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Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

Abstract

The level of clinical disease experienced due to ovine Johne's disease (OJD) appears to vary considerably between infected sheep flocks in Australia, even for flocks in the same locality that appear to have similar characteristics. This has led to speculation on the cause. Risk factors for the severity of OJD were identified in this project. They were related to some farming practices such as fertiliser application, as well as to flock management and soil type. In particular weaner management and nutrition of sheep to hogget stage were important factors that producers can optimise to reduce the impact of OJD. High soil fertility, organic matter and clay content were also important factors associated with higher levels of OJD. There was less OJD associated with sandy soils. Further research is required in order to determine how these soil characteristics affect the prevalence of OJD and how best to manage soil and pasture to mitigate the losses due to OJD.

Executive Summary

The level of clinical disease experienced due to ovine Johne's disease (OJD) appears to vary considerably between infected sheep flocks in Australia, even for flocks in the same locality that appear to have similar characteristics. This has led to considerable speculation about the potential importance of flock management, soil type, pH and micro-nutrients. Sound understanding about factors that influence disease expression will lead to management recommendations that improve on-farm disease control. Consequently the aim of this project was to identify risk factors for OJD expression in infected flocks and improve the understanding of the epidemiology of the infection.

The project consisted of a cross-sectional study on 92 infected properties located in New South Wales, Victoria, Tasmania and Western Australia. The information obtained from each included the OJD prevalence in specific groups of adult sheep measured using pooled faecal culture, details of farm and flock management and soil analyses from paddocks on which the sheep sampled had grazed. Interviews were conducted on each farm and a total of 717 faecal pools each containing faeces from usually 30 or 50 sheep were cultured while 276 soil samples were analysed.

Three different measures of OJD prevalence were derived from PFC results and then univariable and multivariable statistical analyses were used to assess the significance of animal, farm and soil characteristics on these measures of OJD. Some factors were detrimental in that they were associated with a higher level of OJD, while other factors appeared to be protective.

There was a strong relationship between the PFC results and the duration of flock infection, the level of OJD mortality and the trend in OJD mortality, as well as a relation with parent soil type. There was also a consistent but statistically non-significant trend for lower OJD levels in 4-year olds compared to 3-year olds, may be due to deaths of affected sheep from 2 to 3 years of age. Wethers had consistent and statistically significant higher OJD levels than ewes, which strongly supports the anecdotal observation of higher losses in wether mobs. As age, sex and current OJD mortality were likely to confound the evaluation of farm and flock management, they were included in all multivariable models so that their effects could be taken into account and other factors correctly identified. In addition to age, sex and current OJD mortality, parent soil type, also likely to confound evaluation of soil characteristics, was included in all multivariable models for soil variables. A total of 31 significant farm/flock/management and soil variables were found across one or more of the final multivariable models. Some were likely to be a consequence of OJD infection, but the remainder appeared to be potential risk factors for the severity of the disease.

Three variables were likely to be a consequence of OJD infection and were management responses to higher flock infection rates:

- Culling of low body weight sheep as a method to control OJD
- The number of lamb drops vaccinated with Gudair as a method to control OJD
- Sale of high loss mobs as an OJD control method

Eight variables were related to property features and management:

- Severe drought conditions over a sheep lifetime (higher OJD prevalence).

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- Receipt of run-off water along >10% of the property boundary (lower OJD prevalence). Additional water may supplement water sources on the property, may provide a source of clean water and may promote pasture growth.
- Implementation of a worm control program assessed by interviewer as likely to be effective (lower OJD prevalence). Effective worm control is likely to reflect better general health management. Spelling paddocks could lower MAP contamination.
- Presence of a creek that flows intermittently on the study property (higher OJD prevalence). This may reflect a lower water supply, poorer pasture growth and sheep drinking from stagnant pools contaminated by sheep faeces.
- The presence of wildlife other than kangaroos and rabbits on the study property (lower OJD prevalence). The reasons for this are unclear.
- A history of applying fertilizers other than single super, molybdenum super or lime (for example bio-soil) (lower OJD prevalence). This appeared to be very important as it was identified in 7 models ($P \leq 0.001$ in 5).
- A history of applying single or molybdenum super fertilizer on the property more than once per 3 years (higher OJD prevalence).
- A history of applying lime on the property (lower OJD prevalence). Lime is usually applied to acidic soils and may reduce the availability of iron to MAP organisms and subsequently the level of environmental contamination. However, there would also be changes in pasture composition and abundance.

Eight variables related to flock management:

- Movement of sheep along roads shared by neighbours (higher OJD prevalence). This could have exposed sheep to areas with higher MAP contamination.
- Sheep cohorts born in autumn or winter (lower OJD prevalence) than those born in spring. This could reflect the importance of pasture conditions at weaning rather than during lambing.
- Decontamination of the weaner paddock. There was higher OJD prevalence when the paddock was rested for ≥ 8 weeks and lower OJD prevalence when the paddock was rested for < 8 weeks. This requires further analysis of possible outlier effects.
- The total period of growth retardation over the lifetime of sheep (or weight loss as adults) of ≥ 12 weeks (higher OJD prevalence).
- Stocking rates ≥ 8 dse/ha in weaning paddock/s (higher OJD prevalence).
- Sheep with condition score ≥ 3 at weaning (lower OJD prevalence).
- Sheep with condition score ≥ 3 at 1 year old (lower OJD prevalence).
- Sheep weaned at > 15 weeks of age (lower OJD prevalence).

Four of these flock management variables related to the weaner stage. Nutritional stress and higher stocking rates could have exposed sheep to higher MAP levels through grazing short pasture, led to consumption of more contaminated soil and accelerated disease progression by impeding immune function.

Twelve variables related to soil characteristics. Higher OJD prevalence was linked to an increase in cation exchange capacity (CEC), phosphorus buffer index and phosphorus level, soils having higher proportions of silt and clay and lower proportions of sand. This suggests a detrimental affect of soil fertility on OJD level as CEC, phosphorus and phosphorus buffer index are indicators of fertility. CEC is enhanced by organic matter and therefore is considered as an indirect indicator of organic matter in soil. The CEC is dependent on the proportion of clay in the soil and increases as % clay increases. Clay particles are negatively charged and are known to bind *M. paratuberculosis*. This could increase the availability of

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the organism to sheep. In sandy soils the organism may be leached to deeper soil layers and not be available to sheep. Further studies are required to fully elucidate the relationship between higher fertility of soil with the increased OJD in the cohorts.

The factors identified in this study provide insight into some of the factors that interact to modulate the prevalence of OJD in sheep flocks. The findings support those of MLA trials OJD.028 and OJD.023 and suggest that pasture and flock management strategies can be devised to reduce the impact of OJD. This will have immediate impact for the industry by providing alternative and complementary strategies to vaccination for the control of OJD.

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1 Introduction

1.1 Background

The level of clinical disease and mortality rates experienced due to ovine Johne's disease (OJD) appear to vary considerably between infected sheep flocks in Australia, even for flocks in the same locality and which otherwise appear to have similar characteristics. A study in 2002 found OJD mortality ranged from 2.1% to 17.5% on 12 infected flocks located in the southern tablelands of New South Wales (NSW) (MLA OJD.023) (Bush, 2004). Some inter-flock variation is related to differences in the features of the disease epidemic between flocks such as time since infection was introduced to the flock and past history of sheep purchases such as number and source of introductions. However, there appear to be other factors (as yet unidentified or proposed but requiring further investigation) that are capable of affecting the clinical expression of disease on farm. In order to improve understanding of this apparent variation research conducted to elucidate the influence of some proposed factors continues. However, in addition to this scientific work, the inter-flock variation has resulted in considerable speculation by some producers as to the potential importance of several specific factors, such as soil type, pH and micro-nutrients. Sound understanding about factors that strongly influence clinical disease expression and can be manipulated by sheep producers will lead to management recommendations that improve on-farm disease control. Efforts to identify and investigate such factors are driven by the need to enhance producer ability to minimise the impact of OJD in infected flocks, shown by Bush (2004) to result in considerable biological and economic losses in some flocks.

Recommended management practices for disease control based on knowledge about factors related to disease transmission and progression now exist for bovine Johne's disease (BJD). Research undertaken in the Netherlands, United Kingdom and United States identified risk factors for BJD in dairy herds such as cleanliness of calving area/pen, removal of calf after birth, exposure of calves to adult faeces, method of calf feeding and spreading of faeces on pasture (Cetinkaya et al., 1997; Daniels et al., 2002; Johnson-Ifeorulundu and Kaneene, 1998; Muskens et al., 2003; Obasanjo et al., 1997). Dairy producers with infected herds are therefore advised to implement management practices such as calving cows in clean calving areas/pens and removal of calves immediately after birth to reduce herd prevalence. Similar scientifically based management recommendations known to influence disease prevalence are required to assist producers control flock OJD prevalence in Australia.

In comparison, investigation of risk factors for OJD is less advanced. Numerous risk factors are proposed in the literature but research has been limited to a small number of studies in Spain and Australia. Mainar-Jaime and Vazquez-Boland (1998) found practices related to intensive management (such as herd size, foreign breeds, high replacement rate and farmer membership in a professional livestock association) were associated with sheep and goat seroprevalence in the Madrid region. Another Spanish study specifically to investigate soil type (classified by municipality/district) found low pH soils and large herd size were associated with positive sheep and goat herds (Reviriego et al., 2000). The one similar cross-sectional study conducted to date in Australia, a postal survey of affected producers in the central and southern tablelands of NSW undertaken in 2000, sought to investigate the relationship between a range of potential risk factors and clinical OJD (Lugton, 2004). It reported associations with a number of factors (for example, time since flock infection, altitude, breed, management of ill-thrifty and clinical sheep, culling practice, proportion of quality pasture and soil texture), however, these findings were not conclusive due to

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limitations of the study design. An opportunistic investigation of risk factors for 2002 quarterly OJD mortality rate on 12 infected NSW farms (MLA OJD.023) found associations with flock size, proportion of improved pasture, stocking rate and lamb weaning age but interpretation was constrained by the small sample size and lack of a control group for comparison (Toribio et al., 2004). In addition, in MLA project OJD.028, which was an investigation of the impact of potential risk factors including age at first exposure and level of exposure, high levels of pasture contamination and exposure of young sheep were key drivers of OJD expression (Whittington and McGregor, 2005 unpublished).

It is evident that work to date on OJD has produced limited evidence for risk factors and therefore few management recommendations to improve on-farm disease control for Australian sheep producers. Although vaccination appears to provide very effective control, it is relatively expensive and there may be circumstances where control without vaccination would be desirable. Thus a need continues to exist to identify risk factors for expression of disease that provide the opportunity for improved on-farm control of OJD in the absence of vaccination, or as an adjunct to vaccination on some properties. Further identification of important risk factors for OJD expression could also help clarify its ecological niche, the potential for disease spread into areas not currently affected and the likely level of disease that would be experienced in these areas.

1.2 Purpose

The purpose of this project was to identify risk factors for OJD expression in infected flocks that can be manipulated by sheep producers to provide improved on-farm disease control or to support risk-based trading. On completion the identified risk factors may support the development of additional recommendations for on-farm control measures for OJD as an alternative to or an adjunct to vaccination.

1.3 Working hypotheses and assumptions

The working hypotheses are that:

- a range of farm- and cohort-level factors can affect the expression of OJD in infected flocks
- some of these factors could be manipulated to provide improved disease control on some farms, either as an alternative to or in addition to vaccination.

It is also assumed that the prevalence of faecal-shedding (estimated from pooled faecal culture) and the OJD mortality rate in a flock are highly correlated. This assumption is necessary because our primary outcome of interest, losses due to OJD, cannot be objectively measured, and therefore prevalence of faecal-shedding has been used as a surrogate variable.

1.4 Study approach

This project consisted of a cross-sectional study in which information about each study flock was collected during 2 farm visits. The information obtained included the OJD prevalence of a specific age cohort of sheep (based on pooled faecal culture), details of farm and cohort management over the lifetime of cohort sheep, and soil analysis results for three paddocks on which cohort sheep grazed as lambs, weaners and hoggets/adults.

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A cross-sectional study by design can identify associations between potential explanatory factors and a disease outcome but cannot prove causation due particularly to its inability to identify a temporal relationship between factor and outcome. This weakness of cross-sectional studies is accentuated in studies of Johne's disease (JD) by the long time period between infection and clinical disease. However the same disease feature makes cross-sectional studies a time- and cost-effective approach to JD investigation and a number of cross-sectional studies have been conducted in several countries. Most have identified some factor associations but the findings of several are questionable due to reliance on producer reporting of outcome and explanatory factors and/or to small sample size impeding the power of statistical analyses.

Limitations in terms of the time (given the increasing use of Gudair® vaccine) and budget available to study risk factors for disease progression in unvaccinated infected flocks necessitated the conduct of a cross-sectional study in this project. Design features deliberately implemented to maximise the strength of this cross-sectional study include:

- Objective measurement of infection-level based on pooled faecal culture
- Focus on a specified age cohort of sheep on each property known to represent sheep with the highest OJD losses in infected flocks
- Collection of management information from birth to faecal collection on this sheep cohort
- Investigation of explanatory factors with credible linkage to OJD infection or progression based on previous study findings and consultation with experts
- Completion of the study questionnaire via face-to-face interviews with producers conducted by two trained investigators
- Collection of soil samples for analysis from paddocks grazed by cohort sheep during specified life stages
- Collection of information about potential confounders from producers and from official records (e.g. duration of infection)
- Inclusion of identified confounders in multivariable analyses to minimise confounding bias (e.g. duration of infection, OJD mortality rate, cohort age, cohort sex)
- Inclusion of flock as a random effect in some multivariable analyses to minimise effect of disease clustering within flock.

At study onset, despite extensive efforts during design and conduct to minimise bias and enhance study validity, several risks to study success were identified. First, the possibility of insufficient eligible flocks available for enrolment as a result of vaccination uptake and premature culling because of the drought. Second, the possibility that associations with some risk factors (particularly any that are relatively uncommon or that have only a moderate effect) may not be detected because of the relatively small sample size. This risk could have been reduced by further increasing sample size but budgetary and logistic considerations made enrolment of a larger sample size unrealistic. Third, the possibility that confounding due to vaccination in heavily infected flocks (making them ineligible for inclusion in the study) or due to the duration of flock infection (which was difficult to determine) would significantly bias the results. This project was authorised by Meat and Livestock Australia to proceed to completion after deliberation regarding these risks and the inherent weaknesses of cross-sectional study design.

2 Project Objectives

- To survey 100 producers with known OJD-infected sheep flocks.
- To classify flocks as high or low prevalence on the basis of PFC testing results, and collect information on potential risk-factors for OJD.
- To identify using univariate and multivariate analyses factors with a statistically significant relationship with PFC prevalence, and quantify the magnitude of any relationships.
- To identify important potential confounding factors such as time since infection, purchasing history, vaccination history and culling practices, and take these into account in flock selection, and data collection and analysis.
- To identify risk factors for the level of faecal shedding in OJD-infected flocks.

3 Overview of Methods

(For detailed methods please refer to Appendix 1)

The project was undertaken using a cross-sectional study design in which a questionnaire was administered by face-to-face interview to sheep producers. The reference population for this study was OJD-infected sheep flocks in Australia. The study population consisted of OJD-infected sheep flocks that met specific selection criteria. A target sample size of 100 flocks and a minimum sample size of 80 flocks were set for this study.

OJD prevalence/severity was estimated using pooled faecal culture (PFC). It was planned to collect 7 faecal pools of 30 sheep (to make cohort size of 210) from each flock. Faecal pools (1 pellet *per rectum* per sheep) were collected from the sheep cohort by systemic random sampling during one property visit. All pools were preferentially selected from one sex and one age group. However, when 210 sheep of one sex or another age group were not available, the remainder of the pools were collected from the other sex and/or age group, as necessary. Each pooled faecal sample was cultured using a modified BACTEC radiometric culture method (Whittington et al., 2000a). The growth of *M. paratuberculosis* was confirmed using a PCR test to identify the presence of IS900 in positive cultures (Whittington et al., 1998) and a restriction endonuclease analysis (REA) to confirm IS900 (Cousins et al., 1999). In the case of pools which exhibited growth in BACTEC medium but were PCR negative, DNA was purified (Wizard PCR preparations, Promega) and PCR was re-performed. In addition, smears were prepared from BACTEC culture and stained by Gram's stain to check for the presence and level of contaminating microorganisms.

Subsequently, at the time of the producer interview, 3 soil samples were collected from paddocks grazed by the cohort sheep. The soil samples were submitted to the Incitec Pivot Werribee laboratory for standard soil analysis. A list of the analyses reported by soil laboratory is shown in Appendix 5.1. An additional particle size analysis (PSA) to determine proportion of sand, silt and clay in the soil was performed by the University of Sydney Soil Physics Laboratory. The proportion of fine sand, coarse sand, silt and clay was used to determine the soil texture category by using the international soil texture triangle (Leeper and Uren, 1993) (Appendix 5.4) employing the TAL software (ver. 4.2 ©1996-2002) available on line at <http://agri.upm.edu.my/~chris/tal/>.

A relational database was custom built in Microsoft Access 2000 (© Microsoft Corporation) for entry and management of the study data. All the data tables from this database were imported into SAS (SAS release 8.02, © 1999-2001 by SAS Institute Inc., Cary, NC, USA) and this statistical software was used for all further analyses unless indicated otherwise. PFC pool results for each sheep cohort were used to calculate the individual animal OJD prevalence employing the variable pool size method of Williams and Moffitt (2001) using the Pooled Prevalence Calculator (PPC) (Sergeant, 2004) available online at <http://www.ausvet.com.au/pprev>. The resulting cohort OJD animal-level prevalence was categorised to designate each sheep cohort as either a low, medium or high prevalence cohort. This outcome variable was used in univariable and multivariable analyses to achieve Project Objective 3 – to identify factors statistically associated with cohort PFC prevalence and quantify the magnitude of these associations. Two different sets of cut-off figures were designated creating two prevalence category outcome variables (labelled as IPREV and IPREV25). For the first, IPREV, the three cohort prevalence categories were low (<2% prevalence), medium (2-10% prevalence) and high (>10% prevalence). The second, IPREV25, had the same low infection prevalence category (<2% prevalence) but the medium

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and high prevalence categories were those with prevalence 2-5% and >5%, respectively. The PFC result for each faecal pool cultured in this study was also used to create a binary outcome variable representing the OJD status of each pool and labelled MPTB. This outcome variable was analysed as an extension of Project Objective 3 increasing the statistical power to identify factors associated with pool OJD status. Faecal shedding of *M. paratuberculosis* for each faecal pool cultured, calculated by employing the method of Reddacliff et al. (2003), created a continuous outcome variable, the log of the number of *M. paratuberculosis* shed per pool, labelled LOGMAP. This outcome variable was used in analyses to achieve Project Objective 5 - to identify factors associated with the level of MAP faecal shedding in OJD-infected flocks.

The explanatory variables related to history and management investigated in this study including proposed risk factors and confounding factors are listed in Appendix 2. The explanatory variables related to soil sample analyses investigated in this study were the proposed risk factors listed in Appendix 6 Descriptive analyses were conducted using all available data on the outcome and explanatory variables.

In this study, a sheep cohort was defined as group of sheep of the same sex and age group (or year of drop) selected for faecal sample collection from a flock. Due to variation in the number of sheep cohorts between enrolled flocks (due to insufficient numbers of same age and sex in some flocks) three datasets were created to represent different levels of consistency and reliability in the cohort data. The first dataset (labelled FIRST dataset) comprised only sheep cohorts where 7 pools were collected from the same sex and age group. The second dataset (labelled SECOND dataset) comprised all cohorts in the first dataset as well as sheep cohorts where ≥ 4 pools were collected from sheep of the same sex and age group. The third dataset (labelled THIRD dataset) was similar to the second dataset except for flocks where a new combined sheep cohort was created by merging two cohorts with < 7 pools of the same age group but different sex to produce one cohort of the same age but mixed sex. In addition 5-year old sheep cohorts (drop year 1999) were included in this dataset. Another dataset was created with each faecal pool collected in the study (except those from 5-year old sheep) represented once and used to search for factors significant for pool OJD status and pool MAP number shed.

Univariable analyses were performed to investigate the association between each outcome variable and each explanatory variable (including the 71 history and management variables and the 44 soil variables) on an individual basis. Separate univariable analyses using the logistic regression SAS LOGISTIC procedure (Stokes et al., 2000) were conducted for each of the three datasets with cohort OJD prevalence category as the outcome of interest – one set of analyses for IPREV and one for IPREV25. Similarly, univariate analysis was conducted for pool OJD status. In contrast, univariable analyses for pool rate of faecal shedding were performed using linear regression employing the SAS GLM procedure (Armitage et al., 2002). Explanatory variables identified in the univariable analyses for each outcome as unconditionally associated with the outcome variable at $P < 0.25$ were then examined for collinearity and the most appropriate variable (based on our opinion of biological plausibility) was subsequently deleted. All remaining explanatory variables were selected for inclusion in the relevant multivariable model.

Separate ordinal logistic regression models for cohort OJD prevalence were constructed for each of the three datasets using the SAS LOGISTIC procedure (Stokes et al., 2000) and following the same procedure – one set of models for IPREV and one for IPREV25. Three variables were forced into each model as fixed effects – cohort age, cohort sex and current

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mortality. We used a manual stepwise procedure during the construction of ordinal models. Forward variable selection was based on changes in log likelihood (retaining variables with $P < 0.10$), with further individual assessment based on the individual contribution of each selected variable following backward selection as a fixed effect (with removal of variables with $P > 0.10$). Similarly, binary logistic regression models for pool OJD status were constructed using the SAS LOGISTIC procedure (Stokes et al., 2000). Log pool size was also forced into every model in addition to the confounders mentioned above. The procedure followed for model building was the same as described above except for the addition of random effects flock variable using the SAS GLIMMIX procedure to the final model (Anonymous, 2005; Schabenberger, 2005) and then removal of fixed effects with $P > 0.10$ by backward selection. For log pool MAP number shed (LOGMAP), general linear mixed models were constructed employing SAS MIXED procedure (Brown and Prescott, 2000). Similar variables as for pool OJD status were forced into this model, and similarly, random effects were added to the final model, but by using SAS MIXED procedure. First order interaction terms were added to all the final models discussed above and retained when significant at $P < 0.05$ and biologically plausible.

Separate models were constructed for the four sets of soil variables - 3-paddock mean variables (mean of 3 paddock soil parameters), lamb paddock variables, weaner paddock variables and hogget/adult paddock variables. In total 32 models were created to investigate association with the outcome variables cohort OJD prevalence (separate models for IPREV and IPREV25 for each of three datasets), pool OJD status and log pool MAP number shed. These models were constructed following the same procedure outlined above except that parent soil type was also forced into each model as an additional fixed effect.

4 Results

4.1 Study flocks

4.1.1 Enrolment

In total 233 known OJD-infected flocks were investigated to identify eligible flocks that met the revised selection criteria (listed in Appendix 1) and had owner approval for participation in this study. Of these, 97 flocks met the revised criteria and 92 flocks were enrolled in the study by 31 July 2004. Visits to each enrolled flock for faecal sample collection were conducted from 28th April 2004 to 22nd September 2004 (excluding 12 flocks of OJD.033 project for which faecal samples had been collected previously). Producer interviews commenced on 18th August 2004 and were completed by 21st December 2004. Cohort faecal collection was performed prior to the producer interview and the average time between farm visits was 97 days (median 100, range 7 to 180) for the 80 flocks enrolled only in OJD.038 project and 140 days (106, 7 to 621) including the 12 flocks also participating in OJD.033.

4.1.2 General description and management

The 92 study flocks were located in the four states of New South Wales (77 flocks), Victoria (7), Tasmania (6) and Western Australia (2) (Table 4.1). Based on figures for currently infected flocks for NSW, Victoria and Tasmania and total infected flocks for Western Australia published in November 2004 (Citer and Sergeant, 2004), the proportion of infected flocks in each state constituted by the enrolled flocks was 21.9% for NSW, 5.4% for Victoria, 14.6% for Tasmania and 66.7% for Western Australia.

Table 4.1
Location by state and district of the 92 study flocks and 124 sheep cohorts

State / District	Number of study flocks	Number of sheep cohorts
New South Wales	77	104
Central Tablelands	26	33
Goulburn	16	25
Gundagai	7	10
Hume	2	4
Molong	4	7
Yass	10	12
Young	12	13
Tasmania	6	8
Victoria	7	10
Western Australia	2	2
Total	92	124

These 92 flocks were kept on properties with an average area of 1327.9 hectares (median 1031.5, range 81 to 8100) of which on average 95.7% (100%, 40 to 100%) was grazed by sheep and 64.1% (72.5, 0 to 100%) was planted with improved pasture (Table 4.2). Current flock numbers were on average 2397 (1705, 0 to 12324) for ewes and 1073 (600, 0 to 11808) for wethers. The 5-year average production figures for weaning percentage, greasy fleece weight and fibre diameter in adult sheep are shown in Table 4.3. Wool production was the sole enterprise on 19 properties and combined with only either cattle production or cropping on 27 and 8 properties, respectively. For the remaining 38 properties wool was one of three or more enterprises (Table 4.4 and Figure 4.1). Of the 61 properties that kept cattle

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in addition to sheep, the cattle on 9 properties (6 in Tasmania and 3 in NSW) were reported to have known bovine Johne's disease status and all were reported to be negative.

The 92 properties were located at an average altitude of 620.3 metres (median 650, range 20 to 2450) above sea level and had topography described as flat for 5 properties (5.4%), gently undulating for 11 (11.9%), undulating for 46 (50%), undulating hilly for 26 (28.3%) and hilly for 4 (4.3%) properties. A permanent creek flowed through 62 (67.4%) properties and an intermittent creek through 73 (79.3%) properties. Owner/managers reported that on average 36% (30%, 0% to 100%) of the property boundary received run off water from neighbouring properties and land. On average, the proportion of neighbouring properties that ran sheep was 78.4% (95.8%, 0% to 100%) and the proportion of these reported to be OJD-infected and likely to be OJD-infected was 38% (25%, 0% to 100%) and 66.6% (92.8%, 0% to 100%), respectively.

Across the 92 properties during the last 10 years, application was reported for single super on 82 properties, molybdenum super on 42 properties, lime on 60 properties (incorporated on 34 of these) and biosoil on 4 properties (incorporated on 2 of these). The average frequency of fertilizer application on these properties was reported to be 0.63 per year (median 0.66, range 0 to 1) for single super, 0.24 per year (0.20, 0.05 to 1) for molybdenum super, 0.24 per year (0.1, 0 to 1) for lime and 0.15 per year (0.15, 0.1 to 0.2) for biosoil.

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Table 4.2

Descriptive property information for the 92 study flocks

State	District	Number of flocks	Property area			% Area grazed by sheep			% Improved pasture		
			Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
New South Wales		77	1281.4	81	5042	95.4	40.0	100.0	60.3	0.0	100.0
	Central Tablelands	26	1038.1	81	4049	94.5	62.0	100.0	61.8	0.0	100.0
	Goulburn	16	887.1	230	1700	98.6	85.0	100.0	57.4	0.0	100.0
	Gundagai	7	2490.6	253	5042	90.9	40.0	100.0	64.1	4.0	100.0
	Hume	2	1533.0	1532	1534	95.0	90.0	100.0	66.0	52.0	80.0
	Molong	4	876.3	640	1200	100.0	100.0	100.0	63.8	10.0	100.0
	Yass	10	1406.1	685	3000	98.5	90.0	100.0	43.9	0.0	100.0
	Young	12	1618.0	771	3988	91.5	50.0	100.0	70.3	5.0	100.0
Tasmania		6	1119.7	360	1700	93.3	78.0	100.0	93.3	70.0	100.0
Victoria		7	1075.6	340	3150	99.6	97.0	100.0	70.7	0.0	100.0
Western Australia		2	4627.5	1155	8100	100.0	100.0	100.0	100.0	100.0	100.0
Overall		92	1327.9	81	8100	95.7	40.0	100.0	64.1	0.0	100.0

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Table 4.3

Descriptive information about the structure and production of the 92 sheep flocks

State	District	No of flocks	Current ewe number			Current wether number			Average flock weaning %			Average greasy fleece weight			Average fibre diameter		
			Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
New South Wales		77	2252	0	9000	918	0	7280	83.4	60.0	105.0	5.2	3.5	7.5	19.5	17.0	26.0
	Central Tablelands	26	1666	0	6399	719	0	3000	82.5	68.0	100.0	5.1	3.5	7.5	19.4	17.0	21.5
	Goulburn	16	1297	350	4800	270	0	800	82.0	60.0	105.0	5.1	4.0	7.5	19.8	18.0	26.0
	Gundagai	7	4923	1163	9000	2422	0	7280	89.5	80.0	100.0	5.2	4.5	6.0	19.4	18.0	20.5
	Hume	2	2603	1511	3695	1671	1070	2272	89.0	86.0	92.0	5.3	5.1	5.4	19.6	19.0	20.2
	Molong	4	763	100	1200	250	0	1000	87.5	75.0	100.0	5.5	4.5	7.0	20.3	19.7	21.6
	Yass	10	3189	880	6500	1297	0	3585	80.2	69.0	95.0	5.0	4.0	6.0	18.8	18.0	20.0
	Young	12	2894	1140	7430	1118	0	4500	84.3	70.0	95.0	5.4	4.0	6.5	19.0	18.4	21.0
Tasmania		6	2352	1260	3662	842	0	1850	82.8	70.0	97.0	5.0	4.5	6.0	20.0	19.5	20.5
Victoria		7	2593	1000	6000	1590	0	4000	78.0	60.0	85.0	5.3	4.5	6.0	19.9	18.5	21.0
Western Australia		2	7412	2500	12324	5904	0	11808	77.5	76.0	79.0	4.7	3.8	5.5	20.5	20.4	20.5
Overall		92	2397	0	12324	1073	0	11808	82.9	60.0	105.0	5.2	3.5	7.5	19.6	17.0	26.0

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Table 4.4

Enterprises conducted on the properties that ran the 92 study flocks

State	District	No of flocks	Only wool		Wool and Cattle		Wool and Crop		Wool, cattle and crop		Wool, cattle and cross bred		Others	
			No	%	No	%	No	%	No	%	No	%	No	%
New South Wales		77	18	23.4	24	31.2	4	5.2	17	22.1	7	9.1	7	9.1
	Central Tablelands	26	9	34.6	11	42.3	0	0.0	2	7.7	4	15.4	0	0.0
	Goulburn	16	3	18.8	7	43.8	1	6.3	1	6.3	2	12.5	2	12.5
	Gundagai	7	0	0.0	3	42.9	2	28.6	2	28.6	0	0.0	0	0.0
	Hume	2	0	0.0	0	0.0	0	0.0	2	100.0	0	0.0	0	0.0
	Molong	4	0	0.0	1	25.0	0	0.0	2	50.0	0	0.0	1	25.0
	Yass	10	4	40.0	2	20.0	0	0.0	2	20.0	1	10.0	1	10.0
	Young	12	2	16.7	0	0.0	1	8.3	6	50.0	0	0.0	3	25.0
Tasmania		6	0	0.0	3	50.0	0	0.0	0	0.0	1	16.7	2	33.3
Victoria		7	1	14.3	0	0.0	4	57.1	2	28.6	0	0.0	0	0.0
Western Australia		2	0	0.0	0	0.0	0	0.0	1	50.0	0	0.0	1	50.0
Overall		92	19	20.7	27	29.4	8	8.7	20	21.7	8	8.7	10	10.9

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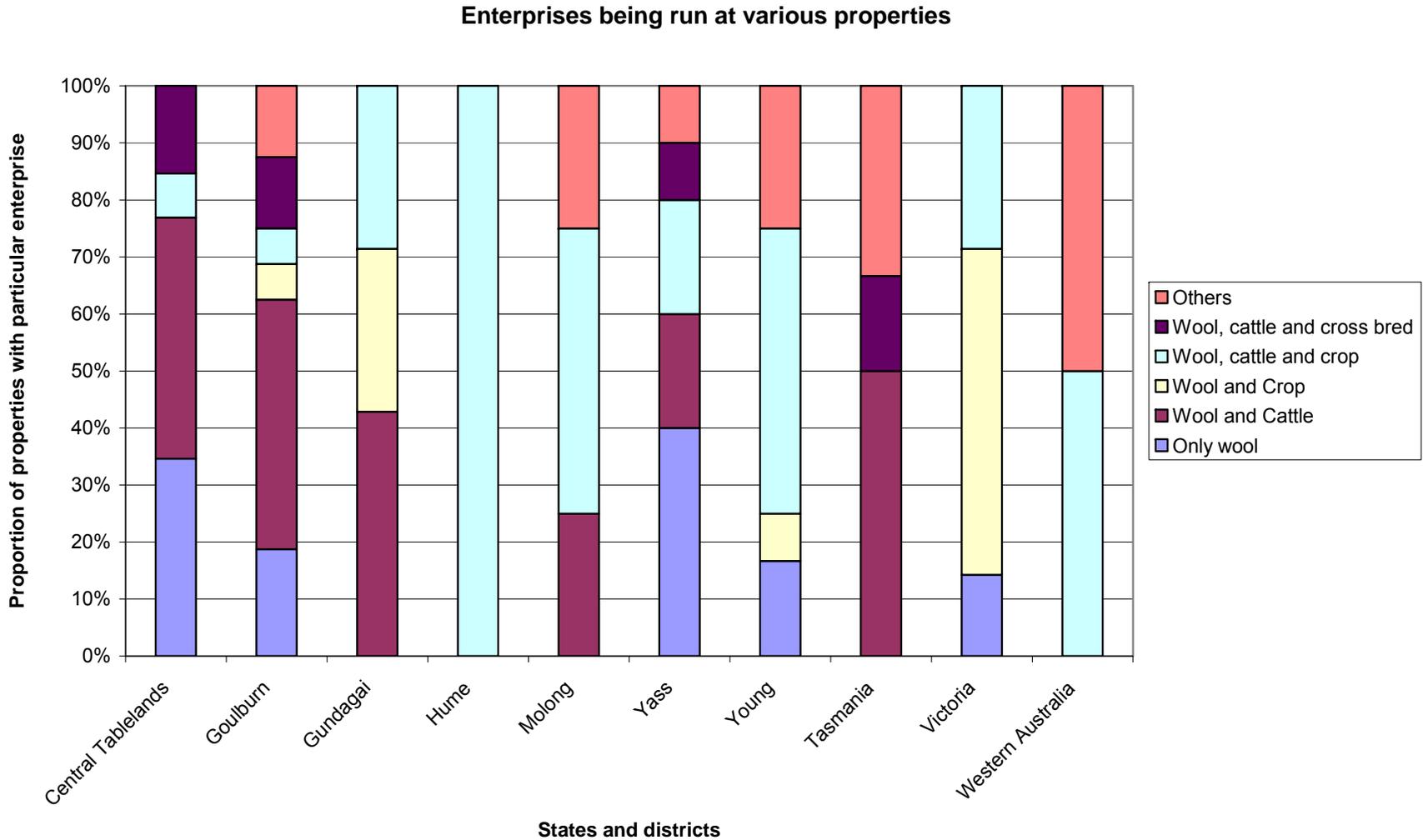


Figure 4.1 Distribution of enterprises conducted on the properties that ran the 92 study flocks by state and district

4.1.3 OJD infection history and control

All study flocks were known to be OJD-infected on the basis of official declaration as either an OJD infected flock (86 flocks) or a suspect flock (6 flocks based on abattoir surveillance for 2, trace back for 1 and observation of clinical sheep for 3). Interviewer estimate of the duration of flock infection was 3 to < 5 years for 11 flocks, 5 to < 7 years for 23 flocks, 7 to < 10 years for 27 flocks, 10 to < 15 years for 19 flocks and ≥ 15 years for 12 flocks. Owners/managers of 73 flocks reported sheep deaths due to OJD and stated that the first observed death occurred on average 4.8 years (median 4, range 0 to 34) ago (Table 4.5). Of 90 flocks officially diagnosed with OJD, flocks were diagnosed as infected on average 4.2 years (4, 0 to 13) ago with the majority (66 flocks) diagnosed by 2001. Diagnosis on the remainder was made by 2002 for 16 flocks, 2003 for 9 flocks and 2004 for 1 flock. Owner reported source of flock OJD infection was a neighbour for 25 (27.1%) flocks, introduction of infected sheep for 24 (26.1%) flocks, both neighbour as well as introduction of infected sheep for 7 (7.61%) flocks, introduction of goats from known infected herd for 1 (1.1%) flock and unknown for 35 (38%) flocks.

Average percentage of adult flock mortality attributed to OJD by the 92 owner/managers for the 12 months prior to interview was reported to be 2.3% (median 0.9%, range 0 to 20%) and for the peak mortality seen in each flock was reported to be 3.0% (1.3%, 0 to 20%) (Table 4.5). Signs of OJD in the 92 flocks reported by the owner/managers were death (73), loss of condition (72) and scouring (55) while managers of 19 flocks reported seeing no signs of the disease.

One or more OJD control procedures were implemented by the owner/managers of 88 study flocks. Vaccination with Gudair® was reported for 79 study flocks with 17 vaccinating lambs since 2001, 37 since 2002, 19 since 2003 and 6 for the first time in 2004. Only 22 managers reported vaccinating sheep as adults. Other control procedures used included sale of high loss mobs (12 study flocks), culling of low body weight sheep (50), destocking of lambing/weaning paddocks (58), handling of young sheep before older sheep (13) and separating young and adult sheep (45). Reported management of clinical OJD sheep included immediate disposal (53 study flocks), isolation from main flock or disposal after isolation (17), and no action (7).

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Table 4.5

Descriptive information about the length and level of infection in the 92 study flocks

State	District	Number of flocks	Number of years since OJD diagnosis			Number of years since 1st mortality			Peak mortality% in ≥ 2 yr old sheep			Current mortality% in ≥ 2 yr old sheep		
			Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
New South Wales		77	4.5	1	13	5.0	0	34	3.0	0.0	20	2.3	0.0	20.0
	Central Tablelands	26	6.2	1	13	8.4	0	34	3.9	0.0	20.0	2.7	0.0	20.0
	Goulburn	16	4.7	2	10	4.1	0	14	3.3	0.0	15.4	2.6	0.0	15.4
	Gundagai	7	3.4	2	6	3.0	0	7	2.4	0.0	6.0	1.4	0.0	6.0
	Hume	2	3.0	2	4	2.0	1	3	4.6	0.2	9.0	2.6	0.2	5.0
	Molong	4	5.0	4	6	5.5	4	8	1.4	0.0	3.0	1.2	0.0	2.5
	Yass	10	2.7	1	5	1.0	0	4	2.0	0.0	15.0	1.5	0.0	15.0
	Young	12	3.0	1	7	3.2	0	14	2.4	0.0	19.2	2.3	0.0	19.2
Tasmania		6	3.6	0	7	4.5	3	7	3.4	0.5	10.7	3.3	0.5	10.7
Victoria		7	2.4	1	4	4.0	0	11	2.3	0.0	8.7	2.3	0.0	8.7
Western Australia		2	1.0	1	1	1.5	0	3	0.4	0.0	0.8	0.4	0.0	0.8
Overall		92	4.2	0	13	4.8	0	34	2.9	0	20	2.3	0	20

4.1.4 General flock management

Introduction of ewes to the study flocks since 1999 was reported for 30 flocks and of rams for 87 flocks. The average number of introductions from 1999 to 2004 was 990 ewes (median 475, range 18 to 3600) and 35 rams (25, 2 to 190) for these flocks. For ewe introductions, the average number of sources was 1.8 (1, 1 to 12) and the average proportion of sources reported as OJD-infected was 19.7%. For ram introductions, the average number of sources was 2.6 (2.0, 1 to 9) and the average proportion of sources reported as OJD-infected was 23.4%.

Sharing of resources (breeding rams, shearing sheds/yards, roads) with neighbours during the last 10 years was reported for rams by 3 flocks, for shearing sheds/yards by 11 and for roads by 34. In these flocks the average frequency of sharing was 0.55 times per year for rams, 1.4 times per year for shearing sheds/yards and 3.5 times per year for roads. A total of 74 study properties were reported to be surrounded by sheep proof fences, however, 61 owner/managers reported straying of sheep between neighbouring properties in the last 10 years (including 43 with sheep proof fences).

Worm control for the study flocks involved drenching adults and lambs in 90 flocks. In addition, faecal egg count tests were used as a component of the control program by 62 owner/managers and drench resistance tests by 51 owner/managers. Based on the worm control program described by each owner/manager, interviewers assessed that the worm control program implemented for 74 flocks was likely to be effective and determined that 61 owner/managers were implementing the recommended control program for their respective district.

Mineral deficiency was reported by owner/managers to affect 57 study flocks. Selenium deficiency (resulting in weaner ill thrift and/or white muscle disease) was reported for 43 flocks and managed by selenium supplementation for 40 of these. Copper deficiency (resulting usually in pigmentation problems) was reported for 8 flocks and managed by providing copper blocks or adding copper to super fertilizer for 3 of these. In addition, magnesium deficiency was reported by 4 producers while cobalt and iodine deficiency was reported by one producer each. Based on owner/manager descriptions interviewers assessed the likelihood of selenium deficiency in the sheep flock to be major for 4 flocks, minor for 17 and nil for 22 flocks. Other mineral deficiencies were assessed to be major for 1 flock, minor for 9 and nil for 4 flocks.

4.1.5 Rainfall 1999-2004

The difference between annual rainfall figures for each property and respective district long-term averages were averaged to provide the state and NSW district figures for mean deviance from long-term average shown in Figures 4.2 and 4.3. The 77 properties in NSW received on average less annual rainfall than the district long-term average during the 4 years (2001 to 2004) that comprise all or most of the cohort lifetime for 3 and 4-year old cohorts, respectively. The mean rainfall deviance per district for all NSW districts was below the long-term average in 2002. The 6 properties in Tasmania received on average less annual rainfall than the district long-term average for 3 consecutive years (2002 to 2004) and the 7 properties in Victoria for the years of 2002 and 2004. In contrast, mean rainfall for the 2 properties in Western Australia was above the district long-term average for 5 year period.

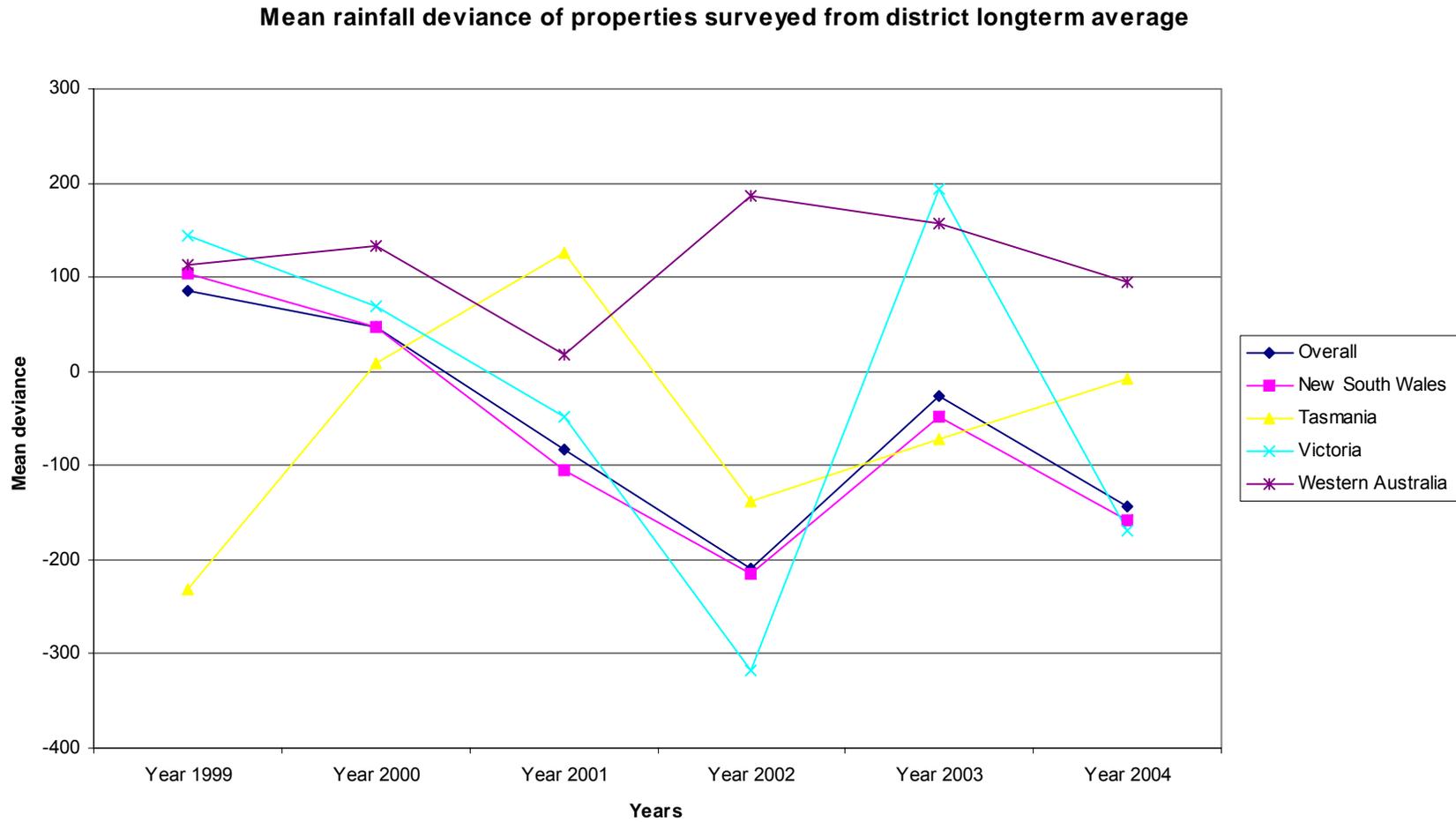


Figure 4.2 Annual mean deviance from district long-term average for 1999 to 2004 for the 92 properties by state

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Mean deviance of rainfall at properties surveyed from the district longterm average

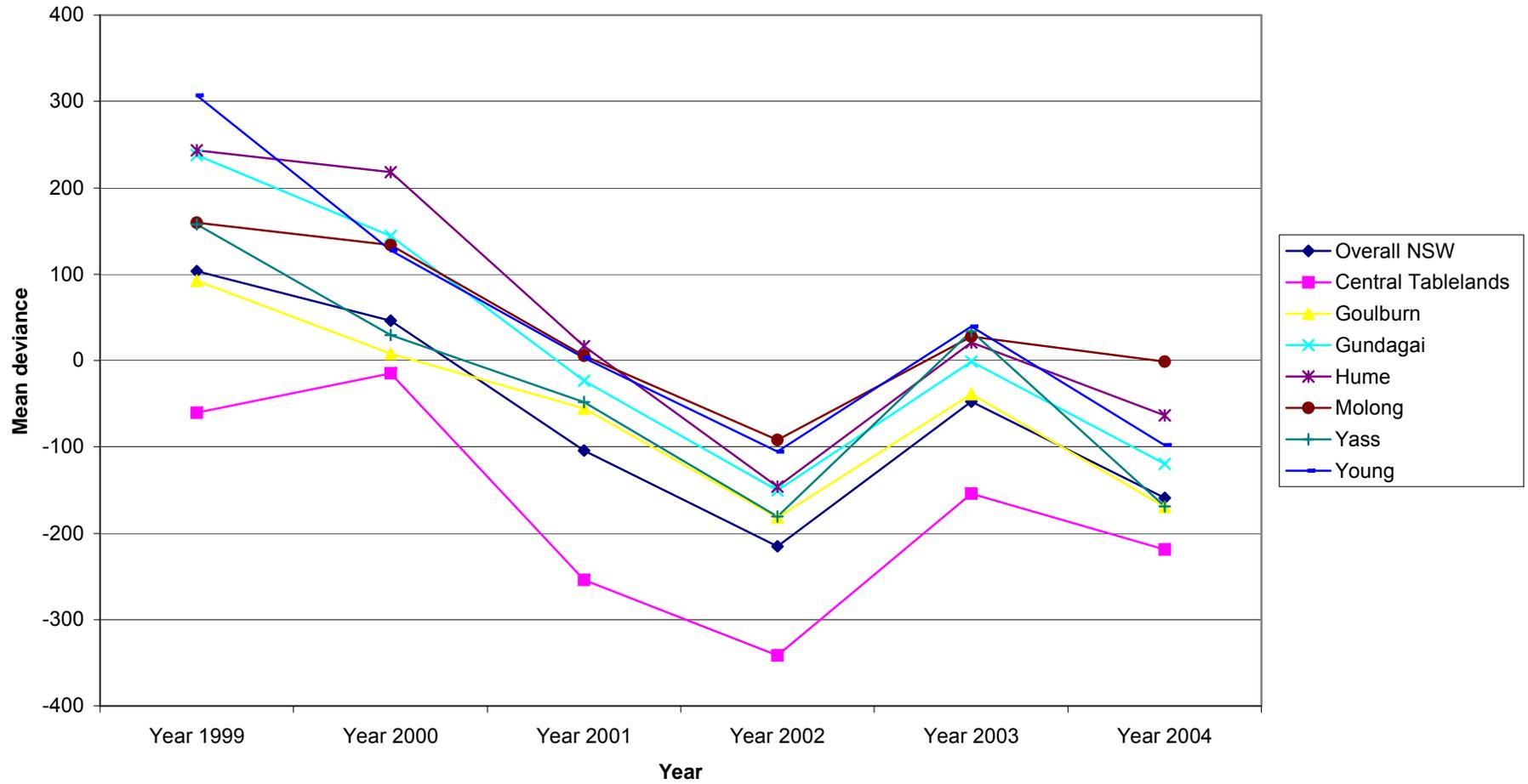


Figure 4.3 Annual mean deviance from district long-term average for 1999 to 2004 for the 77 properties in New South Wales by district

4.2 Sheep cohorts

4.2.1 General description

Faeces and information about 124 sheep cohorts was collected from the 92 study flocks (Table 4.1). Of these cohorts, 66 (53.2%) comprised 3-year old sheep, 46 (37.1%) 4-year old sheep, and 12 (9.7%) 5-year old sheep, and 90 (72.6%) ewes (Table 4.6). Cohort selection in 4 study flocks did not adhere to the sampling protocol and the selected cohorts consisted entirely of 5-year old sheep. For 11 study flocks, both ewe and wether cohorts of the same age (represented by 7 pools of 30 each for 10 and 7 pools of 30 for ewes and 5 pools of 30 for wethers in one flock) were included in this study. At the time of faecal collection the average condition score of sheep within these cohorts was 2.5 (median 2.5, range 1 to 3.5).

Table 4.6
Age and sex distribution of 124 sheep cohorts

Age (years)	Sex	Number	Percentage
3	Ewe	48	38.7
	Wether	18	14.5
4	Ewe	33	26.6
	Wether	13	10.5
5	Ewe	9	7.3
	Wether	3	2.4

4.2.2 Management history

The management history for each sheep cohort was separated into four life stages – lamb (period spent with dams), weaner (from weaning to 12 months old), hogget (from 12 to 24 months old) and adult (> 24 months old). Supplementary feeding was provided to 7 cohorts during lambing, 39 as weaners, 63 as hoggets and 84 as adults. Period of supplementary feeding for these cohorts is summarised in Table 4.7 in addition to information on paddock decontamination, stocking rate, grazing system, water source and supply.

