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Further investigation of identified risk factors for OJD infection-level in sheep flocks

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Abstract

This project, using data from the OJD risk factor study (MLA OJD.038), investigated the combined effect of management and soil risk factors on the level of OJD expression in 92 infected flocks. It sought to clarify the respective importance of management and soil risk factors in order to direct refinement of recommendations for on-farm control of OJD. Several management factors (particularly related to lamb and weaner management and biosecurity) and one soil factor (organic carbon %) were retained in composite models showing that they strongly influence OJD infection level in infected flocks. Producers should be advised of the importance of identified management strategies in on-farm OJD control and the link between soil organic matter content and OJD be investigated further.

Executive Summary

This project further evaluated data from MLA OJD.038, a cross-sectional study of 92 properties affected by ovine Johne's disease (OJD) conducted in 2004-2005, to investigate the combined effect of management and soil risk factors on the level of OJD expression in these infected flocks.

Clarification of the respective importance of management and soil risk factors to OJD expression in infected flocks was important to direct refinement of recommendations for on-farm control of OJD. In addition better understanding about the most influential risk factors might help clarify the potential for disease spread into areas not currently affected in Australia.

This extended work provided opportunity to undertake analyses including evaluation of soil iron content as a risk factor for OJD level; evaluation of most soil risk factors as continuous variables rather than categorical variables (as done previously for OJD.038); assessment of the association of soil risk factors with cohort sex; and identification of the most influential management and soil risk factors by introducing both management and soil variables to each composite model (previously for OJD.038 management and soil risk factors were evaluated in separate models).

Thus this report presents three sets of results:
- Association of soil risk factors with OJD infection level (including iron)
- Association of soil risk factors with cohort sex
- Association of management and soil risk factors with OJD infection level

The principle finding was that both management and soil factors influence OJD infection level in infected flocks. This is the first time that the importance of both management and soil factors has been clearly demonstrated for OJD.

Most prominent among the identified management risk factors were aspects of lamb and weaner management that can be modified to lower lamb exposure to infected pasture and to improve lamb/weaner nutrition. In addition biosecurity was emphasised by the identification of a strong association with movement of sheep along roads shared with neighbours and by the reduction in flock infection achieved in flocks experiencing OJD losses through use of the Guidair vaccine over several years.

The organic matter content of soil was shown consistently to strongly influence OJD infection level in this study. Among the soil risk factors investigated, organic carbon % was the only soil factor to remain in the composite models. It was retained even in the presence of stocking rate proving that it has influence on OJD infection that is beyond a purely confounded relationship with stocking rate. Organic carbon % is an indicator of soil organic matter content but the mechanism by which organic matter aids MAP survival is as yet unknown. It may be direct by providing nutrients that assist MAP survival outside a biological host or indirect by enhancing pasture growth which provides shaded conditions conducive to MAP survival or a combination of the two.

The first analyses of soil risk factors on their own showed that soil clay content also has a strong influence on OJD but provided no evidence of association with soil type and little evidence of association with pH and iron. Although clay was not retained in the composite models, and so is not as influential as management and organic carbon, knowledge that some bacteria adsorp to clay particles raises a hypothesis for investigation that could contribute to our understanding of MAP retention in soil.

We found limited evidence that farmers preferentially place adult ewes on paddocks with better quality soil than adult wethers. However the wether cohorts had higher OJD infection levels than the ewes and this may be explained to some extent by wethers being grazed on paddocks with higher organic carbon %.

Although not specifically investigated in this study, the absence of significant rates of OJD infection in sheep in regions such as the semi arid western division of NSW, might be explained by low soil fertility, specifically organic matter content.

The main benefit to industry from this project is the clarification of the respective contribution of identified management and soil risk factors to the level of OJD-infection on infected properties. On the basis of these findings we recommend that:

- Advisory material be provided to producers related to lamb and weaner management, avoiding nutritional stress in infected flocks, biosecurity and vaccination.
- Research be undertaken to investigate 1) whether organic matter and clay enhance survival and retention of MAP in the top soil; 2) soil composition (particularly organic matter) in regions of NSW with very low OJD prevalence; 3) feeding behaviour of sheep on clay rich and sandy soils with respect to amount of soil ingested; and 4) effect of reduction in stocking rate in infected flocks on economic return.

Contents

1 Background

1.1 Background

A recent cross-sectional study (MLA OJD.038) involving 92 properties affected with OJD located in New South Wales, Victoria, Tasmania and Western Australia (MLA OJD.038) identified several risk factors for the prevalence of ovine Johne's disease (OJD). This study reported 12 management variables (6 related to property features and management and 6 related to flock management) and 12 soil variables associated with OJD prevalence based on pooled faecal culture (PFC) (Toribio et al., 2005).

However, as this study did not proceed beyond separate analyses of management and soil risk factors, there is opportunity to evaluate the relative importance of management and soil risk factors and to define associations between them by introducing management and soil risk factors to a single combined model. Other issues that could be considered in further investigation of the OJD.038 dataset include association between sex and soil risk factors and between soil iron content and OJD prevalence. In particular this further evaluation of the dataset would address concern voiced at the NSW Farmers sponsored meeting on 26 May 2006 about the fact that iron had not been included among the soil risk factors investigated in OJD.038.

