportions in each state can be set by the user;
- For each iteration, a list of flocks is initialised, and each flock is randomly allocated a status of 'no sheep' or 'unexposed' according to the proportions set by the user;
- The index flock is randomly selected, initialised and allocated a status of 'infected';
- Each flock is randomly allocated a purchasing pattern as 'all' or 'rams only' purchased, and whether sheep are purchased from within the region or outside;
- A proportion of flocks purchasing 'rams only' are randomly selected and identified as 'studs' according to the proportions set by the user;
- For small simulations, flock characteristics, neighbour risks and the index flock can all be specified by the user;
- A flock becomes 'infected' when one or more infected animals are present, and returns to 'uninfected' when there are no longer any infected animals present and *M a paratuberculosis* contamination has died out.

- Within an infected flock:
  -the probability of new infection (a) at each time step is calculated as:
  $a = CR \times M_t/N_{total} \times Susceptibility_{age} \times FlockSusceptibility$
  -The probability of progression from LT to LS (b) is calculated as:
  $b = Progress_{age} \times (1 + CR \times M_t/N_{total}) \times (1 – VaccineProgression)$
  and
  -other transitions are calculated as for Model 2.

Table 13: Input parameters and variables for a model of the regional spread of ovine Johne's disease

Variable/ parameter name	Description
CR	See Table 4
a	See Table 4
b	See Table 4
M_t	See Table 8
$M_{neighbour}$	The level of mycobacterial contamination on the neighbouring property (see Model2)
N_{age}	The number of sheep in the flock by age group (lambs, hoggets or adults)
NeighbourRisk	The probability of a sheep having contact with faeces from a neighbouring flock
$Susceptibility_{age}$	See Table 9
FlockSusceptibility	Relative susceptibility of flock due to breed and other factors, compared to a fully susceptible flock
VaccineProgression	See Table 9

6.3.2 Spread between neighbours

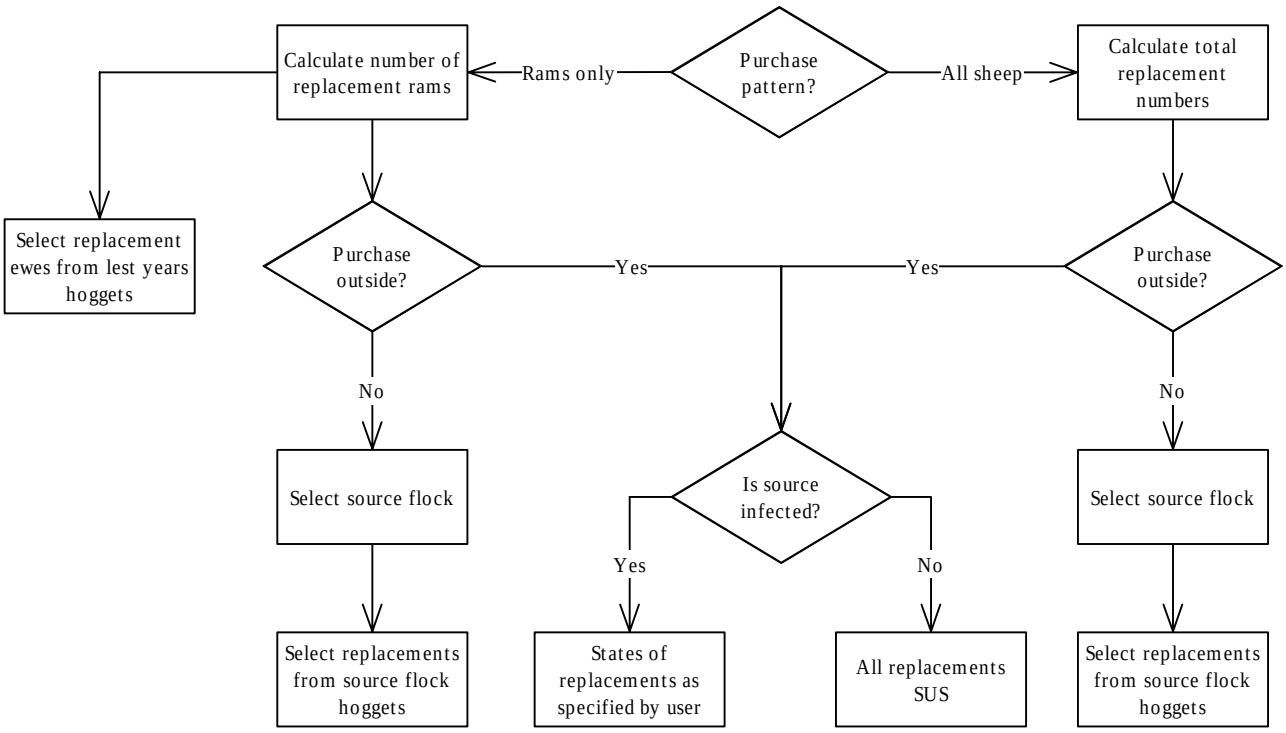
- Flocks are assumed to be on a rectangular grid, so that each flock (except those on the edge of the grid) has eight neighbouring flocks;
- Alternatively, probability of contact between properties can be explicitly specified by the user (for small simulations);
- The probability of a sheep becoming infected from a neighbouring flock depends on the probability of contact, the level of contact on the neighbouring property, the flock size and susceptibility, and is calculated as:
-