Half of the cohorts (57) were born in spring with 33 (28.9%) cohorts born in autumn and 24 (21.1%) in winter. The duration of lambing for these cohorts was on average 6.35 weeks (median 6, range 4 to 12). The average age at marking was 9.0 weeks old (8.6, 2.8 to 28.1) and husbandry procedures performed included vaccination for all cohorts (69 for clostridial diseases, 84 for caseous lymphadenitis (CLA) and 48 for scabby mouth), selenium supplementation for 50 and mulesing for 97 in addition to standard procedures of tail docking and castration. For 24 cohorts mulesing was performed later at an average age of 31.5 weeks old. Weaning was conducted at an average age of 19.1 weeks old (18, 9.8 to 40.1).

The sheep cohorts were mixed with the adult flock at an average age of 22.1 months old (median 24.3, range 5.5 to 38.7). For the 90 ewe cohorts, first lambing occurred at an average age of 22.1 months old (19.3, 15.7 to 36.8) and the average weaning percentage achieved by these ewes was 69.8% (70%, 40% to 102%).

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Table 4.7

Management of 124 study cohorts during the four life stages - lamb (with dams), weaner (weaning to 12 months), hogget (from 12 to 24 months) and adult (> 24 months)

Management practice	Lamb ^a			Weaner			Hogget			Adult		
	Number (%) of cohorts			Number (%) of cohorts			Number (%) of cohorts			Number (%) of cohorts		
Paddock decontamination												
Yes	49 (43.0)			63 (55.3)			14 (12.4)			14 (12.5)		
No	65 (57.0)			51 (44.7)			99 (87.6)			98 (87.5)		
Grazing system												
Set	105 (92.1)			47 (41.2)			49 (43.4)			50 (44.6)		
Rotational	9 (7.9)			67 (58.8)			64 (56.6)			62 (55.4)		
Water source												
Bore	7 (6.1)			6 (5.3)			5 (4.4)			5 (4.5)		
Dam	53 (46.5)			55 (48.2)			50 (44.3)			50 (44.6)		
Creek	4(3.5)			7 (6.1)			7 (6.2)			7 (6.3)		
Combination	50 (43.9)			46 (40.4)			51 (45.1)			50 (44.6)		
Water supply												
Trough	9 (7.9)			9 (7.9)			7 (6.2)			7 (6.2)		
Ground	91 (79.8)			90 (78.9)			91 (80.5)			88 (78.6)		
Combination	14 (12.3)			15 (13.2)			15 (13.2)			17 (15.2)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Stocking rate (dse/ha)	17	3.7	51.4	12.1	2	71.6	9.0	1.5	50	9.6	2.16	37.5
Period of supplementary feeding (weeks)	7.1	0.6	19.4	15.1	1.4	43.8	18.9	2	52.1	32.8	2.3	108.1

a Management implemented for dams of the cohort sheep and the cohort sheep as suckling lambs. Figures for stocking rate and period of supplementary feeding calculated based on information provided about the dams.

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Table 4.8

Disease history of 124 study cohorts during the four life stages - lamb (with dams), weaner (weaning to 12 months), hogget (from 12 to 24 months) and adult (> 24 months)

Disease/health observation	Lamb ^a			Weaner			Hogget			Adult		
	Number (%) of cohorts			Number (%) of cohorts			Number (%) of cohorts			Number (%) of cohorts		
High-level worm burden												
Yes	7(6.1)			25 (21.9)			6 (5.3)			6 (5.3)		
No	107(93.9)			89 (79.1)			107 (94.7)			107 (94.7)		
Scouring												
Yes	18 (15.8)			16 (14.0)			8 (7.1)			8 (7.1)		
No	96 (84.2)			98 (86.0)			105 (92.9)			105 (92.9)		
Other health problems												
Yes	9 (7.9)			13 (11.4)			8 (7.1)			14 (12.4)		
No	105 (92.1)			101 (88.6)			105 (92.9)			99 (87.6)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Condition score at start	3.2	2	4	2.9	2	4	3.0	1.5	4	3.0	1.75	4
Period of growth check (weeks) ^b	0.4	0	8	3.7	0	44	6.7	0	52	9.16	0	65

a Disease observations reported for the dams of the cohort sheep prior to lambing and while the cohort sheep were suckling lambs.

b Growth check for the lamb life stage and the adult life stage referred to loss of body condition in cohort dams and in adult cohort sheep, respectively.

4.2.3 Disease history

Cohort disease history as reported by the owner/managers was recorded for each of the four life stages. Information on reported evidence of high-level worm burden, scouring and other health problems and on average condition score at the start of each life stage and average period of growth cessation (or loss of body condition) during each life stage is summarised in Table 4.8.

4.2.4 Pooled faecal samples

A total of 635 faecal pools were collected from cohort sheep in 80 flocks specifically for this project. A further 82 faecal pools were collected from cohort sheep in 12 flocks for MLA OJD.033. For the remainder of this report the pools collected separately for each project will be considered collectively and results reported for 717 samples.

Table 4.9 presents the age and sex distribution of the 717 faecal pools. Of these pools, 409 (57.0%) from 3-year old sheep, 264 (36.8%) pools were collected from 4-year old sheep and 44 (6.1%) from 5-year old sheep. Faecal samples from ewes comprised 538 (75.0%) of pools.

Most pools (698/717) were of uniform pool size with 619 pools of 30 sheep each collected for this project and 79 pools of 50 sheep each collected for MLA OJD.033. The remaining 19 pools were of variable size including 6 pools made up of pellets from < 20 sheep.

The pooled faecal samples for 74 (59.7%) sheep cohorts (including 2 cohorts of 5-year old sheep) consisted of 7 pools of uniform pool size (67 cohorts with pools of 30 collected for this project and 7 cohorts of 50 pools collected for MLA OJD.033). The remaining 50 cohorts consisted either of 7 pools including one or more pools of variable size or of <7 pools all of uniform size or with one or more pools of variable size. Overall, without considering pool size, 80 cohorts were made up of 7 pools and 44 of <7 pools. After excluding 5 yr old sheep cohorts, 77 cohorts of 3 and 4-year old sheep contained 7 pools. Table 4.10 categories the 124 sheep cohorts by the number of constituent pools and by age and sex and indicates the cohorts that comprise the FIRST, SECOND and THIRD datasets used to investigate the association between cohort OJD prevalence and explanatory variables.

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Table 4.9
Age and sex distribution of 717 faecal pools by pool size

Project	Pool size	Age	Number of pools		
			Ewes	Wethers	Total
MLA.038	30	3	266	98	364
		4	153	63	216
		5	35	4	39
	Total				619
	<30	3	11	0	11
		4	2	1	3
		5	2	0	2
	Total				16
	Total MLA.038				635
	MLA.033	50	3	30	2
4			37	7	44
5			0	3	3
Total				79	
<50		3	1	1	2
		4	1	0	1
		5	0	0	0
Total				3	
Total MLA.033				82	
Grand total				717	

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Table 4.10

The 124 sheep cohorts grouped by number of constituent pools, age and sex that comprise the FIRST, SECOND and THIRD datasets

Number of constituent pools	Age	Ewe	Wether	Total	Cumulative total	
7 pools	4	20	7	27	27	FIRST Dataset
	3	38	12	50	77	
6 pools	4	3		3	80	SECOND dataset
	3	2		2	82	
5 pools	4	3	2	5	87	THIRD dataset
	3	3	1	4	91	
4 pools	4	3	1	4	95	
	3	2	1	3	98	
≤ 3 pools	4	4	3	7	105	
	3	3	4	7	112	
All pools	5	9	3	12	124	
Total		90	34	124	124	

4.3 Soil samples

A total of 276 soil samples, one sample collected from each of three paddocks on every study property, were submitted for laboratory analysis. All except two yellow brown samples were colour classified as brown. Manual assessment of soil texture classified 233 samples as silty loam, 33 samples as sandy loam or loam sand, 7 samples as clay loam and 3 samples as light clay. In comparison, classification for soil texture based on particle size analysis was clay for 1 sample, silty clay for 2 samples, clay loam for 10 samples, silty clay loam for 11 samples, loam for 112 samples, silty loam for 76 samples, sandy loam for 6 samples, sand for 4 samples and loamy sand for 46 samples. Descriptive results for other soil parameters are presented in Table 4.11.

In addition the parent soil type present on each property was basaltic for 8 properties, granite for 28 properties, shale and sandstone for 30 properties, mixed including limestone for 16 properties and mixed without limestone for 10 properties.

Table 4.11
Descriptive information for soil parameters measured in 276 soil samples taken from 3 paddocks on each of the 92 study properties^a

	Minimum	Mean	Median	Maximum
pH (water)	4.50	5.56	5.50	7.90
pH (CaCl ₂)	3.70	4.80	4.60	7.50
Organic carbon%	0.89	2.50	2.30	7.70
Cation exchange capacity	1.95	7.20	5.82	35.1
Phosphorus buffer index	4.86	69.66	57.00	650.0
Sand %	30.37	61.43	62.70	91.91
Silt %	2.67	22.38	21.52	41.44
Clay %	4.05	16.18	14.85	48.74
Nitrate Nitrogen	1	13.86	9.85	76
Sulphate Sulphur	1.4	7.88	6.50	96.0
Phosphorus	6.80	31.30	26.0	200.0
Potassium	0.10	0.58	0.52	2.3
Calcium	0.55	4.66	3.80	29.0
Magnesium	0.29	1.42	0.91	15.0
Aluminium	0.034	0.34	0.22	2.0
Sodium	0.20	0.60	0.44	2.30
Chloride	10.0	36.77	20.0	1100.0
Copper	0.013	0.99	0.51	11.0
Manganese	0.33	36.23	30.0	150.0
Iron	35.0	195.15	190.0	470.0

a Minimum detection limits for specific analyses reported by the Incitec Pivot laboratory were: Nitrate Nitrogen =1, Magnesium=0.2, Aluminium =0.03, Sodium =0.2 and Chloride =10. These factors were assumed to be missing in samples in which these could not be detected due to being lower than the detection limit.

4.4 Outcome variables

4.4.1 Animal-level OJD prevalence for sheep cohorts

Average OJD prevalence based on PFC among the 124 sheep cohorts was 15.3% (median 4.1%, range 0 to 58.9%). The OJD prevalence by age and sex are shown in Table 4.12. The difference in prevalence between age cohorts was not significant ($P=0.52$), however, that between ewe and wether cohorts was significant ($P=0.01$).

Table 4.12
OJD prevalence (%) based on PFC by age and sex for the 124 sheep cohorts

Age (years)	Sex	Minimum	Mean	Median	Maximum
3	Ewe	0	11.05	4.09	58.93
	Wether	0	32.11	53.47	55.03
4	Ewe	0	12.01	3.63	55.03
	Wether	0	13.28	2.78	55.03
5	Ewe	0	19.52	3.60	55.03
	Wether	0	13.46	2.28	38.09

- a Age group means for sheep cohorts of 16.8% for 3 year olds, 12.4% for 4-year olds and 18.0% for 5-year olds.
 b Sex group means for sheep cohorts of 12.3% for ewes and 23.3% for wethers.

The proportion of sheep cohorts categorised as low, medium and high prevalence for each of the cohort prevalence outcome variables (IPREV and IPREV25) is shown in Table 4.13. The difference in number of sheep cohorts per category was nonsignificant for both outcome variables ($P=0.99$ and $P=0.99$, respectively).

Table 4.13
Number of low, medium and high prevalence cohorts based on PFC among 124 sheep cohorts

Outcome variable	Number of cohorts		
	Low	Medium	High
IPREV	34	60	30
IPREV25	34	34	56

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4.4.2 Pool OJD status

Of the 717 pools, 478 (66.7%) were found to be OJD positive and 239 (33.3%) OJD negative based on PFC. Pool OJD status by age and sex are shown in Table 4.14. The difference in proportion of pools between ewe and wether cohorts was significant ($P=0.0001$) but nonsignificant between age cohorts ($P=0.10$).

Table 4.14
Pool OJD status based on PFC by age and sex for 717 pools

Age (years)	Sex	OJD positive		OJD negative	
		Number	Percentage	Number	Percentage
3	Ewe	197	64	111	36
	Wether	89	88.1	12	11.9
4	Ewe	121	62.7	72	37.3
	Wether	44	62	27	38
5	Ewe	23	62.2	14	37.8
	Wether	4	57.1	3	42.9

- a Number (%) of positive faecal pools per age group - 286(69.9%) for 3 year olds, 165 (62.5%) for 4-year olds and 27 (61.4%) for 5-year olds.
b Number (%) of faecal pools per sex group - 341 (63.4%) for ewes and 136 (76.0%) for wethers.

4.4.3 Pool MAP number shed

The mean number of *M. paratuberculosis* excreted per gram of faeces for the 717 faecal pools was 15867.9 (median 166.0, range 0 to 1273503). MAP numbers shed per gram of faeces for the 717 pools by age and sex is shown in Table 4.15. The difference in log MAP number between ewe and wether cohorts was significant ($P = 0.0003$) whereas among age cohorts was nonsignificant ($P = 0.43$).

Table 4.15
Number of MAP shed per gram of faeces based on PFC by age and sex for 717 faecal pools

Age (years)	Sex	Minimum	Mean	Median	Maximum
3	Ewe	0	11713.3	58.5	831763.8
	Wether	0	32834.8	2138.0	831763.8
4	Ewe	0	15127.3	165.9	1273503.1
	Wether	0	11989.7	70.8	231739.5
5	Ewe	0	18038.4	595.7	151356.1
	Wether	0	2149.8	12.9	5011.9

- a Age group means for faecal pools of 16929.1 for 3 year olds, 14283.5 for 4-year olds and 15510.6 for 5-year olds.
b Sex group means for faecal pools of 13373.0 for ewes and 23366.7 for wethers.

4.5 Explanatory variables

Appendix 2 and Appendix 6 present descriptive statistics for the 71 explanatory variables related to history and management and for the 44 explanatory variables related to soil investigated in this study, respectively.

4.6 Association between cohort OJD prevalence and the history and management explanatory variables

4.6.1 Univariable analyses

Complete results for the univariable analyses of each dataset for IPREV and IPREV25 are presented in Appendix 7 and Appendix 8, respectively. Numbers of variables in each category unconditionally associated with IPREV and IPREV25 in FIRST dataset are shown in Table 4.16. Briefly, 29, 27 and 28 variables unconditionally associated with cohort OJD prevalence variable IPREV were selected for multivariable analysis in FIRST, SECOND and THIRD datasets, respectively. The final number of variables unconditionally associated with IPREV25 in the FIRST, SECOND and THIRD datasets included in multivariable analyses were 20, 20 and 21, respectively.

Table 4.16
Number of flock and cohort-level explanatory variables unconditionally associated at P<0.25 with IPREV and IPREV25 in the FIRST dataset

Categories of variables	No of variables in each category		
	Total number	Number with P < 0.25	
		IPREV	IPREV25
<i>Flock-level</i>			
Confounders	7	7	6
OJD control	7	4	4
Lateral spread and purchase risk	12	5	4
Property management	6	4	2
Flock management	2	0	0
Drought and waterlogging	5	2	1
<i>Cohort-level</i>			
General characteristics and management	10	3	2
Dam characteristics and lamb management	5	1	0
Weaner characteristics and management	7	3	4
Hogget characteristics and management	4	1	1
Adult characteristics and management	6	1	0
All categories	71	31	24

4.6.2 Multivariable analyses

Cohort OJD prevalence - IPREV

The logistic regression models for IPREV from the FIRST, SECOND and THIRD datasets are presented in Tables 4.17, 4.18 and 4.19, respectively. All three models demonstrated that the presence of other wildlife (aside from kangaroos and rabbits) and season of cohort birth were strongly associated with cohort OJD prevalence level. Variables associated with cohort prevalence level in models for two datasets included drought over cohort lifetime, the number of age groups vaccinated in the flock, application of fertilizers other than single super, molybdenum super and lime on the property, and the proportion of property boundary receiving run off water. Final models including interaction terms for the SECOND and THIRD datasets are shown in Appendix 16.

Cohort OJD prevalence – IPREV25

The logistic regression models for IPREV25 from the FIRST, SECOND and THIRD datasets are presented in Tables 4.20, 4.21 and 4.22, respectively. All three models demonstrated that culling of low body weight sheep and the application of fertilizers other than single super, molybdenum super

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and lime on the property were strongly associated with cohort OJD prevalence level. Variables associated with cohort prevalence level in models for two datasets included the presence of other wildlife (aside from kangaroos and rabbits) and length of OJD decontamination of the weaner paddock/s. Final models including interaction terms for the SECOND and THIRD datasets are shown in Appendix 16.

Table 4.17

Final logistic model for IPREV (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-10%) and high (>10%)) based on 75 sheep cohorts in the FIRST dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Intercept	-10.1	-16.15	-5.93				
Intercept	-2.7	-5.79	-0.02				
CURRMORT							<0.001
No mortalities				1			
<2% mortalities	3.7	1.66	6.50	42.21	5.27	664.9	
≥ 2% mortalities	5.7	3.10	9.34	312.49	22.19	1000	
SEX							<0.001
Ewes				1			
Wethers	5.3	2.96	8.93	206.85	19.37	1000	
AGEGP							0.83
3 years				1			
4 years	0.2	-1.63	2.03	1.22	0.20	7.64	
DROUGHT							0.07
≤ 150mm lesser OR more than long-term average				1			
>150mm lesser	1.8	-0.15	4.11	6.10	0.86	60.70	
DROPSVACC							<0.001
No drops vaccinated				1			
1 or 2 drops vaccinated	3.5	0.94	6.70	34.12	2.56	812.7	
>2 drops vaccinated	-1.3	-3.96	1.05	0.26	0.02	2.86	
CULL							0.06
No				1			
Yes	1.5	-0.06	3.26	4.54	0.94	26.13	
SUPERFREQ							<0.001
≤ once in three years				1			
>once to ≤ twice in three years	0.5	-1.56	2.58	1.64	0.21	13.18	
> twice to ≤ Every year	1.3	-0.48	3.31	3.67	0.62	27.49	
> Once every year	5.4	2.35	9.39	218.54	10.50	1000	
OTHERWILDLIFE							0.03
No				1			
Yes	-1.7	-3.35	-0.16	0.19	0.04	0.85	
LBGSSN							<0.001
Spring				1			
Autumn	-1.7	-3.79	0.29	0.19	0.02	1.33	
Winter	-2.7	-4.79	-0.89	0.07	0.01	0.41	

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Table 4.18

Final logistic model for IPREV (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-10%) and high (>10%)) based on 97 sheep cohorts in the SECOND dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Intercept	-2.0	-4.03	-0.17				
Intercept	2.4	0.49	4.53				
CURRMORT							<0.001
No mortalities				1			
<2% mortalities	1.8	0.55	3.24	6.31	1.73	25.47	
≥ 2% mortalities	3.3	1.78	4.94	25.89	5.92	139.94	
SEX							0.02
Ewes				1			
Wethers	1.3	0.22	2.52	3.81	1.25	12.47	
AGEGP							0.38
3 years				1			
4 years	-0.5	-1.59	0.58	0.62	0.20	1.79	
DROUGHT							0.01
≤ 150mm lesser OR more than long-term average				1			
>150mm lesser	1.5	0.42	2.77	4.71	1.53	16.01	
HGTCS							0.05
<3				1			
≥3	-1.1	-2.32	0.02	0.33	0.10	1.02	
OTHERWILDLIFE							<0.001
No				1			
Yes	-1.7	-2.87	-0.63	0.18	0.06	0.54	
OTHERFERT							<0.001
No				1			
Yes	-2.4	-4.17	-0.86	0.09	0.02	0.43	
LBGSSN							<0.001
Spring				1			
Autumn	-0.2	-1.40	0.98	0.81	0.25	2.67	
Winter	-2.6	-4.24	-1.15	0.07	0.01	0.32	
SHARING_ROAD							0.05
No sharing				1			
≤ twice per year	1.1	-0.16	2.36	2.93	0.85	10.56	
>twice per year	1.5	0.16	2.89	4.41	1.17	18.03	
RUNOFFWATER							0.02
≤ 10%				1			
>10 to ≤ 30%	-1.1	-2.52	0.33	0.35	0.08	1.39	
>30% to ≤ 60%	-2.2	-3.71	-0.76	0.12	0.02	0.47	
> 60%	-0.98	-2.45	0.42	0.37	0.09	1.52	

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Table 4.19

Final logistic model for IPREV (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-10%) and high (>10%)) based on 109 sheep cohorts in the THIRD dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Intercept	-3.1	-5.16	-1.27				
Intercept	0.7	-1.09	2.57				
CURRMORT							<0.001
No mortalities				1			
<2% mortalities	2.5	1.25	3.79	11.77	3.48	44.23	
≥ 2% mortalities	3.6	2.22	5.16	36.81	9.25	174.54	
SEX							<0.001
Ewes				1			
Wethers	1.8	0.76	2.94	6.11	2.13	19.01	
Mixed sex	-3.95	-6.58	-1.72	0.02	0.00	0.18	
AGEGP							0.42
3 years				1			
4 years	-0.6	-1.65	0.44	0.55	0.19	1.55	
5 years	0.4	-1.52	2.32	1.51	0.22	10.19	
DROPSVACC							0.03
No drops vaccinated				1			
1 or 2 drops vaccinated	1.8	0.29	3.45	6.19	1.33	31.45	
>2 drops vaccinated	0.4	-1.00	1.85	1.49	0.37	6.36	
OTHERWILDLIFE							0.01
No				1			
Yes	-1.3	-2.29	-0.29	0.28	0.10	0.75	
LBGSSN							<0.001
Spring				1			
Autumn	-0.5	-1.53	0.57	0.63	0.22	1.77	
Winter	-2.4	-3.81	-1.06	0.09	0.02	0.35	
OTHERFERT							<0.001
No				1			
Yes	-2.3	-3.86	-0.85	0.10	0.02	0.43	
RUNOFFWATER							0.05
≤ 10%				1			
>10 to ≤ 30%	-0.4	-1.74	0.86	0.65	0.18	2.35	
>30% to ≤ 60%	-1.7	-3.03	-0.43	0.18	0.05	0.65	
> 60%	-0.9	-2.27	0.36	0.40	0.10	1.43	

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Table 4.20

Final logistic model for IPREV25 (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-5%) and high (>5%)) based on 75 sheep cohorts in the FIRST dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Intercept	-7.1	-10.75	-4.25				
Intercept	-4.4	-7.46	-1.91				
CURRMORT							<0.001
No mortalities				1			
<2% mortalities	2.7	1.02	4.72	15.32	2.78	111.75	
≥ 2% mortalities	4.3	2.33	6.75	74.94	10.23	854.09	
SEX							<0.001
Ewes				1			
Wethers	4.2	2.15	6.88	68.92	8.58	973.95	
AGEGP							0.06
3 years				1			
4 years	-1.4	-2.91	0.06	0.26	0.06	1.07	
CULL							
No				1			0.08
Yes	1.3	-0.15	2.84	3.66	0.86	17.05	
DECONT_WNGPDK							0.01
Nil				1			
<8 weeks	-1.2	-3.43	0.84	0.30	0.03	2.32	
8<12 weeks	2.5	0.77	4.46	12.00	2.17	86.88	
≥12weeks	0.8	-1.05	2.80	2.25	0.35	16.45	
OTHERFERT							<0.001
No				1			
Yes	-2.8	-4.78	-1.02	0.06	0.01	0.36	
GROWTHCHK							<0.001
<12 weeks				1			
≥12 weeks	1.9	0.56	3.39	6.68	1.76	29.52	
WNRSR							0.03
<8dse/hac				1			
8 <12 dse/hac	1.5	-0.34	3.39	4.33	0.71	29.69	
≥ 12 dse/hac	2.1	0.46	4.10	8.48	1.59	60.45	
ICREEK							0.11
No				1			
Yes	1.4	-0.29	3.12	3.89	0.75	22.55	

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Table 4.21

Final logistic model for IPREV25 (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-5%) and high (>5%)) based on 97 sheep cohorts in the SECOND dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Intercept	-0.9	-2.71	0.87				
Intercept	1.2	-0.59	2.95				
CURRMORT							<0.001
No mortalities				1			
<2% mortalities	1.0	-0.14	2.20	2.74	0.87	9.04	
≥ 2% mortalities	2.5	1.24	3.95	12.71	3.47	52.00	
SEX							<0.001
Ewes				1			
Wethers	1.8	0.53	3.13	5.84	1.70	22.90	
AGEGP							0.12
3 years				1			
4 years	-0.8	-1.80	0.20	0.46	0.17	1.22	
CULL							<0.001
No				1			
Yes	1.5	0.50	2.51	4.39	1.65	12.36	
DECONT_WNGPDK							0.05
Nil				1			
<8 weeks	-0.2	-1.50	1.19	0.86	0.22	3.29	
8<12 weeks	1.5	0.18	2.96	4.54	1.20	19.34	
≥12weeks	1.2	-0.11	2.56	3.25	0.90	13.00	
OTHERFERT							<0.001
No				1			
Yes	-2.1	-3.64	-0.72	0.12	0.03	0.49	
OTHERWILDLIFE							<0.001
No				1			
Yes	-1.6	-2.65	-0.51	0.21	0.07	0.60	
WNRCS							0.09
<3				1			
≥3	-0.9	-2.03	0.13	0.40	0.13	1.14	
LIME							0.05
No				1			
Yes	-1.0	-2.11	0.01	0.36	0.12	1.01	

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Table 4.22

Final logistic model for IPREV25 (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-5%) and high (>5%)) based on 109 sheep cohorts in the THIRD dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Intercept	-1.2	-2.54	0.03				
Intercept CURRMORT	0.5	-0.71	1.79				<0.001
				1			
				3.51	1.17	11.04	
				14.32	4.23	52.95	
SEX							<0.001
				1			
				4.63	1.57	15.06	
				0.08	0.01	0.61	
AGEGP							0.13
				1			
				0.44	0.18	1.08	
				0.33	0.06	1.97	
CULL							0.02
				1			
				3.02	1.23	7.57	
OTHERFERT							0.03
				1			
				0.25	0.07	0.90	
LBGSSN							0.03
				1			
				0.49	0.18	1.32	
				0.24	0.07	0.73	
OTHERWILDLIFE							0.02
				1			
				0.36	0.14	0.87	

4.7 Association between pool OJD status and the history and management explanatory variables

4.7.1 Univariable analyses

Of the 71 variables investigated, 29 flock-level variables (including 7 of 7 confounders) and 16 cohort-level variables were unconditionally associated with pool OJD status (Table 4.23). The detailed results for the univariable analyses for pool OJD status are presented in Appendix 9.

Table 4.23
Number of flock and cohort-level explanatory variables unconditionally associated at $P < 0.25$ with pool OJD status

Categories of variables	No of variables in each category	
	Total number	Number with $P < 0.25$
<i>Flock-level</i>		
Confounders	7	7
OJD control	7	6
Lateral spread and purchase risk	12	7
Property management	6	3
Flock management	2	2
Drought and waterlogging	5	4
<i>Cohort-level</i>		
General characteristics and management	10	5
Dam characteristics and lamb management	5	1
Weaner characteristics and management	7	5
Hogget characteristics and management	4	3
Adult characteristics and management	6	2
All categories	71	45

4.7.2 Multivariable analyses

The final mixed logistic regression model for pool OJD status is presented in Table 4.24. This model demonstrated that application of fertilizers other than single super, molybdenum super and lime on the property, the proportion of property boundary receiving run off water, culling of low body weight sheep, length of OJD decontamination of the weaner paddock/s and frequency of sharing roads with neighbours were strongly associated with pool OJD status. All these variables except the frequency of sharing roads with neighbours were also associated with cohort OJD prevalence level. The final model including interaction terms is shown in Appendix 16.

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Table 4.24

The final mixed logistic regression model for pool OJD status based on 663 pools

Parameters	<i>b</i>	<i>SE(b)</i>	Adjusted odds ratio	OR LCL	OR UCL	P
Intercept	-3.97	2.44				
Random effects						
PROPERTYID	0.77	0.27				
Fixed effects						
CURRMORT						<0.001
No mortalities			1			
<2% mortalities	0.83	0.37	2.30	1.11	4.76	
≥ 2% mortalities	1.78	0.39	5.92	2.74	12.77	
SEX						0.02
Ewes			1			
Wethers	0.68	0.30	1.98	1.11	3.54	
AGEGP						0.02
3 years			1			
4 years	-0.70	0.30	0.50	0.27	0.90	
LOGPOOLSIZE	1.07	0.71	2.91	0.72	11.82	0.13
CULL						0.003
No			1			
Yes	0.92	0.31	2.52	1.38	4.59	
RUNOFFWATER						0.01
≤ 10%			1			
>10 to ≤ 30%	-0.25	0.40	0.78	0.35	1.71	
>30% to ≤ 60%	-0.98	0.41	0.37	0.17	0.83	
> 60%	-1.22	0.42	0.29	0.13	0.68	
OTHERFERT						0.004
No			1			
Yes	-1.33	0.42	0.27	0.11	0.66	
SHARING_ROAD						0.06
No sharing			1			
≤ twice per year	0.76	0.38	2.13	1.02	4.46	
>twice per year	0.70	0.40	2.02	0.92	4.43	
DECONT_WNGPDK						0.05
Nil			1			
<8 weeks	-0.16	0.44	0.85	0.36	2.01	
8<12 weeks	0.10	0.38	1.10	0.52	2.31	
≥12weeks	1.16	0.45	3.20	1.33	7.71	

4.8 Association between pool MAP number shed and the history and management explanatory variables

4.8.1 Univariable analyses

Of the 71 variables investigated, 29 flock-level variables (including 7 of 7 confounders) and 19 cohort-level variables were unconditionally associated with log MAP number shed per pool (Table 4.25). The detailed results for the univariable analyses for pool MAP number shed are presented in Appendix 10.

Table 4.25
Number of flock and cohort-level explanatory variables unconditionally associated at $P < 0.25$ with log pool MAP number shed

Categories of variables	No of variables in each category	
	Total number	Number with $P < 0.25$
<i>Flock-level</i>		
Confounders	7	7
OJD control	7	6
Lateral spread and purchase risk	12	9
Property management	6	2
Flock management	2	2
Drought and waterlogging	5	3
<i>Cohort-level</i>		
General characteristics and management	10	6
Dam characteristics and lamb management	5	2
Weaner characteristics and management	7	7
Hogget characteristics and management	4	2
Adult characteristics and management	6	2
All categories	71	48

4.8.2 Multivariable analyses

The final mixed logistic regression model for log pool MAP number shed is presented in Table 4.26. This model demonstrated that the application of fertilizers (single super or molybdenum super and other fertilizers (aside from single super, molybdenum super and lime)) on the property, the proportion of property boundary receiving run off water, culling of low body weight sheep, sale of high loss mobs, the period of growth retardation (or weight loss in adult sheep) during cohort lifetime, the length of OJD decontamination of the weaner paddock/s, number of vaccinated drops in flock, implementation of an effective worm control program and cohort age at weaning were strongly associated with log of the number of MAP shed per pool. All these variables were also associated with either cohort OJD prevalence level or pool OJD status with the exception of 3 variables - sale of high loss mobs, implementation of an effective worm control program and cohort age at weaning. The final model including interaction terms is shown in Appendix 16.

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

Table 4.26

The final mixed linear regression model for log pool MAP number shed based on 649 pools

Parameters	<i>b</i>	SE(<i>b</i>)	LCL (<i>b</i>)	UCL (<i>b</i>)	<i>t/z/chi sq</i> ^a	P
Intercept	-2	1.642	-5.23	1.3273	-1.19	0.24
<i>Random Effects</i>						
PROPERTYID	0.2	0.097	0.094	0.6972	7.46	0.006
Residual	2.37	0.141	2.115	2.672	16.77	<0.001
<i>Fixed Effects</i>						
CURRMORT					24.53	<0.001
No mortalities	0					
<2% mortalities	0.78	0.229	0.334	1.2329		
≥2% mortalities	1.2	0.243	0.724	1.6786		
SEX					10.98	0.001
Ewes	0					
Wethers	0.56	0.169	0.228	0.8914		
AGEGP					0.19	0.66
3 years	0					
4 years	-0.1	0.202	-0.48	0.3081		
LOGPOOLSIZE	0.9	0.466	-0.02	1.8108	1.92	0.055
CULL					7	0.008
No	0					
Yes	0.5	0.188	0.128	0.8654		
SELL					5.82	0.016
No	0					
Yes	0.68	0.284	0.127	1.241		
RUNOFFWATER					16.84	0.001
≤ 10%	0					
>10 to ≤ 30%	-0.6	0.242	-1.04	-0.093		
>30% to ≤ 60%	-1	0.243	-1.46	-0.503		
> 60%	-0.6	0.265	-1.1	-0.061		
SUPERFREQ					10.72	0.013
≤ once in three years	0					
>once to ≤ twice in three years	-0.6	0.259	-1.08	-0.063		
> twice to ≤ Every year	0.19	0.256	-0.31	0.6899		
≥ Once every year	-0.1	0.323	-0.75	0.5212		
OTHERFERT					10.95	0.001
No	0					
Yes	-1	0.314	-1.65	-0.422		
GROWTHCHK					9.01	0.003
<12 weeks	0					
≥12 weeks	0.55	0.182	0.189	0.9029		
DECONT_WNGPDK					14.8	0.002
Nil	0					
<8 weeks	0.27	0.277	-0.27	0.8154		
8<12 weeks	0.45	0.221	0.018	0.8869		
≥12weeks	0.96	0.259	0.454	1.4722		

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

DROPSVACC								
	No drops vaccinated	0				9.91		0.007
	1 or 2 drops vaccinated	0.89	0.286	0.329	1.4507			
	>2 drops vaccinated	0.45	0.288	-0.11	1.0209			
WORMCONTROL						5.24		0.02
	No	0						
	Yes	-0.5	0.235	-1	-0.077			
WNGAGE						8.13		0.04
	≤ 15 weeks	0						
	≤ 18 weeks	-0.3	0.251	-0.75	0.2349			
	≤ 21 weeks	-0.8	0.282	-1.35	-0.245			
	>21 weeks	-0.3	0.239	-0.73	0.2144			

a Test of significance: t for intercept and LOGPOOLSIZE; z for residual; LR chi-square for PROPERTYID and Wald chi-square for all other fixed effects

4.9 Association between cohort OJD prevalence and the soil explanatory variables

4.9.1 Univariable analyses

Complete results for the univariable analyses of each dataset for IPREV and IPREV25 are presented in Appendix 11 and Appendix 12, respectively. Briefly, of the 44 soil variables investigated, 24 variables remained for inclusion in multivariable analyses. The final number of variables unconditionally associated with IPREV in the SECOND and THIRD datasets and included in multivariable analyses were 26 and 22, respectively. The final number of variables unconditionally associated with IPREV25 in the FIRST, SECOND and THIRD datasets included in multivariable analyses were 16, 21 and 19, respectively.

4.9.2 Multivariable analyses

Cohort OJD prevalence - IPREV

The logistic regression model including the 3-paddock mean variables for IPREV from the FIRST dataset is presented in Table 4.27 (see Appendix 13 for the models for SECOND and THIRD datasets). The model for the FIRST dataset demonstrated that cation exchange capacity and silt percentage were strongly associated with cohort OJD prevalence level, where as phosphorous buffer index and clay percentage were both identified in models for the SECOND and THIRD datasets.

The separate logistic regression models for IPREV based on the lamb paddock variables, the weaner paddock variables and the hogget/adult paddock variables from the FIRST dataset are presented in Table 4.27 (see Appendix 13 for the models for SECOND and THIRD datasets). All three relevant models for the FIRST, SECOND and THIRD datasets demonstrated that one weaning paddock variable (silt percentage) and one hogget/adult paddock variable (cationic exchange capacity) were strongly associated with cohort OJD prevalence level.

Cohort OJD prevalence – IPREV25

The logistic regression model including the 3-paddock mean variables for IPREV25 from the FIRST dataset is presented in Table 4.28 (see Appendix 13 for the models for SECOND and THIRD datasets). All three models for the FIRST, SECOND and THIRD datasets demonstrated that phosphorus buffer index and silt percentage were associated with cohort OJD prevalence level.

The logistic regression model for IPREV25 based on the weaner paddock variables from the FIRST dataset is shown in Table 4.28. No multivariable models are presented for the lamb paddock variables or hogget/adult paddock variables because no variables were significant on entry to the base model. Models for the SECOND and THIRD datasets are shown in Appendix 13. All three relevant models for the FIRST, SECOND and THIRD datasets demonstrated that one weaning paddock variable (silt percentage) and two hogget/adult paddock variables (silt percentage and sand percentage) were associated with cohort OJD prevalence level.

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

Table 4.27

Final logistic model for IPREV (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-10%) and high (>10%)) for soil variables in the FIRST dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Final model for 3-paddock mean variables (based on 75 cohorts)							
Intercept	-7.87	-11.96	-4.49				
Intercept	-3.25	-6.41	-0.37				
CURRMORT							<0.001
	No mortalities			1			
	<2% mortalities	1.72	0.29	3.27	5.57	1.34	26.24
	≥ 2% mortalities	3.41	1.87	5.20	30.31	6.51	180.92
SEX							<0.001
	Ewes			1			
	Wethers	3.59	2.10	5.36	36.12	8.16	213.08
AGEGP							0.50
	3 years			1			
	4 years	-0.42	-1.70	0.82	0.66	0.18	2.27
PSTYPE							0.15
	Basalt			1			
	Granite	1.17	-1.27	3.74	3.22	0.28	41.97
	Shale and sandstone	2.25	0.00	4.71	9.49	1.00	110.92
	Mixed without limestone	-0.02	-3.15	3.01	0.98	0.04	20.26
	Mixed with limestone	1.09	-1.25	3.57	2.99	0.29	35.35
CEC							0.01
	≤ 6 Meq/100g			1			
	> 6 Meq/100g	1.59	0.36	2.92	4.88	1.43	18.45
SILT							0.03
	≤ 21%			1			
	> 21%	1.42	0.15	2.79	4.12	1.16	16.33

Final model for lambing paddock variables (based on 57 cohorts)

Intercept	-3.96	-6.85	-1.48				
Intercept	0.59	-1.78	2.92				
CURRMORT							<0.001
	No mortalities			1			
	<2% mortalities	1.75	0.11	3.58	5.78	1.11	35.98
	≥ 2% mortalities	3.29	1.50	5.42	26.79	4.47	224.84
SEX							<0.001
	Ewes			1			
	Wethers	4.21	2.31	6.63	67.13	10.08	760.58
AGEGP							0.03

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

PSTYPE	3 years				1			
	4 years	-1.58	-3.19	-0.14	0.21	0.04	0.87	0.15
SAND	Basalt				1			
	Granite	-0.12	-2.41	2.17	0.89	0.09	8.76	
	Shale and sandstone	1.02	-1.07	3.21	2.79	0.34	24.87	
	Mixed without limestone	-2.33	-6.27	1.08	0.10	0.00	2.95	
	Mixed with limestone	0.68	-1.70	3.13	1.97	0.18	22.91	
	≤ 62%				1			
> 62%	-1.60	-3.24	-0.12	0.20	0.04	0.89	0.03	

Final model for weaning paddock variables (based on 50 cohorts)

Intercept		-12.59	-21.44	-6.94				
Intercept		-3.96	-9.20	-0.21				
CURRMORT								<0.001
	No mortalities				1			
	<2% mortalities	3.15	0.56	6.94	23.30	1.75	>999.9	
	≥ 2% mortalities	6.95	3.26	12.97	>999.9	26.18	>999.9	
SEX								<0.001
	Ewes				1			
	Wethers	7.04	3.46	12.58	>999.9	31.88	>999.9	
AGEGP								0.11
	3 years				1			
	4 years	-1.70	-4.17	0.37	0.18	0.02	1.44	
PSTYPE								0.46
	Basalt				1			
	Granite	1.39	-2.05	5.46	4.01	0.13	236.01	
	Shale and sandstone	2.27	-0.92	6.27	9.65	0.40	527.00	
	Mixed without limestone	0.68	-4.82	6.67	1.97	0.01	784.68	
	Mixed with limestone	2.86	-0.77	7.44	17.43	0.46	>999.9	
SILT_WNGPDK								0.00
	≤ 21%				1			
	> 21%	3.47	1.40	6.20	32.00	4.06	494.74	
ALSAST_WNGPDK								0.02
	≤ 2				1			
	>2 to ≤ 5	2.61	-0.23	6.14	13.55	0.80	465.85	
	>5 to ≤ 12	-2.80	-5.85	-0.39	0.06	0.00	0.68	
	>12	0.82	-2.40	4.66	2.26	0.09	105.20	

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

Final model for hogget/adult paddock variables (based on 60 cohorts)

Intercept		-5.76	-9.96	-2.30				
Intercept		-1.41	-4.82	1.83				
CURRMORT								0.00
	No mortalities				1			
	<2% mortalities	1.77	0.14	3.56	5.90	1.15	35.23	
	≥ 2% mortalities	3.24	1.52	5.26	25.58	4.56	191.63	
SEX								
	Ewes				1			0.00
	Wethers	3.19	1.63	5.02	24.27	5.09	151.98	
AGEGP								0.03
	3 years				1			
	4 years	-1.45	-2.97	-0.10	0.23	0.05	0.90	
PSTYPE								0.29
	Basalt				1			
	Granite	-0.17	-3.13	2.79	0.84	0.04	16.27	
	Shale and sandstone	0.64	-2.17	3.52	1.90	0.11	33.89	
	Mixed without limestone	-1.46	-5.11	1.91	0.23	0.01	6.73	
	Mixed with limestone	-0.94	-4.03	2.05	0.39	0.02	7.73	
CEC_ADSDK								0.06
	≤ 6 Meq/100g				1			
	> 6 Meq/100g	1.24	-0.04	2.61	3.47	0.96	13.55	
SILT_ADSDK								0.03
	≤ 21%				1			
	> 21%	1.56	0.14	3.08	4.74	1.15	21.78	

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

Table 4.28

Final logistic model for IPREV25 (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-5%) and high (>5%)) for soil variables in the FIRST dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Final model for 3-paddock mean variables (based on 75 cohorts)							
Intercept	-2.75	-5.37	-0.25				
Intercept	-1.04	-3.57	1.42				
CURRMORT							<0.001
	No mortalities			1			
	<2% mortalities	1.84	0.50	3.31	6.30	1.66	27.34
	≥ 2% mortalities	3.34	1.86	5.03	28.32	6.41	152.81
SEX							<0.001
	Ewes			1			
	Wethers	3.21	1.70	5.02	24.75	5.47	151.71
AGEGP							0.29
	3 years			1			
	4 years	-0.63	-1.82	0.54	0.53	0.16	1.71
PSTYPE							0.10
	Basalt			1			
	Granite	-0.40	-2.58	1.69	0.67	0.08	5.43
	Shale and sandstone	0.49	-1.60	2.51	1.62	0.20	12.24
	Mixed without limestone	-2.61	-5.60	0.14	0.07	0.00	1.15
	Mixed with limestone	-0.25	-2.44	1.89	0.78	0.09	6.62
SILT							0.07
	≤ 21%			1			
	> 21%	1.04	-0.10	2.25	2.83	0.90	9.48

Final model for weaning paddock variables (based on 50 cohorts)

Intercept	-3.74	-6.84	-0.84				
Intercept	-1.58	-4.46	1.20				
CURRMORT							0.006
	No mortalities			1			
	<2% mortalities	1.12	-0.44	2.83	3.06	0.64	16.94
	≥ 2% mortalities	2.78	1.02	4.80	16.16	2.76	121.23
SEX							<0.001
	Ewes			1			
	Wethers	3.46	1.26	6.72	31.79	3.51	832.67
AGEGP							0.29
	3 years			1			
	4 years	-0.81	-2.34	0.68	0.45	0.10	1.97

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

PSTYPE								0.56
	Basalt				1			
	Granite	0.29	-2.28	2.78	1.33	0.10	16.17	
	Shale and sandstone	1.00	-1.34	3.32	2.72	0.26	27.76	
	Mixed without limestone	-1.47	-6.08	2.70	0.23	0.00	14.93	
	Mixed with limestone	1.04	-1.73	3.81	2.84	0.18	44.96	
SILT_WNGPDK								0.006
	≤ 21%				1			
	> 21%	2.08	0.59	3.75	7.98	1.80	42.39	

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

4.10 Association between pool OJD status and the soil explanatory variables

4.10.1 Univariable analyses

Of the 44 variables investigated, 32 variables were selected for inclusion in multivariable analyses. The detailed results for the univariable analyses for pool OJD status are presented in Appendix 14.

4.10.2 Multivariable analyses

The final mixed logistic regression models for pool OJD status based on 3-paddock mean variables and on lamb, weaner and hogget/adult paddock variables are presented in Tables 4.29 and 4.30, respectively. These models demonstrated that phosphorus buffer index (3-paddock mean and weaning paddock), phosphorus content of lamb paddock and silt percentage of adult paddock were associated with pool OJD status.

Table 4.29
Final mixed logistic model for MPTB for 3-paddock mean soil variables based on 659 pools

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Intercept	-1.29	-6.36	3.79				
PROPERTYID (random effect)	1.11						
CURRMORT							<0.001
No mortalities				1			
<2% mortalities	1.18	0.39	1.96	3.25	1.48	7.13	
≥ 2% mortalities	1.91	1.09	2.73	6.75	2.98	15.32	
SEX							0.02
Ewes				1			
Wethers	0.73	0.14	1.32	2.08	1.15	3.75	
AGEGP							0.31
3 years				1			
4 years	-0.31	-0.91	0.29	0.74	0.40	1.34	
LOGPOOLSIZE	0.19	-1.25	1.63	1.21	0.29	5.12	0.79
PSTYPE							0.90
Basalt				1			
Granite	-0.16	-1.43	1.11	0.85	0.24	3.05	
Shale and sandstone	0.15	-1.06	1.35	1.16	0.35	3.85	
Mixed without limestone	-0.36	-1.81	1.09	0.70	0.16	2.97	
Mixed with limestone	-0.15	-1.45	1.15	0.86	0.24	3.16	
PBI							0.02
< 70				1			
≥ 70	0.82	0.11	1.54	2.28	1.12	4.66	

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

Table 4.30
Final mixed logistic models for MPTB for lamb paddock, weaner paddock and hogget/adult paddock soil variables

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Final model for lamb paddock variables (based on 519 pools)							
Intercept	0.16	-5.21	5.53				
PROPERTYID (random effect)	0.99						
CURRMORT							0.01
				1			
	0.81	-0.04	1.66	2.25	0.96	5.28	
						14.3	
	1.76	0.86	2.66	5.81	2.36	0	
SEX							<0.001
				1			
	0.93	0.22	1.64	2.53	1.25	5.13	
AGEGP							0.29
				1			
	-0.44	-1.10	0.22	0.64	0.33	1.24	
LOGPOOLSIZE							
	-0.24	-1.75	1.26	0.78	0.17	3.54	
PSTYPE							0.56
				1			
	-0.29	-1.50	0.91	0.75	0.22	2.49	
	0.13	-1.08	1.34	1.14	0.34	3.84	
	0.11	-1.46	1.68	1.11	0.23	5.36	
	-0.14	-1.52	1.23	0.87	0.22	3.42	
P_LBGPDK							0.01
				1			
	1.00	0.08	1.92	2.72	1.08	6.85	
	0.79	-0.04	1.61	2.20	0.97	5.00	
Final model for weaning paddock variables (based on 452 pools)							
Intercept	0.08	-6.14	6.30				
PROPERTYID	0.94						
CURRMORT							<0.001
				1			
	1.20	0.30	2.10	3.31	1.35	8.13	
	2.04	1.05	3.04	7.73	2.85	20.9	
SEX							0.02
				1			
	0.85	0.13	1.57	2.33	1.14	4.79	
AGEGP							0.16

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

	3 years				1			
	4 years	-0.50	-1.19	0.20	0.61	0.30	1.22	
LOGPOOLSIZE		-0.20	-1.98	1.58	0.82	0.14	4.85	0.82
PSTYPE								0.99
	Basalt				1			
	Granite	-0.29	-1.57	0.99	0.75	0.21	2.68	
	Shale and sandstone	-0.14	-1.37	1.09	0.87	0.26	2.97	
	Mixed without limestone	0.01	-1.57	1.59	1.01	0.21	4.88	
	Mixed with limestone	-0.14	-1.60	1.31	0.87	0.20	3.72	
PBI_WNGPDK								0.01
	< 70				1			
	≥ 70	1.06	0.28	1.84	2.89	1.33	6.31	

Final model for hogget/adult paddock variables (based on 508 Pools)

Intercept		0.08	-5.92	6.07				
PROPERTYID								
CURRMORT								<0.001
	No mortalities				1			
	<2% mortalities	1.23	0.36	2.10	3.44	1.44	8.20	17.2
	≥ 2% mortalities	1.93	1.01	2.85	6.89	2.75	4	
SEX								<0.001
	Ewes				1			
	Wethers	1.34	0.61	2.07	3.84	1.85	7.96	
AGEGP								0.10
	3 years				1			
	4 years	-0.56	-1.23	0.11	0.57	0.29	1.11	
LOGPOOLSIZE		0.00	-1.72	1.72	1.00	0.18	5.59	1.00
PSTYPE								0.36
	Basalt				1			
	Granite	-0.81	-2.40	0.78	0.45	0.09	2.19	
	Shale and sandstone	-0.47	-1.99	1.05	0.63	0.14	2.85	
	Mixed without limestone	-1.17	-2.86	0.52	0.31	0.06	1.69	
	Mixed with limestone	-1.32	-2.95	0.32	0.27	0.05	1.38	
SILT_ADPDK								0.10
	≤ 21%				1			
	> 21%	0.65	-0.13	1.43	1.92	0.88	4.19	

4.11 Association between pool MAP number shed and the soil explanatory variables

4.11.1 Univariable analyses

Of the 44 variables investigated, 31 variables were selected for inclusion in multivariable analyses. The detailed results for the univariable analyses are presented in Appendix 15.

4.11.2 Multivariable analyses

The final mixed logistic regression models for log pool MAP number shed based on 3-paddock mean variables, and on lamb, weaner and hogget/adult paddock variables are presented in Tables 4.31 and 4.32, respectively. These models demonstrated that phosphorus buffer index (3-paddock mean and lamb paddock) and phosphorus content of soil in lambing paddock and cation exchange capacity of soil in adult paddock were associated with the log pool MAP number shed. Three of these variables were also associated with pool OJD status as well as with cohort OJD prevalence level.