Clarification of the respective importance of management and soil risk factors to OJD expression in infected flocks will direct refinement of recommendations for on-farm control of OJD. In addition further understanding about these risk factors might help clarify the potential for disease spread into areas not currently affected.

1.2 Purpose

The purpose of this project was to clarify the relationship between level of OJD expression and management and soil risk factors shown to influence OJD expression in OJD.038.

1.3 Content

The requirement to include soil iron content in the soil risk factors enabled us to reconsider our prior approach to analysis of the soil risk factors. The OJD.038 Final Report presents results for analyses conducted on 44 soil risk factors as categorical variables – 40 variables were based on continuous data that were categorised based on biological plausibility or quartile or median values. Concern about two issues led to a decision to repeat the entire analysis of the soil risk factor dataset. These issues were:

- Previous categorisation of continuous variables may have lead to a loss of information and ability to identify associations
- Previous exclusion of data on several soil characters provided in the soil laboratory result sheets (including iron, boron, calcium, copper, magnesium, manganese, nitrate nitrogen, organic carbon% and zinc) may have meant that influential soil risk factors were not identified.

Therefore this report presents three sets of results:

- Association of soil risk factors with OJD infection level – based on re-analysis of the soil risk factor data including 77 soil variables
- Association of soil risk factors with cohort sex
- Association of management and soil risk factors with OJD infection level.

In the discussion we compare the soil risk factor results reported in the OJD.038 Final Report with the results presented in Section 4.2 of this report.

2 Project Objective

The objective of this project was to quantify the respective contributions of management and soil risk factors to the level of OJD in infected flocks, by 30 June 2007.

To achieve this objective we needed to:

- Create an additional soil risk factor variable based on iron results from the soil sample analyses.
- Perform univariable and multivariable analyses to identify soil risk factors with a statistically significant relationship with PFC prevalence, and to quantify the magnitude of identified relationships.
- Measure the association between sex and each of the soil risk factors.
- Introduce all risk factors (management risk factors and soil risk factors) significant in univariable analyses at $P<0.2$ to one multivariable model. Using a forward stepwise approach to model construction, identify the most influential risk factors and quantify the association between these selected risk factors and OJD infection.

3 Methodology

3.1 Study design, sampling methodology and sample/data collection in OJD.038

Study design and sampling information was described in detail previously in the OJD.038 Final Report (Dhand et al., 2007a, b; Toribio et al., 2005) and therefore will be discussed here only in brief. A target sample size of 100 flocks (and a minimum sample size of 80) was calculated to be essential to identify potential risk factors associated with OJD for this cross-sectional study. At each enrolled farm we identified a cohort of a sheep to collect pooled faecal samples (usually 7 faecal pools of 30 sheep to make cohort size of 210 from each flock; 1 pellet collected *per rectum* per sheep). All pools were preferentially selected from one sex and one age group. However, when 210 sheep of one sex or age group were not available, the remainder of the pools were collected from another sex and/or age group. Each pooled faecal sample was cultured using a modified BACTEC radiometric culture method (Whittington et al., 2000). The growth of *M. paratuberculosis* was confirmed using a PCR test to identify the presence of IS*900* in positive cultures (Whittington et al., 1998) and a restriction endonuclease analysis (REA) to confirm IS*900* (Cousins et al., 1999).

Subsequently, a questionnaire was administered on-farm during a face-to-face interview with the sheep farmers. At the time of interview, 3 soil samples were collected from the paddocks grazed by the cohort sheep during specified life stages (lambing, weaning, yearling/adult) and submitted to a commercial laboratory for standard soil analysis. An additional particle size analysis (PSA) to determine the proportions of sand, silt, and clay in the soil and the texture of soil (Leeper and Uren, 1993) was performed by the University of Sydney Soil Physics Laboratory.

3.2 Data management

All study data were managed using a Microsoft Access relational database (© Microsoft Corporation). Tables from this database were imported into SAS statistical software (release 9.1, © 2002-03, SAS Institute Inc., Cary, NC, USA) which was used for all further analyses unless indicated otherwise. The data set for all analyses presented in this report excluded 5-year-old sheep cohorts (removing 4 flocks) and in addition for cohort OJD prevalence excluded sheep cohorts comprised of ≤ 3 pools (removing 1 further flock).

To investigate the association between cohort sex and soil risk factors (method outlined in Section 3.6), a further 22 sheep cohorts were excluded from the dataset (removing a further 11 flocks). Faecal samples were collected from one cohort of wethers and one of ewes in each of these 11 flocks and as a result sex could not be matched to paddock soil data in these flocks.

3.3 Outcome variables

3.3.1 Cohort OJD prevalence level (CPREV)

PFC pool results for each sheep cohort were used to calculate individual animal level OJD prevalence employing the variable pool size method of Williams and Moffitt (2001). The resulting cohort OJD animal-level prevalence was categorised to designate each sheep cohort as either a low (<2% prevalence), medium (2-10% prevalence) or high (>10% prevalence) prevalence cohort, thus creating CPREV outcome variable. This outcome variable was used in univariable and multivariable analyses to identify factors statistically associated with cohort OJD prevalence and to quantify the magnitude of these associations.

3.3.2 Pool OJD status (PSTATUS)

The PFC result for each faecal pool cultured in this study was also used to create a binary outcome variable, pool OJD status (labelled PSTATUS) representing the OJD status of each pool (positive or negative). We employed it in binomial logistic regression analyses at univariable level and generalised linear mixed model analyses at multivariable level to identify factors statistically associated with positive pool status.