$$P_{infection} = NeighbourRisk \times M_{neighbour}/N_{age} \times Susceptibility_{age} \times FlockSusceptibility$$

6.3.3 Spread with sheep movements

- Flocks which purchase all replacements are assumed to purchase the required number of new adults from a randomly selected flock each year, as shown in Figure 7;
- Flocks which purchase rams only are assumed to purchase sufficient rams (calculated from the ram percentage, the number of adults and the number of years that adults (and rams) are retained) each year from a randomly selected flock that is listed as being a 'stud';
- Flocks which purchase rams only are assumed to replace culled and dead adult ewes with young adults selected from the hoggets in the flock at the end of the preceding year;
- Flocks which purchase from outside the area may purchase from an infected or uninfected flock, according to the prevalence of infected flocks outside the area specified by the user;
- Replacement sheep are allocated a state randomly, according to proportions in each state for the hoggets in the source flock, or for purchases outside the area, according to the proportions specified by the user for these sheep.

Figure 7: Flow diagram of assumed purchasing patterns for replacement sheep in a simulation model for the spread of OJD between flocks



6.3.4 Control programs

- A regional control program, consisting of combinations of surveillance, vaccination and movement restrictions, can be commenced in any year specified by the user;

6.3.4.1 Surveillance

- For surveillance, the user can specify the number of flocks tested per year, the number of sheep tested in each flock, the sensitivity of the test for each infection state, and the percentage of flocks refusing to test;
- The flock-specificity of the test is assumed to be 100% – any positive results are either definitive or are followed-up with a definitive test
- A proportion of flocks specified by the user are identified as 'not testing' and are never subjected to a flock test;
- The required number of flocks are selected at random each year from the flocks that are eligible for testing, and tested as follows;
  - -For each uninfected flock tested, the test result is assumed to be negative;
  - -For each infected flock tested, the required number of sheep (or all sheep if less than the specified sample size) are selected at random from the adult population, and for each sheep tested, the test result is determined by a Bernoulli trial, where the probability of a positive result is the test sensitivity for the state of the sheep;
  - -If ≥1 infected sheep have a positive test result, the flock is detected;

6.3.4.2 Vaccination

- Vaccination is assumed to have two separate effects (see under Model 2). These effects are:

  -Vaccine efficacy, which is the proportion of SUS animals becoming IM following vaccination; and

-Vaccine effect on progression, which is the percentage reduction in the probability that a vaccinated animal will progress from LT to LS in any time period, compared to an unvaccinated animal of the same age.