Table 4.31

Final model of 3-paddock mean soil variables with LOGMAP based on 659 pools

Parameters	<i>b</i>	LCL (<i>b</i>)	UCL (<i>b</i>)	SE (<i>b</i>)	P
Intercept	1.31	-2.23	4.85	1.78	
Random Effects					
PROPERTYID	0.55	0.35	0.99	0.14	<0.001
Residual	2.35	2.10	2.65	0.14	<0.001
Fixed Effects					
CURRMORT					<0.001
No mortalities					
<2% mortalities	1.08	0.55	1.62	0.27	
≥ 2% mortalities	1.57	1.03	2.11	0.28	
SEX					0.00
Ewes					
Wethers	0.54	0.18	0.89	0.18	
AGEGP					0.67
3 years					
4 years	-0.09	-0.49	0.31	0.20	
LOGPOOLSIZE	-0.19	-1.19	0.82	0.51	0.72
PSTYPE					0.68
Basalt					
Granite	-0.08	-0.93	0.77	0.43	
Shale and sandstone	0.20	-0.59	0.99	0.40	
Mixed without limestone	-0.27	-1.23	0.70	0.49	
Mixed with limestone	0.19	-0.67	1.04	0.44	
PBI					0.01
< 70					
≥ 70	0.60	0.13	1.07	0.24	

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Table 4.32
Final mixed logistic models for LOGMAP for lamb paddock, weaner paddock and hogget/adult paddock soil variables

Parameters	b	LCL (b)	UCL(b)	SE (b)	P
Final model for lambing paddock variables (based on 519 pools)					
Intercept	2.32	-1.62	6.25	1.97	
Random Effects					
PROPERTYID	0.60	0.36	1.18	0.18	<0.001
Residual	2.39	2.11	2.74	0.16	<0.001
Fixed Effects					
CURRMORT					
	No mortalities				<0.001
	<2% mortalities	0.87	0.24	1.49	0.32
	≥ 2% mortalities	1.49	0.85	2.13	0.33
SEX					0.00
	Ewes				
	Wethers	0.62	0.20	1.04	0.21
AGEGP					0.62
	3 years				
	4 years	-0.12	-0.59	0.35	0.24
LOGPOOLSIZE					0.38
PSTYPE					0.72
	Basalt				
	Granite	-0.16	-1.02	0.69	0.43
	Shale and sandstone	0.22	-0.63	1.07	0.43
	Mixed without limestone	0.09	-1.01	1.19	0.56
	Mixed with limestone	0.26	-0.71	1.23	0.49
P_LBGPDK					0.09
	<20 mg/kg				
	20-30 mg/kg	0.67	0.02	1.32	0.33
	>30 mg/kg	0.57	-0.02	1.15	0.30
Final model for weaning paddock variables (based on 452 pools)					
Intercept	2.02	-2.47	6.51	2.24	
Random Effects					
PROPERTYID	0.54	0.31	1.14	0.17	<0.001
Residual	2.28	2.00	2.64	0.16	<0.001
Fixed Effects					
CURRMORT					<0.001
	No mortalities				
	<2% mortalities	1.10	0.46	1.73	0.32
	≥ 2% mortalities	1.67	1.00	2.35	0.34
SEX					0.001
	Ewes				
	Wethers	0.72	0.28	1.15	0.22

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AGEGP						0.78
	3 years					
	4 years	-0.07	-0.55	0.41	0.24	
LOGPOOLSIZE		-0.44	-1.73	0.84	0.65	0.50
PSTYPE						0.87
	Basalt					
	Granite	-0.07	-0.96	0.82	0.45	
	Shale and sandstone	0.17	-0.67	1.01	0.43	
	Mixed without limestone	-0.02	-1.08	1.05	0.54	
	Mixed with limestone	0.33	-0.66	1.32	0.50	
PBI_WNGPDK						0.003
	< 70					
	≥ 70	0.80	0.27	1.33	0.27	
Final model for hogget/adult paddock variables (based on 515 pools)						
Intercept		2.43	-1.74	6.60	2.08	
Random Effects						
PROPERTYID		0.50	0.29	1.04	0.16	<0.001
Residual		2.35	2.07	2.69	0.16	<0.001
Fixed Effects						
CURRMORT						
	No mortalities					<0.001
	<2% mortalities	0.85	0.26	1.44	0.30	
	≥ 2% mortalities	1.60	0.99	2.22	0.31	
SEX						<0.001
	Ewes					
	Wethers	0.80	0.41	1.20	0.20	
AGEGP						0.53
	3 years					
	4 years	-0.14	-0.59	0.31	0.23	
LOGPOOLSIZE		-0.43	-1.64	0.78	0.62	0.48
PSTYPE						0.39
	Basalt					
	Granite	-0.40	-1.49	0.69	0.55	
	Shale and sandstone	0.06	-0.98	1.10	0.53	
	Mixed without limestone	-0.59	-1.75	0.56	0.59	
	Mixed with limestone	-0.20	-1.34	0.94	0.58	
CEC_ADPDK						0.01
	≤ 6 Meq/100g					
	> 6 Meq/100g	0.65	0.15	1.14	0.25	

5 Discussion

We conducted a cross-sectional study in order to identify risk factors for OJD expression in infected flocks that could lead to the refinement of recommendations for on-farm control measures for OJD as an alternative to or an adjunct to vaccination. Within the limitations imposed by this study type, substantial efforts were made to maximise the ability of our study to investigate proposed explanatory variables and identify those strongly associated with OJD infection. This discussion presents our current understanding of study strengths and limitations and of significant associations identified by the statistical analyses.

5.1 Issues related to outcome and explanatory variables

5.1.1 Outcome variables

A distinguishing feature of this study, use of PFC to determine the infection status of a specific sheep cohort in infected flocks, is the objective measurement of the disease outcome. It is a notable advance on farmer reporting of disease level, which is known to be influenced by variation in farmer ability to diagnose clinical OJD, and on seroprevalence based on agar-gel diffusion or ELISA tests. PFC is an objective test with considerably enhanced sensitivity compared to serology (Sergeant et al., 2001). It is cheaper as well due to pooling of the samples (Whittington et al., 2000a). The specificity of the PFC is almost 100% and thus has a very high positive predictive value (Sergeant et al., 2001). Secondly, faecal shedding generally starts before immunological response can be detected by ELISA or AGID (Whittington and Sergeant, 2001), and therefore the ability of PFC to detect truly infected animals is higher as compared to immunological tests. In addition the PFC results provided information for three outcome variables – cohort OJD prevalence, pool OJD status and the number of MAP organisms shed per pool. Given the limited number of eligible flocks available for enrolment in this study, the inclusion of pool-level outcome variables in addition to cohort-level outcome was advantageous because it increased the power of statistical analyses.

Collection of faeces from 210 sheep (7 pools of 30 sheep) provided an adequate number of pools to estimate the animal-level prevalence of a sheep cohort and was achieved for 77 (62%) study cohorts. Logistics of sample collection and numbers of 3-5 year old sheep present in the study flocks resulted in variation in the number of pools collected and to a lesser extent size of collected pools for the remainder of study cohorts. We overcame this by use of the Williams and Moffitt (2001) method to estimate individual prevalence from pool results. This method accounts for variable pool size and provides valid results for low and high proportions of positive pools including lower confidence limit above zero for low prevalence estimates. Although it assumes independence between animals within a pool and perfect sensitivity and specificity, it was considered the preferred method due to the lack of information on PFC sensitivity and specificity for the range of pool sizes represented in this study and the respective limitations of alternate methods.

The ability of PFC to designate the infection status of a faecal pool is determined by the test sensitivity and specificity at the respective pool size. PFC sensitivity is known to decrease as pool size increases above about 100 and is dependent on the proportion of multibacillary sheep in the pool. However for practical purposes pool sizes of 50 or less confer a high level of sensitivity compared to serological tests in low prevalence situations (Sergeant et al., 2001). PFC being 100% specific, the only possibility of a false positive test result could be due to cross contamination amongst samples in field or in the lab, or false positive reactions in PCR (Whittington et al., 2000a). However due care was taken in the field as well as in the laboratory and all PCR positive samples were further confirmed by REA and thus there are no reasons to believe that any false positive result was reported in this study. The determination of the number of MAP per pool is influenced by co-growth of irrelevant microbes and clumping of *M. paratuberculosis* organisms in culture and

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likelihood of the organisms to be present in dormant stage (Reddacliff et al., 2003). While acknowledging these limitations of PFC particularly in the presence of paucibacillary cases we are confident that the outcome variables investigated in this study are sound and reflect the true disease status of each study cohort.

Further the selected age groups of cohort sheep (3, 4 and 5-year olds) represent high loss age groups in infected flocks. Of 250 necropsied sheep that died due to OJD on 12 infected properties in 2002, 22% were 3-year olds, 36% 4-year olds and 19% 5-year olds (Toribio et al., 2004). On these same properties, OJD prevalence in 2-year old sheep based on PFC was found to be significantly related to flock annual OJD mortality rate. Given that sheep with clinical and advanced sub-clinical disease (particularly paucibacillary) are faecal shedders of MAP, we consider that OJD prevalence based on PFC of 3 to 4-year old sheep is a credible indicator of OJD severity in infected flocks. In recognition that the level of OJD in 5-year olds is lower than 3 to 4-year olds, data for the 12 5-year old cohorts enrolled in this study were excluded from analytical investigation except for analysis of the THIRD dataset for OJD prevalence where inclusion of these cohorts increased statistical power of the analyses.

Lastly use of the cohort PFC results in this study required that all enrolled flocks are OJD infected. Six study flocks were officially classified as suspect at enrolment based on abattoir surveillance for 2, traceback for 1 and observation of clinical sheep for 3. One or more faecal pools collected from five of these flocks were culture positive confirming infection in these flocks. The sixth flock represented by a single cohort of 3-year old females, 2 pools showed growth in BACTEC media and in both cases the growth index reached up to 999, however, the results could not be confirmed with PCR and REA. The suspect status of this flock was based on abattoir surveillance and thus it is highly likely the flock is infected. However, the owner/manager reported no OJD mortalities indicating a low prevalence of infection in the flock. If this flock is not infected, we consider that there is little adverse consequence for the study results presented as the outcome of interest in all analyses was based on either a cohort or pool result and it is probable that owner management would be similar to that of the 19 other flocks where no OJD mortalities were reported.

5.1.2 Explanatory variables

In contrast, the validity of history and management explanatory variables measured in this study varied. Of these variables, data sourced from or verified by reference to records such as farm inventory and rainfall records, and official OJD data sources were among the most reliable. Others based on owner report were potentially affected by information bias to varying degrees. Data on property characteristics and routine management procedures were considered to be least affected because the majority of sheep producers have sound knowledge and often some documentation of property features (such as paddock area, pasture types, water runoff and creek flow) and of the annual management calendar and enterprise productivity (such as lambing duration, marking date, percentage weaned, fleece weight). However, as producers differ in their ability to diagnose sheep with clinical OJD, the reported OJD mortality rates are likely to be biased and potentially to be more accurate for producers with long-term infected flocks than with recently infected flocks, and for producers with more heavily infected flocks that have gained knowledge about the clinical and gross pathology features of OJD. Specific information about sheep cohorts was expected to be affected by differences in producer recall with data more likely to be incomplete, incorrect or selective the more distant in terms of time from the event of interest and specific to the cohort (such as condition score at weaning, periods of growth retardation and supplementary feeding). Other data based to some degree on producer opinion (such as source of OJD infection and infection status of neighbouring properties) rather than documented evidence were also expected to vary in terms of accuracy but the type of error could not be predicted. To reduce bias in some information (such as duration of flock OJD infection, mineral deficiency and worm control), interviewers asked a series of questions

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and then used criteria to nominate a flock into one of several categories. Further, administration of the questionnaire by personal interview, helped to avoid misinterpretation of questions by producers and enabled the two trained interviewers to clarify answers that were ambiguous, unlikely or inconsistent with previous information or interviewer observation.

Other potential sources of information bias for the history and management explanatory variables related to the timing of producer interviews in this study. Although conducted after faecal sample collection, cohort PFC results were not known by producers and interviewers at time of interview. While 'blind' to cohort status, both producer and interviewer were potentially influenced by available information on flock status including previous test results and producer reported OJD mortality rates. Delays between faecal collection and producer interview, on average 140 days, are unlikely to have had a deleterious effect on producer recall due to the substantial recall periods already required for most variables except for the 6 flocks participating in MLA OJD.033 for which interviews were delayed by 541, 547, 575, 598, 617 and 621 days, respectively.

Information bias is of most concern when there is a difference in measurement of an explanatory variable related to the status of the outcome variable of interest because the direction of resulting bias cannot be predicted (can result in over- or under-estimation of associations). An example of this differential misclassification bias is the potential for producers with more heavily infected flocks to monitor their sheep more closely and therefore to recall information such as details of cohort history more accurately than producers with less infected flocks. By comparison, non-differential misclassification bias (when errors in an explanatory variable are independent of the outcome variable) always directs associations toward the null such that any increased or decrease in disease risk measured will be an underestimate of the true association. In this study differential misclassification bias is unlikely to affect explanatory variables based on records or related to property features and routine management practice. However, several cohort-level variables based on specific details of cohort history (such as hogget condition score) are more likely to be impacted by recall bias, which is often reported to be differential in nature (Dohoo et al., 2004b). To reduce the potential impact of misclassification error on continuous variables most were categorised prior to analysis.

Despite the potential bias introduced by asking producers about past events, current cohort infection status is known to result from previous exposure. Similar to several BJD studies, we therefore collected information about general farm and flock management, and cohort history and management over the lifetime of the cohort sheep rather than current management practices. Further, we focused on the infection status of a particular age group of sheep within each flock rather than flock status to enable investigation of the impact of management during specific life stages on subsequent infection status. These design features again represent an advance on previous OJD studies and align it with superior work conducted on BJD in Michigan, USA (Johnson-Ifearefulundu and Kaneene, 1998).

The soil explanatory variables, based on laboratory analyses and geological maps (for parent soil type), are objective measurements of topsoil composition at the time of collection. Whilst it is very likely that producers correctly identified paddocks for soil sample collection that were grazed by cohort sheep during the specified life stages, some soil components may have changed between the time of grazing and the time of collection in 2004 due to weather conditions and fertilizer application. Advice from a soil scientist is needed to gauge the potential for change in soil composition over 2-4 years. However our efforts to analyse soil samples has provided property specific information that is more accurate than reliance on producer report or reference to regional soil survey data.

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This study sought to investigate a large number of explanatory variables (71 history and management, 44 soil) that far exceeded the standard recommendation of 1 explanatory variable per 10 cases in the analyses for OJD cohort prevalence (IPREV and IPREV25) that included 77 cohorts in the FIRST dataset. This, although not uncommon among cross-sectional studies of JD (Daniels et al., 2002; Johnson-Ifeorunlu and Kaneene, 1998; Lugton, 2004; Obasanjo et al., 1997), introduces potential for the identification of spurious associations. The nature of OJD including its long incubation offers opportunity for a great range of potential factors to influence infection and disease progression. Several strategies to reduce the number of explanatory variables investigated were used in this study. First, explanatory variables were limited to factors with credible links to either OJD infection or disease progression based on prior research or expert opinion. Second, variables with substantial missing values or limited variation were excluded from analyses. Third, when pair wise correlation between explanatory variables was identified one variable was selected for inclusion in analyses based on biological plausibility and reliability of measurement. Fourth, explanatory variables were screened by univariable analysis and only those with unconditional associations of $P < 0.25$ included in multivariable analyses. Lastly, separate models were constructed for the history and management variables and the soil variables as they represent separate subsets of hypothesised predictors and were based on information obtained by different means. As yet simultaneous evaluation of significant history and management variables and soil variables in a composite model, although planned, has not proceeded due to the need for further evaluation of identified associations.

5.1.3 Control of potential confounders and clustering

Among the explanatory variables, 9 history and management variables (7 flock-level and 2 cohort-level) and 1 property-level soil variable were recognised as potential confounders at study commencement, that is, they potentially could bias associations by distorting the relationship between other explanatory variables and the disease outcome of interest. To minimise the impact of these confounders, multivariable models included the most critical confounders and odds ratios adjusted for the presence of these confounders and other model variables were reported. As all proposed flock-level confounders were strongly associated with each outcome variable (with the exception of OJD duration for IPREV25) and significant correlation and associations were found between the OJD mortality percentage, infection level and OJD duration variables, current mortality was selected for inclusion as a fixed effect in all multivariable models. In addition cohort sex and cohort age group were similarly forced into all models, and property soil type into the soil multivariable models.

Inclusion of these confounders accounted for expected associations between cohort infection status and cohort age and sex, and position along the flock epidemic curve at the time of faecal collection. We also anticipated similarity in the infection status of sheep cohorts and faecal pools from the same flock. This disease clustering within flock was accounted for by inclusion of flock as a random effect in the final multivariable models for the two pool outcome variables. Due to the small number of flocks that contributed more than one cohort to the FIRST dataset we decided adjusting for clustering was not required in the final models for OJD cohort prevalence.

5.2 Issues related to flock selection

The 92 flocks in this study, selected because they met specific selection criteria, are a non-representative sample of OJD infected sheep flocks in Australia. The potential selection bias introduced by use of these selection criteria means that study findings should be extrapolated with caution to other types of flocks. Even for self-replacing Merino flocks we acknowledge that the requirement for ≥ 210 unvaccinated 3-5 year old sheep resulted in the exclusion of flocks that experienced high losses during the late 1990s and commenced Gudair® vaccination prior to

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registration. The likely consequence for the study population was particularly to limit the high prevalence cohort category to flocks that have experienced high losses in more recent years.

Extension of the original selection criteria, required to ensure adequate power in the statistical analyses, is considered to potentially have broadened the applicability of study findings in two respects. Firstly, given that management and structure of self-replacing Merino flocks is similar across the known OJD infected districts of Australia, enrolment of flocks from all known infected districts (except Kangaroo Island) strengthens the case for resulting recommendations to be applicable in all OJD infected districts. Secondly, reduction of time since infection from 5 to 3 years increased the number of more recently infected flocks in the study population. However, expert opinion suggests that even recently diagnosed flocks such as those in Western Australia have been infected for many years. With the introduction of risk-based trading it is likely that infection will continue to spread and recommendations from this study will continue to be relevant to newly diagnosed flocks over the coming years.

5.3 History and management variables associated with cohort OJD

5.3.1 Confounding variables

Nine potential confounders were among the history and management variables investigated in this study (7 flock-level and 2 cohort-level).

All proposed flock-level confounders were strongly unconditionally associated with each outcome variable (P-values of at least <0.01 for all variables with the exception of OJD duration for IPREV25 which was non-significant in all 3 datasets). These results confirmed that these variables could confound other associations under investigation and that multivariable models should be used to measure adjusted odds ratios for other explanatory variables after accounting for confounder presence. Each confounder related to the flock epidemic curve from introduction of infection to a flock up to 2004 the year of faecal collection from the cohort sheep. For example, OJD duration indicated the length of the curve, peak OJD mortality the maximum height of the curve, current OJD mortality curve height in 2004, infection level the slope of the curve during the lifetime of the cohort sheep, and age of youngest mortality and observed clinical signs the maximum height of the curve. This study particularly provides sound evidence of the strong relationship between sheep cohort infection level and duration of flock infection, level of OJD mortality and trend in OJD mortality over cohort lifetime.

The 2 proposed cohort-level variables, age and sex, differed in their unconditional association with cohort OJD. Age was not significantly associated with any of the outcome variables (although borderline for OJD pool status at $P=0.05$) but the consistent trend was for lower cohort OJD in 4-year cohorts compared to 3-year old cohorts. This trend is expected as some infected animals born in the 4-year drop would have died as 2-3 year olds and the infected sheep present within enrolled cohorts at the time of faecal collection are likely to be individuals experiencing slower progression of the disease. In contrast, sex was strongly associated with each outcome variable (P-values of at least <0.001 except for IPREV25 where $P=0.05$ using the SECOND dataset and $P=0.02$ using the THIRD dataset) with wethers exhibiting consistently higher cohort OJD levels than ewes. This finding provides strong evidence in support of the anecdotal observation of higher losses in wether mobs. This has been generally attributed to differences in management of wether and ewe mobs, however requires further investigation.

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To account for the influence of these confounders in our investigation of potential history and management risk factors for cohort OJD, the three variables current OJD mortality, cohort age and cohort sex were forced in all multivariable models.

5.3.2 Other explanatory variables

In addition to the confounders, a total of 19 variables were included in one or more of the 8 final multivariable models presented in this report (Table 5.1). These variables, retained in final models due to $P < 0.1$, will be discussed in 3 broad categories (Table 5.2).

Identified associations likely to be a consequence of OJD infection

- *Culling of low body weight sheep* as a method to control OJD was strongly associated with higher cohort OJD. This was understood to be a management response to higher flock infection levels resulting in higher OJD mortalities rather than a cause of higher cohort infection. Two previous studies also identified a significant positive association between culling of clinical or potentially clinical animals and herd/flock status (Lugton, 2004; Muskens et al., 2003) and concluded this practice was a consequence of higher losses not a cause.
- *The number of flock sheep drops vaccinated with Gudair®* as a method to control OJD was associated with higher cohort OJD. Again this was understood to be a management response to higher flock infection rather than cause of it. To be eligible for this study flocks were permitted to have a maximum of four drops vaccinated with Gudair® (that is, sheep born in 2004, 2003, 2001 and 2000) but the cohort sheep had to be unvaccinated. Of 92 flocks, 13 had no vaccinated sheep, 21 had 1 or 2 vaccinated drops, and 58 >2 vaccinated drops. The notable trend for higher odds of higher cohort OJD levels in flocks with 1 or 2 vaccinated drops than flocks with >2 vaccinated drops compared to flocks with no vaccination is likely to reflect 2003/2004 commencement of vaccination in flocks recently experiencing OJD losses (most likely in 3-4 year old sheep) and possibly a reduction in cohort OJD with several drops of younger vaccinated sheep shedding lower MAP and reducing contamination levels across the property.
- *Sale of high loss mobs* as an OJD control method was associated with higher cohort OJD and similarly was understood to be a management response to higher flock infection.

Identified associations related to general property features and management

- Experience of *more severe drought conditions* over the lifetime of the cohort sheep was associated with higher cohort OJD prevalence (in the 2 models for IPREV). This is the first study to investigate the influence of rainfall on OJD and the identified association is likely to represent the effect of poor pasture growth leading to nutritional stress and potentially ingestion of more contaminated soil by cohort sheep.
- Receipt of *run-off water along >10% of the property boundary* was associated with lower cohort OJD. Although run-off from neighbouring infected properties potentially could bring MAP organisms onto the study property and increase property contamination, it is also probable that the additional water supplements water sources on the property and promotes pasture growth which is advantageous particularly during drought conditions.
- Implementation of a *worm control program assessed by interviewer as likely to be effective* was associated with cohort OJD in the one model for pool MAP number shed. Effective worm control is likely to reflect better general disease management in these flocks and potentially a system for

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resting paddocks that could lower MAP contamination across these study properties or in specific paddocks used for lambing ewes and weaners.

- Presence of a *creek that flows intermittently on the study property* was associated with an increase in cohort OJD in one model for IPREV25. Potentially this reflects a lower water supply on these study properties and related poorer pasture growth plus if the creek is not fenced off sheep drinking from stagnant water pools along the creek contaminated by sheep faeces.
- The presence of *wildlife other than kangaroos and rabbits on the study property* was associated with lower cohort OJD in 5 models for cohort OJD prevalence (IPREV and IPREV25). For 29 (32%) study properties the presence of other wildlife species was reported by the owners/managers and included feral pigs for 12 properties, feral goats for 6 properties and wombats for 10 properties. This protective association was an unexpected finding and requires further consideration including consultation with other experts. Although goats and pigs can develop paratuberculosis and shed MAP, goats are widely known to be susceptible to the C strain rather than the S strain (Thoresen and Olsaker, 1994; Whittington et al., 2000b).
- A history of *applying fertilizers such as biosoil, pasture gold, organic manure, reactive phosphorus rock, Mono-ammonium phosphate (MAP), Di-ammonium phosphate (DAP), sewage ash, super potash and pasture special on the property* was associated with lower cohort OJD in 7 models (with $P \leq 0.001$ in 5 models). The owners/managers of 12 (13%) properties reported use of fertilizers other than single super, molybdenum super or lime. The strong protective association of this type of fertilizer application was unexpected and could either be an aberration of the data or a new finding that requires further investigation to identify the influential components of these fertilizers or other factors closely linked to the application of these less common fertilizers.
- A history of *applying single or molybdenum super fertilizer on the property more than once per 3 years* was associated with higher cohort OJD in 2 models. More regular fertilizer application was expected to occur on properties with better pastures and therefore flocks with better nutrition and less OJD disease progression among infected cohort sheep due to less nutritional stress over the cohort lifetime. However, fertilizer application could also be a response to poorer soils and pasture growth or it could lead to higher stocking rates. Further consideration of this finding and assessment of correlation with other explanatory variables is required particularly as two previous studies have found no link between OJD mortality or herd BJD status and fertilizer application (Johnson-Ifearulundu and Kaneene, 1998; Lugton, 2004).
- A history of *applying lime on the property* was associated with lower cohort OJD in 1 model (for IPREV25 using SECOND dataset $P=0.05$). A similar relationship was found by Johnson-Ifearulundu (1999) for BJD herd status in Michigan but none was reported by Lugton (2004) for OJD infected NSW flocks. Lime is usually applied to acidic soils and it has been proposed that the alkalising effect reduces the availability of iron to MAP organisms and subsequently the level of environmental contamination (Johnson-Ifearulundu and Kaneene, 1999).

Identified associations related to flock management

- *Movement of sheep along roads shared by neighbours* was associated with higher cohort OJD in 2 models. Sheep movement along roads accessed by sheep of neighbouring infected flocks could have exposed cohort sheep to areas with higher MAP contamination than present on the study properties. Use of shared roads may also indicate other interactions with neighbouring sheep (although sharing of sheds and frequency of straying sheep were found not to be

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unconditionally associated with cohort OJD in this study) or common property or management features shared with neighbouring properties.

- The association that *sheep cohorts born in autumn or winter* had lower cohort OJD than those born in spring was identified in 4 models. This association was opposite to that expected and could reflect the importance of weather and pasture conditions at weaning rather than during lambing. From weaning the cohort sheep were totally reliant on grazing pasture and where pasture was limited in the weaning paddock/s they could have experienced nutritional stress and consumed more contaminated soil.
- Four models identified that *decontamination of the weaner paddock* was associated with cohort OJD. The identified trend in 3 models was, compared to no decontamination of the weaner paddock/s, lower cohort OJD when the paddock was rested for <8 weeks and higher cohort OJD when the paddock was rested for ≥ 8 weeks. This trend contradicts our belief that longer periods of paddock rest are expected to reduce MAP pasture contamination levels and requires further analysis of the existing data set, particularly an investigation of outliers.
- Over the cohort lifetime, a *total period of growth retardation (or weight loss as adults) of ≥ 12 weeks* was associated with higher cohort OJD in 2 models. Inadequate nutrition and/or severe disease inhibits growth in young sheep and causes weight loss in adult sheep. In particular, nutritional stress could have exposed cohort sheep to higher MAP levels through grazing short pasture and accelerated disease progression in infected sheep by impeding immune function.
- *Stocking rates ≥ 8 dse/ha for cohort sheep in the weaning paddock/s* was associated with higher cohort OJD in 1 model. This association again appears to highlight the importance of pasture conditions for weaners. At higher stocking rates weaners are potentially exposed to higher levels of MAP in the environment and thus higher doses at an age they are more susceptible to infection. Although stocking rate has been investigated in previous cross-sectional studies (Daniels et al., 2002; Lugton, 2004; Reviriego et al., 2000), this is the first study to identify an association of between JD and higher stocking rates. Bush et al. (2004) reported a tentative link between OJD mortality and stocking rate based on finding a reduction in OJD mortality at higher stocking rates.
- Sheep cohorts with a *condition score ≥ 3 at weaning* were associated with lower cohort OJD in 1 model. This is the fourth identified association related to the weaner life stage of cohort sheep. Here sheep cohorts with better body condition at weaning, thus likely to experience less nutritional stress and to have better immune function as weaners, had lower levels of OJD based on PFC at the time of faecal collection.
- Similar to weaner condition score, sheep cohorts with a *condition score ≥ 3 at 1 year old* were associated with lower cohort OJD in 1 model. This indicates that better body condition in hoggets provides some protection against OJD that may relate to lower dose exposure or to the ability of the immune system to impede disease progression.
- Sheep cohorts *weaned at >15 weeks of age* were associated with lower cohort OJD in 1 model (for pool number MAP shed). This finding appears to contradict (Lugton, 2004) who reported OJD mortality at younger age for sheep weaned >5 months of age. However Bush et al. (2004) did report a lower OJD mortality rate for flocks that wean at ≥ 15 weeks based on 12 flocks and this association could be another indicator of the relative importance of the weaner life stage compared to suckling lamb on OJD faecal shedding at 3-4 years old.

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Table 5.1

Description of the effect on cohort OJD of the 19 variables included in one or more of the 8 final multivariable models presented in this report

Variable	Number of models where variable is present	Effect on outcome variables	Description of trend in effect
OTHERFERT	7	Protective	Reduction in cohort OJD in flocks with history of applying fertilisers other than single super, molybdenum super and lime on the property
CULL	6	Detrimental	Increase in cohort OJD in flocks where producers cull low body weight sheep
OTHERWILDLIFE	5	Protective	Reduction in cohort OJD in flocks where wild animals other than kangaroos and rabbits are present on the property
LBGSSN	4	Protective	Reduction in cohort OJD when cohort sheep were born in autumn or winter rather than spring
RUNOFFWATER	4	Protective	Reduction in cohort OJD in flocks where the property receives run off water along >10% of the property boundary
DECONT_WNGPDK	4	Mixed in 3 models Detrimental in 1 model	Reduction in cohort OJD where the cohort weaner paddock was decontaminated for <8 weeks Increase in cohort OJD where the cohort weaner paddock was decontaminated for ≥8 weeks
DROPSVACC	3	Mixed in 1 model Detrimental in 2 models	Increase in cohort OJD in flocks where one or more drops are vaccinated with the Gudair® vaccine
DROUGHT	2	Detrimental	Increase in cohort OJD in flocks that received on average annual total rainfall >150mm below the district long term average over the lifetime of the cohort
SHARING_ROAD	2	Detrimental	Increase in cohort OJD in flocks that move sheep along roads shared by neighbours
SUPERFREQ	2	Mixed in 1 model Detrimental in 1 models	Increase in cohort OJD in flocks with a history of applying single super and molybdenum super fertilizers on the property > once in 3 years
GROWTHCHK	2	Detrimental	Increase in cohort OJD where the cohort sheep experienced growth retardation (or weight loss as adults) for a total period of ≥12 weeks over their lifetime

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WNRSR	1	Detrimental	Increase in cohort OJD when the cohort stocking rate in the weaning paddock/s was ≥ 8 dse/ha
ICREEK	1	Detrimental	Increase in cohort OJD in flocks where an intermittent creek flows onto the property
HGTCS	1	Protective	Reduction in cohort OJD for cohort sheep that had a condition score ≥ 3 at 1 year of age
WNRCS	1	Protective	Reduction in cohort OJD for cohort sheep that had a condition score ≥ 3 at weaning
LIME	1	Protective	Reduction in cohort OJD in flocks with history of lime application on the property
SELL	1	Detrimental	Increase in cohort OJD in flocks where producers sell high loss mobs
WORMCONTROL	1	Protective	Reduction in cohort OJD in flocks where producers implement a worm control program that is likely to be effective
WNGAGE	1	Protective	Reduction in cohort OJD when cohort sheep were weaned at >15 weeks of age

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Table 5.2

P-values for each variable included in the 8 final multivariable models presented in this report

Variables	IPREV			IPREV25			Pool OJD status	Pool MAP number shed
	FIRST dataset	SECOND dataset	THIRD dataset	FIRST dataset	SECOND dataset	THIRD dataset		
<i>Consequence of OJD infection</i>								
CULL	<0.001			0.08	<0.001	0.02	0.003	0.008
DROPVACC	<0.001		0.03					0.007
SELL								0.016
<i>General property features and management</i>								
OTHERFERT		<0.001	<0.001	<0.001	<0.001	0.03	0.004	0.001
OTHERWILDLIFE	0.03	<0.001	0.01		<0.001	0.02		
RUNOFFWATER		0.02	0.05				0.01	0.001
DROUGHT	0.07	0.01						
SUPERFREQ	<0.001							0.013
ICREEK				0.1				
LIME					0.05			
WORMCONTROL								0.02
<i>Flock management</i>								
LBGSSN	<0.001	<0.001	<0.001			0.03		
DECONT_WNGPDK				0.01	0.05		0.05	0.002
SHARING_ROAD		0.05					0.06	
GROWTHCHK				<0.001				0.003
WNRSR				0.03				
HGTCS		0.05						
WNRCS					0.09			
WNGAGE								0.04

5.4 Soil variables associated with cohort OJD

5.4.1 Confounding variable

Parent soil type, the single proposed property-level confounder among the soil explanatory variables, was not significantly unconditionally associated with the 2 cohort OJD prevalence outcome variables (although borderline for IPREV at $P=0.05$ using the SECOND dataset) but had a strong unconditional association with the 2 pool outcome variables ($P<0.001$). However a similar trend was present in all univariable results, where compared to properties with basaltic soil, properties with shale and sandstone soils had higher cohort OJD and properties with granite soil or mixed soils (including and excluding limestone) had lower cohort OJD. Of two previous studies that investigated soil type (based on regional classification of soil type), our result contrasts with the finding of Reviriego et al. (2000) in Spain (who found soil type was 1 of 2 predictor variables included in the final multivariable model for flock seroprevalence) and aligns with that of Johnson-Ifeorlundu and Kanenne (1999) in Michigan (who report a univariable $P=0.651$ for soil type).

We considered parent soil type to be a potential confounder in our investigation of potential soil risk factors for cohort OJD and included it in addition to current OJD mortality, cohort age and cohort sex in all multivariable analyses. Inclusion recognised the close link between soil type and the soil composition variables under investigation and allowed measurement of the effect of these variables after adjustment for soil type, and the potential correlation among properties situated on similar soil (the reason also given by Johnson-Ifeorlundu and Kaneene (1999) for inclusion in multivariable models).

5.4.2 Other explanatory variables

In addition to the confounders, a total of 12 variables were found to be significant ($P<0.1$) in the 32 final multivariable models presented in this report (Table 5.3). P values of these variables are presented in Table 5.4. Overall, soil fertility and OJD status were related as higher cohort OJD prevalence was linked with an increase in cation exchange capacity, phosphorus buffer index and phosphorus level of the soil (both mean of 3 paddocks as well as individual paddocks). Also, higher OJD prevalence was associated with soils having higher proportions of silt and clay and lower proportions of sand. This is apparently contrary to the findings of Lugton (2004) where more OJD was found in flocks raised on light texture soils, but the comparability of the soil descriptors in the two studies is not yet clear.

The cation exchange capacity of the soil (CEC) indicates its ability to hold cations such as calcium, magnesium, potassium and sodium. The greater the CEC, the greater is the ability of the soil to supply these important nutrients to plants. CEC, in turn, is dependent on the proportion of clay in the soil and increases as % clay in the soil increases. Clay particles are negatively charged and have a large surface area and thus are capable of holding huge quantities of cations. Both CEC and % clay of the soil were associated with higher OJD in the cohorts in the present study. Also, CEC and % clay were highly correlated (Spearman correlation coefficient >0.5 , $P < 0.001$ in all 4 datasets). It has been reported previously that *M. avium* binds to clay particles and a similar phenomenon has been inferred for *M. paratuberculosis* (Brooks et al., 1984; Whittington et al., 2003). Attachment of *M. paratuberculosis* to clay particles could increase the availability of the organism to sheep by maintaining it in the upper soil layers rather than allowing it to be leached to deeper layers. The % sand of the soil was associated with lower OJD prevalence in cohorts in the present study. Also, CEC and sand % were negatively correlated (Spearman correlation coefficient < -0.3 , $P < 0.01$ in all 4 datasets). These observations support the hypothesis of higher uptake of organisms in soils with a higher % clay and CEC and a lower uptake in soils with higher % sand.

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The CEC of the soil is also enhanced in the presence of organic matter and therefore is also considered as an indirect indicator of the amount of organic matter in the soil. The latter may favour survival of *M. paratuberculosis* by providing essential nutrients for its continued existence outside biological host and may be one of the reasons for higher OJD on such properties. It may also increase the pasture growth, which may again help survival of the organism. Higher organic matter levels in the soil could also be a confounder for a higher stocking rate where more faeces may get mixed with the soil over time.

A higher phosphorus level of soil and a higher phosphorus buffer index (PBI) - both indicators of better fertility of the soil - were associated with increased OJD prevalence in the cohorts. Higher fertility may be acting as a confounder for some flock management practices or may be associated with better uptake of the organism or greater survivability of the organism (by improved pasture growth resulting in more shade or by some implicit mechanism *hitherto* unknown). However, expert opinion or further studies are required to fully elucidate the relationship between higher fertility of soil with increased OJD in the cohorts.

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Table 5.3

Description of the effect on cohort OJD of the 12 variables included in one or more of the 32 final multivariable models presented in this report

Variable	Number of models where variable is present	Effect on outcome variables	Description of trend in effect
3-paddock mean variables			
PBI	6	Detrimental	Greater risk of OJD in cohorts raised on properties having soil phosphorus buffer index >70
SILT	2	Detrimental	Greater risk of OJD in cohorts raised on properties having soil silt percentage >21%
CLAY	2	Detrimental	Greater risk of OJD in cohorts raised on properties having soil clay percentage >15%
CEC	1	Detrimental	Greater risk of OJD in cohorts raised on properties having soil of cation exchange capacity of >6 Meq/100g
Lambing paddock variables			
P_LBGPDK	4	Detrimental	Greater risk of OJD in cohorts with increase in soil phosphorus level for the paddock on which they were lambed
SAND_LAMBINGPDK	2	Protective	Less risk of OJD in cohorts lambed on soils with sand percentage >62%
Weaner paddock variables			
SILT_WNGPDK	6	Detrimental	Greater risk of OJD in cohorts weaned on paddocks having soil silt percentage >21%
PBI_WNGPDK	2	Detrimental	Greater risk of OJD in cohorts weaned on paddocks having soil phosphorus buffer index >70
ALSAST_WNGPDK	1	Mixed	Greater risk of OJD in cohorts where weaning paddock has aluminium saturation percentage of 2-5 and >12 % while lesser risk in those with aluminium saturation >5-12%, as compared with a reference level of ≤2%
Hogget/adult paddock variables			
CEC_ADPDK	4	Detrimental	Greater risk of OJD in cohorts when the cation exchange capacity of the soil of hogget/adult paddock was >6 Meq/100g
SILT_ADPDK	3	Detrimental	Greater risk of OJD in cohorts where the hogget/adult paddock soil silt percentage was >21%
SAND_ADPDK	3	Protective	Less risk of OJD in cohorts when the sand percentage of the soil of hogget/adult paddock was >62%

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Table 5.4

P-values for each soil variable included in the 32 final multivariable models presented in this report

Variables	IPREV			IPREV25			Pool OJD status	Pool MAP number shed
	FIRST dataset	SECOND dataset	THIRD dataset	FIRST dataset	SECOND dataset	THIRD dataset		
3-paddock mean variables								
CEC	0.01							
PBI		0.06	0.03		0.07	0.04	0.02	0.01
SILT	0.03			0.07				
CLAY		0.01	0.10					
Lambing paddock variables								
P_LBGPDK			0.02			0.08	0.01	0.09
SAND_LAMBINGPDK	0.03	0.07						
Weaner paddock variables								
PBI_WNGPDK							0.01	0.003
ALSAST_WNGPDK	0.02							
SILT_WNGPDK	<0.001	0.01	0.01	0.006	0.01	0.004		
Hogget/adult paddock variables								
CEC_ADPPDK	0.06	0.02	0.05					0.01
SILT_ADPPDK	0.03					0.02	0.10	
SAND_ADPPDK		0.09	0.10		0.07			

5.5 Comparison with the findings of the Lugton study

This cross-sectional study, with outcome variables based on culture of faeces of specific age cohorts of sheep and explanatory variables based on information gathered by personal interview and analysis of soil samples, obtained data that is more reliable than the previous postal survey conducted by Lugton in 2000 (Lugton, 2004). Further several features of the statistical analyses used in this study have produced consistent and potentially more credible results:

- Assessment of potential confounders
- Adjustment for critical confounders by their inclusion as fixed effects in the multivariable models
- Evaluation of explanatory variables by measuring association with cohort outcome and pool outcome variables
- Inclusion of flock as a random effect in some multivariable analyses to minimise effect of disease clustering within flock.

Table 5.5 lists the variables included in final analyses for reported OJD mortality (occurrence of OJD mortalities within flock and incidence of OJD mortalities) and for reported age of youngest OJD mortality within flock published by Lugton (2004) and summarises the associations identified in this previous study and the current study. In addition Lugton reported variables associated with presence of scouring as a clinical sign of OJD and season of reported peak OJD incidence but, as no equivalent outcomes were investigated in this study, these are not considered.

Five variables (breed, altitude, lamb marking percentage, culling age and proportion of quality pasture) identified by Lugton as associated with reported flock OJD mortality were not investigated as explanatory variables in this study. Breed, though recognised as a potential risk factor, was essentially excluded by selection of self-replacing Merino flocks in order to enrol flocks with comparable structure and management. This similarity provided opportunity to investigate more subtle differences in cohort experience over their lifetime. Altitude was not considered a risk factor in its own right but rather to reflect proposed explanatory variables related to soil type and rainfall. The general flock characteristics of average lamb marking percentage and age at culling were considered unlikely to impact the OJD status of 3-4 year old sheep in enrolled flocks. Detailed information on cohort stocking rate and nutrition was obtained in this study to investigate the factors related to proportion of quality pasture proposed by Lugton.

Detrimental associations for duration of OJD infection and removal of clinical sheep identified in both studies were recognised to be a consequence of flock OJD infection. Inadequate nutrition, represented by duration of supplemental feeding, was shown by both studies to adversely impact OJD. The protective effect of sheep introductions identified by Lugton was supported for rams only in this study but requires recategorisation to separate the effect of ram introductions from non-infected and infected sources. The higher risk related to ewe/wether introductions in this study may reflect that ewes and wethers are likely to be introduced in larger numbers and from more sources than rams. The results for weaning age conflict and further consideration of the relative impact of MAP exposure as suckling lambs and as weaners is required to understand the protective association indicated by this and the 12 farm study (MLA OJD.023).

A comparison of soil factors between the two studies will require access to raw data from the Lugton trial and involvement of a soil scientist to ensure that the soil descriptors are comparable between the studies.

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Table 5.5

Description of the associations identified between variables and OJD occurrence in the Lugton study and this study

Variable	Association with OJD occurrence	
	Lugton study	Current study
Duration of OJD infection	Detrimental More OJD with increase in duration of flock infection	Detrimental More OJD with increase in duration of flock infection
Removal of clinical sheep	Detrimental More OJD with removal of clinical sheep	Detrimental More OJD with culling of clinical sheep and sale of high loss mobs
Duration of supplementary feeding	Detrimental More OJD with increase in duration of supplementary feeding	Detrimental ^a More OJD with increase in duration of supplementary feeding
Introduction of sheep to flock	Protective Less OJD with introduction of sheep to flock	Mixed ^a Less OJD with introduction of rams to flock More OJD with introduction of ewes and/or wethers to flock
Higher age at weaning	Detrimental More OJD when sheep weaned at >5 months of age	Protective Less OJD when sheep weaned at >15 weeks of age
Light soil texture	Detrimental More OJD in flocks on properties with light soil texture	Protective More OJD in flocks on properties with fertile soil

a Unconditional association identified only in univariable analyses.

6 Success in Achieving Objectives

The objectives of this study were successfully achieved and findings related to each objective are summarised below:

- **To survey 100 producers with known OJD-infected sheep flocks.**

Ninety-two OJD-infected sheep flocks were enrolled in this study. Data collection for each enrolled flock involved the administration of a questionnaire via a face-to-face interview with the owner/manager plus the collection of faecal samples from 210 cohort sheep and of soil samples from 3 paddocks grazed by the cohort sheep during specified cohort life stages.

To enrol the study flocks, a total of 233 OJD-infected flocks were assessed against the revised selection criteria to identify eligible flocks and then owner approval for study participation sought. The number of eligible flocks identified was less than the target of 100 largely due to the combined impact of increasing use of Gudair® vaccination and of reduction in stock numbers in response to the drought since 2002. Of 97 eligible flocks identified, owner approval for study participation was obtained for 92. Substantial effort was made to identify eligible flocks and obtain owner permission for study participation.

- **To classify flocks as high or low prevalence on the basis of PFC testing results, and collect information on potential risk-factors for OJD.**

A total of 717 pooled faecal samples were collected from 124 sheep cohorts in this study. By applying the Williams and Moffitt method (2001) the PFC results for these pools were used to determine the animal-level OJD prevalence for each sheep cohort. Cohorts were classified into low, medium and high categories using two different sets of cut-off figures (for IPREV, <2% prevalence, 2-10% prevalence and >10% prevalence; for IPREV25, <2% prevalence, 2-5% prevalence and >5% prevalence). The number of sheep cohorts in each category was 34 low, 60 medium and 30 high for IPREV and 34 low, 34 medium and 56 high for IPREV25.

Information obtained about each study flock from the completed questionnaires, specimen advice forms and soil analysis results was used to create the potential explanatory factors for OJD cohort prevalence, pool OJD status and pool Map number shed investigated in this study. The explanatory variables assessed included 71 history and management variables and 44 soil variables.

- **To identify using univariate and multivariate analyses factors with a statistically significant relationship with PFC prevalence, and quantify the magnitude of any relationships.**

The association between proposed explanatory variables and cohort OJD prevalence was investigated using univariable and multivariable analyses. Cohort OJD prevalence was represented by two outcomes IPREV and IPREV25 that classified cohorts into low, medium and high prevalence using the cut-offs <2% prevalence, 2-10% prevalence and >10% prevalence for IPREV and <2% prevalence, 2-5% prevalence and >5% prevalence for IPREV25.

The explanatory variables identified by univariable analyses as unconditionally associated with IPREV with $P < 0.25$ included 31 history and management variables (Table 4.16) and 24 soil variables for the FIRST dataset.

The three final multivariable logistic regression models for IPREV using the FIRST, SECOND and THIRD datasets demonstrated that the presence of other wildlife (aside from kangaroos and rabbits)

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and season of cohort birth were strongly associated with cohort OJD prevalence level. Variables associated with cohort prevalence level in models for two datasets included drought over cohort lifetime, the number of age groups vaccinated in the flock, application of fertilizers other than single super, molybdenum super and lime on the property, and the proportion of property boundary receiving run off water (Tables 4.17, 4.18 and 4.19). The final multivariable models of the soil variables identified associations with 4 3-paddock mean variables (cation exchange capacity and silt percentage for FIRST dataset, phosphorous buffer index and clay percentage for SECOND and THIRD datasets), one weaning paddock variable (silt percentage) and one hogget/adult paddock variable (cationic exchange capacity) (Table 4.27).

For IPREV25, univariable analyses identified unconditional associations $P < 0.25$ for 24 history and management variables (Table 4.16) and 16 soil variables for the FIRST dataset.

Multivariable analyses for IPREV25, in the three final models for the FIRST, SECOND and THIRD datasets, demonstrated that culling of low body weight sheep and the application of fertilizers other than single super, molybdenum super and lime on the property were strongly associated with cohort OJD prevalence level. Variables associated with cohort prevalence level in models for two datasets included the presence of other wildlife (aside from kangaroos and rabbits) and length of OJD decontamination of the weaner paddock/s (requires further analysis) (Tables 4.20, 4.21 and 4.22). Of the soil variables, two 3-paddock mean variables (phosphorus buffer index and silt percentage), one weaning paddock variable (silt percentage) and two hogget/adult paddock variables (silt percentage and sand percentage) were included in the final multivariable models (Table 4.28).

The direction and magnitude of each identified association is discussed in Section 5.

- **To identify important potential confounding factors such as time since infection, purchasing history, vaccination history and culling practices, and take these into account in flock selection, and data collection and analysis.**

There was a strong relationship between the PFC results and the duration of flock infection, the level of OJD mortality and the trend in OJD mortality, as well as a relation with parent soil type. There was also a consistent but statistically nonsignificant trend for lower OJD levels in 4-year olds compared to 3-year olds, may be due to deaths of affected sheep from 2 to 3 years of age. Wethers had consistent and statistically significant higher OJD levels than ewes, which supports the anecdotal observation of higher losses in wether mobs. As these relationships were likely to confound the evaluation of farm and flock management, current OJD mortality, cohort age and cohort sex were forced in all multivariable models evaluating history and management variables and these three plus parent soil type were forced into models evaluating soil variables. This ensured that their effect was taken into account and other factors were correctly identified.

In addition, during study design the potential confounders recent infection, vaccination and breed were considered. Use of selection criteria that restricted study flocks to self-replacing Merino flocks infected for >3 years and with unvaccinated 3-5 year old sheep aided to reduce their influence on the study results.

In this study aspects of purchasing history (e.g. introductions), vaccination history (e.g. number of drops vaccinated) and culling practices (e.g. culling of low body weight sheep), were investigated as potential explanatory variables in order to assess their influence after adjustment for variables considered to be critical confounders.

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- **To identify risk factors for the level of faecal shedding in OJD-infected flocks.**

Two pool-level outcome variables were investigated in this study – pool OJD status (positive or negative) and pool MAP number shed. This enabled identification of explanatory variables associated with whether a pool was infected or not (binary outcome), as well as of variables associated with the log MAP number present in a pool (continuous outcome).

For pool OJD status, history and management variables identified as closely associated in the final mixed logistic regression model included application of fertilizers other than single super, molybdenum super and lime on the property, the proportion of property boundary receiving run off water, culling of low body weight sheep, length of OJD decontamination of the weaner paddock/s (requires further analysis) and frequency of sharing roads with neighbours (Table 4.24). Soil variables found to be associated with pool OJD status included phosphorus buffer index (3-paddock mean and weaning paddock), phosphorus content of lamb paddock and silt percentage of adult paddock (Tables 4.29 and 4.30).

History and explanatory variables found to be associated with log pool MAP number shed in the final model (Table 4.26) included the application of fertilizers (single super or molybdenum super and other fertilizers (aside from single super, molybdenum super and lime)) on the property, the proportion of property boundary receiving run off water, culling of low body weight sheep, sale of high loss mobs, the period of growth retardation (or weight loss in adult sheep) during cohort lifetime, the length of OJD decontamination of the weaner paddock/s (requires further analysis), number of vaccinated drops in flock, implementation of an effective worm control program and cohort age at weaning. Among the soil variables, phosphorus buffer index (3-paddock mean and lamb paddock) and phosphorus content of soil in lambing paddock and cation exchange capacity of soil in adult paddock were associated in the final models with the log pool MAP number shed (Tables 4.31 and 4.32).

7 Impact on Meat and Livestock Industry – now & in five years time

The key findings of this study include the identification of farm and sheep management factors and soil characteristics associated with occurrence of high levels of OJD. Many of these factors can be modified by producers to reduce the impact of OJD. Weaner management and nutrition are important examples. The results are broadly applicable and complement those of experimental OJD transmission in MLA project OJD.028. Given that management and structure of self-replacing Merino flocks is similar across the known OJD infected districts of Australia, enrolment of flocks from the majority of known infected states strengthens the case for resulting recommendations to be applicable in all OJD infected districts. With the introduction of risk-based trading it is likely that infection will continue to spread and the recommendations from this study will continue to be relevant to newly diagnosed flocks over the coming years.