3.3.3 Log pool MAP number (MAPNUM)

The log of the viable number of MAP per gram of faeces for each faecal pool was calculated based on the number of days taken by the sample to reach a cumulative growth index of 1000 (dcgi1000) in the BACTEC media, employing the method of Reddacliff et al. (2003). We utilized this continuous outcome variable in general linear model analyses at univariable level and general linear mixed model analyses at multivariable level to identify factors statistically associated with MAPNUM.

3.3.4 Cohort sex

To investigate the association between sex and soil risk factors, the outcome variable was cohort sex (coded as 1 for wethers and 0 for ewes).

3.4 Explanatory variables

Explanatory variables related to management and soil characteristics investigated in this study are listed in Table 1.

The management risk factors are the same as reported previously except that:

- a composite variable ‘cull low-body-weight sheep or sell sub-flocks experiencing high losses’ was created from two variables ‘cull low-body-weight sheep’ and ‘sell sub-flocks experiencing high losses’;
- a composite variable ‘separate young sheep or handle young sheep first’ was created from two variables ‘separate young sheep’ and ‘handle young sheep first’;
- a variable ‘interviewers' assessment of adoption of worm control recommendations’ was dropped while ‘interviewers' assessment of effectiveness of worm control program’ was retained.

In addition, categories of some variables were merged to increase statistical power.

The soil explanatory variables include 40 variables not reported in the OJD.038 Final Report (10 for each paddock type subset) – aluminium (Meq/100g), boron (mg/Kg), calcium (Meq/100g), copper (mg/kg), iron (mg/Kg), magnesium (Meq/100g), manganese (mg/kg), nitrate nitrogen (mg/kg), organic carbon%, zinc (mg/kg). In this dataset aluminium was investigated as is rather than as aluminium saturation %.

For this work, we examined a total of 77 soil variables including 1 categorical property-level variable (PSTYPE) and 76 paddock-level variables (19 variables each for samples from lamb paddock, weaner paddock and hogget/adult paddock and a further 19 variables that represented the average result for the 3 samples from different paddocks per property). All the paddock-level variables were analysed as continuous variables (in contrast to the analyses reported in the OJD.038 Final Report where these variables were categorical).

The soil variable dataset consisted of 4 subsets of soil variables. Three subsets were based on the results of soil samples taken from the paddocks grazed by cohort sheep either as lambs, weaners or yearling/adults and labelled “lambing paddock”, “weaner paddock” and “adult paddock” variables, respectively. The final subset labelled “3-paddock mean” represent average results for the 3 soil samples analysed per farm. It was considered necessary to evaluate each subset for three reasons:

- The considerable variability among the results for the 3 soil samples collected per farm
- The likelihood that soil characteristics to which the sheep were exposed at various stages during their lifetime could determine their disease status as adults
- The expectation that farmer preference to mark out particular paddocks for lambing or weaning could confound the results.

Descriptive analyses were conducted for all explanatory variables that included frequency distributions for categorical variables and box and whisker plots for continuous variables.

Table 1
Management and soil explanatory variables used to identify epidemiological associations with three outcome variables in the ovine Johne’s disease (OJD) risk factor study conducted in 2004-05 in Australia.

Category	**Management Variables**
Flock-level variables	
Farm and flock management	% of farm area grazed by sheep; Application of fertilizers other than super phosphate and lime; Application of lime; Flock size; Flock stocking rate; Frequency of application of super phosphate fertilizers; Interviewers' assessment of effectiveness of worm control program; Interviewers' assessment of evidence of mineral deficiency in animals.
Drought or water logging	% area prone to water logging; % area with pin rushes (weeds growing on water logged area); Average difference in total annual rainfall from district long-term average in the year of birth of the cohort; Average difference in total rainfall one year prior to sampling from district long term average; Average difference of annual total rainfall from district long term average over the lifetime of the cohort
OJD control	Cull low-body-weight sheep or sell sub-flocks experiencing high losses; Destock lambing and weaning paddocks; Management of OJD clinical sheep; Separate young sheep or handle young sheep first; Years since commencement of OJD vaccination
Lateral spread and purchase risk	Boundary sheep straying amongst neighbours; Intermittent creek flowing onto the farm; Number of likely infected neighbours; Number of rams purchased in past 5 years; Permanent creek flowing onto the farm; Presence of rabbits; Presence of wild animals other than kangaroos and rabbits; Proportion of farm boundary receiving run-off water; Proportion paddocks inhabited by kangaroos; Purchase of ewes/wethers in past 5 years; Sharing of