- Vaccine efficacy and the reduction in progression due to vaccination can be specified by the user, as well as options for vaccine usage;

- Options for vaccine usage include vaccinating:

  -detected flocks;
  -neighbours of detected flocks; and
  -uninfected flocks or undetected infected flocks

- Detected flocks are randomly selected for vaccination at the time of detection, according to the percentage specified by the user, if this option is selected;
- Neighbouring flocks are randomly selected for vaccination at the time of detection of their infected neighbour, according to the percentage specified by the user, if this option is selected;
- Uninfected flocks and undetected infected flocks are randomly selected for vaccination at the start of the control program, according to the percentage specified by the user for each of four ranges of annual mortality rate due to OJD, if this option is selected;
- Uninfected/undetected flocks are vaccinated commencing in the year specified by the user, which must be no earlier than the year the control program commences, if this option is selected;
- Once a flock starts vaccination it is assumed to continue vaccinating each year, indefinitely;

6.3.4.3 Destocking

- A percentage of Infected flocks may destock each year, depending on the percentages specified by the user for each of four categories of annual mortality rate;
- In addition, a percentage of Infected flocks that have been detected by surveillance may destock each year, regardless of their annual mortality rate, at a rate specified by the user;
- Destocked flocks may start restocking a minimum of two years after destocking, at a rate specified by the user.

6.3.4.4 Movement restrictions

- Movement restrictions available include:

  -quarantine of known infected flocks;
  -restricting purchases to tested-negative flocks only; and
  -restricting purchases to vaccinating flocks only.

- All infected flocks that are detected by surveillance are either quarantined or not quarantined, as determined by the user;
- If a flock is quarantined, it is no longer eligible as a source of replacement sheep;
- If a movement test is required, purchases can only be sourced from a flock that has had a negative surveillance test within the period specified;
- If vaccination of source flocks is required, purchases can only be sourced from a flock that is vaccinating and is not in quarantine;
- If quarantine of detected flocks is not required, flocks may still purchase from infected flocks that are vaccinating.

6.3.5 Model testing and validation

6.3.5.1 Estimation of model parameters

Suggested ranges and initial values for key model parameters were estimated as follows:

Program Control	
Number of Years	The number of years to be simulated. Suggested range is 20 – 100 years, default value = 30 years
Number of Iterations	The number of iterations (repetitions) to be done. Suggested range is 2 – 20, default value = 5
Percentiles (Low, High)	Percentiles for summarising output. Suggested values 2.5/97.5%, 5/95% or 25/75%. Default values = 5% & 95%
Use random seed	Flag to use a random seed for the random number generator. If a fixed seed is used, the simulation can be reproduced exactly by repeating with the same seed and input values. If a random seed is used the simulation will be different every time, even if all other inputs are identical. Checked = random seed, unchecked = fixed seed, default = unchecked
Set random number seed	Seed value for the random number generator if "Use random seed" is unchecked. Inactive if "Use random seed" is checked. Default = 1
Use new Index flock for each iteration	Flag to use constant or random index flock. Check to use random index flock for each iteration, uncheck to use the same index flock for each iteration. Default is checked
Use existing file of flock details and contact risks	Flag to use existing text files of flock details and contact risks. Check to use existing files, uncheck to use computer generated flock details and contact risks. Default is unchecked. Use select files button to select input files if checked.
Initial numbers by state on index farm – for adults (IM, LT, LS, HS, CC)	Numbers by initial state for adults in the index flock. Number SUS is calculated by the program, and cannot be entered. Default values = 0, 0, 2, 2, 0.
Initial numbers by state for hoggets (IM, LT, LS, HS, CC)	Numbers by initial state for hoggets in the index flock. Number SUS is calculated by the program, and cannot be entered. Default values = 0, 0, 0, 0, 0.
Distribution of states in infected flocks from out of area (SUS, IM, LT, LS, HS, CC)	Percentage by state for replacements purchased from infected flocks from outside the area. Must total 100%. Default values = 5, 50, 20, 15, 10, 0
Number of properties	The number of properties to be simulated. Suggested range 1000 – 5000, default value is 4,000.
% of properties with no sheep	Percentage of properties with no sheep. Suggested range 5 – 20%, default value is 10%.
Disease parameters	
See details for Model 2.	
Flock Parameters	
Ram percentage	Percentage of rams as a percentage of total number of adults. Suggested range: 2 – 3%, default = 2%.
Years kept (adults)	The number of years that adult sheep are retained. Suggested value and default = 5.
Background mortality rate (Adults, Hoggets, Lambs)	The average mortality rate in uninfected sheep by age group. Suggested values: Adults = 2 – 5%, Hoggets = 2 – 5%, lambs = 5 – 10%. Default values: Adults = 3%, Hoggets = 5%, lambs = 7%.
Sheep movements	
% flocks purchasing all replacements	Percentage of flocks that purchase all their replacement sheep. Flocks that don't purchase all their replacements are assumed to purchase rams only Suggested range = 10 – 50%, default value = 40%.