Some of the findings of the study will require further investigation before they can be verified and understood. An important example is the association between high soil fertility and clay content and increased occurrence of OJD. Although modulation of soil characteristics is possible through application of soil conditioners, the potential benefits of this are as yet unclear. Within five years, future research may be able to assess and clarify the issues related to these soil factors and potentially to provide appropriate recommendations for producers.

8 Conclusions and Recommendations

The outcomes of this study include the identification of risk factors for expression of OJD on farms in Australia that help to explain why the level of clinical disease experienced due to *M. paratuberculosis* appears to vary considerably between infected sheep flocks, even those in the same locality which appear superficially to have similar characteristics. Over recent years the observation of variation between flocks has led to considerable speculation among producers and scientists as to the potential importance of flock management, soil type, pH and micro-nutrients. Sound understanding of the factors that influence disease expression will lead to management recommendations that improve on-farm disease control. This project consisted of a cross-sectional study on 92 infected properties located in New South Wales, Victoria, Tasmania and Western Australia and so the results are broadly applicable.

As expected there was a strong relationship between the PFC results used to estimate prevalence/severity of OJD and the duration of flock infection, the level of OJD mortality and the trend in OJD mortality, as well as a relation with parent soil type. There was also a consistent although statistically nonsignificant trend for lower OJD levels in 4-year olds compared to 3-year olds, due likely to deaths of affected sheep from 2 to 3 years of age. Wethers had consistent and statistically significant higher OJD levels than ewes, which supports the anecdotal observation of higher losses in wether mobs. As parent soil type, age, sex and current OJD mortality were likely to confound the evaluation of farm and flock management, they were included in all multivariable models so that their effects could be taken into account and other factors correctly identified. A total of 31 significant farm/flock/management and soil variables were found across one or more of the final multivariable models. Some were likely to be a consequence of OJD infection, but the remainder appeared to be potential risk factors for the severity of the disease.

Three variables were likely to be a consequence of OJD infection and were management responses to higher flock infection rates: culling of low body weight sheep as a method to control OJD, the number of lamb drops vaccinated with Gudair® as a method to control OJD and the sale of high loss mobs as an OJD control method.

Eight variables were related to property features and management: severe drought conditions over a sheep lifetime (higher OJD prevalence), receipt of run-off water along >10% of the property boundary (lower OJD prevalence); implementation of a worm control program assessed by interviewer as likely to be effective (lower OJD prevalence); presence of a creek that flows intermittently on the study property (higher OJD prevalence); the presence of wildlife other than kangaroos and rabbits on the study property (lower OJD prevalence); a history of applying fertilizers other than single super, molybdenum super or lime (for example biosoil) (lower OJD prevalence) (this appeared to be very important as it was identified in 7 models, $P \leq 0.001$ in 5); a history of applying single or molybdenum super fertilizer on the property more than once per 3 years was associated (higher OJD prevalence); a history of applying lime on the property (lower OJD prevalence).

Eight variables were related to flock management: movement of sheep along roads shared by neighbours (higher OJD prevalence); sheep cohorts born in autumn or winter (lower OJD prevalence) than those born in spring; decontamination of the weaner paddock (requires further analysis); the total period of growth retardation over the lifetime of sheep (or weight loss as adults) of ≥ 12 weeks (higher OJD prevalence); stocking rates ≥ 8 dse/ha in weaning paddock/s (higher OJD prevalence); sheep with condition score ≥ 3 at weaning (lower OJD prevalence); sheep with condition

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score ≥ 3 at 1 year old (lower OJD prevalence); sheep weaned at >15 weeks of age (lower OJD prevalence).

Twelve variables related to soil characteristics. Higher OJD prevalence was linked to an increase in cation exchange capacity (CEC), phosphorus buffer index and phosphorus level, soils having higher proportions of silt and clay and lower proportions of sand. These factors are related to high levels of soil fertility.

The factors identified in this study provide insight into some of the factors that interact to modulate the prevalence of OJD in sheep flocks. The findings support those of MLA trials OJD.028 and OJD.023 and suggest that pasture and flock management strategies can be devised to reduce the impact of OJD. This will have immediate impact for the industry by providing alternative and complementary strategies to vaccination for the control of OJD.

Recommendations:

- 1. It is recommended that there be further evaluation of the following factors through expert consultation and/or further analysis of the data:**
 - The presence of wildlife other than kangaroos and rabbits on the study property as the reasons for this being associated with lower OJD prevalence are unclear.
 - Factors related to pasture improvement: a history of applying fertilizers other than single super, molybdenum super or lime (for example biosoil) and a history of applying lime (lower OJD prevalence), and a history of applying single or molybdenum super fertilizer on the property more than once per 3 years (higher OJD prevalence). All treatments may change pasture composition and abundance. These factors may be correlated with pasture factors that were not assessed in the study.
 - Decontamination of the weaner paddock. There was higher OJD prevalence when the paddock was rested for ≥ 8 weeks and lower OJD prevalence when the paddock was rested for < 8 weeks. This is counterintuitive as MAP viability is believed to decline quickly (90% per month) within faeces and soil on farm. Further analysis of the existing data set should be undertaken to rule out the influence of a small number of properties with atypical prevalence or explanatory variables.
 - Factors related to time of lambing and age at weaning as the results of this study conflict with current management recommendations that are to lamb in spring to optimize lactation and lamb growth and to wean at 12-14 weeks to minimize lactational stress on ewes.

- 2. It is recommended that advisory material be prepared and distributed to producers with the following advice related to identified risk factors where the mechanism is understood:**
 - Maintain effective worm control. This is likely to be reflected in better general health management. Spelling paddocks as a component of worm control may also be beneficial as it could lower MAP contamination. With respect to concurrent recommendations for worm control, infective third stage nematode larvae (L3) develop from ova in faeces in 5 days under optimal conditions (warm, moist) but this may be delayed up to a few weeks in cool dry conditions. The L3 remain viable in moist cool conditions for about 6 months, or until a heavy frost penetrates the detritus layer on soil or until hot dry conditions lead to its desiccation. In a grazing rotation of more than a few days, the most common grazing scenario except for high stocking rate cell grazing systems, many infective larvae would be present on pasture at the

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time of commencement of spelling, so that very prolonged spelling would be required to render the pastures safe. In high stocking rate cell grazing systems where the inter-rotation interval is short, sheep would return to pastures that harbour L3 within the lifespan of the L3 deposited in the previous grazing rotation. Therefore in order to control both internal parasitism and OJD through a reduction in the level of contamination of pasture, regardless of the length of the grazing rotation, prolonged pasture spelling preferably including a full summer is required. A possible disadvantage of this approach for parasite control is the potential loss of sub-populations containing drench-susceptible nematodes, necessitating carefully planned drenching programs.

- Remove access by stock to intermittent creeks. Discourage sheep drinking from stagnant pools contaminated by sheep faeces.
 - Avoid movement of sheep along roads shared by neighbours as this could expose sheep to areas with higher MAP contamination than present on the home farm.
 - Optimise weaner to hogget management
 - Maintain adequate weaner nutrition to avoid periods of growth retardation
 - Avoid stocking rates ≥ 8 dse/ha in weaning paddocks
 - Maintain sheep with condition score ≥ 3 at weaning through to 1 year old
 - Nutritional stress and higher stocking rates could expose sheep to higher MAP levels through grazing short pasture, lead to consumption of more contaminated soil and accelerated disease progression by impeding immune function.
 - Grazing and pasture management recommendations should be harmonised with those from MLA projects OJD.002A and OJD.028 when they are prepared.
- 3. It is recommended that further research be undertaken to explain soil risk factors:**
- Higher OJD prevalence was linked to an increase in cation exchange capacity (CEC), phosphorus buffer index and phosphorus level, soils having higher proportions of silt and clay and lower proportions of sand. This suggests a detrimental affect of soil fertility on OJD level as CEC, phosphorus and phosphorus buffer index are indicators of fertility. CEC is enhanced by organic matter and therefore is considered as an indirect indicator of organic matter in soil. The CEC is dependent on the proportion of clay in the soil and increases as % clay increases. Clay particles are negatively charged and are known to bind *M. paratuberculosis*. This could increase the availability of the organism to sheep. In sandy soils the organism may be leached to deeper soil layers and not be available to sheep. Research is recommended on:
 - The movement of MAP in soil of different composition to explore the hypothesis that MAP remains in the surface layers of soil with high clay content/organic content/CEC, and moves quickly into deeper layers in sandy soils
 - The binding of MAP to clay, organic matter and other soil components
 - The feeding behaviour of sheep on clay rich and sandy soils with respect to amount of soil ingested.

9 Bibliography

- Anonymous 2005. The GLIMMIX Procedure (SAS Institute Inc., Cary, NC).
- Armitage, P., Berry, G., Matthews, J.N.S., 2002, Modelling continuous data, In: Statistical methods in medical research. Blackwell Science, Massachusetts, pp. 312-375.
- Brooks, R.W., George, K.L., Parker, B.C., Falkinham, J.O., 3rd, Gruff, H., 1984, Recovery and survival of nontuberculous mycobacteria under various growth and decontamination conditions. *Can J Microbiol* 30, 1112-1117.
- Brown, H., Prescott, R., 2000, Applied Mixed Models in Medicine. John Wiley and Sons.
- Bush, R., 2004. A study of the biological and economic impact of OJD in affected sheep flocks in NSW. In: *Ovine Johne's Disease: An update of Australian and International Research*, Sydney, Australia, March 2004, pp. 46-48.
- Cameron, A., Sergeant, E., Baldock, C., 2004, Data Management for Animal Health, Vol 1, First Edition. AusVet Animal Health Services, Brisbane, Australia, 185 p.
- Cetinkaya, B., Erdogan, H.M., Morgan, K.L., 1997, Relationships between the presence of Johne's disease and farm and management factors in dairy cattle in England. *Preventive Veterinary Medicine* 32, 253-266.
- Citer, L., Sergeant, E. 2004 (Canberra, Animal Health Australia), p. 13.
- Cousins, D.V., Whittington, R., Marsh, I., Masters, A., Evans, R.J., Kluver, P., 1999, Mycobacteria distinct from *Mycobacterium avium* subsp. *paratuberculosis* isolated from the faeces of ruminants possess IS900-like sequences detectable IS900 polymerase chain reaction: implications for diagnosis. *Molecular & Cellular Probes* 13, 431-442.
- Cowling, D.W., Gardner, I.A., Johnson, W.O., 1999, Comparison of methods for estimation of individual-level prevalence based on pooled samples. *Prev Vet Med* 39, 211-225.
- Daniels, M.J., Hutchings, M.R., Allcroft, D.J., McKendrick, I.J., Greig, A., 2002, Risk factors for Johne's disease in Scotland - the results of a survey of farmers. *Veterinary Record* 150, 135-139.
- Dohoo, I., W., M., Stryhn, H., 2004a, Chapter 3. Questionnaire Design, In: *Veterinary Epidemiologic Research*. AVC Inc., Charlottetown, Prince Edward Island, Canada, p. 320.
- Dohoo, I., W., M., Stryhn, H., 2004b, Chapter 12. Validity in observational studies, In: *Veterinary Epidemiologic Research*. AVC Inc., Charlottetown, Prince Edward Island, Canada, pp. 207-235.
- Johnson-Ifearegulu, Y., Kaneene, J.B., 1999, Distribution and environmental risk factors for paratuberculosis in dairy cattle herds in Michigan. *American Journal of Veterinary Research* 60, 589-596.
- Johnson-Ifearegulu, Y.J., Kaneene, J.B., 1998, Management-related risk factors for *M. paratuberculosis* infection in Michigan, USA, dairy herds. *Preventive Veterinary Medicine* 37, 41-54.
- Kline, R.L., Brothers, T.A., Brookmeyer, R., Zeger, S., Quinn, T.C., 1989, Evaluation of human immunodeficiency virus seroprevalence in population surveys using pooled sera. *J Clin Microbiol* 27, 1449-1452.
- Leeper, G.W., Uren, N.C., 1993, *Soil Science: an introduction*. Melbourne University Press, Melbourne.
- Lugton, I.W., 2004, Cross-sectional study of risk factors for the clinical expression of ovine Johne's disease on New South Wales farms. *Aust Vet J* 82, 355-365.
- Mainar-Jaime, R.C., Vazquez-Boland, J.A., 1998, Factors associated with seroprevalence to *Mycobacterium paratuberculosis* in small-ruminant farms in the Madrid region (Spain). *Preventive Veterinary Medicine* 34, 317-327.

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

- Muskens, J., Elbers, A.R., van Weering, H.J., Noordhuizen, J.P., 2003, Herd management practices associated with paratuberculosis seroprevalence in Dutch dairy herds. *Journal of Veterinary Medicine Series B* 50, 372-377.
- Obasanjo, I., Grohn, Y.T., Mohammed, H.O., 1997, Farm factors associated with the presence of *Mycobacterium paratuberculosis* infection in dairy herds on the New York State paratuberculosis control program. *Preventive Veterinary Medicine* 32, 243-251.
- Reddacliff, L.A., Nicholls, P.J., Vadali, A., Whittington, R.J., 2003, Use of growth indices from radiometric culture for quantification of sheep strains of *Mycobacterium avium* subsp. *paratuberculosis*. *Applied & Environmental Microbiology* 69, 3510-3516.
- Reviriego, F.J., Moreno, M.A., Dominguez, L., 2000, Soil type as a putative risk factor of ovine and caprine paratuberculosis seropositivity in Spain. *Prev Vet Med* 43, 43-51.
- Sacks, J.M., Bolin, S.R., Crowder, S.V., 1989, Prevalence estimation from pooled samples. *Am J Vet Res* 50, 205-206.
- Schabenberger, O., 2005. Introducing the GLIMMIX Procedure for Generalized Linear Mixed Models. In: SAS® USERS GROUP INTERNATIONAL, Philadelphia, April 10-13, 2005.
- Sergeant, E. 2004. Pooled Prevalence Calculator (Aus Vet Animal Health Services).
- Sergeant, E.S.G., Whittington, R.J., More, S.J., 2001, Sensitivity and specificity of pooled faecal culture and serology as flock-screening tests for detection of ovine paratuberculosis in Australia. *Preventive Veterinary Medicine* 52, 199-211.
- Stokes, M.E., Davis, C.S., Koch, G.G., 2000, *Categorical Data Analysis using The SAS system*, Second Edition. SAS Institute Inc., Cary, N.C.
- Thoresen, O.F., Olsaker, I., 1994, Distribution and hybridization patterns of the insertion element IS900 in clinical isolates of *Mycobacterium paratuberculosis*. *Veterinary Microbiology* 40, 293-303.
- Toribio, J.A., Bush, R., Windsor, P. 2004. A study of the biological and economic impact of OJD in affected sheep flocks in NSW. (Sydney, Faculty of Veterinary Science, University of Sydney).
- Tu, X.M., Litvak, E., Pagano, M., 1994, Studies of AIDS and HIV surveillance. Screening tests: can we get more by doing less? *Stat Med* 13, 1905-1919.
- Whitlock, R.H., Rosenberger, A.E., 1990, Fecal culture protocol for *Mycobacterium paratuberculosis*. A recommended procedure. *Proceedings - Annual Meeting of the United States Animal Health Association* 94, 280-285.
- Whittington, R.J., Fell, S., Walker, D., McAllister, S., Marsh, I., Sergeant, E., Taragel, C.A., Marshall, D.J., Links, I.J., 2000a, Use of pooled fecal culture for sensitive and economic detection of *mycobacterium avium* subsp. *paratuberculosis* infection in flocks of sheep. *Journal of Clinical Microbiology* 38, 2550-2556.
- Whittington, R.J., Hope, A.F., Marshall, D.J., Taragel, C.A., Marsh, I., 2000b, Molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis*: IS900 restriction fragment length polymorphism and IS1311 polymorphism analyses of isolates from animals and a human in Australia. *Journal of Clinical Microbiology* 38, 3240-3248.
- Whittington, R.J., Marsh, I., Turner, M.J., McAllister, S., Choy, E., Eamens, G.J., Marshall, D.J., Ottaway, S., 1998, Rapid detection of *Mycobacterium paratuberculosis* in clinical samples from ruminants and in spiked environmental samples by modified BACTEC 12B radiometric culture and direct confirmation by IS900 PCR. *Journal of Clinical Microbiology* 36, 701-707.
- Whittington, R.J., Marsh, I.B., Taylor, P.J., Marshall, D.J., Taragel, C., Reddacliff, L.A., 2003, Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from environmental samples collected from farms before and after destocking sheep with paratuberculosis. *Australian Veterinary Journal* 81, 559-563.
- Whittington, R.J., Sergeant, E.S., 2001, Progress towards understanding the spread, detection and control of *Mycobacterium avium* subsp. *paratuberculosis* in animal populations. *Australian Veterinary Journal* 79, 267-278.

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

Williams, C., Moffitt, C., 2001, A critique of methods of sampling and reporting pathogens in populations of fish. *Journal of Aquatic Animal Health* 13, 300-309.

10 Appendices

Appendix 1 Detailed methodology

A1.1 Study design

A1.1.1 Study type

The project was undertaken using a cross-sectional study design in which a questionnaire was administered by face-to-face interview to enrolled sheep producers and soil samples collected from specific paddocks grazed by cohort sheep during one property visit, and faecal samples from cohort sheep collected during another property visit.

A1.1.2 Reference and study population

The reference population for this study was OJD-infected sheep flocks in Australia.

The study population consists of OJD-infected sheep flocks that met specific selection criteria. As such the study population is a subset of the reference population, but is not representative of it. Selection criteria were required to reduce the effect of factors likely to confound the expression of clinical disease such as enterprise type, past purchasing history, vaccination history and time since infection. It is acknowledged that due to the potential bias introduced by the selection criteria that the study results should be extrapolated to other types of flocks with caution.

The original selection criteria stated for eligible flocks in the proposal document were:

1. Self-replacing Merino flocks
2. Location - Rural Lands Protection Districts (RLPB) of Central Tablelands, Goulburn, Yass and Young in New South Wales (NSW)
3. Flock infected for more than 5 years
4. Non-vaccinated 3 & 4-year old (6-8 tooth) sheep present in flock
5. ≥ 210 sheep in the 3 & 4-year old cohort - 7 pools of 30 sheep to classify flocks as high or low prevalence.

Investigation from March to April 2004 of the 194 known infected flocks present in the four designated RLPB, identified 64 flocks that met these criteria and had owner approval for participation in this study. However this figure fell short of the target sample size of 100. The number of eligible flocks available in the central and southern tablelands of NSW was reduced due, as anticipated, to increased use of the Gudair® vaccine and to reduction in stock numbers resulting from the drought conditions in the four RLPB districts.

As a consequence, in order to achieve the objectives set for OJD.038, the following revised selection criteria were implemented:

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1. Self-replacing Merino flocks
2. All identified OJD infected districts in Australia including NSW, Victoria, Kangaroo Island, Tasmania (including Flinders Island) and Western Australia
3. Flock infected for 3 or more years
4. Non-vaccinated 3-year old, 3 & 4-year old (6-8 tooth) or 4 & 5-year old sheep present in flock
5. ≥ 210 sheep in the 3-year old, 3 & 4-year old or 4 & 5-year old cohort - 7 pools of 30 sheep to classify flocks as high or low prevalence.

A total of 92 flocks were identified that met these revised selection criteria and owner approval gained for participation.

A cohort size of 210 sheep was set because the pooled faecal culture results for 7 pools of 30 sheep was adequate to classify each flock as either high or low prevalence. A larger number of cohort sheep could have given more precise prevalence categorisation but would have reduced the number of flocks that met the selection criteria.

A1.1.3 Sample size

Assuming that there are equal numbers of low- and high-prevalence flocks, the estimated sample sizes required to provide 95% ($\alpha = 0.05$) or 90% ($\alpha = 0.10$) confidence of detecting a significant difference for odds ratios of 3 and 5, assuming 10% or 15% of flocks in the low-prevalence group have the factor of interest were calculated (Table 9.1). Based on these figures a target sample size of 100 flocks and a minimum sample size of 80 flocks were set for this study.

Table A1.1
Calculation of sample size

Odds ratio	% of low-prevalence group with the factor	Sample size per group (Total)	
		$\alpha = 0.05$	$\alpha = 0.10$
3	10	113 (226)	92 (184)
3	15	85 (170)	69 (138)
5	10	49 (98)	40 (80)
5	15	38 (76)	31 (62)

Though it was initially planned to randomly select a sample of 100 flocks from the sampling frame of eligible flocks stratified by district using proportional allocation, this procedure became redundant when the number of eligible flocks was less than the target number.

A1.1.4 Unit of interest

The unit of interest in this study was the flock, specifically the OJD infection status of the cohort sheep sampled in each flock based on pooled faecal culture results.

A1.2 Producer interview

Information about the property and flock management practices and about the sampled cohort sheep was gathered by administer of a questionnaire during a face-to-face interview with each enrolled producer.

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A1.2.1 Questionnaire design and testing

The questionnaire was designed to collect general information about each enrolled flock, general management procedures and detailed information about the history of the cohort sheep. It was structured specifically to obtain data on most of the proposed risk factors and confounding factors listed in Appendix 2. Data on the remaining risk factors and confounding factors were obtained from the specimen advice form submitted with the cohort faecal samples or from the soil sample analysis results. The risk factors for OJD faecal shedding were hypothesised on the basis of previous literature and by consultation with experts. Questions for inclusion in the questionnaire were formulated to gather the best available information on each proposed risk factor and confounder. Guidelines on questionnaire design applicable to questionnaires administered by face-to-face interview were followed during questionnaire design (Cameron et al., 2004; Dohoo et al., 2004a). The questionnaire was piloted with 4 sheep producers in the Central Tablelands RLPB considered similar to producer participants and this resulted in further modification to the questionnaire to aid producer response. The questionnaire consisted of the three sections outlined in Table A1.2. A complete copy of the questionnaire is presented in Appendix 3.

A1.2.2 Questionnaire administration

The questionnaire was administered in a face-to-face interview with the owner/manager of each enrolled flock. Interviews were conducted by two trained interviewers over four months from August to December 2004. One interviewer completed interviews for flocks located in Victoria, Tasmania, Western Australia and the NSW RLPB districts of Central Tablelands, Goulburn, Gundagai, Molong, Yass and Young. The second interviewer completed interviews for flocks located in the Goulburn, Gundagai, Hume, Yass and Young RLPB districts in NSW.

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Table A1.2

Outline of the three part questionnaire

SECTION 1 – Property description, environment and management

- Property data – total area, percent grazed area, altitude, topography
 - Pasture types used for sheep
 - Enterprises run on the property
 - Sheep flock – current flock numbers, long term flock numbers, average production
 - Soil profile of property
 - Fertilizer application history
 - Mineral deficiency history for animals on the property
 - Area of water logging and pin rushes on the property
 - Total monthly rainfall on the property over past six years
 - Worm control practices for sheep flock
-

SECTION 2 - OJD infection history and management

- Duration and level of infection - assessed by the interviewer based on year and method of OJD diagnosis and on owner's view of duration of infection, source of infection and signs of OJD
 - Losses due to OJD in 2-year old and older sheep - first mortality, peak mortality, current mortality, 5-year mean mortality
 - Source of OJD infection - owner's view of the source of infection
 - Risk of lateral spread - number of infected and likely infected sheep neighbours; sharing of rams, roads, sheds/yards with the neighbours and straying of sheep between properties; intermittent and permanent creek and proportion of the property receiving run off water from the neighbours.
 - Wildlife risk - kangaroos, feral goats, rabbits and other wild animals.
 - OJD control strategies - number of drops vaccinated, other OJD control procedures, management of clinical OJD sheep
 - Sheep purchases / introductions - purchase history of sheep and rams
-

SECTION 3 – Cohort History & Management

Data collected for each of the following designated stages of cohort life history: lambs (birth to weaning), weaners (weaning to 12 months), hoggets (12 months to 24 months) and adults (>24 months).

- Date at start
 - Cohort number at start
 - Total area grazed
 - Condition score
 - Length & method of paddock decontamination
 - Pasture type
 - Period of any growth check
 - Grazing Management
 - Water source and water supply
 - Mineral supplementation
 - Grazing of fodder crops
 - Grazing of stubble
 - Supplementary feeding
 - Evidence of high-level worm burden
 - Presence of scouring
 - Other health problems
-

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Additional data specific to a designated stage of cohort life history.

- Husbandry practices at marking
 - Weaner growth after weaning
 - Dates of marking, mulesing, first shearing, separation of sexes (if separated)
 - Age at which mixed with adult flock (if mixed)
 - Management and performance of cohort ewes at joining and lambing
-

A1.3 On-farm sample collection

Faecal samples from cohort sheep were collected during one property visit and subsequently at the time of the producer interview soil samples were collected from paddocks grazed by the cohort sheep during designated stages of the cohort life history.

A1.3.1 Collection of faecal samples from cohort sheep

Faecal sample collection from cohort sheep was performed by the district veterinarian located in the district of each enrolled flock. These veterinarians were requested to adhere to the sampling protocol shown in Figure A1.1 to identify the drop and sex of cohort sheep to be sampled.

Briefly, the sampling protocol was designed to preferably select 3-year old unvaccinated sheep. If the desired numbers of 210 sheep were not available in this drop, the additional animals required to complete seven pools were selected from 4-year old unvaccinated sheep. If no 3-year old unvaccinated sheep were available, a similar procedure was used with preferential selection of 4-year old sheep and then completion of pools from 5-year old sheep as required when <210 4-year olds were available. In addition all pools were preferentially selected from one sex. However, when 210 sheep of one sex were not available, samples were collected from all the animals of one sex (the sex with the greater number of animals) and the balance required to complete seven pools collected from the other sex. In flocks where more than 210 animals of each sex were available for sampling, 7 pools were selected from each sex to allow comparison between sexes. When more sheep were available for sampling than the required number of 210, individual animals were selected from the group using systemic random sampling. As the sheep were run through a race, every i^{th} sheep was selected, where i was calculated by N/n (N - total number available, n - required sample size). For example if 420 ewes were available for sampling then every 2nd ewe was selected ($420/210 = 2$).

One faecal pellet was collected *per rectum* from each selected sheep. The pellets collected from 30 consecutive sheep were placed in a sterile labelled jar. The veterinarians performing faecal collection changed gloves between pools. The pooled samples were stored in a refrigerator at 4 °C until dispatched to the University of Sydney Farm Animal Health laboratory in boxes with ice pack and specimen advice form by overnight courier service. The specimen advice form (shown in Appendix 4) listed the property identification code, district, submitter (district veterinarian) name and contact details, sampling date, number of pools, details of each pool (number of sheep, year of drop, sex), and the condition score of sheep at time of faecal collection. On reaching the laboratory samples were stored at -20 °C until cultured.

This sampling protocol was followed for 80 flocks enrolled in this study. A further 12 flocks were concurrently enrolled in MLA OJD.033 project and the PFC results for faecal samples collected from 3 and 4-year old sheep (usually 7 pools of 50 sheep per flock) from February 2003 to April 2004 were also used in this project. These methods for faecal sample collection, transport and culture were identical to those described for this project.

A1.3.2 Collection of soil samples from identified paddocks

During the course of the face-to-face interview with each enrolled flock owner/manager, the paddocks grazed by the cohort sheep as lambs, weaners, hoggets and adults were identified. In consultation with the flock owner/manager, three paddocks were chosen as representative of paddocks grazed by cohort sheep and designated for soil sample collection.

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A soil sample was collected from each designated paddock by the interviewer on the same day as the interview. A stainless steel probe (Incitec Pivot™) was used to collect samples of the top soil layer to a depth of 10cm. Approximately 30 samples were collected from across each paddock in a zigzag pattern avoiding areas such as fences, roads and dams in order to obtain a representative sample. All the samples collected from one paddock were mixed by hand in a bucket, placed in a bag (supplied by Incitec Pivot™) and stored at 4°C until transported with the soil sample advice form to the Incitec Pivot laboratory by courier (usually once a week). Each sample was identified by a unique soil sample identification number, the paddock name used by the owner/manager and by its GPS coordinates.

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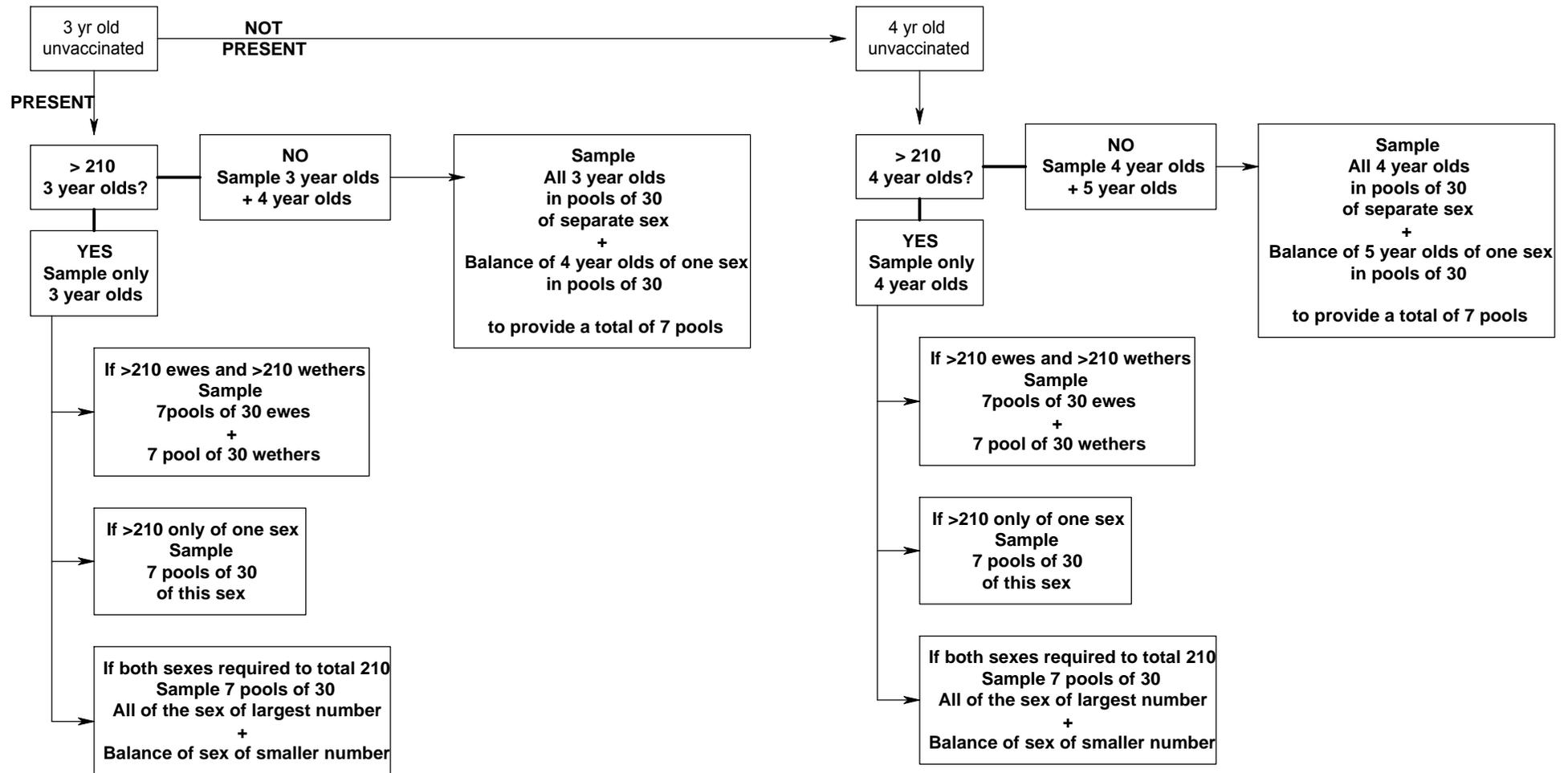


Figure A1.1 Sampling protocol followed to identify cohort sheep for faecal collection

A1.4 Laboratory analyses

A1.4.1 Pooled faecal culture

On arrival at the University of Sydney Farm Animal Health laboratory, each pooled faecal sample was cultured using a modified BACTEC radiometric method (Whittington et al., 2000a). When pooled faecal samples could not be processed within 48 hours of arrival they were stored at -80°C. A brief description of the protocol for pooled faecal culture follows.

Decontamination of pooled faecal samples

The faecal pellets in each pooled sample were thoroughly mixed using a sterile stainless steel homogeniser. This faecal homogenate was cultured following a double incubation preparation (Whitlock and Rosenberger, 1990). Briefly, a small amount (approx. 1 g) of the homogenate was mixed with 10 ml normal saline in a sterile 15 ml tube and allowed to stand for 30 minutes at room temperature after which a 3-5 ml aliquot of surface liquid was transferred to a 35 ml tube containing hexadecylpyridinium chloride and brain heart infusion broth. After 24 hours incubation at 37°C, the tube was centrifuged for 30 min at 900g and the pellet was collected and resuspended in 1ml sterile water with VAN (Vancomycin, Nalidixic acid and Amphotericin B) and incubated for 72 hours at 37°C.

Culture in modified BACTEC radiometric medium

After incubation, 0.1ml of the incubated solution was inoculated in a radiometric BACTEC 12B media supplemented with PANTA PLUS, mycobactin J and egg yolk. The vials were then incubated at 37°C for 12 weeks and growth index was measured weekly. If no growth was detected during the 12 week period then the pool was declared negative.

Confirmation of M. paratuberculosis

The growth of *M. paratuberculosis* was confirmed using a PCR test to identify the presence of IS900 in positive cultures (Whittington et al., 1998) and a restriction endonuclease analysis (REA) to confirm IS900 (Cousins et al., 1999). From each BACTEC vial, 0.2ml of the medium was separated for PCR when the growth index started increasing (i.e. reached >200) and again when growth index reached 999. Samples were prepared for PCR using differential centrifugation method in ethanol. In case of pools which exhibited growth in BACTEC medium but were PCR negative, DNA was purified by wizard clean up procedure (Wizard PCR preparations, Promega) and PCR was re-performed. In addition, smears were prepared from BACTEC culture and stained by Gram's stain to check for presence and level of contaminating micro organisms.

A1.4.2 Soil sample analyses

The soil samples were submitted to the Incitec Pivot Werribee laboratory for standard soil analysis. A list of the analyses reported by soil laboratory is shown in Appendix 5.1. In brief, the physical characteristics of each sample were assessed subjectively and allocated to designated categories described by specific criteria. For example, texture was assessed manually by moistening and rolling soil into a ball and then trying to make a sausage shape or a ribbon. The guidelines used by the laboratory in categorising soil texture are given in Appendix 5.2. The chemical parameters were measured according to standardised procedures using calibrated equipment. Further calculated parameters were created using standard industry formulae presented in Appendix 5.3.

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An additional particle size analysis (PSA) was performed by the University of Sydney Soil Physics Laboratory. This analysis was conducted according to standard procedure using calibrated equipment and provided data on the proportion of fine sand, coarse sand, silt and clay in each soil sample. Briefly, a calibrated soil hydrometer was used to measure the amount of silt and clay in a dispersed suspension of soil while the amount of sand was measured gravimetrically. A weighed amount of soil was mixed with sodium hexametaphosphate and distilled water in a shaking bottle and agitated for 24 hours on a shaking wheel to disperse the soil particles. The contents were then transferred to a measuring cylinder and mixed with requisite amount of distilled water and allowed to stand for 4 min 48 sec (with room temperature maintained at 20-21°C) before the soil hydrometer measured amounts of silt and clay. Another reading was taken, similarly, after 8 hours to measure the amount of clay only. Amount of silt in the soil was obtained from the difference in these two readings. To estimate the amount of coarse sand, the contents of the cylinder were sieved through a 200µ sieve into a weighed beaker, washed repeatedly and dried in hot air oven at 105°C for 24 hours. Weight of fine sand was obtained after subtraction of clay, silt and coarse sand from the initial weight of the soil. Moisture content of the soil, used for correction, was measured by oven drying a weighed amount of soil for 24 hours. Soil texture was estimated from silt, clay and sand percentage of the soil samples employing the TAL software (TAL for windows ver. 4.2 (c)1996-2002 available on line at <http://agri.upm.edu.my/~chris/tal/>) that uses the international soil triangle (shown in Appendix 5.4) (Leeper and Uren, 1993).

A1.5 Database design and data management

A1.5.1 Database design

A relational database OJDRFS (Ovine Johne's Disease Risk Factor Study) was custom built in Microsoft Access 2000 (© Microsoft Corporation) for entry and management of the study data. It consisted of 18 tables and sub-tables linked by unique identifiers for property, sheep cohort and pool faecal sample with the relational structure shown in Figure A1.2. Queries were created for retrieval of data on request. The database was tested with fake data prior to commencement of data entry for this study.

A1.5.2 Data entry

Data from the questionnaires, faecal sample advice forms, faecal sample culture results and soil sample analysis results were entered into the database as soon as available. Specific codes were allocated for missing values and for additional information that did not fit in database fields and recorded in a codebook.

After completion of data entry for all records continuous variables were sorted to identify the ten lowest and highest values for each variable and these were checked against original questionnaire, form or result sheet to identify data entry errors. When a data value was considered improbable or was missing the relevant interviewer or laboratory person was requested to check the value.

A1.5.3 Data export/import

All the data tables were exported from the OJDRFS database and imported into SAS (SAS release 8.02, © 1999-2001 by SAS Institute Inc., Cary, NC, USA). Sub-tables were then merged as required using the unique identifier variables.

FIGURE A1.2

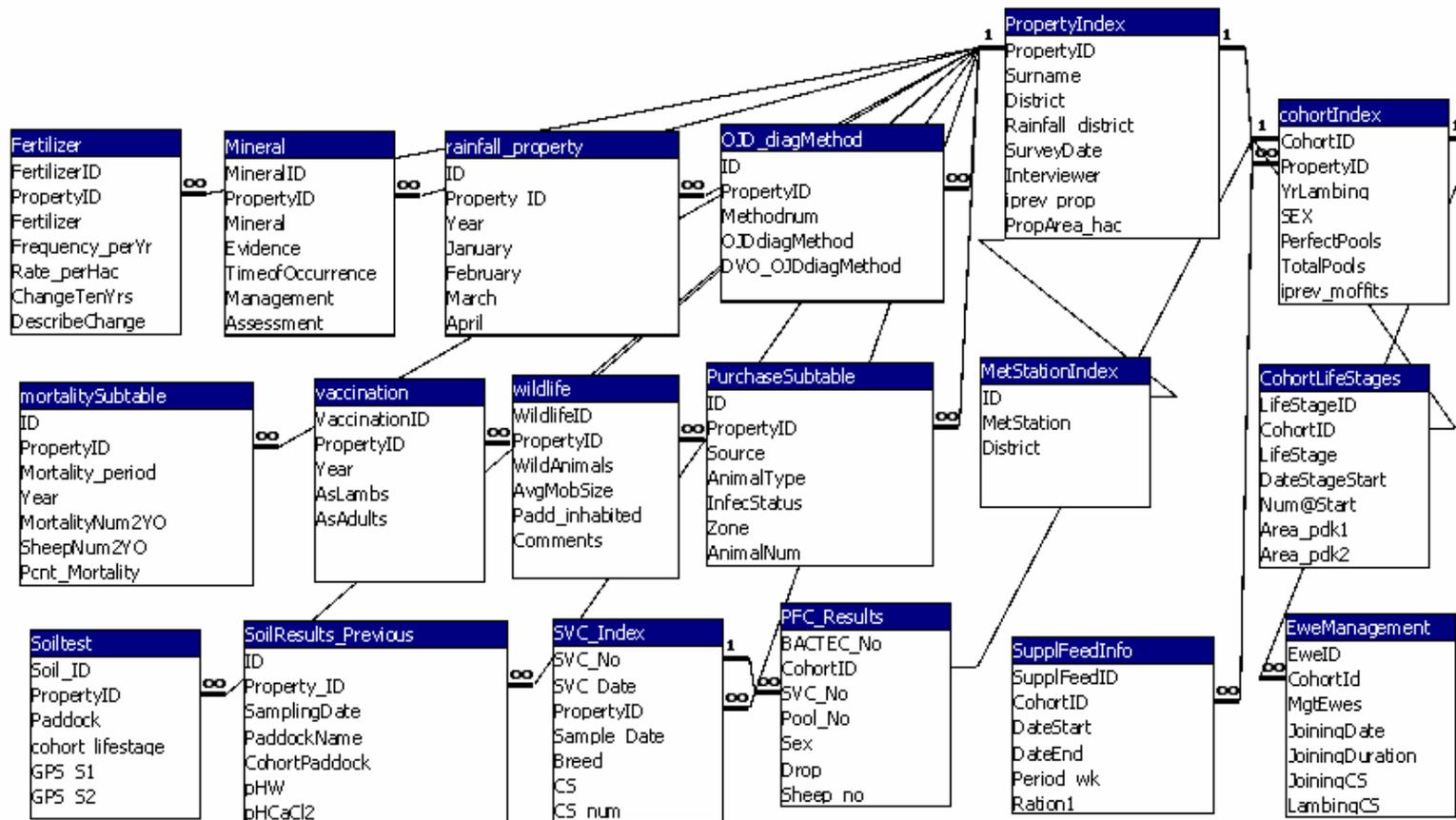


Figure 3.2: Tables in the Microsoft Access database OJDRFS and their relationships

Note: Relationships are denoted by line joining two tables; one to many relationships have '1' sign on parent table 'infinity' symbol on child table. Only a few of the fields of the tables are visible in this graphical presentation of database relationships.

A1.6 Outcome variables

A1.6.1 Animal-level OJD prevalence for sheep cohorts

The PFC pool results for each sheep cohort (that is, a group of sheep of the same sex and age group from which usually 7 faecal pools were collected) were used to calculate the individual animal OJD prevalence of each cohort employing the variable pool size method of Williams and Moffitt (2001). Due to logistics of sample collection, samples of uniform pool size could not be collected for every sheep cohort in the present study. Therefore, this method was used for the calculation of individual animal prevalence as it is the only method available that can incorporate variable pool size in the calculations, even though it assumes perfect sensitivity and specificity – an assumption that does not apply to PFC. Other available methods for calculation of individual animal prevalence were not implemented for the following reasons:

- The Bayesian method available for calculation of individual prevalence (method 7 of Cowling et al., 1999) accounts for imperfect sensitivity and specificity, however, information on PFC sensitivity and specificity for most pool sizes used in the study and for prior prevalence in each sheep cohort were not available.
- The frequentist method available for calculation of individual prevalence when sensitivity and specificity is not certain (method 6 of Cowling et al., 1999) yields invalid results when a very low or high proportion of pools are positive and the lower confidence limit calculated is negative in low prevalence situations.
- The method of Sacks *et al.* (1989) and Kline *et al.* (1989) (method 2 of Cowling et al., 1999) assume fixed pool size and perfect sensitivity and specificity.
- Method 4 of Cowling et al. (1999) based on maximum likelihood estimations (Tu et al., 1994) assumes fixed pool size and imperfect but known sensitivity and specificity.

Thus although not ideal, the Williams and Moffitt method (2001) was the best option available to calculate individual animal prevalence from the PFC pool results available for each sheep cohort in this study.

Briefly, the William and Moffitt method (2001) uses maximum likelihood to estimate individual prevalence from pool results whereby a positive pool indicates that at least one animal in the pool is positive. Confidence intervals are constructed based on large sample statistical theory. The method assumes independence of prevalence status between the animals represented in a given pool meaning that the health of any animal is unrelated to others in the pool. Secondly, it assumes perfect sensitivity and specificity. Though both of these assumptions cannot be met by our data, this is the only method available in which information about variable pool size can be considered in prevalence estimation.

In this study all cohort prevalence calculations using the Williams and Moffitt method (2001) were performed using the Pooled Prevalence Calculator (PPC) (Sergeant, 2004) available online at <http://www.ausvet.com.au/pprev/>. Inputs required by the PPC include pool size and the number of pools tested and number positive for each pool size. PPC outputs include a prevalence point estimate and upper and lower confidence limits for the specified level of confidence.

The resulting cohort OJD animal-level prevalence was a continuous variable. To create an outcome (or dependent) variable, this continuous data was categorised to designate each sheep cohort as either a low, medium or high prevalence cohort. This outcome variable was used in univariable and multivariable analyses to achieve Project Objective 3 – to identify factors statistically associated with cohort PFC prevalence and quantify the magnitude of these associations.

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Cut-off figures for the low/medium/high categories were set on the basis of expert advice on OJD biology and dynamics under Australian circumstances. Two different sets of cut-off figures were designated creating two prevalence category outcome variables (labelled as IPREV and IPREV25). For the first, IPREV, the three cohort prevalence categories were low (<2% prevalence), medium (2-10% prevalence) and high (>10% prevalence). The second, IPREV25, had the same low infection prevalence category (<2% prevalence) but the second cut-off was reduced to 5% individual animal prevalence so that the medium and high prevalence categories were those with prevalence 2-5% and >5%, respectively. Two different categorisations were necessary firstly because information about what individual animal prevalence level that constitutes a high level of OJD cohort prevalence was scarce. Secondly, as no method for calculation of individual animal prevalence ideally suited to the study data was available, only the best option was selected out of available methods – the Williams and Moffit method (2001). When all the tested pools for a sheep cohort were positive, this method calculates a very high prevalence figure with wide confidence limits for the cohort. Cohorts of this 'all positive' type in the first outcome variable, IPREV, constitute the high prevalence category (>10%). In the second outcome variable, IPREV25, these 'all positive' cohorts and cohorts where all but one pool were positive, constitute the high prevalence category (>5%) with the exception of cohorts where the pool size was 50. For these cohorts with pool size of 50 when all but one pool was positive the cohort was still classified in the medium prevalence category as the calculated prevalence figure was less than 5%.

A1.6.2 Pool OJD status

The PFC result for each faecal pool cultured in this study was used to create a binary outcome variable representing the OJD status of each pool and labelled MPTB. This outcome variable was analysed as an extension of Project Objective 3 increasing the statistical power to identify factors associated with pool OJD status.

A1.6.3 Pool MAP number shed

Faecal shedding of *M. paratuberculosis* for each faecal pool cultured, calculated by employing the method of Reddacliff et al.(2003), created a continuous outcome variable, the log of the number of *M. paratuberculosis* shed per pool, labelled LOGMAP. This outcome variable was used in analyses to achieve Project Objective 5 - to identify factors associated with the level of MAP faecal shedding in OJD-infected flocks.

The method of Reddacliff et al. (2003) calculates the number of *M. paratuberculosis* excreted per gram of faeces based on the number of days taken by a pooled faecal sample to reach cumulative growth index (CGI) of 1000 in BACTEC media. In brief, BACTEC reading was recorded every week for 12 weeks from which the commutative reading (CGI) for each week was calculated. Number of days post inoculation taken by the sample to reach the highest CGI (but less than 1000) was counted (d1). Also, the days taken by the typical curve to reach 1000 from that highest CGI were noted from the standard graphs (d2). Days taken by the sample to reach CGI of 1000 (DAYS@CGI1000) were calculated by adding d1 and d2 from which log inoculum size was determined by the following equation:

$$\text{LOG}_{10} \text{ inoculum size} = 9.25 - (0.185 * \text{DAYS@CGI1000})$$

Actual number of organisms (MAPNUM) was calculated by exponentiation of LOG₁₀ inoculum size. All the samples found negative in PCR and REA were assigned MAPNUM of zero. For the purposes of linear regression analysis, each MAPNUM value was increased by the addition of one and then

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logged (\log_{10}) to create the outcome variable (LOGMAP). This was done so as to avoid problem of infinity values ($-\infty$) when calculating \log_{10} of MAPNUM zero for negative samples.

A1.7 Explanatory variables

A1.7.1 History and management

The explanatory variables related to history and management investigated in this study including proposed risk factors and confounding factors are listed in Appendix 2.

All 71 of these explanatory variables investigated were categorical variables with discrete data collected for 20 and continuous data collected for 23 and then categorised based on quartiles (or median where appropriate) or on biological plausibility. A further 28 were categorical composite variables created using information from two or more questions in the questionnaire. For example, the categorical composite variable for weaner health (labelled WNRHLTH) combined data from 4 questions on health of the sheep cohort when they were weaners (growth post weaning, high worm burden, scouring and other health problems) to determine whether or not the cohort experienced any health problems as weaners.

A1.7.2 Soil

The explanatory variables related to soil sample analyses investigated in this study were the proposed risk factors listed in Appendix 6.

All 44 explanatory variables investigated were categorical variables with discrete data collected for 1 and continuous data collected for 40 and then categorised based on biological plausibility or quartiles or median as appropriate. Three variables were discrete composite variables, each created by pooling information from three variables. Of these, 10 variables represent the average result for the 3 soil samples from different paddocks analysed per property (termed the 3-paddock mean variables) and 33 represent the result of a single sample taken from a paddock typical of the paddocks cohort sheep grazed as either lambs, weaners or hoggets/adults (termed either lamb paddock, weaner paddock or hogget/adult paddock variables). One variable (parent soil type) was a property level variable.

A1.8 Descriptive data analyses

All descriptive analyses were performed using SAS System for Windows release 8.02 (SAS Institute, Cary, NC, USA).

Descriptive analyses were conducted using all available data on the outcome and explanatory variables. Standard statistical analyses (percentages for categorical variables; mean, median, percentiles and range for continuous variables) were performed to provide a detailed description of each variable. For the outcome variables, differences between age and sex of cohort sheep were assessed using the F test in PROC GLM of SAS (Armitage et al., 2002). Explanatory variables with > 10% missing values or very little variation (<10%) were identified and not considered in further analyses.

A1.9 Datasets for analytical analyses

A1.9.1 Animal-level OJD prevalence for sheep cohorts

In this study, a sheep cohort was defined as group of sheep of the same sex and age group (or year of drop) selected for faecal sample collection from a flock. The original intention was to collect faecal samples from 210 sheep (7 pools of 30 sheep) of the same sex and age group representing one sheep cohort from each flock. However, where insufficient animals of the same sex and age group were available for sampling, some faecal pools were collected from another sex and/or an older age group of sheep resulting in more than one sheep cohort being selected from some flocks. For each sheep cohort, the PFC results were used to calculate the individual animal OJD prevalence of the cohort by the Williams and Moffitt method (2001) as described in Section 3.6.1. Due to variation in the number of sheep cohorts between enrolled flocks, three datasets were created to represent different levels of consistency and reliability in the cohort data.

The first dataset (labelled FIRST dataset) comprised only sheep cohorts where 7 pools were collected from the same sex and age group. Thus each flock included in this dataset is represented by only one sheep cohort with the exception of flocks where 7 pools were collected from ewes of the same age group and 7 pools were collected from wethers of the same age group.

The second dataset (labelled SECOND dataset) comprised all cohorts in the first dataset as well as sheep cohorts where ≥ 4 pools were collected from sheep of the same sex and age group. Again each flock in this dataset is represented once with the exception of flocks where 7 pools were collected from ewes of the same age group and 7 pools were collected from wethers of the same age group.

The third dataset (labelled THIRD dataset) was similar to the second dataset except for flocks where a new combined sheep cohort was created by merging two cohorts with < 7 pools of the same age group but different sex to produce one cohort of the same age but mixed sex. In addition 5-year old sheep cohorts (drop year 1999) were included in this dataset.

A1.9.2 Pool OJD status

A single dataset was created with each faecal pool collected in the study (except those from 5-year old sheep) represented once.

A1.9.3 Pool MAP number shed

A single dataset was created with each faecal pool collected in the study (except those from 5-year old sheep) represented once.