	roads among neighbours; Sharing of sheds with neighbours.
Cohort level variables	
Overall cohort variables	Cohort age; Cohort sex; Condition score at the start of supplementary feeding; Inclusion of lime in supplementary feed; Likelihood of cohort water source and supply to be contaminated; Method of supplementary feeding; Period of any supplementary feed; Period of fodder- or stubble-grazing; Period of growth-check in the life of the cohort; Provision of mineral supplement.
Lambing variables[a]	Condition score of ewes at start of lambing; Decontamination of the lambing paddock; Lambing paddock stocking rate; Presence of scouring in lactating ewes; Season of lambing.
Weaner variables[b]	Age at weaning; Any health problems experienced by weaners; Condition-score of lambs at weaning; Decontamination of the weaning paddock; Grazing management for weaners; Weaning-paddock stocking rate; Weaning percentage
Yearling-variables[c]	Any health problems experienced by yearlings; Condition-score of sheep at 1 year of age; Grazing management for yearlings; Yearling-paddock stocking rate.
Adult-variables[d]	Adult-paddock stocking rate; Any health problems experienced by adults; Condition- score of adults at 2 year of age; Condition-score of cohort at the time of faecal sample collection; Grazing management for adults; Joining duration of cohort ewes
Soil variables	
Parent soil type. Aluminium (Meq/100g); Boron (mg/Kg); Calcium (Meq/100g); Cation exchange capacity (Meq/100g); Clay %; Copper (mg/kg); Iron (mg/Kg); Magnesium (Meq/100g); Manganese (mg/kg); Nitrate Nitrogen (mg/kg); Organic carbon%; pH (CaCl2);Phosphorus (mg/Kg); Phosphorus buffer index; Potassium (Meq/100g); Sand %; Silt %; Sulphate Sulphur (mg/Kg); Zinc (mg/kg).	

a. Lamb: from birth up to weaning; b. Weaner: from weaning up to 1 year of age; c. Yearling: from 1 to 2 years of age; d. Adult: from 2 years of age until date of faecal sample collection.

3.5 Data analysis to investigate association of soil risk factors with OJD infection level

3.5.1 Univariable analyses

Separate univariable analyses were conducted for all four subsets of the soil explanatory variables to investigate their unconditional association with each outcome using ordinal logistic regression for CPREV, binomial logistic regression for PSTATUS and linear regression for MAPNUM outcomes (Armitage et al., 2002; Hosmer and Lemeshow, 2000; Stokes et al., 2000). Explanatory variables associated with the outcome variables at $P < 0.25$ were subsequently selected for inclusion in the relevant multivariable model.

Linearity of continuous variables was assessed visually by fitting a spline of the variable with the categorical outcome variables CPREV and PSTATUS, facilitated by a macro PSPLINET (available from Vanderbilt University School of Medicine website: http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/SasMacros).

3.5.2 Mulitvariable analyses

3.5.2.1 Ordinal logistic regression analyses for CPREV

Ordinal logistic regression models were constructed for the CPREV outcome variable using the SAS LOGISTIC procedure (Stokes et al., 2000) by a manual forward stepwise approach. Decision on inclusion or exclusion of a variable at each step was based on the individual contribution of each variable using a likelihood-ratio chi-square test (retaining variables with $P < 0.10$). Two confounders (cohort age and cohort sex) were forced into each model as fixed effects. First order interaction terms were then added to all the final models and retained when significant at $P < 0.05$.

Assumption of linearity of continuous variables was again assessed after building the multivariable model. If there were slight departures from linearity, the model was fitted again after creating spline variables as well as after adding a quadratic term (or other appropriate term) to the model. Significance of spline variables or the quadratic term was assessed based on the changes in the log- likelihood and the simpler model based on the linear term was retained if the complex model was not better. However, if there were considerable departures from linearity, then a spline of the non-linear variable was fitted after adjusting for the effect of other variables in the final model (and presented as such).

3.5.2.2 Generalised linear mixed models for PSTATUS

Generalised linear mixed models for PSTATUS were conducted for each variable subset employing SAS GLIMMIX procedure (Anonymous, 2005; Schabenberger, 2005). A random effects flock variable was added and a 'log of the pool size' variable forced (in addition to cohort age and sex) in all the models. Variable selection was based on type III tests of fixed effects (cut-off P-value < 0.10). First order interaction terms were added to all the final models and retained when significant at $P < 0.05$.

Linearity of the continuous variables was tested visually by plotting splines of the variables against log odds of the outcome (without random effects), similar to the CPREV model.

3.5.2.3 Linear mixed models for MAPNUM

General linear mixed models for MAPNUM were constructed using SAS MIXED procedure (Brown and Prescott, 2000) following a procedure similar to that reported for PSTATUS.

3.6 Data analysis to investigate association of soil risk factors with cohort sex

We investigated the association between cohort sex and 2 subsets of the soil explanatory variables – 3-paddock mean variables and adult paddock variables. Analysis of the lamb paddock and weaner paddock subsets was not undertaken because ewes and wethers are less likely to be separated at these ages. About half of the cohorts for which data on the age of separation of male and female sheep were available, had not separated ewes and wethers by one year of age indicating that analysis of data prior to that period would not be appropriate.

3.6.1 Univariable analyses

Separate univariable analyses were conducted for the two subsets of explanatory variables to investigate the unconditional association of each variable with cohort sex using binomial logistic regression.

3.7 Data analysis to investigate association of management and soil risk factors with OJD infection level

To assess the combined effect of management and soil risk factors, we investigated the association between each of three outcome variables (CPREV, PSTATUS and MAPNUM) and 68 management variables and 20 soil variables (1 property-level variable and 19 3-paddock mean variables).

3.7.1 Univariable analyses

Separate univariable analyses using the logistic regression SAS LOGISTIC procedure (Stokes et al., 2000) were conducted for CPREV and PSTATUS outcomes. Similarly, we performed general linear regression analyses employing SAS GLM procedure (Armitage et al., 2002) to assess unconditional association of all explanatory variables with MAPNUM.