% stud flocks	Percentage of flocks that are studs. Studs are a sub-set of flocks that purchase rams only. The percentage of flocks purchasing all replacements, plus the percentage of studs cannot be greater than 100%. Suggested range = 5 – 15%, default value = 10%.
% flocks purchasing from outside area	Percentage of flocks purchasing replacement sheep from outside the simulated region. Suggested range = 0 – 20%, default value = 10%
% infected flocks outside area	Prevalence of infected flocks outside the simulated region. Suggested range = 0 – 50%, default value = 0%
Flock size (Minimum, Most likely, Maximum)	Minimum, most likely and maximum values for a triangular distribution of flock sizes. Suggested values and default values = 500 – 2000 – 5000
Lambing percentage – in absence of OJD (Minimum, Most likely, Maximum)	Minimum, most likely and maximum values for a triangular distribution of lambing marking percentages. Suggested range: 80 – 150%, default values = 80 – 100 – 120%.
Relative susceptibility (Minimum, Most likely, Maximum)	Minimum, most likely and maximum values for a triangular distribution of flock susceptibility, relative to a highly susceptible flock. Flock susceptibility is likely to vary with breed or other factors. Suggested range = 50 – 100%, default values = 80 – 90 – 100%.
Probability of contact with neighbours (Minimum, Most likely, Maximum)	Minimum, most likely and maximum values for a triangular distribution of probability of contact of a sheep with faeces from neighbours sheep. Suggested range = 0 – 20%, default values = 0 – 5 – 10%.

OJD Control Program

Year that controls commence	The simulation year in which the control program commences. If the year started is greater than the number of years simulated the program will never start. If year commenced > years simulated other control options are disabled. Suggested range depends on other assumptions and aims of simulated control. Default value = 999 (no controls).
Surveillance program	
Flocks tested per year	The total number of flocks subjected to surveillance each year. The number selected depends on the total number of flocks, and the coverage desired. Suggested range = 0 – 50% of number of flocks. Default value = 500
% flocks refuse testing	The percentage of flocks refusing or avoiding testing under the surveillance program. These flocks are selected at random and never tested. Suggested range: 0 – 40%, default value = 40%.
Number tested per flock	The number of sheep tested per flock for surveillance. Suggested values: 350 for PFC, 875 for AGID or 500 for abattoir surveillance, default value = 350.
Quarantine positive flocks (0=no quarantine, 1=quarantine	Flag to control quarantine of detected infected flocks. Check to quarantine all detected flocks, uncheck to leave flocks un-quarantined, default value = checked.
Test sensitivity (%) (LT, LS, HS, CC)	Sensitivity of the surveillance test used according to stage of disease. Values will vary according to the test used and the stage of disease. Suggested ranges for serology are: 0 – 5% for LT, 5 – 30% for LS, 20 – 70% for HS and 50 – 95% for CC. Suggested ranges for PFC are: 0 – 5% for LT (default = 0%), 5 – 50% for LS (default = 50%), 50 – 90% for HS (default = 90%) and 90 – 100% for CC (default = 100%).
Vaccination Options	
Vaccine efficacy (%)	The percentage of SUS sheep becoming IM following vaccination. Suggested range = 70 – 95%, default value = 80%.
Reduction in progression (%)	The percentage reduction in the transition rate for LT>LS in vaccinated sheep. Suggested range = 40 – 80%, default value = 50%.
Vaccinate detected flocks (0=no vaccination, 1=use vaccination)	Flag to control vaccination of detected infected flocks. Check to vaccinate a percentage of flocks detected by surveillance, leave unchecked for no vaccination of detected flocks, default = unchecked.
% detected flocks	The percentage of detected flocks vaccinating. Suggested range: 50 –