A1.10 Univariable data analyses

Univariable analyses were performed (following the same procedure with the exception of pool rate of faecal shedding) to investigate the association between each outcome variable and each explanatory variable (including the 71 history and management variables and the 44 soil variables) on an individual basis. Separate univariable analyses were conducted for each of the three datasets with cohort OJD prevalence category as the outcome of interest – one set of analyses for IPREV and one for IPREV25. The unconditional association between outcome and explanatory variables (except for pool rate of faecal shedding) was assessed using the logistic regression SAS LOGISTIC

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procedure (Stokes et al., 2000). The likelihood ratio chi-square was calculated by subtracting the -2 log likelihood of the variable from the intercept and related P-value checked.

In contrast, univariable analyses for pool rate of faecal shedding were performed using linear regression employing the SAS GLM procedure (Armitage et al., 2002). Test of significance was based on F and P-values calculated from Type III sum of squares.

Explanatory variables identified in the univariable analyses for each outcome as unconditionally associated with the outcome variable at $P < 0.25$ were then examined for collinearity. Highly correlated variables (with Spearman rank correlation > 0.80) were further evaluated using either the chi-square analysis or Fisher's Exact test (for categorical data without and with a number of expected cell counts < 5 , respectively). If significant associations ($P < 0.05$) were found, the most appropriate variable (based on our opinion of biological plausibility) was subsequently deleted. All remaining explanatory variables were selected for inclusion in the relevant multivariable model.

A1.11 Multivariable data analyses

A1.11.1 Animal-level OJD prevalence for sheep cohorts

Association between cohort OJD prevalence and history and management explanatory variables

Separate ordinal logistic regression models for cohort OJD prevalence were constructed for each of the three datasets using the SAS LOGISTIC procedure (Stokes et al., 2000) and following the same procedure – one set of models for IPREV and one for IPREV25. Three variables were forced into each model as fixed effects – cohort age, cohort sex and current mortality.

We used a manual stepwise procedure during the construction of ordinal models. Forward variable selection was based on changes in log likelihood (retaining variables with $P < 0.10$), with further individual assessment based on the individual contribution of each selected variable following backward selection as a fixed effect (with removal of variables with $P > 0.10$). First order interaction terms were then added to the model and retained when the change in log likelihood was $P < 0.05$ and the interaction term was biologically plausible.

Association between cohort OJD prevalence and soil explanatory variables

The first multivariable models created used only 3-paddock mean variables. According to the same model building procedure described above, separate models were constructed using these mean variables for each of the three datasets – one set of models for IPREV and one for IPREV25. Following a similar procedure, multivariable models were then created separately using the lamb paddock, the weaner paddock and the hogget/adult paddock variables for each of the three datasets firstly with IPREV as the outcome of interest and then IPREV25. Potential confounders forced into each model as fixed effects included cohort age, cohort sex, current mortality and parent soil type.

Composite multivariable model for cohort OJD prevalence

All variables present in the two final models for IPREV for the FIRST dataset – variables in the model of history and management variables and the model of soil variables – were then entered in a composite model. Four variables were retained in this model as fixed effects – cohort age, cohort sex, current mortality and parent soil type - and the remainder were individually assessed for their contribution as a fixed effect by backward selection (with removal of variables with $P > 0.10$). First

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order interaction terms were then added and retained if the change in log likelihood was $P < 0.05$ and the interaction term was biologically plausible.

A1.11.2 Pool OJD status

Association between pool OJD status and history and management explanatory variables

Binary logistic regression models for pool OJD status were constructed using the SAS LOGISTIC procedure (Stokes et al., 2000). Variables forced into the model as fixed effects were cohort age, cohort sex, current mortality and log pool size. The procedure followed for model building was the same as described above except for the addition of random effects flock variable using the SAS GLIMMIX procedure to the final model (Anonymous, 2005; Schabenberger, 2005) and then removal of fixed effects with $P > 0.10$ by backward selection. First order interaction terms were then added to the model and retained when the change in log likelihood was $P < 0.05$ and the interaction term was biologically plausible.

Association between pool OJD status and soil explanatory variables

Adhering to the same model construction procedure described above, four binary logistic regression models for pool OJD status were created separately for 3-paddock mean variables, lamb paddock variables, weaner paddock variables and hogget/adult paddock variables. Variables forced in every model as fixed effect terms were cohort age, cohort sex, current mortality and parent soil type.

A1.11.3 Pool MAP number shed

Association between pool MAP number shed and history and management explanatory variables

General linear mixed models for log pool MAP number shed (LOGMAP) were constructed employing SAS MIXED procedure (Brown and Prescott, 2000). Variables forced into the model included cohort age, cohort sex, current mortality and log pool size as fixed effects, and flock as a random effect. The procedure followed for model building was similar to that described above with forward variable selection retaining variables based on $P < 0.10$ followed by backward selection of fixed effects to remove variables with $P > 0.10$ and finally addition and retention of first order interaction terms when change in log likelihood was $P < 0.05$ and the interaction term was biologically plausible.

Association between pool MAP numbers shed and soil explanatory variables

The same model building procedure as above was followed to create four separate general linear mixed models for log pool MAP number shed using 3-paddock mean variables, lamb paddock, weaner paddock and hogget/adult paddock variables.

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Appendix 2 Description of history and management explanatory variables

Appendix 2

Description of the 71 explanatory variables related to history and management investigated in this study

Variables	Code	Description and categories	No of flocks	25P	Median	Mean	75P
<i>Flock-level variables</i>							
<i>Confounding variables</i>							
INFLEVEL		Level of flock infection assessed from trend in mortalities	92		Composite variable		
	0	No mortalities	19				
	1	Low mortalities and trend falling or steady	28				
	2	High mortalities but trend falling or steady OR Low mortalities but trend escalating	35				
	3	High mortalities as well as trend escalating	10				

OJD_DURN		Interviewers' assessment of length of flock infection	92		Composite variable		
	0	3<5 years	11				
	1	5<7years	23				
	2	7<10years	27				
	3	10 year or more	31				

PEAKMORT		Peak flock OJD mortality% in adults (>2 yr old)	92	0.1	1.3	3.0	4.3
	0	No mortalities	19				
	1	<2% mortalities	33				
	2	≥ 2% mortalities	40				

CURRMORT		Current flock OJD mortality% in adults (≥2yr old)	92	0.1	0.9	2.3	2.5
	0	No mortalities	20				
	1	<2% mortalities	39				
	2	≥ 2% mortalities	33				

MEANMORT		5 year mean OJD mortality% in adults (>2yr old)	85	0.1	0.8	1.7	2.5
	0	No mortalities	19				
	1	<2% mortalities	38				
	2	≥ 2% mortalities	28				

YNGAGEMORT		Age of youngest mortality in the flock	91	24.0	24.0	29.0	36.0

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	0	No mortalities	19				
	1	<24 months	11				
	2	24m to < 36 months	37				
	3	≥ 36 months	24				
<hr/>							
OJDSIGNS		Signs of OJD in the flock observed by producer	92				Discrete variable
	0	Nil	19				
	1	Death and/or Loss of condition	18				
	2	Death, loss of condition and scouring	55				
<hr/>							
OJD Control							
DROPSVACC		Number of sheep drops vaccinated in the flock	92	2.0	3.0	2.7	4.0
	0	No drops vaccinated	13				
	1	1 or 2 drops vaccinated	21				
	2	>2 drops vaccinated	58				
<hr/>							
CLINICALMGT		Management of OJD clinical sheep	92				Composite variable
	0	No mortalities observed	15				
	1	Immediately dispose off	53				
	2	Dispose off later	17				
	3	Do nothing	7				
<hr/>							
CULL		Control methods: Cull low body weight sheep	92				Discrete variable
	0	No	42				
	1	Yes	50				
<hr/>							
SELL		Control methods: Sell high loss mob	92				Discrete variable
	0	No	80				
	1	Yes	12				
<hr/>							
YOUNGSEPARATE		Control methods: Separate young sheep	92				Discrete variable
	0	No	47				
	1	Yes	45				
<hr/>							
YOUNGFIRST		Control methods: Handle young sheep first	92				Discrete variable
	0	No	79				
	1	Yes	13				
<hr/>							
DESTOCK		Control methods: Destock lambing and weaning paddocks	92				Discrete variable
	0	No	34				
	1	Yes	58				

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Lateral Spread and purchase risk

INFNBRS		Percentage likely infected neighbours	89	29.1	92.8	66.6	100.0
	0	≤ 25%	23	Composite variable			
	1	>25% to ≤ 75%	21				
	2	> 75%	45				
<hr style="border-top: 1px dashed black;"/>							
SHSTRAY		Frequency and number of boundary sheep straying amongst neighbours	92	Composite variable			
	0	No straying	31				
	1	<10 sheep stray annually OR stray not even once per year	33				
	2	10-20 sheep stray annually	17				
	3	>20 sheep stray annually OR stray more than once annually	11				
<hr style="border-top: 1px dashed black;"/>							
SHARING_ROAD		Frequency of sharing of roads with neighbours	92	1.0	2.0	3.5	3.5
	0	No sharing	58	Composite variable			
	1	≤ twice per year	19				
	2	>twice per year	15				
<hr style="border-top: 1px dashed black;"/>							
SHARING_SHED		Sharing of sheds/yards with neighbours	92	Discrete variable			
	0	No sharing	81				
	1	Sharing	11				
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RUNOFFWATER		Proportion of property boundary receiving run off water	92	10.0	30.0	36.0	60.0
	0	≤ 10%	29				
	1	>10 to ≤ 30%	21				
	2	>30% to ≤ 60%	23				
	3	> 60%	19				
<hr style="border-top: 1px dashed black;"/>							
PCREEK		Permanent creek flowing onto the property	92	Discrete variable			
	0	No	30				
	1	Yes	62				
<hr style="border-top: 1px dashed black;"/>							
ICREEK		Intermittent creek flowing onto the property	92	Discrete variable			
	0	No	19				
	1	Yes	73				
<hr style="border-top: 1px dashed black;"/>							
KANGAROO		Percent paddocks inhabited by kangaroos	92	20.0	50.0	53.7	100.0
	0	≤ 20%	29				
	1	>20% to ≤ 50%	27				

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

	2	>50%	36				
<hr/>							
RABBIT		Percent paddocks inhabited by rabbits	92	4.0	9.5	25.7	40.0
	0	Nil	29				
	1	≤ 5%	38				
	2	>5%	25				
<hr/>							
OTHERWILDLIFE		Wild animals other than kangaroos and rabbits present on the property	92				Discrete variable
	0	No	63				
	1	Yes	29				
<hr/>							
RAMRISK		Purchase of rams from infected sources	92				Composite variable
	1	No	55				
	2	Yes	37				
<hr/>							
EWERISK		Purchase of ewes					Discrete variable
	0	No	62				
	1	Yes	30				
<hr/>							
Property management							
<hr/>							
GRAZEDAREA		Area of the property grazed by sheep	92	623.5	965.5	1243.4	1401.1
	1	≤ 965 hectares	46				
	2	> 965 hectares	46				
<hr/>							
FLOCKSIZE		Long term number of ≥2 year old adult sheep in the flock	92	1955	3061.5	4135.6	5250
	1	≤ 3000	46				
	2	> 3000	46				
<hr/>							
SUPERFREQ		Sum of frequency of application of single super and molybdenum super fertilizers on the property	90	0.3	0.7	0.7	1.0
	0	≤ once in three years	25				Composite variable
	1	>once to ≤ twice in three years	22				
	2	> twice to ≤ Every year	32				
	3	> Once every year	11				
<hr/>							
LIME		Application of lime on the property	92				Discrete variable
	0	No	32				
	1	Yes	60				
<hr/>							

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OTHERFERT		Application of fertilizers other than single super, molybdenum super and lime on the property	92	Discrete variable			
	0	No	80				
	1	Yes	12				
<hr/>							
MINERALDEF		Evidence of mineral deficiency in animals and soil	92	Composite variable			
	0	No evidence	65				
	1	Some evidence	27				
<hr/>							
<i>Flock management</i>							
WORMCONTROL		Likelihood of worm control program to be effective	92	Composite variable			
	0	No	18				
	1	Yes	74				
<hr/>							
WORMRECOMM		Producer follows worm control recommendations	92	Composite variable			
	0	No	31				
	1	Yes	61				
<hr/>							
<i>Drought and water logging</i>							
WATERLOG		Percent of property grazing area prone to become boggy in a wet season	92	1.0	8.0	18.8	30.0
	0	<1%	22				
	1	1% to <10%	24				
	2	10% to <30%	20				
	3	≥ 30%	26				
<hr/>							
PINRUSH		Percent property area having pin rushes	92	0.0	0.3	5.7	5.0
	0	Nil	38				
	1	<1%	14				
	2	1 to ≤ 5%	22				
	3	>5%	18				
<hr/>							
DROUGHT		Average difference of annual total rainfall from district long term average over the lifetime of the cohort	92	-	-62.1	-55.0	19.2
				151.1			
	0	≤150mm lesser OR more than long-term average	69	Composite variable			
	1	>150mm lesser	23				
<hr/>							
DROUGHT_SAMPLEYR		Difference in total rainfall one year prior to sampling from district long term average	92	-	-133.0	-	-12.0
				228.0	110.7		

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

	0	up to 132mm lesser OR more than long-term average	45	Composite variable			
	1	> 132 mm to 228mm lesser	24				
	2	> 228 mm lesser	23				
<hr/>							
DROUGHT_YRLAMBING		Difference in total annual rainfall from district long-term average in the year of birth of the cohort	124	-	-14.5	-9.6	96.0
				112.5			
	0	up to 15mm lesser OR more than long-term average	62	Composite variable			
	1	>15 to 112mm lesser	31				
	2	>112mm lesser	31				
<hr/>							
Cohort-level factors							
General							
AGEGP		Age group of the cohort	124	Discrete variable			
	3	3 years	66				
	4	4 years	46				
	5	5 years	12				
<hr/>							
SEX		Sex of the cohort	124	Discrete variable			
	0	Ewes	90				
	1	Wethers	34				
	2	Mixed sex	0				
<hr/>							
GROWTHCHK		Period of any growth check during the lifetime of the cohort	114	0.0	12.0	19.7	30.0
	0	<12 weeks	53	Composite variable			
	1	≥12 weeks	61				
<hr/>							
MINSUPPL		Provision of mineral supplement during the lifetime of the cohort	114	Composite variable			
	0	No	62				
	1	Yes	52				
<hr/>							
FODSTUB		Period of any fodder or stubble grazing by the cohort	114	0.0	0.0	9.3	15.0
	0	Not grazed	61	Composite variable			
	1	Grazed for <16 weeks	27				
	2	Grazed for ≥ 16 weeks	26				
<hr/>							
WATER		Likelihood of cohort water source and supply to be contaminated	114	Composite variable			
	1	Less	6				
	2	Average	25				
	3	High	83				

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

SUPPLFEED		Period of any supplementary feed fed to cohort (weeks)	110	15.0	38.6	41.4	58.6
	0	No	15	Composite variable			
	1	≤ 26	23				
	2	26 to 52	37				
	3	>52	35				
<hr/>							
SUPPLFEED_CS		Condition score at the start of supplementary feeding	94	2.0	2.5	2.4	3.0
	1	<3	75	Composite variable			
	2	≥ 3	19				
<hr/>							
SUPPLFEED_METHOD		Method of supplementary feeding	95	Composite variable			
	0	On ground	85				
	1	Some or all in trough	10				
<hr/>							
SUPPLFEED_LIME		Included lime with supplementary feed	95	Composite variable			
	0	No	71				
	1	Yes, with some or all feeding	24				
<hr/>							
Lambing variables							
DAMSR		Estimated stocking rate in lambing paddock/s	113	10.0	14.0	17.0	23.0
	1	<8dse/hac	17				
	2	8 <12 dse/hac	17				
	3	≥12 dse/hac	79				
<hr/>							
DAMCS		Condition score of ewes at start of lambing	113	3.0	3.0	3.2	3.5
	1	<3	15				
	2	3<4	82				
	3	≥4	16				
<hr/>							
DAM_SCOUR		Presence of scouring in lactating ewes	114	Discrete variable			
	0	No	96				
	1	Yes	18				
<hr/>							
DECONT_LBGPDK		Length of any OJD decontamination of the lambing paddock	114				
	0	Nil	65	0.0	0.0	5.0	6.0
	1	<8 weeks	21				
	2	8<12 weeks	14				
	3	≥12weeks	14				
<hr/>							
LBGSSN		Season of lambing	114	Composite variable			
	0	Spring	57				

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

	1	Autumn	33				
	2	Winter	24				
<hr/>							
Weaner variables							
DECONT_WNGPDK		Length of any OJD decontamination of the weaning paddock	114	0.0	4.0	12.8	8.0
	0	Nil	51				
	1	<8 weeks	19				
	2	8<12 weeks	24				
	3	≥12weeks	20				
<hr/>							
WNRGMGT		Grazing management during weaning	114		Discrete variable		
	1	Set	47				
	2	Rotational	67				
<hr/>							
WNRSR		Estimated stocking rate in weaning paddock/s	111	6.9	10.0	12.1	13.8
	1	<8dse/hac	36				
	2	8 <12 dse/hac	35				
	3	≥ 12 dse/hac	40				
<hr/>							
WNRCS		Condition score of lambs at weaning	113	2.5	3.0	2.9	3.0
	1	<3	42				
	2	≥3	71				
<hr/>							
WNRHLTH		Any health problems experienced by weaners	114		Composite variable		
	0	Some problems	41				
	1	No problems	73				
<hr/>							
WNGAGE		Age at weaning		15.4	18.0	19.1	21.8
	0	≤ 15 weeks	28		Composite variable		
	1	≤ 18 weeks	29				
	2	≤ 21 weeks	23				
	3	>21 weeks	34				
<hr/>							
WNGPCNT		Weaning percentage	103	68.5	79.0	79.5	91.7
	0	<70%	27		Composite variable		
	1	70<80%	25				
	2	80<90%	23				
	3	>90%	28				
<hr/>							
Hogget variables							
HGTGMGT		Grazing management for hoggets	113		Discrete variable		
	1	Set	49				

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

	2	Rotational	64				
HGTSR		Estimated stocking rate of hoggets	108	5.0	7.3	9.0	10.8
	1	<8dse/hac	57				
	2	8 <12 dse/hac	30				
	3	≥ 12 dse/hac	21				
HGTCS		Condition score of hoggets at 1 year of age	112	3.0	3.0	3.0	3.5
	1	<3	27				
	2	≥3	85				
HGTHLTH		Any health problems experienced by hoggets	113				Composite variable
	0	some problems	14				
	1	No problems	99				
Adult variables							
ADCS		Condition score of adults at 2 years of age	111	2.5	3.0	3.0	3.5
	1	<3	31				
	2	≥3	80				
ADSR		Estimated stocking rate of adults	107	5.6	9.0	9.6	13.0
	1	<8dse/hac	47				
	2	8 <12 dse/hac	29				
	3	≥ 12 dse/hac	31				
ADGMGT		Grazing management for adults	112				Discrete variable
	1	Set	50				
	2	Rotational	62				
ADHLTH		Any health problems experienced by adults	112				Composite variable
	0	No problems	91				
	1	Yes, some problems	21				
JOININGDURN_1		Joining duration of cohort ewes	82	6.0	6.0	6.3	6.0
	0	<6 weeks	13				
	1	6-7 weeks	54				
	2	>7 weeks	15				
SVC_CS		Condition score of cohort at the time of faecal sample collection	80	2.0	2.5	2.5	3.0
	1	<2	8				
	2	2<3	45				
	3	>3	27				

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

Appendix 3 Questionnaire

Ovine Johne's Disease Risk Factor Study

Property ID	Surname	Date of visit
<input type="text"/>	<input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>
District/State	Interviewer	Drop/s sampled _____
<input type="text"/>	<input type="text"/>	Soil samples taken? Y / N

SECTION 1 – Property Description, Environment and Management

1. Property data

Total area (hectare)	<input type="text"/>	% Grazed	<input type="text"/>
Altitude	<input type="text"/>	Topography	<input type="text"/>

2. Pasture types used for sheep

Fertilised introduced perennial species	%
<input type="text"/>	<input type="text"/>
Fertilised native species (P + legumes)	<input type="text"/>
<input type="text"/>	<input type="text"/>
Unfertilised native species	<input type="text"/>
<input type="text"/>	<input type="text"/>

3. Enterprises

Please tick (✓) the enterprises run on the property		Stock numbers (2 yrs +)		Comments
		Current	Long term mean	
<input type="checkbox"/> Wool	Ewe	<input type="text"/>	<input type="text"/>	5 yr avg figures for adult ewes Av GFW _____ Av FD _____ Av Weaning % _____
	Wether	<input type="text"/>	<input type="text"/>	
<input type="checkbox"/> Cross bred ewes		<input type="text"/>	<input type="text"/>	
<input type="checkbox"/> Sheep trading		<input type="text"/>	<input type="text"/>	
<input type="checkbox"/> Cattle	Breeders	<input type="text"/>	<input type="text"/>	BJD status known? <input type="checkbox"/> Yes <input type="checkbox"/> No If yes, inform whether: <input type="checkbox"/> BJD +ve <input type="checkbox"/> BJD -ve
	Finishers	<input type="text"/>	<input type="text"/>	
<input type="checkbox"/> Cropping (other than fodder crops)		Area cropped _____ ha		Crop types:
Other				Specify:

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

4. Soil profile of property

What soil types/parent materials exist on this property?

If you have soil test data from the previous 3 yrs please summarise below

Date	Cohort Paddock?	pH	Colwell P	CEC	AI%

5. Fertilizer application

Cohort Paddocks	Single Super	Mo Super	Lime	Bio Soil
Frequency of application (<i>eg 1 in 3 yrs</i>)				
Rate (<i>kg/ha</i>)				
Has this changed in the last 10 years?				
Incorporated or top dressed				

6. Mineral deficiencies

Mineral deficiency [Tick (✓) if Yes]	Evidence (eg soil tests, clinical disease)	Time of occurrence	Management	Interviewer Assessment: Likelihood of deficiency/toxicity? (<i>Major, Minor, Nil</i>)
-----------------------------------------	-----------------------------------------------	--------------------	------------	--------------------------------------------------------------------------------------------

Selenium
(weaner ill thrift, white muscle disease)

Other (*eg Cu deficiency/toxicity, Cobalt, Iodine*)

7. Water Logging

What % of the grazing area is prone to become boggy in a wet season?

Area (ha) of pin rushes on property

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

8. Rainfall

Can you supply the average annual rainfall figures for the last 5 yrs?

Month	2004	2003	2002	2001	2000	1999
Jan						
Feb						
Mar						
Apr						
May						
Jun						
Jul						
Aug						
Sep						
Oct						
Nov						
Dec						

Or location of the closest Met Station _____
(see list)

9. Worm control

When do you drench?

Adults		
Lambs		
What drench do you use?	<input type="checkbox"/> White (BZ) <input type="checkbox"/> Ivomec (ML)	<input type="checkbox"/> Clear (LEV) <input type="checkbox"/> Other - Specify
Do you conduct FEC tests and when?		
Any resistance testing and results?		
Do you move drenched sheep to spelled paddocks?	<input type="checkbox"/> Yes	<input type="checkbox"/> No
Do you think this has been effective?	<input type="checkbox"/> Yes	<input type="checkbox"/> No

Interviewer Assessment:

The producer follows recommended practice.	<input type="checkbox"/> Yes	<input type="checkbox"/> No
The program is likely to be effective.	<input type="checkbox"/> Yes	<input type="checkbox"/> No

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

SECTION 2: OJD infection history and management

1. Duration and level of Infection

In which year was the OJD diagnosed in your flock?

How was OJD diagnosed? In which order?

- Clinical signs & Post mortem
- Histopathology
- Abattoir Surveillance
- Blood test (AGID or ELISA)
- Faecal test (PFC test)
- Other (please specify)

Do you agree to us obtaining copies? Yes/No

From

How long ago, do you suspect, your flock became infected with OJD & why?

What signs of OJD do you see in your flock?

- Deaths
- Loss of condition
- Scouring
- Nil
- Other – Specify

Interviewer assessment:

Duration of infection in flock

- 3 years to less than 5 years
- 5 years to less than 7 years
- 7 years to less than 10 years
- 10 years to less than 15 years
- More than 15 years

2. Losses due to OJD:

	Year	<i>OJD mortalities of 2yo+ sheep</i> (actual numbers if possible)
First Mortalities		
Peak Mortalities		
Current Mortalities	Last 12 months	
5 yr mean	1999-2004	

Age of youngest Mortality

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

3. Source of OJD infection

In your opinion, what was the **source of OJD infection** on this property?

- Neighbour
- Introduction of infected sheep
- Unknown
- Others – Give details

Risk of lateral spread

No. of neighbours

No. of neighbours running sheep

No. of neighbours known infected

No. of neighbours likely infected

[Please Tick (✓) if following is true]

- Sharing of rams with neighbours
- Sharing of sheds/yards with neighbours
- Sharing of roads with neighbours
- Straying of sheep between properties

Details: Frequency over last 10 yrs

Do you have a sheep proof fence around your property?

What proportion of property boundary receives run off water?

Does an *intermittent* creek flow onto your property?

Does a *permanent* creek flow onto your property?

Water source in holding yards (source & delivery)

<input type="checkbox"/> Yes	<input type="checkbox"/> No
%	
<input type="checkbox"/> Yes	<input type="checkbox"/> No
<input type="checkbox"/> Yes	<input type="checkbox"/> No

4. Wildlife risk

Do you have the following animals on your property?

[PI Tick (✓) if yes]

- Kangaroos
- Feral Goats
- Rabbits
- Others (Pl. specify)_____

Average mob size/No active warrens	% of paddocks inhabited

Identification of Risk Factors for OJD Infection-Level in Sheep Flocks

5. OJD control strategies

What sheep drops are currently vaccinated on this property?

	2004	2003	2002	2001	2000	1999	1998+
Vaccinated as lambs							
Vaccinated as adults							

What other management procedures do you use to control OJD on your property?

- Sell high loss mobs
- Cull low body weight sheep
- Separate young sheep
- Handle young sheep first
- Destock lambing/weaning paddocks
- Other [specify] _____

What do you do with clinical OJD sheep?

- Nothing
- Dispose off immediately
- Isolate from main flock e.g. hospital pdk
- Other [specify] _____

Sheep purchases / introductions

Have you regularly introduced **rams** over the last 5 years?

Yes No

From how many sources have you purchased rams **from 1999 to 2004**?

Source	Infection status (Infected, Uninfected, Unknown)	District (PZ, CZ, RZ)	Total number of rams sourced 1999-2004

Have you introduced **other sheep** over the last 5 years?

Yes No

From how many sources have you purchased these sheep **from 1999-2004**?

Source	Infection status (Infected, Uninfected, Unknown)	District (PZ, CZ, RZ)	Total number of sheep sourced 1999-2004

8 Owners view on why losses are low/high?

SECTION 3 – Cohort History & Management (Questions relate to the cohort of sheep sampled. If this varies from normal management please note)

USE DIFFERENT FORMS FOR TWO DIFFERENT AGE GROUPS

1. General Management

YEAR OF LAMBING	
Date of marking	
Husbandry practices at marking	<input type="checkbox"/> Clostridial vaccination <input type="checkbox"/> +Selenium? <input type="checkbox"/> Booster given? <input type="checkbox"/> CLA vaccination (6 in 1) <input type="checkbox"/> Scabby mouth vaccination <input type="checkbox"/> Mulesing <input type="checkbox"/> Other -----
Date of mulesing (If not at marking)	
When are the sexes separated?	
Age at which mixed with adult flock	Wethers _____ Ewes _____
Date of first shearing	

2. Management during different age groups

Stage of Life	Lambing Ewes	Weaners WNG to 12 m	Hoggets 12 m to 24 m	Adults >24 m
Date Start of stage				
Duration of lambing				
Number @ start of stage	Num ewes joined/lambed	Num lambs weaned	Num hoggets into paddocks	
Total area grazed Paddocks 1 Paddocks 2				
Est'd Stock rate (shp/ha) Paddocks 1 Paddocks 2				
CS @ start of stage	@ start of lambing	@ weaning	@12 m	@24 m
Did weaners continue to grow after weaning				
Length (weeks) & method of any OJD decontamination				
Pasture (Imp/FN/Nat)				
Period (weeks) of any growth check				
Grazing M'gt (Set,				

Stage of Life	Lambing Ewes	Weaners WNG to 12 m	Hoggets 12 m to 24 m	Adults >24 m
<i>Rotational, Cell – include mean rest period)</i>				
Water source (<i>bore, dam, creek</i>)				
Water supply (<i>trough/ground</i>)				
Did you give mineral supplement?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If Yes , What type? Provide details				
Did animals graze fodder crops?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If Yes , for how long (wks)?				
Did animals graze stubble?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If Yes , for how long (wks)?				
Any evidence of high- level worm burden?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If Yes , provide details or copies FEC reports				
Scouring present?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If Yes , Cause of scouring? (eg nutrition, worms, OJD)				
Did these animals experience other health problems?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If Yes , provide details				

3. Management of ewes	Date & duration	CS	Weaning %
First Joining			
First Lambing			
Second Joining			
Second Lambing			

4. Did you provide supplementary feed to the cohort sheep?

If yes: shade & number events on management calendar.

Period ID	Date start	Date End	No of weeks	Ration	Quantity (kg/head/day)	Included Lime?	Method feeding (G, T)	CS at start	Reasons for supplementary feeding
						<input type="checkbox"/>			
						<input type="checkbox"/>			
						<input type="checkbox"/>			
						<input type="checkbox"/>			

Management Calender

Operation	YEAR 1 =																							
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	
Paddock																								
Breeding																								
Shearing																								
Feeding																								
Nutritional stress																								
Flock																								
Paddock																								
Breeding																								
Shearing																								
Feeding																								
Nutritional stress																								
Flock																								
Paddock																								
Breeding																								
Shearing																								
Feeding																								
Nutritional stress																								

Appendix 4 Specimen advice form

The University of Sydney

Faculty of Veterinary Science
Farm Animal Health Laboratory

Specimen Advice- Risk Factor Trial 038

For Laboratory Use Only

SVC _____
Date _____
Officer _____

Flock Code ID _____ Sample Date _____

Property Name _____ District _____

Submitter _____ Address _____

Phone No _____

Fax _____

E-mail _____

Analysis required for: **OJD**

Research Project Details: **Risk Factor Trial 038**

Pool Number	Sex	Drop	Number of sheep	Results [For lab use only]

Breed: _____ Condition Score _____

Comments:

Signature _____ Date _____

Appendix 5 Soil sample analyses

Appendix 5.1

List of soil sample analysis results provided by the Incitec Pivot laboratory

Colour	Chloride
Texture	Electrical conductivity
pH (1:5 Water)	Copper
pH (1:5 CaCl ₂)	Zinc
Organic carbon%	Manganese
Nitrate Nitrogen	Iron
Sulphate Sulphur	Boron
Phosphorus	Cation exchange capacity
Potassium	Calcium Magnesium Ratio
Calcium	Electrical conductivity (Sat Ext)
Magnesium	Aluminium saturation%
Aluminium	Sodium % of cation
Sodium	Phosphorus buffer index

Appendix 5.2

Guidelines used by the Incitec Pivot laboratory for classification of soil texture

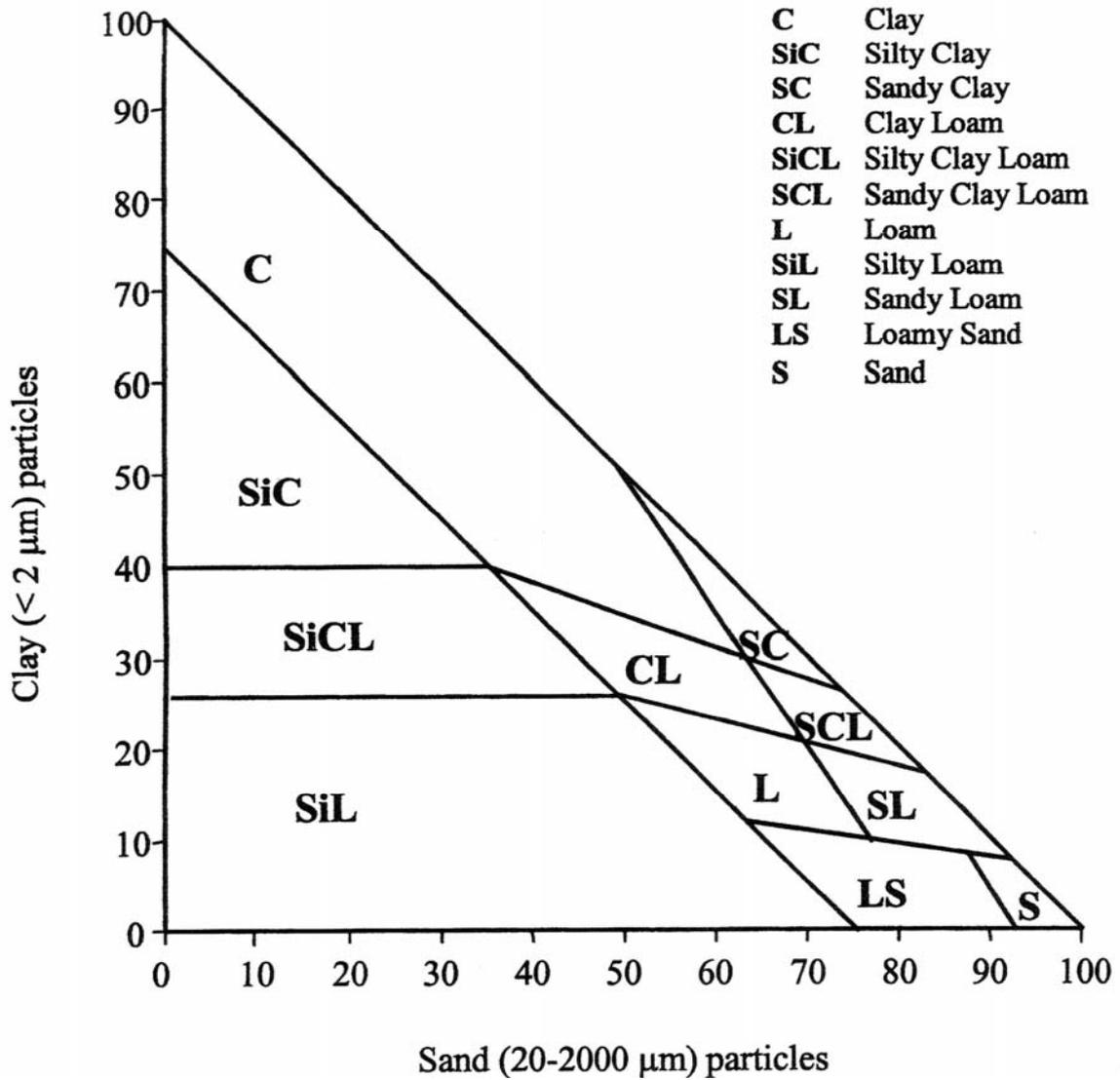
Classification	Description	Ribbon Length
Sand	Coherence nil to very slight, cannot be moulded, single sand grains adhere to fingers	Less than 5 mm
Loamy Sand	Slight coherence, discolours fingers with dark organic stain	Up to 5mm
Sandy Loam	Bolus just coherent but very sandy to touch	15-25mm
Silty Loam	Coherent bolus, very smooth to silky when manipulated; will not form solid rods or ribbons.	25mm
Clay Loam	Coherent plastic bolus, smooth to manipulate, slight adherence to fingers, forms rods	40-50mm
Sandy Clay Loam	Strong coherent bolus, sandy to touch; medium size sand grains visible in finer matrix, will form rods that will break easily due to sand content	25-40mm
Sandy Clay	Plastic bolus; fine to medium sand can be felt or heard in clayey matrix	50-75mm
Light Clay	Plastic bolus; smooth to touch, slight resistance to shearing between thumb and forefinger	50-75mm
Medium Clay	Smooth plastic bolus; handles like plasticine and can be moulded into rods without fracture; has some resistance to ribboning shear	75+mm
Heavy Clay	Smooth plastic bolus; handles like stiff plasticine; can be moulded into rods without fracture; has firm resistance to ribboning shear	75+mm

Appendix 5.3

Calculations for some chemical soil analysis results provided by the Incitec Pivot laboratory and their reporting limits

Results	Calculations	Reporting Limit
Ca (MEQ/100g)	$Ca(\text{MEQ}/100\text{g}) = Ca (\text{mg}/\text{kg}) \times 0.00499$	0.1 MEQ/100g
K (MEQ/100g)	$K(\text{MEQ}/100\text{g}) = K (\text{mg}/\text{kg}) \times 0.00256$	0.01 MEQ/100g
Mg (MEQ/100g)	$Mg(\text{MEQ}/100\text{g}) = Mg (\text{mg}/\text{kg}) \times 0.00823$	0.2 MEQ/100g
Na (MEQ/100g)	$Na(\text{MEQ}/100\text{g}) = Na (\text{mg}/\text{kg}) \times 0.00435$	0.02 MEQ/100g
CEC (MEQ/100g)	$CEC = Ca(\text{MEQ}/100\text{g}) +$ $K(\text{MEQ}/100\text{g}) +$ $Mg(\text{MEQ}/100\text{g}) +$ $Na(\text{MEQ}/100\text{g})$	
Ca/Mg Ratio	$\frac{Ca(\text{MEQ}/100\text{g})}{Mg(\text{MEQ}/100\text{g})}$	
Al (MEQ/100g)	$Al(\text{MEQ}/100\text{g}) = Al (\text{mg}/\text{kg}) \times 0.01112$	0.03 MEQ/100g
CEC Al (MEQ/100g)	$CEC Al = Ca(\text{MEQ}/100\text{g}) +$ $K(\text{MEQ}/100\text{g}) +$ $Mg(\text{MEQ}/100\text{g}) +$ $Na(\text{MEQ}/100\text{g}) +$ $Al(\text{MEQ}/100\text{g})$	
Al % of Cations	$\frac{Al(\text{MEQ}/100\text{g})}{CEC Al(\text{MEQ}/100\text{g})} \times 100$	
Na % of Cations	$\frac{Na(\text{MEQ}/100\text{g})}{CEC Al(\text{MEQ}/100\text{g})} \times 100$	
PBI Colwell	$\frac{(1000 - (10 \times P) + ColP)}{P^{0.41}}$ where P is the concentration of Phosphorus remaining in solution in mg/L.	
Electrical Conductivity	Expressed as dS/cm	0.01

Appendix 5.4 International soil texture triangle



Appendix 6 Description of soil explanatory variables

Appendix 6

Description of the 44 explanatory variables related to soil sample analyses investigated in this study

Variables	Code	Description and categories	No of flocks	25P	Median	Mean	75P
Property-level variables							
PSTYPE		Parent soil type on the property	92		Discrete variable		
	1	Basaltic	8				
	2	Granite	28				
	3	Shale and sandstone	30				
	4	Mixed without limestone	10				
	5	Mixed with limestone	16				
3-paddock mean variables							
Ph		pH (1:5 CaCl ₂) of soil	92	4.43	4.68	4.80	5.07
	0	<4.6	34				
	1	4.6 - 5.2	41				
	2	>5.2	17				
CEC ¹		Cation exchange capacity of soil- Meq/100g	92	4.74	5.94	7.21	8.80
	1	≤ 6	47				
	2	> 6	45				
P		Phosphorus (Colwell) content of soil- mg/kg	92	21.00	28.33	31.29	40.00
	0	<20	21				
	1	20-30	30				
	2	>30	41				
PBI ²		Phosphorus buffer index (PBI-Col) of soil	90	47.00	61.50	69.66	81.67
	0	< 70	56				
	1	≥ 70	34				
S		Sulphate Sulphur KCl40 content of the soil -mg/kg	92	4.65	7.03	7.88	9.17
	0	<4	18				
	1	4--8	38				
	2	>8	36				

¹ CEC is the capacity of the soil to interact with and hold elements for release into the soil solution for subsequent plant use.

² Phosphorus buffer index (PBI) is the ability of the soil to fix and hold on phosphorus

K	Potassium (Amm-acet.) content of soil Meq/100g	92	0.39	0.54	0.58	0.71
1	<0.4	25				
2	>0.4	67				
ALSAT	Aluminium saturation %	92	1.39	4.27	6.32	9.95
0	≤ 2	28				
1	>2 to ≤ 5	23				
2	>5 to ≤ 12	26				
3	>12	15				
SAND	Percent of coarse and fine sand particles in soil	90	54.75	62.01	61.53	69.02
1	≤ 62%	45				
2	> 62%	45				
SILT	Percent of silt particles in the soil	90	17.97	21.80	22.32	26.13
1	≤ 21%	42				
2	> 21%	48				
CLAY	Percent of clay particles in the soil	90	12.62	15.15	16.14	18.58
1	≤ 15%	44				
2	> 15%	46				
Lamb paddock variables						
PH_LBGPDK	pH (1:5 CaCl ₂) of soil samples	71	4.40	4.60	4.81	5.10
0	<4.6	30				
1	4.6 - 5.2	27				
2	>5.2	14				
CEC_LBGPDK	Cation exchange capacity of soil- Meq/100g	71	4.58	6.32	7.48	8.41
1	≤ 6	32				
2	> 6	39				
P_LBGPDK	Phosphorus (Colwell) content of soil- mg/kg	71	18.00	26.00	33.78	41.00
0	<20	22				
1	20-30	21				
2	>30	28				
PBI_LBGPDK	Phosphorus buffer index (PBI- Col) of soil	70	41.33	57.25	63.63	81.00
0	< 70	48				
1	≥ 70	22				

S_LBGPDK	Sulphate Sulphur KCl40 content of the soil -mg/kg	71	4.30	6.60	7.78	9.70
	0 <4	14				
	1 4--8	33				
	2 >8	24				
K_LBGPDK	Potassium (Amm-acet.) content of soil Meq/100g	71	0.37	0.55	0.62	0.86
	1 <0.4	21				
	2 >0.4	50				
ALSAT_LBGPDK	Aluminium saturation %	71	0.77	3.14	5.90	10.31
	0 ≤ 2	30				
	1 >2 to ≤ 5	16				
	2 >5 to ≤ 12	12				
	3 >12	13				
SAND_LBGPDK	Percent of coarse and fine sand particles in soil	69	54.50	62.58	62.19	69.11
	1 ≤ 62%	33				
	2 > 62%	36				
SILT_LBGPDK	Percent of silt particles in the soil	69	16.55	21.01	22.05	27.95
	1 ≤ 21%	34				
	2 > 21%	35				
CLAY_LBGPDK	Percent of clay particles in the soil	69	12.00	14.84	15.77	19.26
	1 ≤ 15%	37				
	2 > 15%	32				

Weaner paddock variables

PH_WNGPDK	pH (1:5 CaCl ₂) of soil samples	63	4.40	4.80	4.90	5.10
	0 <4.6	22				
	1 4.6 - 5.2	29				
	2 >5.2	12				
CEC_WNGPDK	Cation exchange capacity of soil- Meq/100g	63	4.35	6.16	7.83	9.10
	1 ≤ 6	30				
	2 > 6	33				

P_WNGPDK	Phosphorus (Colwell) content of soil- mg/kg	63	21.00	30.00	34.29	42.00
	0 <20	11				
	1 20-30	22				
	2 >30	30				
PBI_WNGPDK	Phosphorus buffer index (PBI-Col) of soil	61	50.00	62.00	67.23	87.00
	0 < 70	36				
	1 ≥ 70	25				
S_WNGPDK	Sulphate Sulphur KCl40 content of the soil -mg/kg	63	4.95	7.60	10.32	10.00
	0 <4	9				
	1 4--8	28				
	2 >8	26				
K_WNGPDK	Potassium (Amm-acet.) content of soil Meq/100g	63	0.39	0.55	0.61	0.78
	1 <0.4	16				
	2 >0.4	46				
ALSAT_WNGPDK	Aluminium saturation %	63	0.69	1.92	5.09	5.76
	0 ≤ 2	32				
	1 >2 to ≤ 5	10				
	2 >5 to ≤ 12	14				
	3 >12	7				
SAND_WNGPDK	Percent of coarse and fine sand particles in soil	60	46.59	60.33	58.86	68.93
	1 ≤ 62%	34				
	2 > 62%	26				
SILT_WNGPDK	Percent of silt particles in the soil	60	18.49	24.28	23.97	30.98
	1 ≤ 21%	26				
	2 > 21%	34				
CLAY_WNGPDK	Percent of clay particles in the soil	60	11.32	16.04	17.17	21.08
	1 ≤ 15%	29				
	2 > 15%	31				

Hogget/Adult paddock variables

PH_ADPPDK	pH (1:5 CaCl ₂) of soil samples	68	4.40	4.60	4.77	5.03
	0 <4.6	29				
	1 4.6 - 5.2	27				
	2 >5.2	12				

CEC_ADSDK	Cation exchange capacity of soil- Meq/100g	68	4.43	5.63	7.11	8.69
1	≤ 6	37				
2	> 6	31				
P_ADSDK	Phosphorus (Colwell) content of soil- mg/kg	68	18.25	27.00	29.45	38.00
0	<20	23				
1	20-30	17				
2	>30	28				
PBI_ADSDK	Phosphorus buffer index (PBI-Col) of soil	67	46.00	62.00	69.76	84.00
0	< 70	41				
1	≥ 70	26				
S_ADSDK	Sulphate Sulphur KCl40 content of the soil -mg/kg	68	4.02	6.08	6.71	8.55
0	<4	16				
1	4--8	32				
2	>8	20				
K_ADSDK	Potassium (Amm-acet.) content of soil Meq/100g	68	0.35	0.50	0.57	0.69
1	<0.4	20				
2	>0.4	47				
ALSAT_ADSDK	Aluminium saturation %	68	0.99	3.54	7.04	11.46
0	≤ 2	26				
1	>2 to ≤ 5	15				
2	>5 to ≤ 12	11				
3	>12	16				
SAND_ADSDK	Percent of coarse and fine sand particles in soil	67	52.32	62.97	60.85	70.58
1	≤ 62%	32				
2	> 62%	35				
SILT_ADSDK	Percent of silt particles in the soil	67	17.40	21.79	22.28	25.40
1	≤ 21%	31				
2	> 21%	36				
CLAY_ADSDK	Percent of clay particles in the soil	67	12.03	14.98	16.86	19.93
1	≤ 15%	35				
2	> 15%	32				

Appendix 7 Univariable results: History and management variables for IPREV

Cohort OJD prevalence – IPREV

Results for the 71 variables investigated are presented in the table Appendix 7.2. Of these, 22 flock-level variables (including 7 of 7 confounders) and 9 cohort-level variables were unconditionally associated with IPREV in the FIRST dataset (Appendix 7.1). After deletion of highly correlated variables (as described in Appendix 1 Section 3.10) a total of 29 variables remained for inclusion in multivariable analyses. The final number of variables unconditionally associated with IPREV in the SECOND and THIRD datasets and included in multivariable analyses were 27 and 22, respectively. The 17 variables identified as unconditionally associated with IPREV in all three datasets and used in multivariable analyses were:

- Interviewers' assessment of length of flock OJD infection (OJD_DURN)
- Current flock OJD mortality% in adults (≥ 2 yr old) (CURRMORT)
- Age of youngest OJD mortality in the flock (YNGAGEMORT)
- Number of age groups vaccinated in the flock (DROPSVACC)
- Management of OJD clinical sheep (CLINICALMGT)
- Culling of low body weight sheep (CULL)
- Proportion of property boundary receiving run off water (RUNOFFWATER)
- Permanent creek flowing onto the property (PCREEK)
- Percent paddocks inhabited by kangaroos (KANGAROO)
- Presence of other wildlife (aside from kangaroos and rabbits) (OTHERWILDLIFE)
- Application of fertilizers other than single super, molybdenum super and lime on the property (OTHERFERT)
- Drought over cohort lifetime (DROUGHT)
- Sex of the cohort (SEX)
- Period of any growth check during the lifetime of the cohort (GROWTHCHK)
- Season of cohort birth (LBGSSN)
- Condition score of hoggets at 1 year of age (HGTCS)
- Condition score of cohort at the time of faecal sample collection (SVC_CS).