Explanatory variables identified in the univariable analyses for each outcome as unconditionally associated with the outcome variable at $P < 0.20$ were then examined for collinearity and the most appropriate variable (based on our opinion of biological plausibility) was subsequently deleted. We also assessed these variables for missing values and excluded those with > 10% missing values from multivariable analyses. All the remaining explanatory variables were selected for inclusion in the relevant multivariable model.

3.7.2 Multivariable analyses

We developed ordinal logistic regression models for CPREV using SAS LOGISTIC procedure (Stokes et al., 2000), generalised linear mixed models for PSTATUS using SAS GLIMMIX procedure (Anonymous, 2005; Schabenberger, 2005) and general linear mixed models for MAPNUM using SAS MIXED procedure (Brown and Prescott, 2000). Three variables (cohort age, cohort sex and current OJD mortality) were forced into each model as fixed effects. In addition, log of pool size and flock level random effect variables were added to models for pool level outcomes, PSTATUS and MAPNUM.

A manual stepwise procedure was used to build all multivariable models based on cut-off P-value of <0.1. Facilitated by in-house developed SAS macros, all the variables were tested at each step and the most significant variable was entered into the model or the least significant ($P \geq 0.1$) removed from the model until all the variables in the model were significant. First order interaction terms were then added to all the final models and retained when significant at $P < 0.05$ and biologically plausible. All removed variables were retested by entering to the multivariable model one by one.

Assumption of linearity of continuous variables in the final model was tested by fitting a spline of the variable against outcome variable CPREV and PSTATUS using PSPLINET macro (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/SasMacros). For MAPNUM outcome, linearity was checked by plotting residuals against the continuous variables. Other model assumptions and influential diagnostics were also checked by examining residuals.

4 Results

4.1 Study flocks

A total of 233 known OJD-infected flocks were investigated to identify eligible flocks, of which 32 (13.7%) refused to participate and of the remainder, 92 flocks that met the study selection criteria were enrolled. One or more OJD control procedures were implemented by the owners or managers of 88 study flocks including vaccination of sheep with killed MAP vaccine, Gudair™ (79), sale of high loss sub-flocks (12 flocks), culling of low body weight sheep (50), destocking of lambing or weaning paddocks (58), handling of young sheep before older sheep (13) and separating young and adult sheep (45).

Details about OJD prevalence, MAP numbers per gram of faeces and frequency of OJD positive pools in these flocks were presented in the OJD.038 Final Report (Toribio et al., 2005), and therefore are not repeated here.

4.2 Association of soil risk factors with OJD infection level

4.2.1 Soil samples

Descriptive information for the 276 soil samples (3 per farm) collected during the risk factor study is presented in Table 2. 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.

4.2.2 Ordinal logistic regression analyses for cohort OJD prevalence (CPREV model)

Of the 20 soil variables investigated for unconditional association in each of the four subsets, 14, 15, 13 and 13 were significant in the univariable models ($P<0.25$) of 3-paddock mean, lambing, weaning and yearling/adult variable subsets, respectively. Final model results for 3-paddock mean and weaning paddock variable subsets are presented in Table 3. The association of the outcome with organic carbon % and with sand %, the only continuous variables in these models, was linear (Figure 1 a, b).

Models for the lambing paddock variable subset had a continuous variable (sulphate sulphur) that had a non-linear association with CPREV. A quadratic term of the variable was significant but its addition did not help in making the association linear. Sulphur categorised according to quartiles was also significant but we preferred to present the results graphically to let the data speak for themselves, rather than masking the association by categorisation. The association appears relatively linear except at low sulphur content in Fig. 1c. However, deletion of one outlier (with the sulphur content of 49 mg/kg) highlighted the non-linear nature of the association (Fig 1 d). The other variable in this model, nitrate nitrogen, had an approximately linear association (Fig. 1 e).

Similar to the lambing paddock, the adult paddock variable subset also included one variable, pH (CaCl2), which had significant departures from linearity. As soil pH variable was not significant when categorised (according to quartiles), and the model fit did not improve with inclusion of a quadratic term, we present the results of soil pH graphically after adjusting for other variables (Fig. 1 f).

Table 2
Descriptive information for continuous soil variables measured in 276 soil samples collected from 92 OJD infected farms (3 samples per farm) in 2004-05 in Australia.