vaccinating	100%, default = 100%.
Vaccinate neighbouring flocks (0=no vaccination, 1=use vaccination)	Flag to control vaccination of neighbours of detected infected flocks. Check to vaccinate a percentage of neighbouring flocks, leave unchecked for no vaccination of neighbouring flocks, default = unchecked.
% neighbouring flocks vaccinating	The percentage of neighbouring flocks vaccinating. Suggested range = 50 – 100%, default value = 10%.
Vaccinate undetected flocks (0=no vaccination, 1=use vaccination)	Flag to control vaccination of undetected or uninfected flocks. Check to vaccinate a percentage of undetected/uninfected flocks, leave unchecked for no vaccination of undetected/uninfected flocks default = unchecked.
Year undetected flocks commence vaccinating	The year vaccination of undetected/uninfected flocks commences. Must be greater then the year the control program starts. If greater than the number of years simulated, vaccination will never start, default = 999 (no vaccination).
% undetected flocks vaccinating, by annual % deaths (<=2%, 2-5%, 5-10%, >10%)	The percentage of undetected infected flocks vaccinating. The values should increase with the annual mortality rate in infected flocks. Suggested range = 0 – 100%, default values = 0, 50, 90, 99.
Destocking	
Percentage destocking – by annual % deaths (<=2%, 2-5%, 5-10%, >10%)	The percentage of infected flocks destocking each year. The values should increase with the annual mortality rate in infected flocks. Suggested range = 0 – 100%, default values = 0, 10, 20, 50%.
Percentgae of destocking – detected flocks	The percentage of detected infected flocks destocking each year. Suggested range = 0 – 100%, default value = 50%.
Percentage of restocking each year	The percentage of destocked flocks restocking, each year, after a minimum of 2 years fully destocked. Suggested range = 0 – 100%, default value = 20.
Movement restrictions	
Movement test required (0=no test, 1=test required	Flag to allow purchases only from tested flocks. Check to require testing, uncheck for no testing requirement, default = unchecked. If there are no tested flocks available, purchases will continue from untested flocks until there are tested flocks available.
Years since test	The number of years since the last surveillance test for eligibility to sell sheep. Suggested range: 1 – 2 years, default value = 2. Flocks that have not tested within this period will be excluded from providing replacement sheep.
Purchase vaccinates (0=no vaccination, 1=vaccination required	Flag to allow purchases only from vaccinating flocks. Check to require vaccination, uncheck for no vaccination requirement, default = unchecked. If there are no vaccinated flocks available, purchases will continue from unvaccinated flocks until there are some vaccinated flocks available.
Year purchase of vaccinates commences	The year that the vaccination requirement for purchases commences. Must be greater than the year the control program starts, and if it is greater than the number of years being simulated the requirement will never start. Default value = 999 (purchase vaccinates never starts).

6.3.5.2 Validation of the model

There is very little data available against which to validate this model. Based on an analysis of abattoir surveillance data, the estimated prevalence in NSW was 6 – 10% (of about 32,000 flocks) at 31 December 2001 (Sergeant and Baldock, in press). In the residual zone the estimated prevalence was 29 – 39% of about 3,400 flocks. Although other areas of NSW had a lower prevalence, there is no reliable information on when the infection was introduced to these areas, making comparisons difficult.