Appendix 7.1

The P-values from univariable analyses of FIRST, SECOND and THIRD datasets for history and management variables unconditionally associated with IPREV (P<0.25 – shown in regular text) in one or more datasets and included in subsequent multivariable analyses for the respective dataset/s

Variables	FIRST Dataset	SECOND Dataset	THIRD Dataset
OJD_DURN	<0.001	0.01	0.01
CURRMORT	<0.001	<0.001	<0.001
YNGAGEMORT	<0.001	<0.001	<0.001
DROPSVACC	<0.001	0.02	0.14
CLINICALMGT	0.09	0.01	<0.001
CULL	0.01	0.01	<0.001
SELL	0.13	0.16	0.32
YOUNGSEPARATE	0.51	0.14	0.01
YOUNGFIRST	0.89	0.59	0.17
SHARING_ROAD	0.09	0.1	0.28
RUNOFFWATER	0.14	0.21	0.22
PCREEK	0.04	0.14	0.2
ICREEK	0.25	0.33	0.06
KANGAROO	0.09	0.23	0.12
OTHERWILDLIFE	0.12	0.02	0.17
SUPERFREQ	0.04	0.36	0.06
LIME	0.19	0.25	0.62
OTHERFERT	0.03	0.03	0.03
MINERALDEF	0.22	0.28	0.91
DROUGHT	<0.001	<0.001	<0.001
DROUGHT_YRLAMBING	0.06	0.19	0.38
AGEGP	0.13	0.5	0.64
SEX	<0.001	0.01	0
GROWTHCHK	0.04	0.03	0.08
MINSUPPL	0.28	0.22	0.25
SUPPLFEED	0.32	0.27	0.15
LBGSSN	0.14	0.07	0.07
DECONT_WNGPDK	0.04	0.16	0.33
WNRGMGT	0.05	0.15	0.45
WNRSR	0.23	0.24	0.45
WNRHLTH	0.09	0.4	0.5
HGTSR	0.29	0.14	0.51
HGTCS	0.01	0.01	0.05
SVC_CS	0.08	0.04	0.03
Total no of variables for inclusion in multivariable analyses	29	27	22

Appendix 7.2

Unconditional associations between history and management flock- and cohort-level variables and IPREV (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-10%) and high (>10%)) for 77 cohorts in the FIRST Dataset, 98 cohorts in the SECOND Dataset and 117 cohorts in the THIRD Dataset

Variables and categories	Code	FIRST Dataset				SECOND Dataset				THIRD Dataset			
		OR	LCL	UCL	P	OR	LCL	UCL	P	OR	LCL	UCL	P
<i>Flock-level variables</i>													
<i>Confounding variables</i>													
INFLEVEL					<0.001				<0.001				<0.001
No mortalities	0	1				1				1			
Low mortalities and trend falling or steady	1	4.26	1.23	15.87		3.51	1.16	11.13		3.99	1.44	11.60	
High mortalities but trend falling or steady OR Low mortalities but trend escalating	2	14.45	3.97	58.86		15.73	4.97	54.78		13.32	4.81	39.62	
High mortalities as well as trend escalating	3	13.06	2.88	65.12		12.93	3.02	59.53		7.09	1.92	27.50	

OJD_DURN					<0.001				0.01				0.01
3<5 years	0	1				1				1			
5<7years	1	3.38	0.86	13.82		2.99	0.85	10.78		2.17	0.68	7.19	
7<10years	2	1.00	0.26	3.86		1.37	0.41	4.62		1.14	0.37	3.60	
10 year or more	3	5.43	1.42	22.17		5.60	1.67	19.68		4.82	1.56	15.54	

PEAKMORT					<0.001				<0.001				<0.001
No mortalities	0	1				1				1			
<2% mortalities	1	6.97	2.06	25.64		5.72	1.94	17.91		5.85	2.16	16.77	
≥2% mortalities	2	9.57	2.93	34.35		9.95	3.44	30.96		8.70	3.34	24.21	

CURRMORT					<0.001				<0.001				<0.001

No mortalities	0	1			1				1		
<2% mortalities	1	5.52	1.74	18.82	5.21	1.85	15.47	5.31	2.05	14.46	
≥2% mortalities	2	10.96	3.25	40.59	10.23	3.48	32.25	8.67	3.30	24.16	

MEANMORT					<0.001			<0.001			<0.001
No mortalities	0	1			1			1			
<2% mortalities	1	10.15	2.88	40.00	7.88	2.65	25.27	8.90	3.26	26.03	
≥2% mortalities	2	8.25	2.30	32.83	8.48	2.73	28.41	8.16	2.92	24.33	

YNGAGEMORT					<0.001			<0.001			<0.001
No mortalities	0	1			1			1			
<2years	1	7.09	1.58	34.45	10.87	2.66	47.13	11.76	3.27	44.84	
2 to < 3 yrs	2	8.72	2.66	31.25	8.93	3.06	27.97	8.79	3.30	24.96	
≥3 years	3	7.98	2.03	34.11	5.13	1.61	17.30	4.44	1.55	13.40	

OJDSIGNS					<0.001			<0.001			<0.001
Nil	0	1			1			1			
Death and/or Loss of condition	1	4.81	1.20	20.62	4.40	1.30	15.76	4.82	1.60	15.26	
Death, loss of condition and scouring	2	7.73	2.49	26.02	9.58	3.44	28.77	8.61	3.40	23.31	

OJD Control											
DROPSVACC					<0.001			0.02			0.14
No drops vaccinated	0	1			1			1			
1 or 2 drops vaccinated	1	9.25	2.18	42.16	5.32	1.48	20.00	3.19	1.02	10.33	
>2 drops vaccinated	2	1.84	0.53	6.49	1.74	0.58	5.33	1.87	0.69	5.14	

CLINICALMGT					0.09			0.01			<0.001
No mortalities observed	0	1			1			1			
Immediately dispose off	1	4.62	1.42	16.08	6.29	2.22	18.95	6.90	2.63	19.31	
Dispose off later	2	3.57	0.82	16.36	3.40	0.90	13.36	4.76	1.43	16.63	
Do nothing	3	3.57	0.50	26.67	4.16	0.72	25.09	2.64	0.60	11.80	

CULL					0.01			0.01			<0.001
No	0	1			1			1			

Yes	1	3.08	1.26	7.93		3.03	1.37	6.93		3.06	1.51	6.39
SELL					0.13				0.16			0.32
No	0	1				1				1		
Yes	1	2.45	0.77	8.09		2.17	0.73	6.58		1.65	0.62	4.49
YOUNGSEPARATE					0.51				0.14			0.01
No	0	1				1				1		
Yes	1	1.33	0.57	3.16		1.77	0.83	3.86		2.38	1.19	4.85
YOUNGFIRST					0.89				0.59			0.17
No	0	1				1				1		
Yes	1	1.09	0.33	3.60		1.35	0.45	4.06		1.96	0.74	5.25
DESTOCK					0.34				0.40			0.62
No	0	1				1				1		
Yes	1	0.65	0.27	1.57		0.72	0.32	1.56		0.84	0.41	1.70

Lateral Spread and purchase risk

INFNBRS					0.98				0.89			0.87
≤25%	0	1				1				1		
>25% to ≤75%	1	0.88	0.28	2.77		0.81	0.28	2.31		0.84	0.32	2.20
> 75%	2	0.91	0.31	2.66		1.00	0.39	2.57		1.04	0.45	2.43
SHSTRAY					0.32				0.95			0.74
No straying <10 sheep stray annually OR stray not even once per year	0	1				1				1		
10-20 sheep stray annually	1	1.61	0.55	4.77		1.10	0.44	2.78		0.83	0.36	1.88
>20 sheep stray annually OR stray more than once annually	2	2.60	0.76	9.18		1.38	0.47	4.15		0.58	0.21	1.55
	3	2.93	0.76	11.67		1.20	0.35	4.13		0.93	0.30	2.89
SHARING_ROAD					0.09				0.10			0.28
No sharing ≤twice per year	0	1				1				1		
	1	1.88	0.66	5.53		1.93	0.74	5.15		1.90	0.81	4.54

>twice per year	2	3.34	1.08	10.77		2.85	1.01	8.29		1.59	0.62	4.11
<hr/>												
SHARING_SHED					0.90					0.74		0.60
No sharing	0	1				1				1		
Sharing	1	1.09	0.29	4.11		0.83	0.26	2.58		0.76	0.27	2.11
<hr/>												
RUNOFFWATER					0.14					0.21		0.22
≤10%	0	1				1				1		
>10 to ≤ 30%	1	0.70	0.23	2.13		0.64	0.23	1.72		0.77	0.31	1.91
>30% to ≤ 60%	2	1.11	0.35	3.53		0.71	0.26	1.97		0.58	0.23	1.44
> 60%	3	0.23	0.06	0.89		0.28	0.09	0.91		0.36	0.13	0.97
<hr/>												
PCREEK					0.04					0.14		0.20
No	0	1				1				1		
Yes	1	0.38	0.14	0.96		0.54	0.24	1.22		0.63	0.30	1.29
<hr/>												
ICREEK					0.25					0.33		0.06
No	0	1				1				1		
Yes	1	1.92	0.63	5.98		1.61	0.62	4.24		2.19	0.96	5.13
<hr/>												
KANGAROO					0.09					0.23		0.12
≤ 20%	0	1				1				1		
>20% to ≤ 50%	1	2.58	0.91	7.65		2.09	0.79	5.63		2.21	0.92	5.41
>50%	2	2.93	1.02	8.81		1.99	0.80	5.03		2.12	0.94	4.87
<hr/>												
RABBIT					0.76					0.90		0.57
Nil	0	1				1				1		
≤ 5%	1	0.69	0.25	1.90		1.00	0.40	2.50		1.56	0.68	3.60
>5%	2	0.88	0.28	2.76		0.82	0.31	2.21		1.37	0.56	3.38
<hr/>												
OTHERWILDLIFE					0.12					0.02		0.17
No	0	1				1				1		
Yes	1	0.48	0.18	1.20		0.39	0.17	0.88		0.61	0.30	1.23
<hr/>												
RAMRISK					0.68					0.72		0.59
None purchased/ none from infected sources	1	1				1				1		

Some/all purchased from infected sources	2	0.83	0.34	1.99		0.87	0.40	1.88		0.83	0.41	1.66
<hr/>												
EWERISK					0.34				0.40			0.87
No	0	1				1				1		
Yes	1	1.54	0.63	3.78		1.40	0.63	3.14		1.06	0.51	2.21
<hr/>												
Property management												
GRAZEDAREA					0.54				0.39			0.37
≤ 965 hectares	1	1				1				1		
> 965 hectares	2	0.76	0.31	1.84		0.72	0.33	1.53		0.73	0.37	1.44
<hr/>												
FLOCKSIZE					0.96				0.78			0.71
≤ 3000	1	1				1				1		
> 3000	2	1.02	0.43	2.42		0.90	0.42	1.92		0.88	0.44	1.73
<hr/>												
SUPERFREQ					0.04				0.36			0.06
≤ once in three years	0	1				1				1		
>once to ≤ twice in three years	1	2.50	0.70	9.20		1.56	0.53	4.62		1.72	0.66	4.50
> twice to ≤ Every year	2	4.61	1.45	15.53		2.48	0.92	6.89		3.36	1.39	8.39
>Once every year	3	5.39	1.20	25.48		1.76	0.48	6.51		1.72	0.52	5.68
<hr/>												
LIME					0.19				0.25			0.62
No	0	1				1				1		
Yes	1	0.56	0.23	1.35		0.63	0.28	1.39		0.84	0.41	1.70
<hr/>												
OTHERFERT					0.03				0.03			0.03
No	0	1				1				1		
Yes	1	0.26	0.07	0.85		0.27	0.08	0.87		0.31	0.10	0.90
<hr/>												
MINERALDEF					0.22				0.28			0.91
No evidence	0	1				1				1		
Some evidence	1	0.56	0.22	1.41		0.64	0.28	1.44		0.96	0.46	1.99

Flock management

WORMCONTROL					0.88				0.38			0.34
No	0	1				1				1		
Yes	1	0.92	0.29	2.87		0.65	0.25	1.68		0.66	0.28	1.53

WORMRECOMM					0.88				0.38			0.34
No	0	1				1				1		
Yes	1	1.27	0.50	3.23		0.92	0.41	2.07		1.15	0.56	2.37

Drought and water logging

WATERLOG					0.71				0.64			0.53
<1%	0	1				1				1		
1% to <10%	1	1.53	0.47	5.13		1.61	0.55	4.73		1.89	0.71	5.08
10% to <30%	2	1.15	0.33	4.00		0.98	0.34	2.87		1.40	0.54	3.68
≥ 30%	3	0.76	0.23	2.47		0.80	0.28	2.26		1.00	0.39	2.55

PINRUSH					0.57				0.52			0.26
Nil	0	1				1				1		
<1%	1	0.42	0.10	1.71		0.39	0.11	1.37		0.35	0.12	0.99
1 to < 5%	2	0.59	0.20	1.70		0.73	0.27	1.92		0.82	0.34	1.97
>5%	3	0.64	0.19	2.12		0.72	0.26	1.97		0.90	0.36	2.25

DROUGHT					<0.001				<0.001			<0.001
≥150mm lesser OR more than long-term average	0	1				1				1		
>150mm lesser	1	5.88	2.17	17.30		4.19	1.73	10.58		3.84	1.73	8.79

DROUGHT_SAMPLEYR					0.93				0.88			0.89
up to 132mm lesser OR more than long-term average	0	1				1				1		
> 132 mm to 228mm lesser	1	1.642	0.588	4.664		1.252	0.501	3.151		0.819	0.362	1.845
> 228 mm lesser	2	1.081	0.374	3.138		1	0.393	2.547		0.953	0.406	2.233

DROUGHT_YRLAMBING					0.06				0.19			0.38
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up to 15mm lesser OR
more than long-term
average

0	1				1				1		
1	1.11	0.34	3.71		0.91	0.35	2.35		1.28	0.56	2.97
2	3.07	1.16	8.54		2.09	0.85	5.23		1.79	0.79	4.13

Cohort level factors

General

AGEGP					0.13				0.50			0.64
3 years	3	1				1				1		
4 years	4	0.50	0.20	1.23		0.77	0.35	1.67		0.71	0.34	1.47
5 years	5									0.74	0.21	2.59

SEX					<0.001				0.01			0.00
Ewes	0	1				1				1		
Wethers	1	8.10	2.72	26.37		3.53	1.39	9.28		3.09	1.33	7.39
Mixed sex	2									0.20	0.03	1.02

GROWTHCHK					0.04				0.03			0.08
<12 weeks	0	1				1				1		
≥12 weeks	1	2.59	1.07	6.50		2.37	1.08	5.31		1.99	0.93	4.34

MINSUPPL					0.28				0.22			0.25
No	0	1				1				1		
Yes	1	0.62	0.25	1.48		0.62	0.29	1.33		0.64	0.30	1.35

FODSTUB					0.52				0.49			0.76
No	0	1				1				1		
<16 weeks	1	1.83	0.64	5.31		1.68	0.66	4.35		1.42	0.55	3.65
≥16 weeks	2	1.39	0.50	3.94		1.49	0.59	3.80		1.11	0.45	2.72

WATER					0.83				0.83			0.61
Less	1	1				1				1		
Average	2	1.38	0.24	8.25		1.41	0.25	8.15		1.25	0.22	7.17
High	3	1.60	0.31	8.39		1.60	0.32	8.17		1.79	0.36	9.16

SUPPLFEED					0.32				0.27			0.15
No	0	1				1				1		
≤ 6months	1	0.98	0.21	4.45		0.95	0.26	3.49		1.388	0.376	5.174
												11.35
6 months to 1 year	2	2.74	0.68	11.42		2.30	0.71	7.63		3.352	1.021	5
> 1year	3	1.55	0.39	6.22		2.10	0.65	6.91		2.57	0.794	8.531

SUPPLFEED_CS					0.93				1			0.6
<3	1	1				1				1		
≥ 3	2	1.05	0.33	3.37		1	0.36	2.79		1.3	0.49	3.50

SUPPLFEED_METHOD					0.60				0.96			0.48
All on ground	0	1				1				1		
Some in trough	1	0.69	0.17	2.72		0.96	0.26	3.60		0.63	0.18	2.25

SUPPLFEED_LIME					1.00				0.78			0.73
No	0	1				1				1		
Yes, in some feeds	1	1.00	0.35	2.86		1.15	0.44	3.00		1.18	0.47	2.95

Lambing variables												
DAMSR					0.96				0.94			0.62
<8dse/hac	1	1				1				1		
8 <12 dse/hac	2	1.23	0.25	6.14		0.98	0.24	4.01		0.56	0.14	2.19
≥12 dse/hac	3	1.16	0.37	3.64		1.16	0.40	3.39		0.93	0.33	2.61

DAMCS					0.56				0.92			0.95
<3	1	1				1				1		
3<4	2	1.91	0.54	6.97		1.29	0.38	4.37		1.16	0.37	3.60
≥4	3	1.36	0.28	6.69		1.29	0.30	5.66		1.24	0.31	5.07

DAM_SCOUR					0.55				0.35			0.54
No	0	1				1				1		
Yes	1	1.40	0.46	4.33		1.64	0.58	4.65		1.36	0.51	3.69

DECONT_LBGPDK					0.76				0.82			0.92
Nil	0	1				1				1		
<8 weeks	1	1.23	0.38	3.97		1.17	0.42	3.29		1.37	0.53	3.57

8<12 weeks	2	0.55	0.14	2.13	0.62	0.19	2.08	1.01	0.35	2.94
≥12weeks	3	0.78	0.22	2.76	0.82	0.26	2.54	1.20	0.41	3.58

LBGSSN					0.14			0.07		0.07
Spring	0	1			1			1		
Autumn	1	0.81	0.29	2.25	0.68	0.28	1.66	0.65	0.28	1.50
Winter	2	0.32	0.10	1.00	0.30	0.11	0.85	0.33	0.12	0.85

Weaner variables										

DECONT_WNGPDK					0.04			0.16		0.33
Nil	0	1			1			1		
<8 weeks	1	0.22	0.05	0.91	0.66	0.20	2.10	1.41	0.51	3.94
8<12 weeks	2	1.10	0.37	3.23	1.08	0.41	2.84	1.13	0.45	2.85
≥12weeks	3	2.47	0.74	8.49	2.87	0.97	8.79	2.63	0.93	7.68

WNRGMGT					0.05			0.15		0.45
Set	1	1			1			1		
Rotational	2	0.41	0.16	0.99	0.57	0.26	1.22	0.76	0.37	1.56

WNRSR					0.23			0.24		0.45
<8dse/hac	1	1			1			1		
8 <12 dse/hac	2	2.06	0.67	6.46	1.51	0.57	4.10	1.43	0.57	3.60
≥ 12 dse/hac	3	2.41	0.85	7.05	2.28	0.88	6.03	1.77	0.73	4.38

WNRCS					0.40			0.46		0.54
<3	1	1			1			1		
≥3	2	0.69	0.29	1.64	0.75	0.34	1.62	0.80	0.38	1.65

WNRHLTH					0.09			0.40		0.50
Some problems	0	1			1			1		
No problems	1	0.45	0.17	1.14	0.71	0.32	1.58	0.77	0.36	1.64

WNGAGE					0.63			0.62		0.52
≤ 15 weeks	0	1			1			1		
≤ 18 weeks	1	0.55	0.18	1.66	0.66	0.24	1.85	0.75	0.28	2.02
≤ 21 weeks	2	0.57	0.15	2.10	0.48	0.15	1.45	0.53	0.19	1.50

>21 weeks	3	0.53	0.16	1.67		0.66	0.24	1.85		0.52	0.19	1.38
WNGPCNT					0.53				0.54			0.33
<70%	0	1				1				1		
70<80%	1	0.64	0.18	2.22		0.51	0.16	1.59		0.45	0.15	1.28
80<90%	2	0.42	0.13	1.38		0.48	0.16	1.42		0.56	0.19	1.62
>90%	3	0.52	0.15	1.85		0.62	0.20	1.84		1.01	0.36	2.85
Hogget variables												
HGTGMGT					0.33				0.57			0.77
Set	1	1				1				1		
Rotational	2	0.65	0.27	1.54		0.80	0.37	1.71		0.90	0.43	1.88
HGTSR					0.29				0.14			0.51
<8dse/hac	1	1				1				1		
8 <12 dse/hac	2	2.27	0.78	6.82		2.24	0.89	5.75		1.69	0.70	4.16
≥12 dse/hac	3	1.70	0.55	5.39		2.22	0.78	6.46		1.25	0.45	3.52
HGTCS					0.01				0.01			0.05
<3	1	1				1				1		
≥3	2	0.28	0.10	0.78		0.33	0.13	0.79		0.40	0.16	0.98
HGTHLTH					0.92				0.92			0.60
Some problems	0	1				1				1		
No problems	1	0.92	0.20	4.34		0.94	0.27	3.21		1.43	0.37	5.52
Adult variables												
ADCS					0.80				0.59			0.47
<3	1	1				1				1		
≥3	2	1.12	0.45	2.86		1.26	0.55	2.89		1.35	0.60	3.05
ADSR					0.47				0.34			0.88
<8dse/hac	1	1				1				1		
8 <12 dse/hac	2	1.82	0.64	5.26		1.71	0.66	4.52		1.14	0.45	2.94
≥ 12 dse/hac	3	1.61	0.55	4.80		1.88	0.74	4.89		1.26	0.51	3.13

ADGMGT						0.77				0.78		0.77
Set	1	1					1			1		
Rotational	2	0.88	0.37	2.08			0.90	0.42	1.91	0.90	0.43	1.88

ADHLTH						0.40				0.89		1.00
No problems	0	1					1			1		
some problems	1	0.588	0.166	2.04			1.071	0.393	2.922	1	0.369	2.711

JOININGDURN_1						1.00				0.87		0.91
<6 weeks	0	1					1			1		
6-7 weeks	1	1.06	0.28	4.08			0.97	0.27	3.46	1.30	0.38	4.49
>7 weeks	2	1.06	0.21	5.41			0.73	0.16	3.29	1.13	0.26	4.93

SVC_CS						0.08				0.04		0.03
<2	1	1					1			1		
2<3	2	0.26	0.06	1.01			0.29	0.08	1.05	0.40	0.12	1.33
>3	3	0.61	0.14	2.57			0.79	0.20	3.06	1.20	0.33	4.40
=====												

Appendix 8 Univariable results: History and management variables for IPREV25

Cohort OJD prevalence – IPREV25

Full results for the 71 variables investigated are presented in the table Appendix 8.2. Analysis of the FIRST dataset found 17 flock-level variables (including 6 of 7 confounders) and 7 cohort-level variables were unconditionally associated with IPREV25. After deletion of highly correlated variables, a total of 20 variables were remained for inclusion in multivariable analyses (Appendix 8.1). The final number of variables unconditionally associated with IPREV25 in the SECOND and THIRD datasets included in multivariable analyses were 20 and 21, respectively. The 13 variables identified in all three datasets as unconditionally associated with IPREV25 (of which 10 were similarly identified for IPREV) were:

- Current flock OJD mortality% in adults (≥ 2 yr old) (CURRMORT)
- Age of youngest OJD mortality in the flock (YNGAGEMORT)
- Management of OJD clinical sheep (CLINICALMGT)
- Culling of low body weight sheep (CULL)
- Sale of high loss mobs (SELL)
- Proportion of property boundary receiving run off water (RUNOFFWATER)
- Application of fertilizers other than single super, molybdenum super and lime on the property (OTHERFERT)
- Drought over cohort lifetime (DROUGHT)
- Sex of the cohort (SEX)
- Period of any growth check during the lifetime of the cohort (GROWTHCHK)
- Length of OJD decontamination of the weaning paddock (DECONT_WNGPDK)
- Condition score of lambs at weaning (WNRCS)
- Condition score of hoggets at 1 year of age (HGTCS).

Appendix 7.1

The P-values from univariable analyses of FIRST, SECOND and THIRD datasets for history and management variables unconditionally associated with IPREV25 (P<0.25 – shown in regular text) in one or more datasets and included in subsequent multivariable analyses for the respective dataset/s

Variables	FIRST Dataset	SECOND Dataset	THIRD Dataset
OJD_DURN	0.28	0.21	0.14
CURRMORT	<0.001	<0.001	<0.001
YNGAGEMORT	<0.001	<0.001	<0.001
DROPSVACC	0.14	0.16	0.28
CLINICALMGT	0.13	0.03	<0.001
CULL	<0.001	<0.001	<0.001
SELL	0.01	<0.001	0.01
YOUNGSEPARATE	0.33	0.23	0.04
YOUNGFIRST	0.68	0.51	0.21
RUNOFFWATER	0.22	0.16	0.2
PCREEK	0.19	0.23	0.36
ICREEK	0.2	0.46	0.08
KANGAROO	0.1	0.3	0.25
OTHERWILDLIFE	0.37	0.08	0.2
SUPERFREQ	0.08	0.34	0.07
LIME	0.36	0.23	0.61
OTHERFERT	0.01	0.01	0.03
DROUGHT	0.04	0.05	0.03
SEX	<0.001	0.05	0.02
GROWTHCHK	0.04	0.06	0.21
SUPPLFEED	0.51	0.32	0.17
LBGSSN	0.42	0.21	0.11
DECONT_WNGPDK	0.01	0.07	0.25
WNRSR	0.1	0.27	0.36
WNRCS	0.06	0.09	0.13
WNGPCNT	0.22	0.29	0.41
HGTCS	0.09	0.07	0.17
Total no of variables for inclusion in multivariable analyses	20	20	21

Appendix 8.2

Unconditional associations between flock- and cohort-level variables and IPREV25 (cohort OJD animal-level prevalence categorised as Low (<2%), Medium (2-5%) and High (>5%)) for 77 cohorts in the FIRST Dataset, 98 cohorts in the SECOND Dataset and 117 cohorts in the THIRD Dataset

Variables and categories	Code	FIRST Dataset				SECOND Dataset				THIRD Dataset			
		OR	LCL	UCL	P	OR	LCL	UCL	P	OR	LCL	UCL	P
<i>Flock-level variables</i>													
<i>Confounding variables</i>													
INFLEVEL					<0.001				<0.001				<0.001
No mortalities	0	1				1				1			
Low mortalities and trend falling or steady	1	3.11	0.96	10.64		2.48	0.87	7.38		2.89	1.08	7.38	
High mortalities but trend falling or steady OR Low mortalities but trend escalating	2	11.38	3.31	43.24		11.07	3.73	35.35		10.48	3.88	35.35	
High mortalities as well as trend escalating	3	53.34	7.59	1000.00		55.74	8.29	1000.00		16.90	3.95	1000.00	

OJD_DURN					0.28				0.21				0.14
3<5 years	0	1				1				1			
5<7years	1	1.22	0.31	4.74		1.45	0.42	5.07		1.14	0.34	5.07	
7<10years	2	0.67	0.16	2.67		0.92	0.27	3.11		0.80	0.24	3.11	
10 year or more	3	2.09	0.53	8.26		2.45	0.73	8.36		2.16	0.67	8.36	

PEAKMORT					<0.001				<0.001				<0.001
No mortalities	0	1				1				1			
<2% mortalities	1	4.47	1.43	14.96		3.59	1.30	10.44		3.95	1.51	10.44	
≥ 2% mortalities	2	12.34	3.77	44.54		11.10	3.87	34.17		9.37	3.59	34.17	

CURRMORT					<0.001				<0.001				<0.001
No mortalities	0	1				1				1			
<2% mortalities	1	3.74	1.26	11.74		3.31	1.24	9.20		3.63	1.45	9.20	
≥ 2% mortalities	2	11.65	3.46	43.42		9.77	3.37	30.28		8.43	3.21	30.28	

MEANMORT										
					<0.001				<0.001	<0.001
No mortalities	0	1				1			1	
<2% mortalities	1	5.28	1.69	17.82		4.20	1.53	12.14	5.30	2.05 12.14
≥ 2% mortalities	2	14.27	3.86	59.86		13.41	4.19	47.13	10.30	3.64 47.13
YNGAGEMORT										
					<0.001				<0.001	<0.001
No mortalities	0	1				1			1	
<2years	1	18.17	3.37	147.5		23.51	4.72	181.1	19.77	4.78 181.1
				3				7		7
2 to < 3 yrs	2	6.28	2.04	20.79		6.14	2.22	17.98	6.24	2.43 17.98
≥ 3 years	3	5.96	1.62	23.93		3.92	1.30	12.46	3.92	1.39 12.46
OJDSIGNS										
					<0.001				<0.001	<0.001
Nil	0	1				1			1	
Death and/or Loss of condition	1	4.19	1.11	17.05		3.43	1.07	11.46	3.72	1.28 11.46
Death, loss of condition and scouring	2	6.97	2.36	22.17		7.81	2.94	22.05	7.61	3.06 22.05
OJD Control										
DROPSVACC										
					0.14				0.16	0.28
No drops vaccinated	0	1				1			1	
1 or 2 drops vaccinated	1	3.82	0.99	15.51		3.26	0.96	11.45	2.37	0.78 11.45
>2 drops vaccinated	2	2.01	0.61	6.86		1.91	0.66	5.63	2.04	0.77 5.63
CLINICALMGT										
					0.13				0.03	<0.001
No mortalities observed	0	1				1			1	
Immediately dispose off	1	3.80	1.21	12.66		4.67	1.70	13.45	5.59	2.16 13.45
Dispose off later	2	4.21	0.97	20.20		3.63	0.98	14.34	4.48	1.35 14.34
Do nothing	3	2.44	0.38	17.06		2.62	0.51	14.32	1.94	0.47 14.32
CULL										
					<0.001				<0.001	<0.001
No	0	1				1			1	
Yes	1	4.33	1.79	10.88		4.11	1.90	9.21	3.66	1.81 9.21
SELL										
					0.01				<0.001	0.01
No	0	1				1			1	
Yes	1	5.97	1.45	40.72		7.16	1.80	48.04	5.19	1.55 48.04

YOUNGSEPARATE						0.33				0.23		0.04
No	0	1					1			1		
Yes	1	1.53	0.65	3.62			1.58	0.75	3.36	2.04	1.03	3.36

YOUNGFIRST						0.68				0.51		0.21
No	0	1					1			1		
Yes	1	1.29	0.39	4.64			1.44	0.49	4.60	1.86	0.70	4.60

DESTOCK						0.36				0.56		0.80
No	0	1					1			1		
Yes	1	0.67	0.27	1.59			0.80	0.37	1.71	0.91	0.45	1.71

Lateral Spread and purchase risk												

INFNBRS						0.94				0.95		0.97
≤ 25%	0	1					1			1		
>25% to ≤ 75%	1	1.23	0.40	3.85			1.12	0.39	3.21	1.12	0.42	3.21
> 75%	2	1.07	0.38	3.04			0.96	0.38	2.38	1.01	0.44	2.38

SHSTRAY						0.78				0.97		0.73
No straying	0	1					1			1		
<10 sheep stray annually OR stray not even once per year	1	1.02	0.35	2.95			0.93	0.38	2.31	0.71	0.31	2.31
10-20 sheep stray annually	2	1.55	0.46	5.44			1.23	0.42	3.69	0.58	0.21	3.69
>20 sheep stray annually OR stray more than once annually	3	1.71	0.45	6.95			0.99	0.30	3.39	0.81	0.26	3.39

SHARING_ROAD						0.58				0.48		0.44
No sharing	0	1					1			1		
≤ twice per year	1	1.37	0.49	3.92			1.49	0.59	3.92	1.75	0.74	3.92
>twice per year	2	1.73	0.59	5.41			1.70	0.63	4.84	1.20	0.48	4.84

SHARING_SHED						0.50				0.45		0.37
No sharing	0	1					1			1		
Sharing	1	0.66	0.19	2.30			0.66	0.22	1.97	0.64	0.24	1.97

RUNOFFWATER					0.22				0.16		0.20
≤ 10%	0	1				1			1		
>10 to ≤ 30%	1	0.60	0.20	1.80		0.53	0.20	1.40	0.67	0.27	1.40
>30% to ≤ 60%	2	0.92	0.29	2.97		0.76	0.27	2.14	0.70	0.27	2.14
> 60%	3	0.26	0.07	0.99		0.28	0.09	0.89	0.34	0.12	0.89

PCREEK					0.19				0.23		0.36
No	0	1				1			1		
Yes	1	0.54	0.21	1.35		0.61	0.27	1.36	0.71	0.34	1.36

ICREEK					0.20				0.46		0.08
No	0	1				1			1		
Yes	1	2.03	0.69	6.05		1.43	0.55	3.65	2.06	0.91	3.65

KANGAROO					0.10				0.30		0.25
≤ 20%	0	1				1			1		
>20% to ≤ 50%	1	2.93	1.03	8.75		2.14	0.82	5.79	2.10	0.87	5.79
>50%	2	2.26	0.82	6.42		1.54	0.64	3.73	1.58	0.71	3.73

RABBIT					0.65				0.85		0.33
Nil	0	1				1			1		
≤ 5%	1	1.03	0.38	2.74		1.28	0.53	3.12	1.80	0.80	3.12
>5%	2	1.62	0.52	5.22		1.23	0.47	3.25	1.69	0.70	3.25

OTHERWILDLIFE					0.37				0.08		0.20
No	0	1				1			1		
Yes	1	0.66	0.26	1.66		0.49	0.22	1.08	0.63	0.31	1.08

RAMRISK					0.39				0.70		0.61
None purchased/ none from infected sources	1	1				1			1		
Some/all purchased from infected sources	2	0.69	0.29	1.63		0.86	0.41	1.84	0.84	0.42	1.84

EWERISK					0.72				0.63		0.95
No	0	1				1			1		
Yes	1	1.17	0.49	2.83		1.21	0.56	2.67	1.03	0.50	2.67

Property management

GRAZEDAREA					0.97				0.75			0.80
≤ 965 hectares	1	1				1				1		
> 965 hectares	2	0.99	0.41	2.35		0.89	0.42	1.86		0.92	0.47	1.81
<hr/>												
FLOCKSIZE					0.90				0.75			0.71
≤ 3000	1	1				1				1		
> 3000	2	0.95	0.40	2.22		0.89	0.42	1.86		0.88	0.45	1.73
<hr/>												
SUPERFREQ					0.08				0.34			0.07
≤ once in three years	0	1				1				1		
>once to ≤ twice in three years	1	3.33	0.95	12.34		2.30	0.79	6.88		2.61	1.00	6.88
> twice to ≤ Every year	2	3.67	1.23	11.43		2.21	0.85	5.84		2.96	1.26	5.84
> Once every year	3	4.28	1.02	20.49		1.67	0.49	5.90		1.76	0.56	5.90
<hr/>												
LIME					0.36				0.23			0.61
No	0	1				1				1		
Yes	1	0.67	0.27	1.59		0.62	0.28	1.36		0.83	0.41	1.36
<hr/>												
OTHERFERT					0.01				0.01			0.03
No	0	1				1				1		
Yes	1	0.21	0.06	0.66		0.24	0.07	0.72		0.32	0.11	0.72
<hr/>												
MINERALDEF					0.85				0.63			0.89
No evidence	0	1				1				1		
Some evidence	1	0.92	0.36	2.37		0.82	0.37	1.86		1.05	0.51	1.86

Flock management

WORMCONTROL					0.81				0.27			0.32
No	0	1				1				1		
Yes	1	0.87	0.27	2.68		0.59	0.21	1.51		0.65	0.27	1.51
<hr/>												
WORMRECOMM					0.81				0.27			0.32
No	0	1				1				1		
Yes	1	1.35	0.54	3.36		0.94	0.42	2.07		1.18	0.58	2.07

Drought and water logging

WATERLOG					0.87				0.65			0.91
<1%	0	1				1				1		
1% to <10%	1	0.81	0.24	2.70		0.83	0.28	2.42		1.10	0.41	2.42
10% to <30%	2	0.62	0.18	2.10		0.52	0.18	1.48		0.78	0.30	1.48
≥ 30%	3	0.68	0.20	2.29		0.69	0.23	2.00		0.92	0.35	2.00

PINRUSH					0.36				0.51			0.43
Nil	0	1				1				1		
<1%	1	0.46	0.11	1.94		0.53	0.15	1.96		0.43	0.14	1.96
1 to ≤ 5%	2	0.50	0.17	1.45		0.62	0.24	1.62		0.67	0.28	1.62
>5%	3	0.41	0.13	1.32		0.52	0.20	1.38		0.64	0.26	1.38

DROUGHT					0.04				0.05			0.03
≤ 150mm lesser OR more than long-term average	0	1				1				1		
>150mm lesser	1	2.69	1.07	7.17		2.32	1.01	5.62		2.39	1.11	5.62

DROUGHT_SAMPLEYR					0.78				0.97			0.76
up to 132mm lesser OR more than long-term average	0	1				1				1		
> 132 mm to 228mm lesser	1	1.094	0.396	3.083		0.935	0.384	2.299		0.74	0.33	1.655
> 228 mm lesser	2	0.743	0.264	2.1		0.896	0.358	2.261		0.895	0.383	2.107

DROUGHT_YRLAMBING					0.32				0.42			0.50
up to 15mm lesser OR more than long-term average	0	1				1				1		
>15 to 112mm lesser	1	1.25	0.39	4.21		1.03	0.41	2.64		1.26	0.55	2.64
>112mm lesser	2	2.06	0.80	5.42		1.75	0.73	4.30		1.63	0.72	4.30

Cohort level factors

General

AGEGP					0.27				0.62			0.53
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3 years	3	1				1				1		
4 years	4	0.61	0.25	1.48		0.82	0.39	1.77		0.73	0.35	1.77
5 years	5									0.57	0.17	6.82

SEX						<0.001				0.05		0.02
Ewes	0											
Wethers	1	5.56	1.81	21.16		2.53	1.02	6.82		2.30	0.99	4.43
Mixed sex	2									0.22	0.03	1.77

GROWTHCHK						0.04				0.06		0.21
<12 weeks	0	1				1				1		
≥12 weeks	1	2.47	1.04	5.97		2.05	0.96	4.43		1.60	0.76	3.21

MINSUPPL						0.89				0.64		0.53
No	0	1				1				1		
Yes	1	1.06	0.45	2.57		0.84	0.39	1.77		0.79	0.38	1.64

FODSTUB						0.89				0.82		0.91
No	0	1				1				1		
<16 weeks	1	1.28	0.46	3.65		1.27	0.51	3.21		1.21	0.48	3.12
≥16 weeks	2	1.13	0.41	3.15		1.27	0.51	3.21		1	0.42	2.42

WATER						0.65				0.70		0.53
Less	1	1				1				1		
Average	2	1.58	0.29	8.71		1.50	0.28	8.11		1.35	0.25	5.32
High	3	1.99	0.41	9.66		1.84	0.39	8.77		1.97	0.41	7.76

SUPPLFEED						0.51				0.32		0.17
No	0	1				1				1		
≤ 6months	1	1.63	0.37	7.35		1.49	0.42	5.32		1.963	0.553	7.162
6 months to 1 year	2	2.70	0.71	10.60		2.48	0.81	7.76		3.582	1.145	11.62
> 1year	3	1.63	0.44	6.09		2.48	0.81	7.75		2.535	0.831	7.956

SUPPLFEED_CS						0.30				0.61		0.37
<3	1	1				1				1		
≥ 3	2	1.94	0.56	6.69		1.3	0.47	3.92		1.60	0.57	4.4

SUPPLFEED_METHOD						0.48				0.75		0.39
On ground	0	1				1				1		

Some in trough	1	0.62	0.16	2.42		0.82	0.23	3.06		0.58	0.17	5.52
SUPPLFEED_LIME					0.97				0.71			0.73
No	0	1				1				1		
Yes, in some	1	0.98	0.35	2.88		1.20	0.47	3.24		1.18	0.47	2.92
Lambing variables												
DAMSR					0.95				0.91			0.95
<8dse/hac	1	1				1				1		
8 <12 dse/hac	2	1.31	0.26	6.93		1.30	0.32	5.52		0.81	0.20	3.12
≥12 dse/hac	3	1.09	0.34	3.30		1.03	0.35	2.92		0.87	0.30	3.36
DAMCS					0.56				0.93			0.97
<3	1	1				1				1		
3<4	2	1.35	0.35	4.92		0.92	0.25	3.12		0.92	0.28	4.38
≥4	3	0.74	0.15	3.49		0.78	0.17	3.36		0.85	0.21	3.07
DAM_SCOUR					0.61				0.44			0.60
No	0	1				1				1		
Yes	1	1.34	0.45	4.35		1.49	0.54	4.38		1.30	0.49	6.46
DECONT_LBGPDK					0.63				0.77			0.77
Nil	0	1				1				1		
<8 weeks	1	1.06	0.35	3.39		1.10	0.41	3.07		1.37	0.54	2.02
8<12 weeks	2	2.42	0.49	17.92		1.55	0.42	6.46		1.64	0.54	1.76
≥12weeks	3	0.69	0.21	2.33		0.69	0.24	2.02		0.95	0.34	1.10
LBGSSN					0.42				0.21			0.11
Spring	0	1				1				1		
Autumn	1	0.90	0.33	2.48		0.74	0.31	1.76		0.65	0.28	1.54
Winter	2	0.47	0.15	1.47		0.40	0.14	1.10		0.38	0.15	7.76
Weaner variables												
DECONT_WNGPDK					0.01				0.07			0.25
Nil	0	1				1				1		
<8 weeks	1	0.26	0.06	1.00		0.52	0.18	1.54		0.88	0.34	5.01
8<12 weeks	2	4.22	1.25	17.05		2.58	0.93	7.76		2.39	0.89	2.01

≥12weeks	3	1.61	0.52	5.22		1.70	0.61	5.01		1.61	0.60	4.97
WNRGMGT					0.58				0.90			0.81
Set	1	1				1				1		
Rotational	2	0.79	0.33	1.84		0.95	0.45	2.01		1.09	0.54	5.20
WNRSR					0.10				0.27			0.36
<8dse/hac	1	1				1				1		
8 <12 dse/hac	2	3.26	1.05	10.89		1.86	0.71	4.97		1.82	0.74	1.12
≥ 12 dse/hac	3	2.29	0.85	6.40		2.05	0.82	5.20		1.71	0.72	2.01
WNRCS					0.06				0.09			0.13
<3	1	1				1				1		
≥3	2	0.44	0.18	1.05		0.51	0.23	1.12		0.56	0.27	2.56
WNRHLTH					0.28				0.86			0.93
Some problems	0	1				1				1		
No problems	1	0.60	0.23	1.50		0.93	0.43	2.01		0.97	0.46	1.79
WNGAGE					0.99				0.71			0.76
≤ 15 weeks	0	1				1				1		
≤ 18 weeks	1	0.91	0.30	2.73		0.94	0.34	2.56		0.97	0.37	3.32
≤ 21 weeks	2	0.91	0.25	3.38		0.61	0.21	1.79		0.61	0.22	2.67
>21 weeks	3	0.86	0.28	2.71		1.18	0.42	3.32		0.91	0.34	1.07
WNGPCNT					0.22				0.29			0.41
<70%	0	1				1				1		
70<80%	1	1.00	0.27	3.80		0.81	0.25	2.67		0.63	0.21	1.60
80<90%	2	0.35	0.11	1.08		0.38	0.13	1.07		0.43	0.15	1.93
>90%	3	0.51	0.15	1.76		0.55	0.19	1.60		0.76	0.27	4.41
Hogget variables												
HGTGMGT					0.66				0.82			0.97
Set	1	1				1				1		
Rotational	2	0.83	0.35	1.93		0.92	0.43	1.93		0.99	0.47	3.66
HGTSR					0.33				0.46			0.80
<8dse/hac	1	1				1				1		

8 <12 dse/hac	2	2.30	0.77	7.54		1.75	0.72	4.41		1.22	0.52	1.06
≥ 12 dse/hac	3	1.19	0.40	3.63		1.33	0.50	3.66		0.85	0.32	4.58
<hr/>												
HGTCS						0.09				0.07		0.17
<3	1											
≥3	2	0.42	0.14	1.14		0.45	0.18	1.06		0.55	0.22	2.02
<hr/>												
HGTHLTH						0.69				0.51		0.34
Some problems	0	1				1				1		
No problems	1	1.34	0.30	5.71		1.46	0.46	4.58		1.84	0.52	4.63
<hr/>												
Adult variables												
ADCS						0.86				0.78		0.92
<3	1	1				1				1		
≥3	2	0.92	0.36	2.31		0.89	0.39	2.02		0.96	0.42	3.21
<hr/>												
ADSR						0.30				0.52		0.61
<8dse/hac	1	1				1				1		
8 <12 dse/hac	2	2.35	0.80	7.38		1.74	0.68	4.63		1.43	0.55	2.15
≥ 12 dse/hac	3	1.26	0.45	3.62		1.30	0.53	3.21		0.87	0.37	3.13
<hr/>												
ADGMGT						1.00				0.96		0.97
Set	1	1				1				1		
Rotational	2	1.00	0.42	2.33		1.02	0.49	2.15		0.99	0.47	2.37
<hr/>												
ADHLTH						0.50				0.70		0.66
No problems	0	1				1				1		
some problems	1	0.658	0.192	2.296		0.829	0.32	2.189		0.809	0.312	2.132
<hr/>												
JOININGDURN_1						0.90				0.83		0.96
<6 weeks	0	1				1				1		
6-7 weeks	1	0.77	0.19	2.87		0.68	0.18	2.37		0.91	0.26	1.66
>7 weeks	2	0.94	0.18	4.75		0.71	0.15	3.21		1.05	0.24	2.44
<hr/>												
SVC_CS						0.77				0.48		0.35
<2	1	1				1				1		
2<3	2	0.61	0.14	2.28		0.47	0.12	1.66		0.53	1.74	0.15
>3	3	0.71	0.16	2.88		0.64	0.15	2.44		0.91	3.23	0.24

Appendix 9 Univariable results: History and management variables for pool OJD status

Results for the 71 variables investigated are presented in the table Appendix 9.2. Of these, 29 flock-level variables (including 7 of 7 confounders) and 16 cohort-level variables were unconditionally associated with pool OJD status. After deletion of highly correlated variables (as described in Appendix 1 Section 3.10) a total of 41 variables remained for inclusion in multivariable analyses (Appendix 9.1). Of these the 18 variables with the strongest association ($P < 0.001$) were:

- Interviewers' assessment of length of flock OJD infection (OJD_DURN)
- Current flock OJD mortality% in adults (≥ 2 yr old) (CURRMORT)
- Age of youngest OJD mortality in the flock (YNGAGEMORT)
- Number of age groups vaccinated in the flock (DROPSVACC)
- Management of OJD clinical sheep (CLINICALMGT)
- Culling of low body weight sheep (CULL)
- Sale of high loss mobs (SELL)
- Frequency of sharing of roads with neighbours (SHARING_ROAD)
- Proportion of property boundary receiving run off water (RUNOFFWATER)
- Permanent creek flowing onto the property (PCREEK)
- Presence of other wildlife (aside from kangaroos and rabbits) (OTHERWILDLIFE)
- Frequency of application of single super and molybdenum super fertilizers on the property (SUPERFREQ)
- Application of fertilizers other than single super, molybdenum super and lime on the property (OTHERFERT)
- Drought over cohort lifetime (DROUGHT)
- Drought during year of cohort birth (DROUGHT_YRLAMBING)
- Sex of the cohort (SEX)
- Period of any growth check during the lifetime of the cohort (GROWTHCHK)
- Condition score of hoggets at 1 year of age (HGTCS).