Variables[a]	Num	Min	25P	Mean	Median	75P	Max
Aluminium (Meq/100g)	231	0.03	0.1	0.3	0.2	0.4	2.0
Boron (mg/Kg)	270	0.2	0.3	0.6	0.4	0.6	5.4
Calcium (Meq/100g)	276	0.6	2.4	4.7	3.8	6.0	29.0
Cation exchange capacity (Meq/100g)	276	2.0	4.2	7.2	5.8	8.4	35.1
Chloride (mg/kg)[b]	197	10.0	14.0	36.8	20.0	33.0	1100.0
Clay %	268	4.0	11.8	16.2	14.8	19.5	48.7
Copper (mg/kg)	276	0.01	0.3	1.0	0.5	1.0	11.0
Iron (mg/Kg)	276	35.0	130.0	195.2	190.0	250.0	470.0
Magnesium (Meq/100g)	274	0.3	0.6	1.4	0.9	1.6	15.0
Manganese (mg/kg)	276	0.3	19.0	36.2	30.0	47.5	150.0
Nitrate Nitrogen (mg/kg)	268	1.0	5.4	13.9	9.9	19.0	76.0
Organic carbon%	270	0.9	1.7	2.5	2.3	3.0	7.7
pH (CaCl2)	276	3.7	4.4	4.8	4.6	5.1	7.5
Phosphorus (mg/Kg)	276	6.8	18.0	31.3	26.0	38.5	200.0
Phosphorus buffer index	270	4.9	46.0	69.7	57.0	83.0	650.0
Potassium (Meq/100g)	276	0.1	0.3	0.6	0.5	0.7	2.3
Sand %	268	30.4	52.7	61.4	62.7	69.8	91.9
Silt %	268	2.7	17.1	22.4	21.5	27.4	41.4
Sodium (Meq/100g) [b]	51	0.04	0.2	0.5	0.4	0.7	2.3
Sulphate Sulphur (mg/Kg)	276	1.4	4.3	7.9	6.5	9.3	96.0
Zinc (mg/kg)	276	0.2	0.9	1.7	1.3	1.9	33.0

a. Chemical compounds were assumed to be missing if present at a level lower than the minimum detection limit of the laboratory method. Minimum detection limits reported by the laboratory were: Aluminium =0.03Meq/100g, Chloride =10mg/kg, Magnesium=0.2Meq/100g, Nitrate nitrogen =1mg/kg and Sodium =0.2Meq/100g;
b. Excluded from further analysis due to large number of missing values.

Table 3
Final ordinal logistic regression models of 2 variable subsets[a] for cohort OJD animal level prevalence (CPREV) categorised as low (<2%), medium (2-10%) and high (>10%) based on faecal pools collected from sheep flocks in Australia in 2004-05.

Parameters and categories	*3-paddock mean variables (n= 96)*					*Weaning paddock variables (n = 69)*				
	b	*SE(b)*	OR and 95% CL		*P*	*b*	*SE(b)*	OR and 95% CL		*P*
Constant										
>10% versus 2-10% and <2%	-0.46	1.49	-	-	-	-0.99	1.60	-	-	-
>10% and 2-10% versus <2%	2.59	1.53	-	-	-	2.05	1.62	-	-	-
Cohort age	-	-	-	-	0.7	-	-	-	-	0.5
3 years	-	-	1.0	-	-	-	-	1.0	-	-
4 years	-0.17	0.42	0.8	0.4, 2.0	-	-0.36	0.50	0.7	0.3, 1.9	-
Cohort sex	-	-	-	-	0.009	-	-	-	-	0.03
Ewes	-	-	1.0	-	-	-	-	1.0	-	-
Wethers	1.37	0.52	3.9	1.4, 11.6	-	1.44	0.63	4.2	1.2, 16.5	-
Organic carbon%	0.89	0.26	2.4	1.5, 4.2	<0.001	0.89	0.32	2.4	1.4, 4.7	0.001
Sand%	-0.06	0.02	0.9	0.9, 1.0	0.003	-0.05	0.02	1.0	0.9, 1.0	0.01

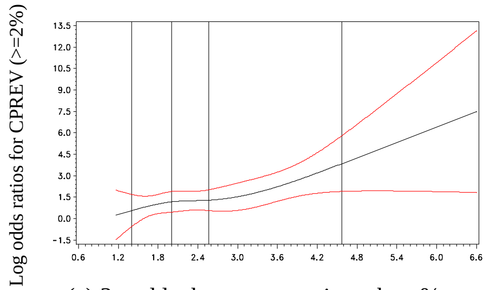
a. Results of lambing paddock and adult paddock variables are presented separately in Fig. 1 because the effects of some variables were not linear.

4.2.3 Generalised linear mixed models for pool OJD status (PSTATUS model)

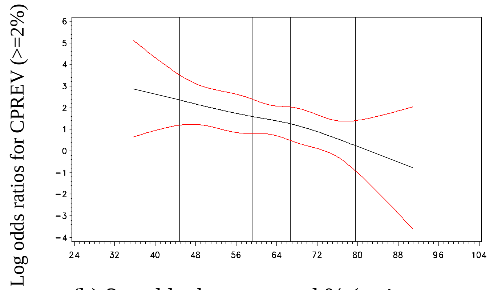
Of the 20 soil variables investigated in each subset, 18, 15, 16 and 16 were significant in the univariable binary logistic models (P<0.25) of 3-paddock mean, lambing, weaning and yearling/adult variable subsets, respectively. The multivariable models for 3 variable subsets (3-paddock mean, weaning and adult paddock) are presented in Table 4. The association of clay % was linear as was that of the other continuous variables (Fig. 1 g, h). For the lambing paddock variable subset, the assumption of linearity of sulphur content of the soil was not satisfied. The association was similar to that in the CPREV model (Figure 1 c, d) therefore not presented here.