Two simulations were run for comparison of the model with the estimated prevalence in New South Wales. For these simulations, it was assumed that OJD was first introduced into NSW in about 1955 (Sergeant, 2001a), so that it had been present for about 45 years at the end of 2000. Although quarantine of known infected flocks was introduced in 1996, this would have had only a marginal impact by 2000,

and therefore hasn't been specifically modelled in this evaluation. For the first simulation, 4,000 properties were simulated, 90% of which had sheep, approximately representing the Residual zone. For the second simulation, 30,000 properties were simulated, representing all of NSW. Each simulation was run for 10 iterations.

Using the default input-values, with 4,000 properties simulated, the median percentage of properties that were infected after 45 years was 31.1% (range: 13.3 – 48.7%). For 30,000 properties simulated, using the same input values, the median prevalence was 8% (range: 4.2 – 20.6%). The predicted outcomes from the model are very widely distributed, with a range of about 35% for 4000 properties after 45 years. While this variability is unfortunate, it probably represents the real impact of chance events on the likely spread of disease in a region. The variability in prevalence after 45 years was closely associated with the percentage of simulated studs that were infected.

The majority of iterations for the Residual zone simulation were in the range 20 – 40%, and the median prevalence was within the estimated 95% probability interval for prevalence for both the residual zone and for the whole of NSW. Therefore, despite the apparent variability in outcome, this model appears capable of providing a reasonable representation of spread of OJD in a region.

6.3.5.3 Sensitivity analysis

A sensitivity analysis was undertaken to examine the importance of varying input values on the output distributions. For this analysis, the value of each variable was increased or decreased by a variable amount, depending on the default value and its assumed likely range of realistic values. Each simulation was run for 10 iterations of 25 years each and all other variables were held constant at the default values. Except where indicated otherwise, a seed value of 1 was used. A series of 45-year simulations were also run to examine the effect of varying the initial seed value for the random number generator and of using a fixed rather than random index flock between iterations. The output variable for the sensitivity analysis was the percentage of infected flocks at the end of the simulation. The results of the sensitivity analysis are summarised in Table 14.

Varying the initial seed value resulted in some variation in the median and range of the output distribution, with the median varying from about 29% to 35%.

For the main sensitivity analysis, disease parameters such as probability and rate of progression, contact rate, age-susceptibility, rate of shedding and bacterial survival all had substantial effects on the proportion of infected flocks after 25 years. This was at least partly because these values were fixed across all flocks, and therefore any effect was repeated for every infected flock that occurred, amplifying the effect across the whole simulation. Use of a range of values for these inputs would reduce the sensitivity to changes in these values, but would further increase the overall variability in the model, as well as increasing model complexity.

The remaining input variables all had a moderate effect on prevalence after 25 years, except for the percentage with no sheep, the percentage purchasing all replacements and the percentage purchasing sheep from outside the simulated region, which had little, if any, apparent effect. Keeping the values of the four variables that were input as distributions (flock size, lambing percentage, flock susceptibility and risk from neighbours) constant at their most likely values reduced the width of the output distribution, but did not affect the estimated median prevalence. Increasing the initial number of infected sheep on the infected flock resulted in a faster progression of the outbreak and a higher prevalence at 25 years, depending on the number of infected sheep.

Table 14: Summary of results of a sensitivity analysis for and OJD regional spread model.