Appendix 9.1

The P-values for variables unconditionally associated with pool OJD status and included in multivariable analyses

Variables	P-value
OJD_DURN	<0.001
CURRMORT	<0.001
YNGAGEMORT	<0.001
DROPSVACC	<0.001
CLINICALMGT	<0.001
CULL	<0.001
SELL	<0.001
YOUNGSEPARATE	0.10
DESTOCK	0.18
SHARING_ROAD	<0.001
SHARING_SHED	0.11
RUNOFFWATER	<0.001
PCREEK	<0.001
ICREEK	0.05
KANGAROO	0.02
OTHERWILDLIFE	<0.001
SUPERFREQ	<0.001
LIME	0.05
OTHERFERT	<0.001
WORMCONTROL	0.01
WORMRECOMM	0.01
WATERLOG	0.24
PINRUSH	0.23
DROUGHT	<0.001
DROUGHT_YRLAMBING	<0.001
AGEGP	0.05
SEX	<0.001
GROWTHCHK	<0.001
FODSTUB	0.22
SUPPLFEED	0.01
LBGSSN	0.09
DECONT_WNGPDK	0.08
WNRGMGT	0.21
WNRSR	0.01
WNRCS	0.01
WNGPCNT	0.10
HGTSR	0.01
HGTCS	<0.001
HGTHLTH	0.19
ADSR	0.11
SVC_CS	0.04

Appendix 9.2

Unconditional associations between flock- and cohort-level variables and pool OJD status for 673 pools

Variables and categories	Code	Odds ratio	LCL	UCL	P
<i>Flock-level variables</i>					
<i>Confounding Variables</i>					
INFLEVEL					<0.0001
No mortalities	0	1			
Low mortalities and trend falling or steady	1	2.38	1.55	3.68	
High mortalities but trend falling or steady OR Low mortalities but trend escalating	2	6.55	4.17	10.45	
High mortalities as well as trend escalating	3	7.19	3.82	14.32	

OJD_DURN					0.00
3<5 years	0	1			
5<7years	1	1.29	0.77	2.15	
7<10years	2	1.04	0.63	1.70	
10 year or more	3	2.42	1.44	4.07	

PEAKMORT					<0.0001
No mortalities	0	1			
<2% mortalities	1	3.15	2.07	4.82	
≥ 2% mortalities	2	5.78	3.77	8.96	

CURRMORT					<0.0001
No mortalities	0	1			
<2% mortalities	1	2.96	1.98	4.45	
≥ 2% mortalities	2	5.66	3.64	8.91	

MEANMORT					<0.0001
No mortalities	0	1			
<2% mortalities	1	3.37	2.24	5.13	
≥ 2% mortalities	2	5.86	3.67	9.54	

YNGAGEMORT					<0.0001
No mortalities	0	1			
<2years	1	8.23	4.17	17.62	
2 to < 3 yrs	2	4.40	2.90	6.74	
≥ 3 years	3	3.19	2.02	5.07	

OJDSIGNS					<0.0001
Nil	0	1			
Death and/or Loss of condition	1	3.03	1.85	5.00	
Death, loss of condition and scouring	2	5.08	3.42	7.59	

OJD Control

DROPSVACC					0.00
No drops vaccinated	0	1			
1 or 2 drops vaccinated	1	2.64	1.56	4.51	
>2 drops vaccinated	2	1.67	1.06	2.62	

CLINICALMGT					<0.0001
No mortalities observed	0	1			
Immediately dispose off	1	3.48	2.30	5.29	
Dispose off later	2	3.13	1.83	5.44	
Do nothing	3	2.15	1.10	4.29	

CULL					<0.0001
No	0	1			
Yes	1	2.57	1.85	3.58	

SELL					<0.0001
No	0	1			
Yes	1	2.78	1.62	5.04	

YOUNGSEPARATE					0.10
No	0	1			
Yes	1	1.31	0.95	1.81	

YOUNGFIRST					0.40
No	0	1			
Yes	1	1.23	0.76	2.06	

DESTOCK					0.18
No	0	1			
Yes	1	0.79	0.56	1.11	

Lateral Spread and purchase risk

INFNBRS					0.36
≤ 25%	0	1			
>25% to ≤ 75%	1	0.98	0.62	1.55	
> 75%	2	0.78	0.52	1.16	

SHSTRAY					0.77
No straying	0	1			
<10 sheep stray annually OR stray not even once per year	1	0.94	0.64	1.39	
10-20 sheep stray annually	2	1.11	0.69	1.80	
>20 sheep stray annually OR stray more than once annually	3	1.21	0.72	2.06	

SHARING_ROAD					0.00
No sharing	0	1			

≤ twice per year	1	1.93	1.25	3.04	
>twice per year	2	1.97	1.26	3.15	
<hr/>					0.11
SHARING_SHED					
No sharing	0	1			
Sharing	1	0.67	0.42	1.10	
<hr/>					<0.0001
RUNOFFWATER					
≤ 10%	0	1			
>10 to ≤ 30%	1	0.62	0.40	0.97	
>30% to ≤ 60%	2	0.79	0.50	1.25	
> 60%	3	0.33	0.21	0.52	
<hr/>					0.00
PCREEK					
No	0	1			
Yes	1	0.59	0.41	0.84	
<hr/>					0.05
ICREEK					
No	0	1			
Yes	1	1.48	0.99	2.18	
<hr/>					0.02
KANGAROO					
≤ 20%	0	1			
>20% to ≤ 50%	1	1.62	1.08	2.43	
>50%	2	1.57	1.08	2.31	
<hr/>					0.71
RABBIT					
Nil	0	1			
≤ 5%	1	1.18	0.80	1.73	
>5%	2	1.08	0.72	1.63	
<hr/>					0.00
OTHERWILDLIFE					
No	0	1			
Yes	1	0.60	0.43	0.85	
<hr/>					0.51
RAMRISK					
None purchased/ none from infected sources	1	1			
Some/all purchased from infected sources	2	0.90	0.65	1.25	
<hr/>					0.34
EWERISK					
No	0	1			
Yes	1	1.18	0.84	1.66	
<hr/>					
Property management					
GRAZEDAREA					0.67
≤ 965 hectares	1	1			
> 965 hectares	2	0.93	0.67	1.29	
<hr/>					0.79
FLOCKSIZE					

≤ 3000	1	1			
> 3000	2	0.96	0.69	1.32	
<hr/>					
SUPERFREQ					0.00
≤ once in three years	0	1			
>once to ≤ twice in three years	1	1.96	1.24	3.10	
> twice to ≤ Every year	2	2.46	1.62	3.75	
> Once every year	3	1.48	0.87	2.52	
<hr/>					
LIME					0.05
No	0	1			
Yes	1	0.71	0.50	1.00	
<hr/>					
OTHERFERT					0.00
No	0	1			
Yes	1	0.38	0.24	0.62	
<hr/>					
MINERALDEF					0.33
No evidence	0	1			
Some evidence	1	0.84	0.59	1.20	
<hr/>					
Flock management					
WORMCONTROL					0.01
No	0	1			
Yes	1	0.57	0.36	0.89	
<hr/>					
WORMRECOMM					0.01
No	0	1			
Yes	1	1.10	0.78	1.55	
<hr/>					
Drought and water logging					
WATERLOG					0.24
<1%	0	1			
1% to <10%	1	1.10	0.69	1.76	
10% to <30%	2	1.06	0.66	1.72	
≥ 30%	3	0.74	0.47	1.14	
<hr/>					
PINRUSH					0.23
Nil	0	1			
<1%	1	0.71	0.43	1.21	
1 to ≤ 5%	2	0.75	0.50	1.14	
>5%	3	0.67	0.44	1.03	
<hr/>					
DROUGHT					<0.0001
≤ 150mm lesser OR more than long-term average	0	1			
>150mm lesser	1	2.49	1.66	3.82	
<hr/>					
DROUGHT_SAMPLEYR					0.81

up to 132mm lesser OR more than long-term average	0	1			
> 132 mm to 228mm lesser	1	0.919	0.629	1.349	
> 228 mm lesser	2	0.881	0.589	1.326	
<hr/>					0.00
DROUGHT_YRLAMBING					
up to 15mm lesser OR more than long-term average	0	1			
>15 to 112mm lesser	1	1.03	0.69	1.55	
>112mm lesser	2	2.02	1.36	3.04	
<hr/>					
Cohort-level factors					
General					
AGEGP					0.05
3 years	3	1			
4 years	4	0.72	0.52	0.99	
5 years	5				
<hr/>					
SEX					0.00
Ewes	0	1			
Wethers	1	1.96	1.33	2.96	
Mixed sex	2				
<hr/>					
GROWTHCHK					0.00
<12 weeks	0	1			
≥12 weeks	1	1.85	1.34	2.58	
<hr/>					
MINSUPPL					0.66
No	0	1			
Yes	1	0.93	0.67	1.30	
<hr/>					
FODSTUB					0.22
No	0	1			
<16 weeks	1	1.25	0.85	1.88	
≥ 16 weeks	2	1.39	0.93	2.09	
<hr/>					
WATER					0.98
Less	1	1			
Average	2	0.99	0.47	2.02	
High	3	1.03	0.52	1.99	
<hr/>					
SUPPLFEED					0.01
No	0	1			
≤ 6months	1	1.406	0.824	2.402	
6 months to 1 year	2	2.264	1.374	3.735	
> 1year	3	1.888	1.139	3.134	
<hr/>					
SUPPLFEED_CS					0.08
<3	1	1			
≥ 3	2	1.51	0.95	2.44	

<hr/>					
SUPPLFEED_METHOD					0.38
On ground	0	1			
Some in trough	1	0.78	0.45	1.37	
<hr/>					
SUPPLFEED_LIME					0.82
No	0	1			
Yes, in some	1	1.05	0.70	1.60	
<hr/>					
Lambing variables					
<hr/>					
DAMSR					0.94
<8dse/hac	1	1			
8 <12 dse/hac	2	1.11	0.62	2.00	
≥ 12 dse/hac	3	1.05	0.67	1.62	
<hr/>					
DAMCS					0.52
<3	1	1			
3<4	2	1.29	0.79	2.09	
≥ 4	3	1.11	0.61	2.01	
<hr/>					
DAM_SCOUR					0.29
No	0	1			
Yes	1	1.28	0.81	2.06	
<hr/>					
DECONT_LBGPDK					0.67
Nil	0	1			
<8 weeks	1	1.26	0.82	1.97	
8<12 weeks	2	1.15	0.67	2.01	
≥ 12 weeks	3	0.93	0.57	1.53	
<hr/>					
LBGSSN					0.09
Spring	0	1			
Autumn	1	0.72	0.49	1.05	
Winter	2	0.67	0.44	1.03	
<hr/>					
Weaner variables					
<hr/>					
DECONT_WNGPDK					0.08
Nil	0	1			
<8 weeks	1	0.79	0.49	1.29	
8<12 weeks	2	1.32	0.87	2.03	
≥ 12 weeks	3	1.55	0.98	2.50	
<hr/>					
WNRGMGT					0.21
Set	1	1			
Rotational	2	0.81	0.58	1.13	
<hr/>					
WNRSR					0.01
<8dse/hac	1	1			
8 <12 dse/hac	2	1.38	0.92	2.08	
≥ 12 dse/hac	3	1.92	1.29	2.87	

-----						0.01
WNRCS						
<3	1	1				
≥3	2	0.62	0.44	0.87		
-----						0.65
WNRHLTH						
Some problems	0	1				
No problems	1	0.92	0.65	1.30		
-----						0.25
WNGAGE						
≤ 15 weeks	0	1				
≤ 18 weeks	1	0.78	0.50	1.22		
≤ 21 weeks	2	0.61	0.38	0.99		
>21 weeks	3	0.76	0.49	1.19		
-----						0.10
WNGPCNT						
<70%	0	1				
70<80%	1	0.71	0.44	1.17		
80<90%	2	0.56	0.35	0.89		
>90%	3	0.67	0.42	1.07		

Hogget variables						

HGTGMGT						0.54
Set	1	1				
Rotational	2	0.90	0.65	1.25		
-----						0.01
HGTSR						
<8dse/hac	1	1				
8 <12 dse/hac	2	1.67	1.12	2.53		
≥ 12 dse/hac	3	1.67	1.08	2.63		
-----						0.00
HGTCS						
<3	1	1				
≥3	2	0.55	0.36	0.81		
-----						0.19
HGTHLTH						
Some problems	0	1				
No problems	1	1.39	0.85	2.24		

Adult variables						

ADCS						0.42
<3	1	1				
≥3	2	1.16	0.81	1.66		
-----						0.11
ADSR						
<8dse/hac	1	1				
8 <12 dse/hac	2	1.27	0.85	1.91		
≥ 12 dse/hac	3	1.53	1.02	2.32		
-----						0.87
ADGMGT						

Set	1	1			
Rotational	2	0.97	0.70	1.35	
<hr/>					
ADHLTH					0.60
No problems	0	1			
Some problems	1	0.891	0.583	1.376	
<hr/>					
JOININGDURN_1					0.99
<6 weeks	0	1			
6-7 weeks	1	0.96	0.57	1.58	
>7 weeks	2	0.95	0.51	1.80	
<hr/>					
SVC_CS					0.04
<2	1	1			
2<3	2	0.51	0.27	0.93	
>3	3	0.72	0.36	1.34	

Appendix 10 Univariable results: History and management variables for pool MAP number shed

Results for the 71 variables investigated are presented in the table Appendix 10.2. Of these, 29 flock-level variables (including 7 of 7 confounders) and 19 cohort-level variables were unconditionally associated with log MAP number shed per pool. After deletion of highly correlated variables, a total of 44 variables remained for inclusion in multivariable analyses (Appendix 10.1). Of these the 15 variables with the strongest association ($P < 0.001$) were:

- Interviewers' assessment of length of flock OJD infection (OJD_DURN)
- Current flock OJD mortality% in adults (≥ 2 yr old) (CURRMORT)
- Number of age groups vaccinated in the flock (DROPSVACC)
- Management of OJD clinical sheep (CLINICALMGT)
- Culling of low body weight sheep (CULL)
- Sale of high loss mobs (SELL)
- Proportion of property boundary receiving run off water (RUNOFFWATER)
- Age of youngest mortality in the flock (YNGAGEMORT)
- Presence of other wildlife (aside from kangaroos and rabbits) (OTHERWILDLIFE)
- Frequency of application of single super and molybdenum super fertilizers on the property (SUPERFREQ)
- Application of fertilizers other than single super, molybdenum super and lime on the property (OTHERFERT)
- Drought over cohort lifetime (DROUGHT)
- Decontamination of weaning paddock (DECONT_WNGPDK)
- Sex of the cohort (SEX)
- Period of any growth check during the lifetime of the cohort (GROWTHCHK)

Appendix 10.1

The P-values for variables unconditionally associated with log pool MAP number shed and included in multivariable analyses

Flock level variables	P-value	Cohort level variables	P-value
OJD_DURN	<0.001	SEX	<0.001
CURRMORT	<0.001	POOLSIZE	0.21
YNGAGEMORT	<0.001	GROWTHCHK	<0.001
DROPSVACC	0.001	FODSTUB	0.008
CLINICALMGT	<0.001	WATER	0.20
CULL	<0.001	SUPPLFEED	0.11
SELL	<0.001	DAMCS	0.14
YOUNGSEPARATE	0.003	LBGSSN	0.16
DESTOCK	0.011	DECONT_WNGPDK	0.001
SHSTRAY	0.06	WNRGMGT	0.04
SHARING_ROAD	0.002	WNRSR	0.05
SHARING_SHED	0.12	WNRCS	0.009
RUNOFFWATER	<0.001	WNRHLTH	0.06
PCREEK	0.002	WNGAGE	0.11
ICREEK	0.05	WNGPCNT	0.10
KANGAROO	0.003	HGTSR	0.006
OTHERWILDLIFE	<0.001	HGTCS	0.016
RAMRISK	0.07	ADSR	0.08
SUPERFREQ	<0.001	SVC_CS	0.001
OTHERFERT	<0.001		
WORMCONTROL	0.11		
WORMRECOMM	0.21		
PINRUSH	0.012		
DROUGHT	<0.001		
DROUGHT_YRLAMBING	0.006		

Appendix 10.2

Unconditional associations between flock- and cohort-level variables and pool MAP number shed

Parameters	Levels	<i>b</i>	LCL (<i>b</i>)	UCL(<i>b</i>)	SE (<i>b</i>)	P
Flock-level variables						
Confounding Variables						
INFLEVEL						<0.001
	No mortalities					
	Low mortalities and trend falling or steady	0.86	0.50	1.23	0.18	
	High mortalities but trend falling or steady OR Low mortalities but trend escalating	1.69	1.34	2.03	0.18	
	High mortalities as well as trend escalating	1.90	1.45	2.36	0.23	

OJD_DURN						<0.001
	3<5 years					
	5<7years	0.48	0.03	0.94	0.23	
	7<10years	0.24	-0.21	0.69	0.23	
	10 year or more	0.97	0.53	1.41	0.22	

PEAKMORT						<0.001
	No mortalities					
	<2% mortalities	1.13	0.78	1.48	0.18	
	≥ 2% mortalities	1.65	1.31	1.99	0.17	

CURRMORT						<0.001
	No mortalities					
	<2% mortalities	1.09	0.75	1.43	0.17	
	≥ 2% mortalities	1.64	1.29	1.99	0.18	

MEANMORT						<0.001
	No mortalities					
	<2% mortalities	1.23	0.88	1.57	0.17	
	≥ 2% mortalities	1.61	1.24	1.97	0.19	

YNGAGEMORT						<0.001
	No mortalities					
	<2years	2.11	1.63	2.59	0.24	
	2 to < 3 yrs	1.47	1.13	1.81	0.17	
	≥ 3 years	0.99	0.61	1.37	0.19	

OJDSIGNS						<0.001
	Nil					
	Death and/or Loss of condition	1.18	0.76	1.59	0.21	

	Death, loss of condition and scouring	1.50	1.17	1.82	0.17	
OJD Control						
DROPSVACC						0.001
	No drops vaccinated					
	1 or 2 drops vaccinated	0.83	0.37	1.29	0.23	
	>2 drops vaccinated	0.39	-0.02	0.80	0.21	

CLINICALMGT						<0.001
	No mortalities observed					
	Immediately dispose off	1.24	0.88	1.60	0.18	
	Dispose off later	1.10	0.64	1.55	0.23	
	Do nothing	0.90	0.30	1.49	0.30	

CULL						<0.001
	No					
	Yes	0.80	0.52	1.07	0.14	

SELL						<0.001
	No					
	Yes	0.91	0.52	1.31	0.20	

YOUNGSEPARATE						0.003
	No					
	Yes	0.42	0.14	0.70	0.14	

YOUNGFIRST						0.80
	No					
	Yes	0.05	-0.37	0.47	0.21	

DESTOCK						0.01
	No					
	Yes	-0.38	-0.67	-0.09	0.15	
Lateral Spread and purchase risk						
INFNBRS						0.83
	≤ 25%					
	>25% to ≤ 75%	-0.07	-0.46	0.32	0.20	
	> 75%	-0.11	-0.45	0.24	0.18	

SHSTRAY						0.06
	No straying					
	<10 sheep stray annually					
	OR stray not even once per year	-0.35	-0.69	-0.01	0.17	
	10-20 sheep stray annually	-0.12	-0.53	0.29	0.21	
	>20 sheep stray annually					
	OR stray more than once	0.19	-0.25	0.64	0.23	

annually

-----					0.002
SHARING_ROAD					
	No sharing				
	≤ twice per year	0.57	0.21	0.93	0.18
	>twice per year	0.47	0.10	0.84	0.19
-----					0.12
SHARING_SHED					
	No sharing				
	Sharing	-0.34	-0.78	0.09	0.22
-----					<0.001
RUNOFFWATER					
	≤ 10%				
	>10 to ≤ 30%	-0.40	-0.77	-0.03	0.19
	>30% to ≤ 60%	-0.24	-0.62	0.13	0.19
	> 60%	-0.92	-1.32	-0.52	0.20
-----					0.00
PCREEK					
	No				
	Yes	-0.47	-0.77	-0.17	0.15
-----					0.05
ICREEK					
	No				
	Yes	0.36	0.01	0.71	0.18
-----					0.003
KANGAROO					
	≤ 20%				
	>20% to ≤ 50%	0.61	0.26	0.96	0.18
	>50%	0.35	0.02	0.68	0.17
-----					0.43
RABBIT					
	Nil				
	≤ 5%	-0.21	-0.54	0.12	0.17
	>5%	-0.19	-0.54	0.17	0.18
-----					<0.001
OTHERWILDLIFE					
	No				
	Yes	-0.55	-0.85	-0.25	0.15
-----					0.07
RAMRISK					
	None purchased/ none from infected sources				
	Some/all purchased from infected sources	-0.27	-0.55	0.02	0.15
-----					0.38
EWERISK					
	No				
	Yes	0.13	-0.16	0.43	0.15

Property and flock management

GRAZEDAREA						0.60
	≤ 965 hectares					
	> 965 hectares	0.08	-0.21	0.36	0.14	

FLOCKSIZE						0.49
	≤ 3000					
	> 3000	0.1	-0.18	0.38	0.14	

SUPERFREQ						<0.001
	≤ once in three years					
	>once to ≤ twice in three years	0.47	0.07	0.87	0.20	
	> twice to ≤ Every year	0.87	0.51	1.23	0.18	
	> Once every year	0.44	-0.03	0.91	0.24	

LIME						0.57
	No					
	Yes	-0.09	-0.38	0.21	0.15	

OTHERFERT						<0.001
	No					
	Yes	-1.06	-1.49	-0.63	0.22	

MINERALDEF						0.75
	No evidence					
	Some evidence	-0.05	-0.36	0.26	0.16	

WORMCONTROL						0.11
	No					
	Yes	-0.29	-0.66	0.07	0.18	

WORMRECOMM						0.21
	No					
	Yes	0.19	-0.11	0.50	0.15	

Drought and water logging

WATERLOG						0.66
	<1%					
	1% to <10%	0.13	-0.27	0.53	0.21	
	10% to <30%	0.01	-0.41	0.42	0.21	
	≥ 30%	-0.12	-0.50	0.27	0.20	

PINRUSH						0.01
	Nil					
	<1%	-0.51	-0.97	-0.05	0.23	
	1 to ≤ 5%	-0.43	-0.78	-0.07	0.18	

	>5%	-0.50	-0.87	-0.13	0.19	

DROUGHT						<0.001
	≤ 150mm lesser OR more than long-term average					
	>150mm lesser	0.79	0.48	1.11	0.16	

DROUGHT_SAMPLEYR						0.64
	up to 132mm lesser OR more than long-term average					
	> 132 mm to 228mm lesser	-0.05	-0.38	0.29	0.17	
	> 228 mm lesser	-0.17	-0.53	0.18	0.18	

DROUGHT_YRLAMBING						0.01
	up to 15mm lesser OR more than long-term average					
	>15 to 112mm lesser	-0.07	-0.43	0.29	0.18	
	>112mm lesser	0.48	0.15	0.81	0.17	
<hr/>						
Cohort level factors						
General						
AGEGP						0.28
	3 years					
	4 years	-0.16	-0.44	0.13	0.15	
	5 years					

SEX						<0.001
	Ewes					
	Wethers	0.64	0.32	0.96	0.16	
	Mixed sex					

GROWTHCHK						<0.001
	<12 weeks					
	≥12 weeks	0.58	0.30	0.86	0.14	

MINSUPPL						0.42
	No					
	Yes	-0.12	-0.41	0.17	0.15	

FODSTUB						0.008
	No					
	<16 weeks	0.54	0.20	0.89	0.18	
	≥16 weeks	0.12	-0.23	0.46	0.18	

WATER						0.20
	Less					
	Average	0.48	-0.16	1.12	0.32	

High	0.53	-0.05	1.12	0.30	
<hr/>					
SUPPLFEED					0.11
No					
≤ 6months	0.33	-0.16	0.82	0.25	
6 months to 1 year	0.51	0.06	0.95	0.23	
> 1year	0.51	0.05	0.96	0.23	
<hr/>					
SUPPLFEED_CS					0.93
<3					
≥ 3	0.016	-0.37	0.40	0.19	
<hr/>					
SUPPLFEED_METHOD					0.49
On ground					
Some in trough	-0.17	-0.67	0.32	0.25	
<hr/>					
SUPPLFEED_LIME					0.97
No					
Yes, in some	0.01	-0.35	0.36	0.18	
<hr/>					
Lambing variables					
<hr/>					
DAMSR					0.77
<8dse/hac					
8 <12 dse/hac	-0.02	-0.54	0.49	0.26	
≥12 dse/hac	0.10	-0.28	0.49	0.20	
<hr/>					
DAMCS					0.14
<3					
3<4	0.17	-0.26	0.60	0.22	
≥4	-0.22	-0.75	0.31	0.27	
<hr/>					
DAM_SCOUR					0.50
No					
Yes	0.13	-0.26	0.53	0.20	
<hr/>					
DECONT_LBGPDK					0.38
Nil					
<8 weeks	0.31	-0.06	0.68	0.19	
8<12 weeks	0.21	-0.26	0.68	0.24	
≥12weeks	0.01	-0.43	0.45	0.22	
<hr/>					
LBGSSN					0.16
Spring					
Autumn	-0.19	-0.52	0.14	0.17	
Winter	-0.35	-0.73	0.02	0.19	
<hr/>					
Weaner variables					
<hr/>					
DECONT_WNGPDK					0.001
Nil					

	<8 weeks	-0.12	-0.55	0.32	0.22	
	8<12 weeks	0.47	0.11	0.83	0.18	
	≥12weeks	0.65	0.27	1.04	0.20	

WNRGMGT						0.04
	Set					
	Rotational	-0.30	-0.58	-0.01	0.15	

WNRSR						0.05
	<8dse/hac					
	8 <12 dse/hac	0.40	0.04	0.76	0.18	
	≥ 12 dse/hac	0.37	0.03	0.71	0.17	

WNRCS						0.01
	<3					
	≥3	-0.39	-0.68	-0.10	0.15	

WNRHLTH						0.06
	Some problems					
	No problems	-0.28	-0.58	0.02	0.15	

WNGAGE						0.11
	≤ 15 weeks					
	≤ 18 weeks	-0.19	-0.57	0.19	0.19	
	≤ 21 weeks	-0.34	-0.76	0.08	0.21	
	>21 weeks	-0.45	-0.83	-0.07	0.19	

WNGPCNT						0.10
	<70%					
	70<80%	-0.26	-0.68	0.16	0.21	
	80<90%	-0.52	-0.93	-0.11	0.21	
	>90%	-0.24	-0.64	0.16	0.21	

Hogget variables						
						0.35
HGTGMGT						
	Set					
	Rotational	-0.14	-0.42	0.15	0.15	

HGTSR						0.01
	<8dse/hac					
	8 <12 dse/hac	0.56	0.21	0.91	0.18	
	≥ 12 dse/hac	0.30	-0.08	0.68	0.19	

HGTCS						0.02
	<3					
	≥3	-0.40	-0.72	-0.07	0.16	

HGTHLTH						0.71
	Some problems					
	No problems	0.08	-0.35	0.52	0.22	

Adult variables

ADCS						0.85
	<3					
	≥3	0.03	-0.29	0.35	0.16	
<hr/>						
ADSR						0.08
	<8dse/hac					
	8 <12 dse/hac	0.30	-0.06	0.66	0.18	
	≥ 12 dse/hac	0.37	0.01	0.72	0.18	
<hr/>						
ADGMGT						0.56
	Set					
	Rotational	-0.08	-0.37	0.20	0.15	
<hr/>						
ADHLTH						0.63
	No problems					
	Some problems	0.09	-0.29	0.47	0.19	
<hr/>						
JOININGDURN_1						0.75
	<6 weeks					
	6-7 weeks	-0.16	-0.61	0.28	0.23	
	>7 weeks	-0.09	-0.64	0.47	0.28	
<hr/>						
SVC_CS						0.001
	<2					
	2<3	-0.81	-1.30	-0.33	0.25	
	>3	-0.41	-0.92	0.09	0.26	

Appendix 11 Univariable results: Soil variables for IPREV

Cohort OJD prevalence - IPREV

Results for the 44 soil variables investigated are presented in the table Appendix 11.2. Of these, 1 property-level variable, 6 3-paddock mean variables, 3 lamb paddock variables, 7 weaner paddock variables and 7 hogget/adult paddock variables were unconditionally associated with IPREV in the FIRST dataset. After deletion of highly correlated variables (as described in Appendix 1 Section 3.10) a total of 24 variables remained for inclusion in multivariable analyses (Appendix 11.1). The final number of variables unconditionally associated with IPREV in the SECOND and THIRD datasets and included in multivariable analyses were 26 and 22, respectively. The 19 variables identified as unconditionally associated with IPREV in all three datasets and used in multivariable analyses were:

- Parent soil type (PSTYPE)
- Mean cation exchange capacity of soil in 3 paddocks (CEC)
- Mean phosphorus buffer index of soil in 3 paddocks (PBI)
- Mean sulphate sulphur content of soil in 3 paddocks (S)
- Mean clay % in the soil from 3 paddocks (CLAY)
- Phosphorus content of soil in lambing paddock (P_LBGPDK)
- Phosphorus buffer index of soil in lambing paddock (PBI_LBGPDK)
- Sand % of the soil in lambing paddock (SAND_LBGPDK)
- Phosphorus buffer index of soil in weaning paddock (PBI_WNGPDK)
- Sulphate sulphur content of soil in weaning paddock (S_WNGPDK)
- Sand % of soil in weaning paddock (SAND_WNGPDK)
- Silt % of soil in weaning paddock (SILT_WNGPDK)
- Cation exchange capacity of the soil in hogget/adult paddock (CEC_ADPDK)
- Phosphorus content of soil in hogget/adult paddock (P_ADPDK)
- Phosphorus buffer index of soil in hogget/adult paddock (PBI_ADPDK)
- Sulphate sulphur content of soil in hogget/adult paddock (S_ADPDK)
- Sand % of soil in hogget/adult paddock (SAND_ADPDK)
- Silt % of soil in hogget/adult paddock (SILT_ADPDK)
- Clay % of soil in hogget/adult paddock (CLAY_ADPDK).

Appendix 11.1

The P-values from univariable analyses of FIRST, SECOND and THIRD datasets for soil variables unconditionally associated with IPREV ($P < 0.25$ – shown in regular text) in one or more datasets and included in subsequent multivariable analyses for the respective dataset/s

Variables	FIRST Dataset	SECOND Dataset	THIRD Dataset
PSTYPE	0.08	0.05	0.09
CEC	0.02	0.01	0.02
P	0.38	0.29	0.04
PBI	0.01	0.002	0.001
S	0.06	0.004	<0.001
SAND	0.07	0.05	0.3
SILT	0.13	0.18	0.41
CLAY	0.03	0.01	0.12
CEC_LBGPDK	0.25	0.18	0.25
P_LBGPDK	0.12	0.04	0.01
PBI_LBGPDK	0.2	0.13	0.06
S_LBGPDK	0.58	0.26	0.03
SAND_LBGPDK	0.01	0.01	0.01
CEC_WNGPDK	0.16	0.16	0.35
PBI_WNGPDK	0.01	0.02	0.01
S_WNGPDK	0.14	0.09	0.01
ALSAT_WNGPDK	0.02	0.14	0.42
SAND_WNGPDK	0.003	0.005	0.02
SILT_WNGPDK	0.001	0.001	0.01
CLAY_WNGPDK	0.03	0.1	0.27
CEC_ADPDK	0.03	0.01	0.05
P_ADPDK	0.05	0.06	0.07
PBI_ADPDK	0.001	0.002	0.01
S_ADPDK	0.06	0.07	0.02
ALSAT_ADPDK	0.6	0.24	0.16
SAND_ADPDK	0.04	0.02	0.07
SILT_ADPDK	0.04	0.04	0.12
CLAY_ADPDK	0.09	0.18	0.24
Total no of variables for inclusion in multivariable analyses	24	26	22

Appendix 11.2

Unconditional associations between soil variables and IPREV (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-10%) and high (>10%)) for 77 cohorts in the FIRST Dataset, 98 cohorts in the SECOND Dataset and 117 cohorts in the THIRD Dataset

Variables and categories	Code	FIRST Dataset				SECOND Dataset				THIRD Dataset			
		Odds ratio	LCL	UCL	P	Odds ratio	LCL	UCL	P	Odds ratio	LCL	UCL	P
<i>Property-level variables</i>													
PSTYPE					0.08				0.05				0.09
	Basaltic	1	1			1				1			
	Granite	2	0.72	0.14	3.65	0.45	0.10	1.91		0.53	0.14	1.96	
	Shale and sandstone	3	3.30	0.65	17.37	2.00	0.48	8.52		1.73	0.48	6.32	
	Mixed without limestone	4	0.79	0.08	7.89	1.00	0.16	6.08		0.70	0.16	3.09	
	Mixed with limestone	5	1.24	0.22	7.19	0.70	0.14	3.40		0.64	0.15	2.69	
<i>3-paddock mean variables</i>													
Ph					0.52				0.39				0.46
	<4.6	0	1			1				1			
	4.6 - 5.2	1	1.61	0.62	4.21	1.56	0.67	3.64		1.35	0.64	2.88	
	>5.2	2	1.82	0.53	6.37	2.03	0.68	6.21		1.84	0.69	5.00	
CEC					0.02				0.01				0.02
	≤ 6	1	1			1				1			
	> 6	2	2.88	1.19	7.27	2.90	1.33	6.58		2.35	1.17	4.80	
P					0.38				0.29				0.04
	<20	0	1			1				1			
	20-30	1	0.92	0.25	3.31	1.37	0.48	3.95		2.29	0.91	5.87	
	>30	2	1.76	0.58	5.44	2.12	0.80	5.73		3.21	1.31	8.10	
PBI					0.01				0.002				0.001
	< 70	0	1			1				1			
	≥ 70	1	3.23	1.30	8.48	3.76	1.64	9.00		3.46	1.65	7.47	

S						0.06				0.004		0.0002	
	<4	0	1				1				1		
	4-8	1	1.04	0.34	3.21		1.00	0.36	2.78		1.61	0.65	4.08
	>8	2	3.10	0.97	10.40		4.10	1.40	12.52		6.36	2.40	17.65

K						0.59				0.65		0.50	
	<0.4	1	1				1				1		
	>0.4	2	1.31	0.50	3.44		1.21	0.53	2.80		1.29	0.62	2.69

ALSAT						0.60				0.50		0.44	
	≤ 2	0	1				1				1		
	>2 to ≤ 5	1	0.94	0.28	3.15		1.04	0.37	2.98		1.02	0.40	2.62
	>5 to ≤ 12	2	0.52	0.16	1.62		0.52	0.18	1.46		0.52	0.21	1.29
	>12	3	0.56	0.17	1.87		0.63	0.21	1.85		0.93	0.34	2.51

SAND						0.07				0.05		0.30	
	≤ 62%	1	1				1				1		
	> 62%	2	0.44	0.17	1.06		0.46	0.21	1.01		0.70	0.35	1.38

SILT						0.13				0.18		0.41	
	≤ 21%	1	1				1				1		
	> 21%	2	1.95	0.81	4.82		1.70	0.79	3.73		1.34	0.67	2.67

CLAY						0.03				0.01		0.12	
	≤ 15%	1	1				1				1		
	> 15%	2	2.72	1.12	6.91		2.78	1.26	6.33		1.72	0.86	3.47

Lamb paddock variables													
PH_LBGPKD						0.88				0.93		0.61	
	<4.6	0	1				1				1		
	4.6 - 5.2	1	0.88	0.29	2.71		0.83	0.31	2.19		0.64	0.26	1.54
	>5.2	2	1.22	0.34	4.40		0.91	0.29	2.84		0.82	0.30	2.20

CEC_LBGPKD						0.25				0.18		0.25	
	≤ 6	1	1				1				1		
	> 6	2	1.79	0.66	4.94		1.81	0.76	4.38		1.58	0.73	3.44

P_LBGPDK					0.12				0.04			0.01
	<20	0	1			1				1		
	20-30	1	1.71	0.39	7.66	2.28	0.74	7.26		2.55	0.96	6.96
	>30	2	3.76	0.94	16.08	4.00	1.35	12.49		4.46	1.70	12.27

PBI_LBGPDK					0.20				0.13			0.06
	< 70	0	1			1				1		
	≥ 70	1	2.08	0.68	6.57	2.09	0.80	5.63		2.29	0.98	5.48

S_LBGPDK					0.58				0.26			0.03
	<4	0	1			1				1		
	4--8	1	1.52	0.43	5.44	1.56	0.51	4.82		2.20	0.80	6.25
	>8	2	2.04	0.53	8.11	2.73	0.81	9.51		4.49	1.48	14.25

K_LBGPDK					0.68				0.48			0.36
	<0.4	1	1			1				1		
	>0.4	2	1.25	0.43	3.64	1.40	0.56	3.52		1.46	0.65	3.30

ALSAT_LBGPDK					0.81				0.68			0.62
	≤ 2	0	1			1				1		
	>2 to ≤ 5	1	1.67	0.48	5.93	1.90	0.63	5.85		1.39	0.50	3.87
	>5 to ≤ 12	2	0.80	0.18	3.45	0.96	0.29	3.22		1.04	0.36	3.03
	>12	3	1.10	0.24	5.03	1.18	0.33	4.26		2.01	0.67	6.17

SAND_LBGPDK					0.01				0.01			0.01
	≤ 62%	1	1			1				1		
	> 62%	2	0.24	0.08	0.68	0.29	0.11	0.71		0.35	0.15	0.76

SILT_LBGPDK					0.25				0.32			0.39
	≤ 21%	1	1			1				1		
	> 21%	2	1.79	0.66	4.96	1.56	0.66	3.75		1.41	0.65	3.07

CLAY_LBGPDK					0.70				1.00			0.56
	≤ 15%	1	1			1				1		
	> 15%	2	1.21	0.45	3.28	1.00	0.42	2.40		0.79	0.36	1.73

Weaner paddock variables

PH_WNGPDK					0.78				0.93			0.96
	<4.6	0	1			1				1		
	4.6 - 5.2	1	1.49	0.48	4.70	1.22	0.45	3.30		1.08	0.44	2.66
	>5.2	2	1.35	0.25	7.51	1.12	0.28	4.49		1.19	0.37	3.79

CEC_WNGPDK					0.16				0.16			0.35
	≤ 6	1	1			1				1		
	> 6	2	2.14	0.75	6.34	1.94	0.78	4.97		1.47	0.65	3.35

P_WNGPDK					0.80				0.89			0.64
	<20	0	1			1				1		
	20-30	1	1.08	0.20	5.92	1.25	0.33	4.80		1.68	0.53	5.44
	>30	2	1.50	0.36	6.45	1.36	0.39	4.78		1.60	0.53	4.85

PBI_WNGPDK					0.01				0.02			0.01
	< 70	0	1			1				1		
	≥ 70	1	4.09	1.34	13.85	3.19	1.22	8.85		2.89	1.23	7.05

S_WNGPDK					0.14				0.09			0.01
	<4	0	1			1				1		
	4-8	1	0.54	0.12	2.36	0.85	0.21	3.44		1.08	0.29	3.99
	>8	2	1.78	0.39	8.38	2.56	0.62	11.05		3.75	1.00	14.72

K_WNGPDK					0.91				0.69			0.66
	<0.4	1	1			1				1		
	>0.4	2	0.93	0.28	3.08	1.23	0.45	3.40		1.23	0.50	3.06

ALSAT_WNGPDK					0.02				0.14			0.42
	≤ 2	0	1			1				1		
	>2 to ≤ 5	1	4.25	0.92	21.94	1.84	0.52	6.71		1.01	0.30	3.36
	>5 to ≤ 12	2	0.24	0.05	0.97	0.35	0.10	1.15		0.48	0.17	1.32
	>12	3	1.00	0.20	4.95	1.24	0.30	5.05		1.34	0.37	4.89

SAND_WNGPDK					0.003				0.005			0.02
	≤ 62%	1	1			1				1		
	> 62%	2	0.17	0.05	0.55	0.24	0.08	0.66		0.35	0.14	0.83

SILT_WNGPDK						0.001				0.001		0.01
	≤ 21%	1	1				1				1	
	> 21%	2	7.93	2.36	32.41		5.18	1.88	15.73		3.37	1.40 8.49

CLAY_WNGPDK						0.03				0.10		0.27
	≤ 15%	1	1				1				1	
	> 15%	2	3.29	1.10	10.64		2.20	0.86	5.80		1.59	0.69 3.70

Hogget/Adult paddock variables												

PH_ADPDK						0.32				0.32		0.25
	<4.6	0	1				1				1	
	4.6 - 5.2	1	1.93	0.66	5.83		1.38	0.53	3.62		1.21	0.50 2.94
	>5.2	2	2.35	0.64	8.89		2.49	0.76	8.42		2.53	0.85 7.81

CEC_ADPDK						0.03				0.01		0.05
	≤ 6	1	1				1				1	
	> 6	2	3.04	1.14	8.59		3.07	1.27	7.78		2.21	0.99 5.05

P_ADPDK						0.05				0.06		0.07
	<20	0	1				1				1	
	20-30	1	0.52	0.13	2.00		0.59	0.19	1.80		0.84	0.30 2.33
	>30	2	2.42	0.81	7.58		2.20	0.81	6.19		2.46	0.98 6.33

PBI_ADPDK						0.001				0.002		0.01
	< 70	0	1				1				1	
	≥ 70	1	5.61	1.95	18.14		4.11	1.64	11.03		3.17	1.38 7.54

S_ADPDK						0.06				0.07		0.02
	<4	0	1				1				1	
	4-8	1	1.97	0.60	6.68		2.02	0.68	6.10		2.21	0.82 6.05
	>8	2	5.37	1.33	23.25		4.14	1.24	14.58		5.10	1.68 16.24

K_ADPDK						0.67				0.79		0.92
	<0.4	1	1				1				1	
	>0.4	2	1.28	0.41	4.02		1.14	0.44	2.99		0.96	0.40 2.28

ALSAT_ADPDK						0.60				0.24		0.16

	≤ 2	0	1			1			1		
	>2 to ≤ 5	1	0.72	0.15	3.30	0.32	0.09	1.14	0.30	0.10	0.88
	>5 to ≤ 12	2	0.72	0.19	2.68	0.63	0.18	2.08	0.62	0.19	1.98
	>12	3	0.44	0.13	1.43	0.40	0.13	1.22	0.48	0.17	1.34

SAND_ADSDK					0.04				0.02		0.07
	≤ 62%	1	1			1			1		
	> 62%	2	0.35	0.12	0.94	0.34	0.14	0.83	0.48	0.21	1.06

SILT_ADSDK					0.04				0.04		0.12
	≤ 21%	1	1			1			1		
	> 21%	2	2.91	1.08	8.32	2.54	1.05	6.37	1.88	0.84	4.26

CLAY_ADSDK					0.09				0.18		0.24
	≤ 15%	1	1			1			1		
	> 15%	2	2.32	0.87	6.42	1.80	0.76	4.38	1.62	0.73	3.66

Appendix 12 Univariable results: Soil variables for IPREV25

Cohort OJD prevalence – IPREV25

Full results for the 44 soil variables investigated are presented in the table Appendix 12.2. Analysis of the FIRST dataset found 1 property-level variable, 4 3-paddock mean variables, 1 lamb paddock variable, 5 weaner paddock variables and 5 hogget/adult paddock variables were unconditionally associated with IPREV25. After deletion of highly correlated variables, a total of 16 variables were remained for inclusion in multivariable analyses (Appendix 12.1). The final number of variables unconditionally associated with IPREV25 in the SECOND and THIRD datasets included in multivariable analyses were 21 and 19, respectively. The 11 variables identified in all three datasets as unconditionally associated with IPREV25 (of which 10 were similarly identified for IPREV) were:

- Parent soil type (PSTYPE)
- Mean cation exchange capacity of soil in 3 paddocks (CEC)
- Mean Phosphorus buffer index of soil in 3 paddocks (PBI)
- Sand % of the soil in lambing paddock (SAND_LBGPDK)
- Phosphorus buffer index of soil in weaning paddock (PBI_WNGPDK)
- Sand % of the soil in weaning paddock (SAND_WNGPDK)
- Silt % of soil in weaning paddock (SILT_WNGPDK)
- Clay % of soil in weaning paddock (CLAY_WNGPDK)
- Phosphorus buffer index of soil in hogget/adult paddock (PBI_ADPDK)
- Sand % of the soil in hogget/adult paddock (SAND_ADPDK)
- Silt % of soil in hogget/adult paddock (SILT_ADPDK).

Appendix 12.1

The P-values from univariable analyses of FIRST, SECOND and THIRD datasets for soil variables unconditionally associated with IPREV25 (P<0.25 – shown in regular text) in one or more datasets and included in subsequent multivariable analyses for the respective dataset/s

Variables	FIRST Dataset	SECOND Dataset	THIRD Dataset
PSTYPE	0.23	0.15	0.09
CEC	0.2	0.09	0.08
P	0.85	0.75	0.19
PBI	0.02	0	0.002
S	0.41	0.06	0.01
SAND	0.25	0.21	0.53
SILT	0.14	0.24	0.45
CLAY	0.21	0.08	0.26
P_LBGPDK	0.32	0.09	0.02
S_LBGPDK	0.73	0.58	0.14
SAND_LBGPDK	0.21	0.16	0.17
CEC_WNGPDK	0.28	0.13	0.2
PBI_WNGPDK	0.03	0.02	0.01
S_WNGPDK	0.45	0.38	0.11
ALSAT_WNGPDK	0.23	0.21	0.41
SAND_WNGPDK	0.01	0.01	0.02
SILT_WNGPDK	0.001	0.001	0.003
CLAY_WNGPDK	0.02	0.02	0.05
CEC_ADPDK	0.23	0.13	0.25
P_ADPDK	0.22	0.18	0.26
PBI_ADPDK	0.004	0.01	0.01
S_ADPDK	0.31	0.37	0.21
ALSAT_ADPDK	0.78	0.17	0.11
SAND_ADPDK	0.07	0.03	0.08
SILT_ADPDK	0.1	0.05	0.07
Total no of variables for inclusion in multivariable analyses	16	21	19

Appendix 12.2

Unconditional associations between soil variables and IPREV25 (cohort OJD animal-level prevalence categorised as Low (<2%), Medium (2-5%) and High (>5%)) for 77 cohorts in the FIRST Dataset, 98 cohorts in the SECOND Dataset and 117 cohorts in the THIRD Dataset

Variables and categories	Code	FIRST Dataset				SECOND Dataset				THIRD Dataset			
		Odds ratio	LCL	UCL	P	Odds ratio	LCL	UCL	P	Odds ratio	LCL	UCL	P
Property-level variables													
PSTYPE					0.23				0.15				0.09
	Basaltic	1	1			1				1			
	Granite	2	0.55	0.09	2.82	0.31	0.06	1.38		0.39	0.09	1.51	
	Shale and sandstone	3	1.61	0.28	8.36	0.90	0.17	3.95		1.04	0.24	4.00	
	Mixed without limestone	4	0.33	0.04	2.80	0.31	0.05	1.75		0.30	0.06	1.34	
	Mixed with limestone	5	0.92	0.14	5.40	0.47	0.08	2.35		0.50	0.10	2.23	
3-paddock mean variables													
Ph					0.86				0.77				0.79
	<4.6	0	1			1				1			
	4.6 - 5.2	1	1.25	0.48	3.24	1.35	0.59	3.12		1.30	0.61	2.78	
	>5.2	2	0.95	0.29	3.14	1.11	0.39	3.19		1.15	0.45	3.02	
CEC					0.20				0.09				0.08
	≤ 6	1	1			1				1			
	> 6	2	1.75	0.75	4.14	1.91	0.90	4.09		1.83	0.93	3.66	
P					0.85				0.75				0.19
	<20	0	1			1				1			
	20-30	1	0.78	0.21	2.84	1.14	0.40	3.24		1.81	0.72	4.61	
	>30	2	1.04	0.33	3.18	1.42	0.54	3.72		2.29	0.94	5.69	
PBI					0.02				0.003				0.002

	< 70	0	1				1			1		
	≥ 70	1	2.83	1.16	7.23		3.34	1.50	7.79	3.17	1.52	6.82

S						0.41				0.06		0.01
	<4	0	1				1			1		
	4--8	1	0.76	0.24	2.33		0.56	0.19	1.57	1.00	0.39	2.54
	>8	2	1.46	0.45	4.71		1.53	0.51	4.51	2.96	1.12	7.96

K						0.95				0.64		0.84
	<0.4	1	1				1			1		
	>0.4	2	1.03	0.39	2.68		0.82	0.35	1.88	0.93	0.44	1.94

ALSAT						0.58				0.75		0.53
	≤ 2	0	1				1			1		
	>2 to ≤ 5	1	1.10	0.33	3.81		0.95	0.34	2.65	0.96	0.38	2.43
	>5 to ≤ 12	2	0.53	0.17	1.58		0.60	0.22	1.62	0.61	0.25	1.48
	>12	3	0.99	0.29	3.46		0.88	0.30	2.63	1.24	0.45	3.50

SAND						0.25				0.21		0.53
	≤ 62%	1	1				1			1		
	> 62%	2	0.60	0.25	1.43		0.62	0.29	1.31	0.81	0.41	1.59

SILT						0.14				0.24		0.45
	≤ 21%	1	1				1			1		
	> 21%	2	1.92	0.81	4.69		1.57	0.74	3.36	1.30	0.66	2.58

CLAY						0.21				0.08		0.26
	≤ 15%	1	1				1			1		
	> 15%	2	1.73	0.73	4.15		1.97	0.92	4.25	1.48	0.75	2.95

Lamb paddock variables												
PH_LBGPDK						0.42				0.61		0.33
	<4.6	0	1				1			1		
	4.6 - 5.2	1	0.50	0.16	1.54		0.62	0.24	1.59	0.52	0.21	1.23
	>5.2	2	0.96	0.26	3.71		0.79	0.25	2.45	0.68	0.25	1.85

CEC_LBGPDK						0.67				0.59		0.72

	≤ 6	1	1				1			1		
	> 6	2	1.24	0.46	3.33		1.26	0.54	2.93	1.15	0.54	2.48

P_LBGPDK						0.32				0.09		0.02
	<20	0	1				1			1		
	20-30	1	2.43	0.57	10.95		2.76	0.92	8.66	2.76	1.05	7.47
	>30	2	2.76	0.73	10.87		2.93	1.05	8.46	3.45	1.36	9.08

PBI_LBGPDK						0.54				0.38		0.26
	< 70	0	1				1			1		
	≥ 70	1	1.41	0.48	4.45		1.52	0.60	4.01	1.61	0.70	3.79

S_LBGPDK						0.73				0.58		0.14
	<4	0	1				1			1		
	4-8	1	1.16	0.33	4.04		0.89	0.28	2.74	1.33	0.48	3.75
	>8	2	1.66	0.43	6.58		1.48	0.43	5.10	2.77	0.90	8.80

K_LBGPDK						0.51				0.36		0.54
	<0.4	1	1				1			1		
	>0.4	2	0.69	0.22	2.05		0.65	0.25	1.64	0.77	0.33	1.77

ALSAT_LBGPDK						0.38				0.53		0.39
	≤ 2	0	1				1			1		
	>2 to ≤ 5	1	2.89	0.79	12.34		2.29	0.77	7.30	1.76	0.64	5.02
	>5 to ≤ 12	2	0.96	0.23	4.06		1.30	0.40	4.39	1.29	0.45	3.79
	>12	3	1.92	0.41	10.62		1.40	0.41	5.05	2.49	0.82	8.27

SAND_LBGPDK						0.21				0.16		0.17
	≤ 62%	1	1				1			1		
	> 62%	2	0.53	0.19	1.43		0.55	0.23	1.28	0.58	0.26	1.25

SILT_LBGPDK						0.53				0.54		0.55
	≤ 21%	1	1				1			1		
	> 21%	2	1.38	0.51	3.75		1.30	0.56	3.07	1.27	0.59	2.75

CLAY_LBGPDK						1.00				0.85		0.44
	≤ 15%	1	1				1			1		
	> 15%	2	1.00	0.37	2.71		0.92	0.39	2.18	0.74	0.34	1.60

Weaner paddock variables

PH_WNGPDK					0.47				0.33			0.55
	<4.6	0	1			1				1		
	4.6 - 5.2	1	1.74	0.57	5.43	1.76	0.67	4.76		1.57	0.64	3.90
	>5.2	2	0.79	0.16	3.79	0.77	0.21	2.74		0.99	0.33	2.99

CEC_WNGPDK					0.28				0.13			0.20
	≤ 6	1	1			1				1		
	> 6	2	1.76	0.64	4.99	1.97	0.81	4.85		1.69	0.76	3.82

P_WNGPDK					0.68				0.57			0.31
	<20	0	1			1				1		
	20-30	1	1.95	0.37	10.81	1.94	0.54	7.10		2.23	0.73	6.98
	>30	2	1.73	0.44	6.90	1.71	0.53	5.61		2.08	0.73	6.10

PBI_WNGPDK					0.03				0.02			0.01
	< 70	0	1			1				1		
	≥ 70	1	3.34	1.15	10.30	3.01	1.20	7.93		3.22	1.37	7.87

S_WNGPDK					0.45				0.38			0.11
	<4	0	1			1				1		
	4-8	1	0.50	0.10	2.17	0.69	0.17	2.74		0.84	0.22	3.12
	>8	2	0.96	0.20	4.31	1.35	0.32	5.50		2.09	0.55	7.96

K_WNGPDK					0.93				0.77			0.62
	<0.4	1	1			1				1		
	>0.4	2	0.95	0.29	3.05	1.16	0.43	3.12		1.25	0.51	3.06

ALSAT_WNGPDK					0.23				0.21			0.41
	≤ 2	0	1			1				1		
	>2 to ≤ 5	1	2.34	0.53	12.59	1.95	0.56	7.49		1.19	0.36	4.17
	>5 to ≤ 12	2	0.36	0.08	1.47	0.44	0.13	1.37		0.50	0.18	1.35
	>12	3	1.07	0.24	5.16	1.24	0.33	5.01		1.33	0.38	5.08

SAND_WNGPDK					0.01				0.01			0.02
	≤ 62%	1	1			1				1		

	> 62%	2	0.24	0.08	0.71		0.29	0.11	0.73		0.36	0.15	0.83
SILT_WNGPDK						0.001				0.001			0.003
	≤ 21%	1	1				1				1		
	> 21%	2	6.80	2.23	22.78		4.88	1.88	13.40		3.63	1.54	8.86
CLAY_WNGPDK						0.02				0.02			0.05
	≤ 15%	1	1				1				1		
	> 15%	2	3.46	1.19	10.54		2.87	1.15	7.42		2.33	1.01	5.49
Hogget/Adult paddock variables													
PH_ADPPDK						0.60				0.80			0.74
	<4.6	0	1				1				1		
	4.6 - 5.2	1	1.77	0.59	5.55		1.29	0.50	3.40		1.23	0.51	2.99
	>5.2	2	1.30	0.38	4.76		1.40	0.45	4.57		1.51	0.52	4.54
CEC_ADPPDK						0.23				0.13			0.25
	≤ 6	1	1				1				1		
	> 6	2	1.80	0.69	4.84		1.94	0.83	4.64		1.58	0.72	3.53
P_ADPPDK						0.22				0.18			0.26
	<20	0	1				1				1		
	20-30	1	0.31	0.07	1.25		0.40	0.13	1.21		0.61	0.22	1.68
	>30	2	0.83	0.26	2.58		0.98	0.35	2.70		1.41	0.56	3.59
PBI_ADPPDK						0.004				0.01			0.01
	< 70	0	1				1				1		
	≥ 70	1	4.36	1.57	13.03		3.46	1.43	8.77		2.92	1.29	6.85
S_ADPPDK						0.31				0.37			0.21
	<4	0	1				1				1		
	4-8	1	2.33	0.71	7.86		2.04	0.69	6.10		2.06	0.77	5.61
	>8	2	2.45	0.65	9.66		2.10	0.66	6.84		2.51	0.87	7.49
K_ADPPDK						0.39				0.69			1.00
	<0.4	1	1				1				1		
	>0.4	2	1.63	0.53	4.98		1.21	0.46	3.13		0.99	0.41	2.35

ALSAT_ADPDK					0.78				0.17			0.11
	≤ 2	0	1			1				1		
	>2 to ≤ 5	1	0.52	0.12	2.27	0.27	0.08	0.90		0.28	0.09	0.80
	>5 to ≤ 12	2	0.88	0.23	3.58	0.85	0.25	3.05		0.89	0.27	3.14
	>12	3	0.62	0.18	2.11	0.53	0.17	1.63		0.60	0.21	1.71

SAND_ADPDK					0.07				0.03			0.08
	≤ 62%	1	1			1				1		
	> 62%	2	0.41	0.15	1.09	0.38	0.16	0.90		0.49	0.22	1.09

SILT_ADPDK					0.10				0.05			0.07
	≤ 21%	1	1			1				1		
	> 21%	2	2.29	0.86	6.25	2.40	1.01	5.83		2.07	0.93	4.65

CLAY_ADPDK					0.34				0.38			0.36
	≤ 15%	1	1			1				1		
	> 15%	2	1.60	0.61	4.28	1.46	0.62	3.47		1.46	0.66	3.27
=====												

Appendix 13 Final models for IPREV and IPREV25 for soil variables in SECOND and THIRD datasets

Appendix 13.1

Final logistic model for IPREV (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-10%) and high (>10%)) for soil variables in the SECOND dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Final model for 3-paddock mean variables (based on 96 cohorts)							
Intercept	-5.46	-8.09	-3.08				
Intercept	-1.83	-4.06	0.32				
CURRMORT							<0.001
	No mortalities			1			
	<2% mortalities	1.58	0.43	2.79	4.85	1.54	16.33
	≥ 2% mortalities	2.91	1.66	4.30	18.39	5.24	73.82
SEX							0.001
	Ewes			1			
	Wethers	1.82	0.72	3.00	6.17	2.06	20.00
AGEGP							0.72
	3 years			1			
	4 years	-0.17	-1.07	0.74	0.85	0.34	2.09
PSTYPE							0.61
	Basalt			1			
	Granite	0.19	-1.74	2.12	1.21	0.18	8.35
	Shale and sandstone	1.00	-0.68	2.74	2.73	0.51	15.54
	Mixed without limestone	0.63	-1.54	2.83	1.88	0.21	16.97
	Mixed with limestone	0.36	-1.49	2.23	1.43	0.22	9.31
PBI							0.06
	< 70			1			
	≥ 70	0.96	-0.04	1.99	2.60	0.96	7.35
CLAY							0.01
	≤ 15%			1			
	> 15%	1.20	0.25	2.20	3.33	1.28	9.06