4.2.4 General linear mixed model analyses for pool MAP number (MAPNUM model)

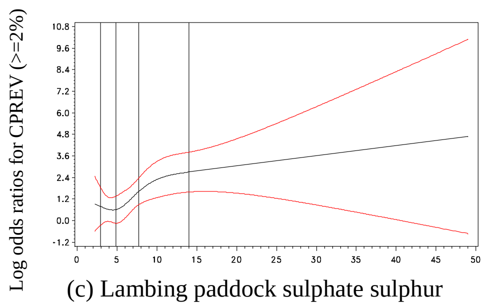
Of the 20 soil variables investigated in each subset, 18, 16, 16 and 18 were unconditionally associated with the MAPNUM models of 3-paddock mean, lambing, weaning, and yearling/adult variable subsets, respectively. Final model results are shown in Table 5; none of the continuous variables had significant departures from linearity. Some deviations from normality were observed in the histograms of studentised residuals, but the studentised conditional residuals were approximately normal.



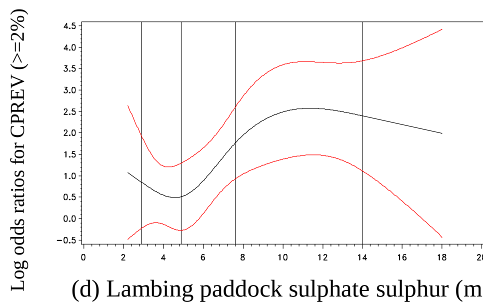
(a) 3-paddock mean organic carbon % (estimates adjusted for sand%, cohort age and cohort sex)



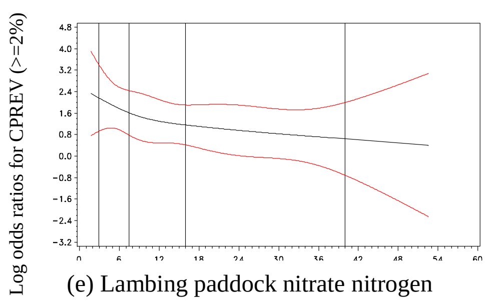
(b) 3-paddock mean sand % (estimates adjusted for organic carbon %, cohort age and cohort sex)



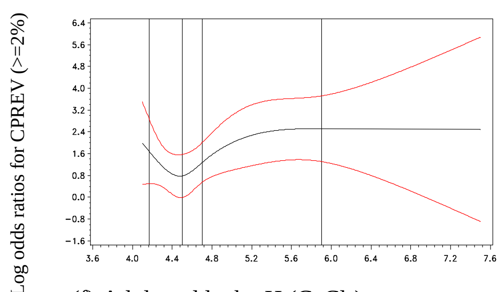
(c) Lambing paddock sulphate sulphur (mg/kg) (estimates adjusted for nitrate nitrogen (mg/kg), cohort age and cohort sex)



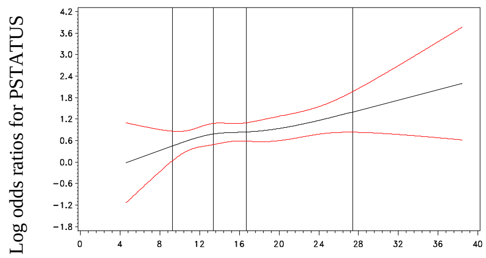
(d) Lambing paddock sulphate sulphur (mg/kg) after excluding one outlier (estimates adjusted for nitrate nitrogen, cohort age and cohort sex)



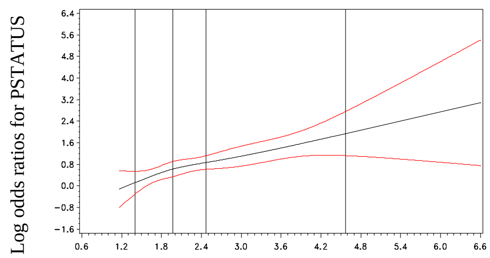
(e) Lambing paddock nitrate nitrogen (mg/kg) (estimates adjusted for sulphate sulphur (mg/kg), cohort age and cohort sex)



(f) Adult paddock pH ($CaCl_2$) (estimates adjusted for organic carbon %, sand %, cohort age and cohort sex)



(g) 3-paddock mean clay % (estimates adjusted for organic carbon %, cohort age, cohort sex and cohort age × cohort sex)



(h) 3-paddock mean organic carbon % (estimates adjusted for clay %, cohort age, cohort sex and cohort age × cohort sex)

Figure 1 Estimated spline transformation and 95% confidence limits of the association of some continuous soil variables with OJD based on the study conducted in Australia in 2004-05.

Table 4. Final generalised linear mixed model of 3 variable subsets of explanatory variables for pool OJD status (PSTATUS) based on culture results of faecal pools (positive or negative) collected from sheep flocks in Australia in 2004-05.