Simulation	Years	Median	Range	Comments
Default, Seed=1	45	31.1	13.3 - 48.7	
Default, Seed=2	45	29.1	7.7 - 45.2	
Default, Seed=5	45	33.2	10.4 - 51.9	
Default, Seed=9	45	30.4	3.3 - 54.5	
Default, random seed	45	35.1	12.3 - 48.0	
Seed = 1, index flock constant	45	27.2	9.0 - 44.6	

Seed = 1	25	3.0	1.1 - 6.0	
Seed = 2	25	4.3	1.3 - 7.5	
Seed = 5	25	4.6	1.1 - 8.0	
Seed = random	25	3.4	2.2 - 5.7	
% No sheep = 0	25	3.4	1.3 - 6.6	
% No sheep = 20	25	3.5	1.5 - 7.3	
Initial number LS = 20	25	5.9	3.2 - 10.1	about 5 years earlier
Initial number LS = 50	25	12.1	9.6 - 18.3	about 10 years earlier
LS>HS = 6	25	6.0	3.8 - 10.5	
LS>HS = 12	25	2.8	0 - 4.2	1 or more iterations failed to establish
HS>CC = 3	25	3.0	0.8 - 4.5	
HS>CC = 9	25	3.2	2.1 - 9.1	
CC>Death = 1	25	1.2	0 - 3.6	1 or more iterations failed to establish
CC>Death = 6	25	17.9	6.9 - 31.1	
LT>IM = 12	25	0.3	0 - 2.5	1 or more iterations failed to establish
LT>IM = 24	25	9.1	4.0 - 16.3	
CR = 80	25	0.6	0 - 2.3	1 or more iterations failed to establish
CR = 120	25	11.0	2.0 - 19.0	
Progress(adults) = 15	25	0.7	0 - 2.4	1 or more iterations failed to establish
Progress(adults) = 35	25	7.8	3.2 - 14.4	
Progress(hoggets) = 0	25	1.1	0.5 - 4.2	
Progress(hoggets) = 10	25	10.8	0 - 27.3	1 or more iterations failed to establish
Progress(lambs) = 0	25	4.8	1.2 - 6.2	
Progress(lambs) = 5	25	9.8	3.4 - 18.5	
Susceptibility(adults) = 0	25	0.3	0.1 - 1.2	
Susceptibility(adults) = 20	25	12.3	5.7 - 21.7	
Susceptibility(hoggets) = 25	25	1.3	0.4 - 3.0	
Susceptibility(hoggets) = 75	25	8.8	1.6 - 20.6	
HS shedding = 0	25	1.3	0.3 - 4.0	
HS shedding = 20	25	8.4	4.6 - 13.0	
LS shedding = 0	25	3.6	0.6 - 10.6	
LS shedding = 10	25	13.9	9.8 - 21.6	
Mptb survival = 0	25	1.6	0.4 - 3.6	
Mptb survival = 10	25	8.7	4.7 - 15.5	
Ram % = 1	25	2.1	0.9 - 5.5	
Ram % = 3	25	4.9	2.7 - 15.8	
% Purchase all = 20	25	3.7	0.5 - 6	
% Purchase all = 40	25	4.6	0.3 - 17.3	
% Purchase all = 40	25	3.7	2.2 - 7.3	seed = 5
% Studs = 5	25	4.2	0.7 - 12.3	
% Studs = 20	25	2.3	1.3 - 3.8	
% Purchase out of area = 0	25	3.1	1.7 - 6.5	
% Purchase out of area = 20	25	4.0	1.3 - 6.9	
Flock size = 500	25	1.7	1.1 - 2.8	
Flock size = 5000	25	3.5	1.3 - 8.7	
Lambing % = 80%	25	9.4	5.1 - 12.4	
Lambing % = 120%	25	1.5	0 - 2.6	1 or more iterations failed to establish
Flock susceptibility = 80%	25	1.9	0.7 - 4.9	
Flock susceptibility = 100%	25	6.8	3.5 - 16	
Neighbour risk = 0%	25	1.6	0 - 2.5	1 or more iterations failed to establish
Neighbour risk = 10%	25	4.6	2.0 - 7.8	
Flock size=2000,	25	3.0	0 - 5.5	1 or more iterations failed to establish
Lambing%=100,	25	3.5	2.9 - 5.4	seed=9
Flock susceptibility=90%, Neighbour risk=5%	25	4.3	2.5 - 6.6	random seed

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