Final model for lambing paddock variables (based on 75 cohorts)

Intercept	-2.86	-4.90	-0.95				
Intercept	0.64	-1.17	2.48				
CURRMORT							<0.001
	No mortalities			1			
	<2% mortalities	1.19	-0.05	2.49	3.29	0.95	12.11
	≥ 2% mortalities	2.67	1.30	4.20	14.46	3.67	66.58
SEX							<0.001

AGEGP	Ewes				1			
	Wethers	2.54	1.19	4.04	12.62	3.30	56.92	0.29
PSTYPE	3 years				1			
	4 years	-0.54	-1.57	0.46	0.58	0.21	1.59	0.75
SAND_LBGPDK	Basalt				1			
	Granite	-0.60	-2.49	1.25	0.55	0.08	3.48	
	Shale and sandstone	0.32	-1.36	2.01	1.37	0.26	7.45	
	Mixed without limestone	-0.28	-2.60	2.01	0.76	0.08	7.48	
	Mixed with limestone	0.06	-1.98	2.11	1.06	0.14	8.23	
	≤ 62%				1			0.07
> 62%	-1.05	-2.23	0.08	0.35	0.11	1.08		

Final model for weaning paddock variables (based on 66 cohorts)

Intercept		-4.87	-7.74	-2.36				
Intercept		-0.89	-3.18	1.34				
CURRMORT	No mortalities				1			<0.001
	<2% mortalities	1.34	0.00	2.76	3.80	1.00	15.79	
	≥ 2% mortalities	3.33	1.70	5.24	27.99	5.47	189.28	
SEX								<0.001
AGEGP	Ewes				1			
	Wethers	2.84	1.31	4.58	17.05	3.69	97.58	0.32
PSTYPE	3 years				1			
	4 years	-0.57	-1.71	0.54	0.57	0.18	1.72	0.61
SILT_WNGPDK	Basalt				1			
	Granite	-0.75	-2.88	1.35	0.47	0.06	3.87	
	Shale and sandstone	0.02	-1.87	1.90	1.02	0.15	6.68	
	Mixed without limestone	0.40	-2.11	2.97	1.50	0.12	19.46	
	Mixed with limestone	0.57	-1.62	2.80	1.76	0.20	16.52	
	≤ 21%				1			0.01
> 21%	1.62	0.39	2.97	5.05	1.48	19.51		

Final model for hogget/adult paddock variables (based on 75 cohorts)

Intercept		-4.16	-6.99	-1.56			
Intercept		-0.55	-3.03	1.96			

CURRMORT	No mortalities				1			<0.001
	<2% mortalities	1.37	0.02	2.78	3.93	1.02	16.15	
	≥ 2% mortalities	2.85	1.43	4.45	17.36	4.16	85.35	
SEX	Ewes				1			<0.001
	Wethers	2.03	0.80	3.39	7.64	2.23	29.57	
AGEGP	3 years				1			0.35
	4 years	-0.50	-1.58	0.54	0.60	0.21	1.72	
PSTYPE	Basalt				1			0.52
	Granite	0.16	-2.33	2.63	1.18	0.10	13.84	
	Shale and sandstone	0.85	-1.44	3.13	2.33	0.24	22.89	
	Mixed without limestone	0.08	-2.50	2.64	1.08	0.08	14.00	
	Mixed with limestone	-0.49	-2.96	1.92	0.61	0.05	6.83	
CEC_ADSDK	≤ 6 Meq/100g				1			0.02
	> 6 Meq/100g	1.32	0.21	2.50	3.76	1.23	12.18	
SAND_ADSDK	≤ 62%				1			0.09
	> 62%	-1.06	-2.34	0.17	0.35	0.10	1.19	

Appendix 13.2

Final logistic model for IPREV (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-10%) and high (>10%)) for soil variables in the THIRD dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Final model for 3-paddock mean variables (based on 115 cohorts)							
Intercept	-4.55	-6.74	-2.51				
Intercept	-1.44	-3.41	0.47				
CURRMORT							<0.001
	No mortalities			1			
	<2% mortalities	1.81	0.76	2.93	6.12	2.13	18.77
	≥ 2% mortalities	2.91	1.77	4.14	18.32	5.89	63.11
SEX							<0.001
	Ewes			1			
	Wethers	1.67	0.68	2.71	5.31	1.98	15.10
	Mixed	-2.68	-4.92	-0.81	0.07	0.01	0.44
AGEGP							0.30
	3 years			1			
	4 years	-0.36	-1.20	0.47	0.70	0.30	1.60
	5 years	-1.04	-2.47	0.34	0.35	0.09	1.40
PSTYPE							0.80
	Basalt			1			
	Granite	0.16	-1.56	1.89	1.17	0.21	6.65
	Shale and sandstone	0.56	-0.94	2.08	1.74	0.39	8.03
	Mixed without limestone	0.14	-1.72	1.99	1.15	0.18	7.31
	Mixed with limestone	-0.18	-1.84	1.50	0.84	0.16	4.48
PBI							0.03
	< 70			1			
	≥ 70	1.04	0.11	2.00	2.82	1.12	7.37
CLAY							0.10
	≤ 15%			1			
	> 15%	0.74	-0.13	1.63	2.09	0.88	5.09

Final model for lambing paddock variables (based on 93 cohorts)

Intercept	-4.65	-7.00	-2.50				
Intercept	-1.45	-3.50	0.53				
CURRMORT							<0.001
	No mortalities			1			
	<2% mortalities	1.54	0.38	2.77	4.64	1.46	15.95
	≥ 2% mortalities	2.87	1.59	4.28	17.72	4.93	72.10
SEX							0.001
	Ewes						

AGEGP	Wethers	1.98	0.79	3.26	7.22	2.21	26.01	0.67
	Mixed	-2.16	-5.52	0.54	0.12	0.00	1.71	
PSTYPE	3 years				1			0.28
	4 years	-0.41	-1.38	0.53	0.66	0.25	1.71	
	5 years	-0.42	-1.98	1.11	0.66	0.14	3.04	
P_LBGPDK	Basalt				1			0.02
	Granite	-0.52	-2.08	1.03	0.59	0.12	2.79	
	Shale and sandstone	0.73	-0.84	2.32	2.07	0.43	10.17	
	Mixed without limestone	0.35	-1.66	2.37	1.42	0.19	10.67	
	Mixed with limestone	-0.24	-2.02	1.54	0.79	0.13	4.67	
	<20 mg/kg				1			
20-30 mg/kg	1.65	0.41	2.96	5.18	1.50	19.23		
>30 mg/kg	1.57	0.35	2.86	4.81	1.41	17.54		

Final model for weaning paddock variables (based on 79 cohorts)

Intercept		-4.43	-6.79	-2.30				
Intercept		-1.06	-3.04	0.85				
CURRMORT	No mortalities				1			<0.001
	<2% mortalities	1.90	0.66	3.24	6.68	1.93	25.66	
	≥ 2% mortalities	3.61	2.14	5.30	37.06	8.46	199.83	
SEX	Ewes				1			<0.001
	Wethers	1.92	0.62	3.32	6.80	1.86	27.53	
	Mixed	-3.69	-7.11	-0.93	0.03	<0.001	0.39	
AGEGP	3 years				1			0.40
	4 years	-0.70	-1.76	0.33	0.50	0.17	1.39	
	5 years	-0.47	-2.15	1.18	0.63	0.12	3.25	
PSTYPE	Basalt				1			0.82
	Granite	-0.41	-2.20	1.40	0.67	0.11	4.05	
	Shale and sandstone	-0.14	-1.82	1.52	0.87	0.16	4.58	
	Mixed without limestone	0.23	-1.79	2.27	1.26	0.17	9.69	
	Mixed with limestone	0.54	-1.46	2.58	1.72	0.23	13.20	
SILT_WNGPDK	≤ 21%				1			0.01
	> 21%	1.46	0.38	2.62	4.32	1.46	13.79	

Final model for hogget/adult paddock variables (based on 87cohorts)

Intercept		-4.42	-7.09	-1.99				
Intercept		-0.95	-3.30	1.33				
CURRMORT								<0.001
	No mortalities				1			
	<2% mortalities	1.73	0.47	3.06	5.62	1.60	21.35	
	≥ 2% mortalities	3.42	2.03	4.98	30.45	7.58	145.21	
SEX								0.005
	Ewes				1			
	Wethers	1.72	0.59	2.94	5.58	1.80	18.87	
	Mixed	-1.69	-5.30	1.30	0.18	0.01	3.68	
AGEGP								0.40
	3 years				1			
	4 years	-0.59	-1.61	0.40	0.55	0.20	1.49	
	5 years	-0.84	-2.58	0.81	0.43	0.08	2.24	
PSTYPE								0.28
	Basalt				1			
	Granite	0.46	-1.83	2.77	1.58	0.16	15.98	
	Shale and sandstone	1.17	-0.92	3.31	3.22	0.40	27.41	
	Mixed without limestone	0.37	-1.86	2.63	1.45	0.16	13.89	
	Mixed with limestone	-0.43	-2.70	1.83	0.65	0.07	6.23	
CEC_ADPDK								0.05
	≤ 6 Meq/100g				1			
	> 6 Meq/100g	1.09	0.01	2.22	2.98	1.01	9.19	
SAND_ADPDK								0.10
	≤ 62%				1			
	> 62%	-0.96	-2.12	0.17	0.39	0.12	1.19	

Appendix 13.3

Final logistic model for IPREV25 (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-5%) and high (>5%)) for soil variables in the SECOND dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Final model for 3-paddock mean variables (based on 96 cohorts)							
Intercept	-1.51	-3.52	0.57				
Intercept	0.12	-1.83	2.20				
CURRMORT							<0.001
	No mortalities <2%			1			
	mortalities ≥ 2%	1.20	0.13	2.32	3.31	1.14	10.14
	mortalities	2.63	1.44	3.93	13.93	4.24	50.73
SEX							0.005
	Ewes			1			
	Wethers	1.56	0.46	2.76	4.77	1.59	15.86
AGEGP							0.61
	3 years			1			
	4 years	-0.23	-1.12	0.65	0.79	0.33	1.92
PSTYPE							0.56
	Basalt			1			
	Granite	-1.02	-3.00	0.77	0.36	0.05	2.17
	Shale and sandstone	-0.38	-2.26	1.30	0.68	0.11	3.66
	Mixed without limestone	-1.50	-3.76	0.59	0.22	0.02	1.80
	Mixed with limestone	-0.71	-2.70	1.15	0.49	0.07	3.15
PBI							0.07
	< 70			1			
	≥ 70	0.92	-0.07	1.94	2.50	0.93	6.99

Final model for weaning paddock variables (based on 66 cohorts)

Intercept	-1.74	-4.05	0.55				
Intercept	0.27	-1.96	2.57				
CURRMORT							0.003
	No mortalities <2%			1			
	mortalities ≥ 2%	0.76	-0.48	2.05	2.14	0.62	7.74
	mortalities	2.32	0.91	3.87	10.20	2.48	47.85
SEX							0.01
	Ewes			1			

AGEGP	Wethers	1.99	0.58	3.59	7.33	1.79	36.36	0.16
	3 years				1			
PSTYPE	4 years	-0.76	-1.88	0.31	0.47	0.15	1.36	0.77
	Basalt				1			
SILT_WNGPDK	Granite	-1.16	-3.33	0.87	0.31	0.04	2.40	0.01
	Shale and sandstone	-0.72	-2.75	1.16	0.49	0.06	3.20	
	Mixed without limestone	-0.96	-3.44	1.42	0.38	0.03	4.13	
	Mixed with limestone	-0.30	-2.57	1.90	0.74	0.08	6.70	
	≤ 21%				1			
	> 21%	1.54	0.40	2.75	4.66	1.49	15.59	

Final model for hogget/adult paddock variables (based on 75 cohorts)

Intercept		0.79	-1.74	4.02				
Intercept		2.44	-0.12	5.73				
CURRMORT	No mortalities <2%				1			<0.001
	mortalities ≥ 2%	1.27	0.03	2.57	3.57	1.03	13.06	
SEX	mortalities	2.80	1.38	4.36	16.39	3.99	78.51	0.001
	Ewes				1			
AGEGP	Wethers	2.10	0.81	3.57	8.20	2.26	35.49	0.14
	3 years				1			
PSTYPE	4 years	-0.76	-1.81	0.26	0.47	0.16	1.30	0.14
	Basalt				1			
SAND_ADPDK	Granite	-2.28	-5.66	0.36	0.10	0.00	1.44	0.07
	Shale and sandstone	-1.54	-4.83	0.94	0.21	0.01	2.56	
	Mixed without limestone	-3.09	-6.60	-0.31	0.05	0.00	0.74	
	Mixed with limestone	-2.54	-5.91	0.10	0.08	0.00	1.10	
	≤ 62%				1			
	> 62%	-1.02	-2.19	0.10	0.36	0.11	1.10	

Appendix 13.4

Final logistic model for IPREV25 (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-5%) and high (>5%)) for soil variables in the THIRD dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Final model for 3-paddock mean variables (based on 115 cohorts)							
Intercept	-1.64	-3.49	0.23				
Intercept	-0.10	-1.90	1.76				
CURRMORT							<0.001
	No mortalities <2%			1			
	mortalities ≥ 2%	1.54	0.52	2.62	4.67	1.68	13.78
	mortalities	2.86	1.72	4.09	17.40	5.59	60.03
SEX							<0.001
	Ewes			1			
	Wethers	1.63	0.60	2.76	5.10	1.82	15.74
	Mixed	-1.94	-4.02	-0.24	0.14	0.02	0.79
AGEGP							0.12
	3 years			1			
	4 years	-0.39	-1.25	0.46	0.68	0.29	1.58
	5 years	-1.38	-2.77	-0.04	0.25	0.06	0.96
PSTYPE							0.46
	Basalt			1			
	Granite	-0.78	-2.55	0.86	0.46	0.08	2.35
	Shale and sandstone	-0.35	-2.03	1.20	0.71	0.13	3.32
	Mixed without limestone	-1.48	-3.46	0.36	0.23	0.03	1.43
	Mixed with limestone	-0.81	-2.61	0.91	0.45	0.07	2.48
PBI							0.04
	< 70			1			
	≥ 70	0.97	0.02	1.95	2.64	1.02	7.05

Final model for lambing paddock variables (based on 93 cohorts)

Intercept	-1.87	-3.97	0.22				
Intercept	-0.21	-2.25	1.86				
CURRMORT							<0.001
	No mortalities <2%			1			
	mortalities ≥ 2%	0.95	-0.15	2.09	2.59	0.86	8.12
	mortalities	2.64	1.40	3.99	14.05	4.04	54.27
SEX							0.03
	Ewes			1			

	Wethers	1.30	0.18	2.49	3.66	1.20	12.07	
	Mixed	-1.62	-4.95	1.10	0.20	0.01	2.99	
AGEGP								0.31
	3 years				1			
	4 years	-0.56	-1.50	0.37	0.57	0.22	1.45	
	5 years	-1.00	-2.53	0.49	0.37	0.08	1.63	
PSTYPE								0.49
	Basalt				1			
	Granite	-0.94	-2.69	0.68	0.39	0.07	1.97	
	Shale and sandstone	-0.01	-1.76	1.65	0.99	0.17	5.18	
	Mixed without limestone	-0.67	-2.76	1.33	0.51	0.06	3.78	
	Mixed with limestone	-0.69	-2.60	1.14	0.50	0.07	3.14	
P_LBGPDK								0.08
	<20 mg/kg				1			
	20-30 mg/kg	1.36	0.16	2.61	3.88	1.18	13.59	
	>30 mg/kg	0.95	-0.20	2.15	2.59	0.82	8.54	

Final model for weaning paddock variables (based on 79 cohorts)

	Intercept	-2.04	-4.08	-0.05				
	Intercept	-0.22	-2.17	1.75				
CURRMORT								<0.001
	No mortalities <2% mortalities				1			
	≥ 2% mortalities	1.31	0.15	2.53	3.71	1.16	12.61	
SEX								0.02
	Ewes				1			
	Wethers	1.41	0.14	2.78	4.09	1.16	16.16	
	Mixed	-2.45	-5.78	0.24	0.09	0.00	1.27	
AGEGP								0.20
	3 years				1			
	4 years	-0.91	-1.98	0.11	0.40	0.14	1.12	
	5 years	-0.77	-2.43	0.91	0.47	0.09	2.49	
PSTYPE								0.84
	Basalt				1			
	Granite	-0.78	-2.62	0.98	0.46	0.07	2.68	
	Shale and sandstone	-0.56	-2.36	1.15	0.57	0.09	3.16	
	Mixed without limestone	-0.73	-2.76	1.23	0.48	0.06	3.43	
	Mixed with limestone	-0.04	-2.11	2.02	0.97	0.12	7.50	
SILT_WNGPDK								0.004
	≤ 21%				1			
	> 21%	1.53	0.47	2.65	4.60	1.60	14.19	

Final model for hogget/adult paddock variables (based on 87cohorts)

Intercept		-1.26	-3.68	1.31				
Intercept		0.46	-1.92	3.03				
CURRMORT								<0.001
	No mortalities <2%				1			
	mortalities ≥ 2%	1.67	0.48	2.94	5.32	1.61	18.99	
	mortalities	3.25	1.90	4.76	25.91	6.70	116.71	
SEX								0.003
	Ewes				1			
	Wethers	1.91	0.73	3.22	6.78	2.07	25.04	
	Mixed	-1.45	-4.97	2.05	0.23	0.01	7.78	
AGEGP								0.08
	3 years				1			
	4 years	-0.82	-1.85	0.17	0.44	0.16	1.18	
	5 years	-1.63	-3.35	0.00	0.20	0.04	1.00	
PSTYPE								0.11
	Basalt				1			
	Granite	-1.56	-4.06	0.64	0.21	0.02	1.90	
	Shale and sandstone	-0.87	-3.26	1.21	0.42	0.04	3.37	
	Mixed without limestone	-2.28	-4.84	-0.02	0.10	0.01	0.98	
	Mixed with limestone	-2.19	-4.72	0.06	0.11	0.01	1.06	
SILT_ADPODK								0.02
	≤ 21%				1			
	> 21%	1.22	0.18	2.32	3.40	1.20	10.17	

Appendix 14 Univariable results: Soil variables for pool OJD status

Results for the 44 soil variables investigated are presented in the table Appendix 14.2. Of these, 1 property-level variable, 7 3-paddock mean variables, 6 lamb paddock variables, 9 weaner paddock variables and 9 hogget/adult paddock variables were unconditionally associated with pool OJD status. After deletion of highly correlated variables (as described in Appendix 1 Section 3.10) a total of 32 variables remained for inclusion in multivariable analyses (Appendix 14.1). Of these the 11 variables with the strongest association ($P < 0.001$) were:

- Parent soil type (PSTYPE)
- Mean cation exchange capacity of soil in 3 paddocks (CEC)
- Mean phosphorus buffer index of soil in 3 paddocks (PBI)
- Mean sulphate sulphur content of soil in 3 paddocks (S)
- Phosphorus content of the soil in lambing paddock (P_LBGPDK)
- Phosphorus buffer index of soil in weaning paddock (PBI_WNGPDK)
- Aluminium saturation % of soil in weaning paddock (ALSAT_WNGPDK)
- Sand % of soil in weaning paddock (SAND_WNDPDK)
- Silt % of soil in weaning paddock (SILT_WNGPDK)
- Phosphorus buffer index of soil in hogget/adult paddock (PBI_ADSDK)
- Aluminium saturation % of soil in hogget/adult paddock (ALSAT_ADSDK).

Appendix 14.1

The P-values for soil variables unconditionally associated with pool OJD status and included in multivariable analyses

3-paddock mean variables	P-value	Lamb paddock variables	P-value
PH	0.05	CEC_LBGPDK	0.16
CEC	<0.001	P_LBGPDK	<0.001
P	0.01	PBI_LBGPDK	0.03
PBI	<0.001	S_LBGPDK	0.03
S	<0.001	K_LBGPDK	0.23
SAND	0.1	SAND_LBGPDK	0.008
CLAY	0.03	SAND_WNGPDK	<0.001
PSTYPE ^a	<0.001		

Weaner paddock variables	P-value	Hogget/adult paddock variables	P-value
PH_WNGPDK	0.22	PH_ADSDK	0.15
CEC_WNGPDK	0.003	CEC_ADSDK	0.006
P_WNGPDK	0.05	P_ADSDK	0.005
PBI_WNGPDK	<0.001	PBI_ADSDK	<0.001
S_WNGPDK	0.007	S_ADSDK	0.01
ALSAT_WNGPDK	<0.001	ALSAT_ADSDK	0.001
SILT_WNGPDK	<0.001	SAND_ADSDK	0.006
CLAY_WNGPDK	0.003	SILT_ADSDK	0.03
		CLAY_ADSDK	0.14

a Property-level variable

Appendix 14.2

Unconditional associations between soil variables and pool OJD status for 673 pools

Variables and categories	Code	Odds ratio	LCL	UCL	P
Property-level variables					
PSTYPE					<0.001
Basaltic	1	1			
Granite	2	0.63	0.32	1.18	
Shale and sandstone	3	1.56	0.79	3.01	
Mixed without limestone	4	0.63	0.29	1.36	
Mixed with limestone	5	0.72	0.35	1.42	
3-paddock mean variables					
Ph					0.05
<4.6	0	1			
4.6 - 5.2	1	1.56	1.09	2.23	
>5.2	2	1.31	0.84	2.08	
CEC					<0.001
≤ 6	1	1			
> 6	2	1.79	1.29	2.50	
P					0.01
<20	0	1			
20-30	1	1.37	0.88	2.12	
>30	2	1.81	1.21	2.70	
PBI					<0.001
< 70	0	1			
≥ 70	1	2.49	1.75	3.58	
S					<0.001
<4	0	1			
4-8	1	0.89	0.58	1.36	
>8	2	2.05	1.30	3.25	
K					0.81
<0.4	1	1			
>0.4	2	0.96	0.67	1.36	
ALSAT					0.27
≤ 2	0	1			
>2 to ≤ 5	1	0.85	0.54	1.33	
>5 to ≤ 12	2	0.66	0.43	1.02	

	>12	3	0.93	0.58	1.51	

SAND						0.10
	≤ 62%	1	1			
	> 62%	2	0.76	0.55	1.06	

SILT						0.68
	≤ 21%	1	1			
	> 21%	2	1.07	0.77	1.48	

CLAY						0.03
	≤ 15%	1	1			
	> 15%	2	1.45	1.05	2.01	

Lamb paddock variables						

PH_LBGPDK						0.48
	<4.6	0	1			
	4.6 - 5.2	1	0.80	0.53	1.21	
	>5.2	2	1.03	0.63	1.71	

CEC_LBGPDK						0.16
	≤ 6	1	1			
	> 6	2	1.30	0.90	1.88	

P_LBGPDK						<0.001
	<20	0	1			
	20-30	1	2.11	1.31	3.43	
	>30	2	2.40	1.54	3.76	

PBI_LBGPDK						0.03
	< 70	0	1			
	≥ 70	1	1.62	1.06	2.53	

S_LBGPDK						0.03
	<4	0	1			
	4-8	1	1.38	0.86	2.20	
	>8	2	2.03	1.20	3.45	

K_LBGPDK						0.22
	<0.4	1	1			
	>0.4	2	0.78	0.51	1.17	

ALSAT_LBGPDK						0.32
	≤ 2	0	1			
	>2 to ≤ 5	1	1.63	0.97	2.79	
	>5 to ≤ 12	2	1.07	0.65	1.78	
	>12	3	1.13	0.67	1.93	

SAND_LBGPDK						0.01
	≤ 62%	1	1			

	> 62%	2	0.60	0.41	0.87	

SILT_LBGPDK						0.73
	≤ 21%	1	1			
	> 21%	2	1.07	0.73	1.56	

CLAY_LBGPDK						0.64
	≤ 15%	1	1			
	> 15%	2	0.91	0.63	1.33	

Weaner paddock variables						

PH_WNGPDK						0.22
	<4.6	0	1			
	4.6 - 5.2	1	1.46	0.96	2.23	
	>5.2	2	1.24	0.70	2.24	

CEC_WNGPDK						0.003
	≤ 6	1	1			
	> 6	2	1.80	1.22	2.67	

P_WNGPDK						0.05
	<20	0	1			
	20-30	1	1.80	1.02	3.17	
	>30	2	1.89	1.13	3.18	

PBI_WNGPDK						<0.001
	< 70	0	1			
	≥ 70	1	2.36	1.56	3.62	

S_WNGPDK						0.01
	<4	0	1			
	4-8	1	0.90	0.51	1.58	
	>8	2	1.76	0.96	3.17	

K_WNGPDK						0.39
	<0.4	1	1			
	>0.4	2	1.21	0.78	1.84	

ALSAT_WNGPDK						<0.001
	≤ 2	0	1			
	>2 to ≤ 5	1	1.75	0.98	3.28	
	>5 to ≤ 12	2	0.45	0.28	0.73	
	>12	3	1.16	0.63	2.22	

SAND_WNGPDK						<0.001
	≤ 62%	1	1			
	> 62%	2	0.46	0.31	0.69	

SILT_WNGPDK						<0.001
	≤ 21%	1	1			

	> 21%	2	2.43	1.62	3.64	
<hr/>						
CLAY_WNGPDK						0.003
	≤ 15%	1	1			
	> 15%	2	1.83	1.23	2.74	
<hr/>						
Hogget/Adult paddock variables						
PH_ADPPDK						0.15
	<4.6	0	1			
	4.6 - 5.2	1	1.10	0.73	1.67	
	>5.2	2	1.68	0.99	2.94	
<hr/>						
CEC_ADPPDK						0.01
	≤ 6	1	1			
	> 6	2	1.70	1.17	2.50	
<hr/>						
P_ADPPDK						0.01
	<20	0	1			
	20-30	1	0.53	0.33	0.87	
	>30	2	1.12	0.72	1.75	
<hr/>						
PBI_ADPPDK						<0.001
	< 70	0	1			
	≥ 70	1	2.35	1.59	3.52	
<hr/>						
S_ADPPDK						0.01
	<4	0	1			
	4-8	1	1.86	1.17	2.97	
	>8	2	2.02	1.21	3.39	
<hr/>						
K_ADPPDK						0.80
	<0.4	1	1			
	>0.4	2	0.95	0.62	1.44	
<hr/>						
ALSAT_ADPPDK						0.001
	≤ 2	0	1			
	>2 to ≤ 5	1	0.38	0.23	0.63	
	>5 to ≤ 12	2	0.93	0.53	1.68	
	>12	3	0.62	0.38	1.02	
<hr/>						
SAND_ADPPDK						0.01
	≤ 62%	1	1			
	> 62%	2	0.59	0.40	0.86	
<hr/>						
SILT_ADPPDK						0.02
	≤ 21%	1	1			
	> 21%	2	1.54	1.06	2.24	
<hr/>						
CLAY_ADPPDK						0.14
	≤ 15%	1	1			
	> 15%	2	1.33	0.91	1.93	

Appendix 15 Univariable results: Soil variables for pool MAP number shed

Results for the 44 soil variables investigated are presented in the table Appendix 15.2. Of these, 1 property-level variable, 7 3-paddock mean variables, 7 lamb paddock variables, 9 weaner paddock variables and 7 hogget/adult paddock variables were unconditionally associated with log MAP number shed per pool. After deletion of highly correlated variables, a total of 31 variables remained for inclusion in multivariable analyses (Appendix 15.1). Of these the 15 variables with the strongest association ($P < 0.001$) were:

- Parent soil type (PSTYPE)
- Mean cation exchange capacity of soil in 3 paddocks (CEC)
- Mean phosphorus buffer index of soil in 3 paddocks (PBI)
- Mean sulphate sulphur content of soil in 3 paddocks (S)
- Phosphorus content of the soil in lambing paddock (P_LBGPDK)
- Sand % of lambing paddock (SAND_LBGPDK)
- Cation exchange capacity of soil in weaning paddock (CEC_WNGPDK)
- Phosphorus buffer index of soil in weaning paddock (PBI_WNGPDK)
- Sulphur content of soil in weaning paddock (S_WNGPDK)
- Clay % of soil in weaning paddock (CLAY_WNGPDK)
- Sand % of soil in weaning paddock (SAND_WNDPDK)
- Silt % of soil in weaning paddock (SILT_WNGPDK)
- Cation exchange capacity of soil in hogget/adult paddock (CEC_ADSDK)
- Phosphorus buffer index of soil in hogget/adult paddock (PBI_ADSDK)
- Sand % of soil in hogget/adult paddock (SAND_ADSDK).

Appendix 15.1

The P-values for soil variables unconditionally associated with the number of MAP shed per pool and included in multivariable analyses

3-paddock mean variables	P-value	Lamb paddock variables	P-value
CEC	0.0001	PH_LBGPDK	0.061
P	0.039	CEC_LBGPDK	0.086
PBI	0.0001	P_LBGPDK	0.0001
S	0.0004	PBI_LBGPDK	0.0061
SAND	0.0013	S_LBGPDK	0.0038
SILT	0.078	ALSAT_LBGPDK	0.046
CLAY	0.0019	SAND_LBGPDK	0.001
PSTYPE ^a	0.0001		

Weaner paddock variables	P-value	Hogget/adult paddock variables	P-value
CEC_WNGPDK	0.0007	CEC_ADSDK	0.0001
P_WNGPDK	0.22	P_ADSDK	0.012
PBI_WNGPDK	0.0001	PBI_ADSDK	0.0001
S_WNGPDK	0.0001	S_ADSDK	0.023
K_WNGPDK	0.19	SAND_ADSDK	0.0001
ALSAT_WNGPDK	0.002	SILT_ADSDK	0.012
SAND_WNGPDK	0.0001	CLAY_ADSDK	0.062
SILT_WNGPDK	0.0001		
CLAY_WNGPDK	0.0001		

a Property-level variable

Appendix 15.2

Unconditional association of soil variables with the LOGMAP (Log number of organisms shed) for 673 pools.

Parameters	Levels	<i>b</i>	LCL (<i>b</i>)	UCL (<i>b</i>)	SE (<i>b</i>)	P
<i>Property-level variables</i>						
PSTYPE						<0.001
	Basaltic					
	Granite	-0.57	-1.11	-0.02	0.28	
	Shale and sandstone	0.42	-0.12	0.96	0.28	
	Mixed without limestone	-0.47	-1.13	0.19	0.34	
	Mixed with limestone	-0.04	-0.63	0.55	0.30	
<i>3-paddock mean variables</i>						
Ph						0.25
	<4.6					
	4.6 - 5.2	0.26	-0.05	0.57	0.16	
	>5.2	0.18	-0.22	0.58	0.20	
CEC						<0.001
	≤ 6					
	> 6	0.61	0.33	0.89	0.14	
P						0.04
	<20					
	20-30	0.16	-0.23	0.55	0.20	
	>30	0.43	0.08	0.79	0.18	
PBI						<0.001
	< 70					
	≥ 70	0.90	0.62	1.18	0.14	
S						<0.001
	<4					
	4--8	-0.05	-0.43	0.33	0.19	
	>8	0.55	0.16	0.93	0.20	
K						0.54
	<0.4					
	>0.4	0.10	-0.21	0.41	0.16	
ALSAT						0.37
	≤ 2					
	>2 to ≤ 5	-0.02	-0.40	0.37	0.19	

	>5 to ≤ 12	-0.31	-0.69	0.07	0.19	
	>12	-0.08	-0.49	0.32	0.21	
<hr/>						
SAND						0.001
	≤ 62%					
	> 62%	-0.46	-0.75	-0.18	0.14	
<hr/>						
SILT						0.08
	≤ 21%					
	> 21%	0.26	-0.03	0.54	0.14	
<hr/>						
CLAY						0.002
	≤ 15%					
	> 15%	0.45	0.17	0.73	0.14	
<hr/>						
Lamb paddock variables						
PH_LBGPDK						0.06
	<4.6					
	4.6 - 5.2	-0.43	-0.79	-0.07	0.18	
	>5.2	-0.11	-0.53	0.31	0.21	
<hr/>						
CEC_LBGPDK						0.09
	≤ 6					
	> 6	0.28	-0.04	0.60	0.16	
<hr/>						
P_LBGPDK						<0.001
	<20					
	20-30	0.59	0.17	1.01	0.21	
	>30	0.83	0.44	1.21	0.20	
<hr/>						
PBI_LBGPDK						0.01
	< 70					
	≥ 70	0.50	0.14	0.85	0.18	
<hr/>						
S_LBGPDK						0.004
	<4					
	4-8	0.37	-0.04	0.79	0.21	
	>8	0.76	0.31	1.22	0.23	
<hr/>						
K_LBGPDK						0.88
	<0.4					
	>0.4	-0.03	-0.38	0.32	0.18	
<hr/>						
ALSAT_LBGPDK						0.05
	≤ 2					
	>2 to ≤ 5	0.54	0.10	0.97	0.22	
	>5 to ≤ 12	0.02	-0.42	0.46	0.22	
	>12	0.41	-0.04	0.87	0.23	
<hr/>						
SAND_LBGPDK						0.001

	≤ 62%					
	> 62%	-0.54	-0.86	-0.22	0.16	
<hr/>						
SILT_LBGPDK						0.99
	≤ 21%					
	> 21%	0.00	-0.32	0.33	0.17	
<hr/>						
CLAY_LBGPDK						0.77
	≤ 15%					
	> 15%	0.28	-0.04	0.60	0.16	
<hr/>						
Weaner paddock variables						
PH_WNGPDK						0.66
	<4.6					
	4.6 - 5.2	-0.02	-0.38	0.35	0.19	
	>5.2	-0.22	-0.72	0.28	0.26	
<hr/>						
CEC_WNGPDK						<0.001
	≤ 6					
	> 6	0.57	0.24	0.90	0.17	
<hr/>						
P_WNGPDK						0.22
	<20					
	20-30	0.26	-0.24	0.76	0.25	
	>30	0.40	-0.06	0.87	0.24	
<hr/>						
PBI_WNGPDK						<0.001
	< 70					
	≥ 70	0.81	0.48	1.15	0.17	
<hr/>						
S_WNGPDK						<0.001
	<4					
	4-8	0.15	-0.34	0.64	0.25	
	>8	0.83	0.33	1.33	0.25	
<hr/>						
K_WNGPDK						0.19
	<0.4					
	>0.4	0.25	-0.12	0.62	0.19	
<hr/>						
ALSAT_WNGPDK						0.002
	≤ 2					
	>2 to ≤ 5	0.54	0.08	1.00	0.23	
	>5 to ≤ 12	-0.51	-0.94	-0.08	0.22	
	>12	0.04	-0.48	0.55	0.26	
<hr/>						
SAND_WNGPDK						<0.001
	≤ 62%					
	> 62%	-0.79	-1.13	-0.45	0.17	
<hr/>						
SILT_WNGPDK						<0.001

	≤ 21%					
	> 21%	0.82	0.48	1.16	0.17	
<hr/>						
CLAY_WNGPDK						<0.001
	≤ 15%					
	> 15%	0.57	0.24	0.90	0.17	
<hr/>						
Hogget/Adult paddock variables						
PH_ADPDK						0.26
	<4.6					
	4.6 - 5.2	0.24	-0.12	0.60	0.18	
	>5.2	0.31	-0.13	0.75	0.22	
<hr/>						
CEC_ADPDK						<0.001
	≤ 6					
	> 6	0.71	0.39	1.03	0.16	
<hr/>						
P_ADPDK						0.01
	<20					
	20-30	-0.45	-0.89	-0.02	0.22	
	>30	0.17	-0.20	0.54	0.19	
<hr/>						
PBI_ADPDK						<0.001
	< 70					
	≥ 70	0.94	0.63	1.26	0.16	
<hr/>						
S_ADPDK						0.03
	<4					
	4--8	0.51	0.10	0.93	0.21	
	>8	0.56	0.11	1.01	0.23	
<hr/>						
K_ADPDK						0.25
	<0.4					
	>0.4	0.21	-0.15	0.57	0.18	
<hr/>						
ALSAT_ADPDK						0.31
	≤ 2					
	>2 to ≤ 5	-0.34	-0.79	0.10	0.23	
	>5 to ≤ 12	-0.19	-0.65	0.28	0.24	
	>12	-0.34	-0.76	0.07	0.21	
<hr/>						
SAND_ADPDK						<0.001
	≤ 62%					
	> 62%	-0.63	-0.96	-0.31	0.16	
<hr/>						
SILT_ADPDK						0.01
	≤ 21%					
	> 21%	0.42	0.09	0.74	0.17	
<hr/>						
CLAY_ADPDK						0.06
	≤ 15%					
	> 15%	0.31	-0.02	0.63	0.17	

Appendix 16 Final models with interaction terms

Appendix 16.1

Final logistic model (including interaction terms) for IPREV (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-10%) and high (>10%)) based on 97 sheep cohorts in the SECOND dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Intercept-2	-2.42	-4.67	-0.32				
Intercept-1	2.67	0.45	5.12				
Main effects							
CURRMORT							<0.001
	No mortalities						
	<2% mortalities						
	1.51	-0.01	3.13				
	≥ 2% mortalities						
	4.44	2.59	6.60				
SEX							0.06
	Ewes						
	Wethers						
	1.12	-0.05	2.36	3.07	0.96	10.58	
AGEGP							0.96
	3 years						
	4 years						
	-0.04	-1.29	1.19	0.96	0.27	3.30	
DROUGHT							0.19
	≤ 150mm lesser OR more than long-term average						
	>150mm lesser						
	2.09	-1.00	5.47				
HGTCS							0.02
	<3						
	≥3						
	-1.46	-2.83	-0.19	0.23	0.06	0.82	
OTHERWILDLIFE							0.18
	No						
	Yes						
	-0.90	-2.26	0.40				
OTHERFERT							<0.001
	No						
	Yes						
	-3.07	-5.10	-1.29	0.05	0.01	0.28	
LBGSSN							0.002
	Spring						
	Autumn						
	-0.52	-1.79	0.73	0.60	0.17	2.07	
	Winter						
	-2.91	-4.83	-1.23	0.05	0.01	0.29	
SHARING_ROAD							0.03
	No sharing						
	≤ twice per year						
	1.55	0.19	3.02	4.70	1.21	20.41	
	>twice per year						
	1.40	-0.02	2.94	4.05	0.98	19.00	
RUNOFFWATER							0.004
	≤ 10%						
	>10 to ≤ 30%						
	-1.24	-2.83	0.26	0.29	0.06	1.29	
	>30% to ≤ 60%						
	-2.90	-4.73	-1.29	0.06	0.01	0.28	
	> 60%						
	-1.50	-3.24	0.05	0.22	0.04	1.05	
Interaction terms							
DROUGHT*OTHERWILDLIFE							0.01

	>150mm lesser rainfall * other wildlife present	-3.62	-6.51	-0.94	
CURRMORT*DROUGHT					0.03
	<2% mortalities* >150mm lesser rainfall	2.67	-1.02	6.43	
	≥ 2% mortalities* >150mm lesser rainfall	-0.87	-4.65	2.78	

Appendix 16.2

Final logistic model (including interaction terms) for IPREV (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-10%) and high (>10%)) based on 109 sheep cohorts in the THIRD dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Intercept-2	-3.44	-5.76	-1.27				
Intercept-1	0.92	-1.17	3.02				
Main effects							
CURRMORT							<0.001
	No mortalities						
	<2% mortalities	1.93	0.23	3.74			
	≥ 2% mortalities	3.90	1.91	6.13			
SEX							<0.001
	Ewes			1*			
	Wethers	2.08	0.93	3.36	8.03	2.54	28.66
	Mixed sex	-4.48	-7.13	-2.13	0.01	<0.001	0.12
AGEGP							0.15
	3 years			1*			
	4 years	-1.03	-2.20	0.06	0.36	0.11	1.06
	5 years	0.23	-2.00	2.38	1.26	0.14	10.82
DROPSVACC							0.02
	No drops vaccinated			1*			
	1 or 2 drops vaccinated	2.25	0.53	4.12	9.53	1.71	61.76
	>2 drops vaccinated	0.73	-0.79	2.31	2.07	0.45	10.04
OTHERWILDLIFE							0.008
	No			1*			
	Yes	-1.42	-2.54	-0.36	0.24	0.08	0.69
LBGSSN							0.01
	Spring						
	Autumn	-0.68	-3.40	1.81			
	Winter	-3.74	-6.64	-1.22			
OTHERFERT							0.001
	No			1*			
	Yes	-2.63	-4.38	-1.10	0.07	0.01	0.33
RUNOFFWATER							0.17
	≤ 10%			1*			
	>10 to ≤ 30%	-0.15	-1.60	1.29	0.86	0.20	3.62
	>30% to ≤ 60%	-1.46	-2.95	-0.05	0.23	0.05	0.95
	> 60%	-0.63	-2.18	0.87	0.53	0.11	2.39
Interaction terms							
CURRMORT*LBGSSN							0.005
	<2% mortalities* autumn	2.21	-0.90	5.56			
	<2% mortalities* winter	0.62	-2.67	4.11			

≥ 2% mortalities*			
autumn	-1.30	-4.54	2.00
≥ 2% mortalities*			
winter	2.44	-0.65	5.76

Appendix 16.3

Final logistic model (including interaction terms) for IPREV25 (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-5%) and high (>5%)) based on 97 sheep cohorts in the SECOND dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Intercept-2	0.68	-1.53	2.91				
Intercept-1	3.19	0.89	5.66				
Main effects							
CURRMORT							0.42
	No mortalities <2% mortalities						
	≥ 2% mortalities	-1.20	-3.49	0.98			
		-0.10	-2.30	2.11			
SEX							<0.001
	Ewes						
	Wethers	4.73	2.36	7.84			
AGEGP							0.33
	3 years						
	4 years	0.94	-0.94	2.97			
CULL							0.01
	No			1*			
	Yes	1.49	0.37	2.67	4.42	1.45	14.39
DECONT_WNGPDK							0.01
	Nil			1*			
	<8 weeks	-1.07	-2.68	0.49	0.35	0.07	1.64
	8<12 weeks	1.91	0.43	3.57	6.74	1.53	35.60
	≥12weeks	1.16	-0.38	2.78	3.19	0.68	16.07
OTHERFERT							0.003
	No			1*			
	Yes	-2.43	-4.24	-0.81	0.09	0.01	0.44
OTHERWILDLIFE							0.06
	No						
	Yes	-1.19	-2.48	0.03			
WNRCS							0.04
	<3			1*			
	≥3	-1.32	-2.61	-0.09	0.27	0.07	0.92
LIME							0.001
	No						
	Yes	-3.90	-6.67	-1.48			
Interaction terms							
SEX*OTHERWILDLIFE							0.001
	Wethers* other wildlife present	-4.88	-8.57	-1.82			
CURRMORT*LIME							0.001
	<2% mortalities* lime applied	4.46	1.51	7.75			

	≥ 2% mortalities* lime applied	5.30	2.27	8.66	
AGEGP*LIME					0.01
	4years* lime applied	-3.06	-5.68	-0.69	

Appendix 16.4

Final logistic model (including interaction terms) for IPREV25 (cohort OJD animal-level prevalence categorised as low (<2%), medium (2-5%) and high (>5%)) based on 109 sheep cohorts in the THIRD dataset

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	Adjusted odds ratios	OR LCL	OR UCL	P
Intercept-2	-1.33	-2.79	0.06				
Intercept-1	0.58	-0.83	1.97				
Main effects							
CURRMORT							<0.001
No mortalities <2%							
mortalities ≥ 2%	0.97	-0.35	2.36				
mortalities ≥ 2%	3.16	1.76	4.70				
SEX							0.30
Ewes							
Wethers	1.65	-0.42	3.85				
Mixed sex	-3.95	-7.15	-1.56				
AGEGP							0.26
3 years				1*			
4 years	-0.77	-1.79	0.21	0.46	0.17	1.23	
5 years	-0.76	-2.57	1.05	0.47	0.08	2.86	
CULL							0.01
No				1*			
Yes	1.28	0.32	2.27	3.58	1.38	9.68	
OTHERFERT							0.02
No				1*			
Yes	-1.63	-3.02	-0.29	0.20	0.05	0.75	
LBGSSN							0.01
Spring				1*			
Autumn	-0.74	-1.82	0.31	0.48	0.16	1.36	
Winter	-1.72	-2.94	-0.56	0.18	0.05	0.57	
OTHERWILDLIFE							0.01
No				1*			
Yes	-1.14	-2.10	-0.22	0.32	0.12	0.80	
Interaction terms							
CURRMORT*SEX							0.03
<2% mortalities * wethers	0.96	-1.79	3.83				
<2% mortalities * mixed sex	3.89	-0.01	7.86				
≥ 2% mortalities * wethers	-2.45	-5.48	0.62				

Appendix 16.5

The final mixed logistic regression model (including interaction terms) for pool OJD status based on 663 pools

Parameters	<i>b</i>	<i>b</i> LCL	<i>b</i> UCL	SE(<i>b</i>)	Adjusted odds ratio	OR LCL	OR UCL	P
Intercept	-3.24	-8.13	1.66	2.46				
Random effects								
PROPERTYID	0.80			0.29				
Fixed main effects								
CURRMORT								<0.001
No mortalities					1*			
<2% mortalities	0.53	-0.23	1.29	0.39	1.70	0.80	3.63	
≥ 2% mortalities	1.61	0.83	2.39	0.40	5.00	2.28	10.95	
SEX								0.02
Ewes								
Wethers	0.98	0.02	1.95	0.49				
AGEGP								<0.001
3 years								
4 years	-0.33	-0.98	0.32	0.33				
LOGPOOLSIZE	0.90	-0.51	2.31	0.72	2.46	0.60	10.03	0.21
CULL								<0.001
No								
Yes	0.64	-0.01	1.30	0.33				
RUNOFFWATER								0.02
≤ 10%					1*			
>10 to ≤ 30%	-0.27	-1.09	0.54	0.41	0.76	0.34	1.72	
>30% to ≤ 60%	-0.93	-1.74	-0.11	0.42	0.40	0.18	0.90	
> 60%	-1.21	-2.05	-0.36	0.43	0.30	0.13	0.70	
OTHERFERT								0.002
No					1*			
Yes	-1.46	-2.39	-0.52	0.48	0.23	0.09	0.59	
SHARING_ROAD								0.04
No sharing					1*			
≤ twice per year	0.73	-0.03	1.48	0.38	2.07	0.98	4.40	
>twice per year	0.86	0.06	1.67	0.41	2.37	1.06	5.31	
DECONT_WNGPDK								0.07
Nil					1*			
<8 weeks	-0.06	-0.93	0.81	0.44	0.94	0.40	2.25	
8<12 weeks	0.14	-0.62	0.90	0.39	1.15	0.54	2.46	
≥12weeks	1.16	0.27	2.05	0.45	3.18	1.31	7.75	
Interaction terms								
SEX*CULL								0.03
wethers* cull	1.54	0.19	2.90	0.69				
wethers*don't cull	0.00							
ewes* cull	0.00							
ewes*don't cull	0.00							
SEX*AGEGP								0.003
wethers* 4-years age group	-1.93	-3.21	-0.64	0.65				

wethers* 3-year age group	0.00
ewes* 4-years age group	0.00
ewes* 3-year age group	0.00

Appendix 16.6

The final mixed linear regression model (including interaction terms) for log pool MAP number shed based on 649 pools

Parameters	<i>b</i>	SE(<i>b</i>)	LCL (<i>b</i>)	UCL (<i>b</i>)	P
Intercept	-1.33	1.67	-4.67	2.01	
Random effects					
PROPERTYID	0.14	0.09	0.05	0.93	0.06
Residual	2.34	0.14	2.09	2.64	<0.001
Fixed main effects					
CURRMORT					0.04
No mortalities					
<2% mortalities	0.39	0.54	-0.67	1.44	
≥2% mortalities	0.09	0.51	-0.91	1.09	
SEX					0.002
Ewes					
Wethers	0.90	0.22	0.46	1.33	
AGEGP					0.21
3 years					
4 years	0.10	0.21	-0.30	0.51	
LOGPOOLSIZE	0.91	0.46	0.01	1.82	0.05
CULL					<0.001
No					
Yes	0.62	0.18	0.27	0.97	
SELL					0.04
No					
Yes	0.56	0.27	0.03	1.08	
RUNOFFWATER					<0.001
≤ 10%					
>10 to ≤ 30%	-0.65	0.23	-1.10	-0.20	
>30% to ≤ 60%	-0.98	0.23	-1.44	-0.52	
> 60%	-0.63	0.25	-1.13	-0.14	
SUPERFREQ					0.004
≤ once in three years					
>once to ≤ twice in three years	-0.60	0.25	-1.09	-0.12	
> twice to ≤ Every year	0.22	0.25	-0.27	0.71	
> Once every year	-0.23	0.31	-0.83	0.37	
OTHERFERT					0.003
No					
Yes	-1.77	0.42	-2.60	-0.95	
GROWTHCHK					0.01
<12 weeks					
≥12 weeks	0.48	0.18	0.12	0.84	
DECONT_WNGPDK					<0.001
Nil					
<8 weeks	0.24	0.27	-0.29	0.77	
8<12 weeks	0.38	0.22	-0.05	0.80	
≥12weeks	1.05	0.26	0.54	1.55	

DROPSVACC						0.01
	No drops vaccinated					
	1 or 2 drops vaccinated	0.84	0.28	0.30	1.38	
	>2 drops vaccinated	0.68	0.28	0.13	1.24	
WORMCONTROL						0.001
	No					
	Yes	-1.31	0.44	-2.19	-0.44	
WNGAGE						0.04
	≤ 15 weeks					
	≤ 18 weeks	-0.41	0.27	-0.94	0.12	
	≤ 21 weeks	-0.94	0.29	-1.51	-0.36	
	>21 weeks	-0.49	0.24	-0.96	-0.01	
Interaction terms						
AGEGP*SEX						0.03
	4years*wethers	-0.75	0.34	-1.41	-0.09	
	4years*ewes	0.00				
	3years*wethers	0.00				
	3years*ewes	0.00				
CURRMORT*WORMCONTROL						0.04
	≥2% mortalities*					
	effective worm control	1.28	0.55	0.20	2.36	
	≥2% mortalities *					
	ineffective worm control	0.00				
	<2% mortalities *					
	effective worm control	0.35	0.60	-0.82	1.53	
	<2% mortalities *					
	ineffective worm control	0.00				
OTHERFERT*WNGAGE						0.03
	other fertilizer applied *					
	>21 weeks weaning					
	age	1.73	0.66	0.44	3.02	
	other fertilizer applied *					
	≤ 18 weeks weaning					
	age	0.84	0.70	-0.53	2.20	
	other fertilizer not					
	applied* > 21 weeks					
	weaning age	0.00				
	other fertilizer not					
	applied* ≤ 18 weeks					
	weaning age	0.00				