Parameters and categories	*3-paddock mean variables (n= 659)*					*Weaning paddock variables (n = 466)*					*Adult paddock variables (n = 529)*				
	b	*SE(b)*	OR and 95% CL		P	*b*	*SE(b)*	OR and 95% CL		*P* [b]	*b*	*SE(b)*	OR and 95% CL		*P* [b]
Random effects															
Flock variable	1.47	0.38				1.47	0.47				1.31	0.39			
Fixed Effects															
Intercept	-2.27	2.74				-1.22	3.50				1.68	3.14			
Cohort sex	-	-	-	-	0.1	-	-	-	-	0.4	-	-	-	-	0.01
Ewes						-	-	-	-	-	-	-	-	-	-
Wethers	1.40	0.44				1.66	0.51	-	-	-	7.43	3.02			
Cohort age	-	-	-	-	0.03	-	-	-	-	0.004	-	-	-	-	0.3
3 years						-	-		-	-	-	-	1.0	-	-
4 years	0.11	0.34				-0.11	0.40				-0.34	0.35	0.7	0.4, 1.4	
Log of pool size	0.15	0.76	1.2	0.3, 5.2	0.8	0.69	1.00	2.0	0.3, 14.3	0.5	-0.50	0.91	0.6	0.1, 3.6	0.6
Organic carbon%	0.62	0.21	1.9	1.2, 2.8	0.004	0.68	0.25	2.0	1.2, 3.2	0.007	0.67	0.24	2.0	1.2, 3.2	0.01
Clay %	0.05	0.03	1.1	1.0, 1.1	0.08	-	-	-	-	-	-	-	-	-	-
Sand%	-	-	-	-	-	-0.04	0.02	1.0	0.9, 1.0	0.04	-0.01	0.02			0.005
Effect modifications															
Cohort age (4 years)× Cohort sex (Wether)	-1.79	0.62	0.17	0.05, 0.6	0.004	-2.63	0.81	0.1	0.01, 0.4	0.001	-	-	-	-	-
Sand% × Cohort sex (Wether)	-	-	-	-	-	-	-	-	-	-	-0.10	0.04	0.9	0.8,1.0	0.03

a. Results of lambing paddock variables are discussed separately because the effect of some variables was not linear

Table 5. Final linear mixed model for log pool MAP number (MAPNUM) based on faecal pools collected from sheep flocks in Australia in 2004-05.

Parameters and Categories	3-paddock mean variables (n=659)				Lambing paddock variables (n=540)				Weaning paddock variables (n=466)				Adult paddock variables (n=529)			
	b	SE (b)	95% CL of b	P	b	SE(b)	95% CL of b	P	b	SE(b)	95% CL of b	P	b	SE(b)	95% CL of b	P
Random effects																
Flock variable	0.74	0.17	0.5, 1.2	<0.001	0.74	0.19	0.5, 1.4	<0.001	0.75	0.21	0.5, 1.4	<0.001	0.83	0.21	0.5, 1.5	<0.001
Residual	2.32	0.14	2.1, 2.61	<0.001	2.37	0.15	2.1, 2.7	<0.001	2.22	0.16	1.9, 2.6	<0.001	2.37	0.16	2.1, 2.7	<0.001
Fixed Effects																
Constant	-0.11	1.93	-4.0, 3.7	-	3.9	2.23	-0.5, 8.3	-	-0.98	2.51	-6.0, 4.0		3.51	2.31	-1.1, 8.1	
Cohort sex	-	-	-	0.09	-	-	-	0.006	-	-	-	0.2				0.002
Ewes	0.00	-	-	-	0.00	-	-	-	0.00	-	-	-	0.00	-	-	-
Wethers	0.77	0.23	0.3, 1.2	-	0.76	0.27	0.2, 1.3	-	0.95	0.26	0.4, 1.5		0.67	0.21	0.3, 1.1	
Cohort age	-	-	-	0.3	-	-	-	0.3	-	-	-	0.2				0.9
3 years	0.00	-	-	-	0.00	-	-	-	0.00	-	-		0.00	-	-	-
4 years	0.20	0.23	-0.3, 0.7	-	0.24	0.26	-0.3, 0.8	-	0.22	0.27	-0.3, 0.8		-0.04	0.26	-0.6, 0.5	
Log of pool size	-0.06	0.53	-1.1, 1.0	0.9	-0.9	0.6	-2.1, 0.3	0.2	0.14	0.69	-1.2, 1.5	0.8	-0.38	0.66	-1.7, 0.9	0.6
Iron	0.00	0.00	-0.0, 0.01	0.07	0.00	0.00	0.00, 0.01	0.04	0.00	0.00	0.00, 0.01	0.05	-	-	-	-
Nitrogen	-	-	-	-	-0.02	0.01	-0.04, 0.00	0.07	-	-	-	-	-	-	-	-
Organic carbon%	0.40	0.12	0.2, 0.6	0.00	0.29	0.14	0.01, 0.6	0.04	0.35	0.15	0.06, 0.6	0.02	0.42	0.15	0.1, 0.7	0.01
Clay %	0.04	0.02	-0.0, 0.01	0.05	-	-	-	-	0.05	0.02	0.01, 0.09	0.01	-	-	-	-
Sand %	-	-	-	-	-	-	-	-	-	-	-	-	-0.02	0.01	-0.04, 0.0	0.04
Effect modifications																
Cohort age (4 years) × Cohort sex (Wether)	-0.91	0.36	-1.63	0.01	-1.09	0.42	-1.9, -0.3	0.01	-1.29	0.48	-2.2, -0.4	0.01	-	-	-	-

4.3 Association of soil risk factors with cohort sex

Of the 19 3-paddock mean variables and 19 adult paddock variables investigated, 10 and 5 respectively were unconditionally associated with cohort sex at P value <0.1 (Table 6).

Table 6
Unconditional associations of the soil explanatory variables based on the mean of 3-paddocks (76 cohorts) and on the adult paddock (59 cohorts) with cohort sex.

Parameters	OR	OR LCL	OR UCL	*P*
3-paddock mean variables				
Organic carbon %	2.17	1.24	4.14	0.006
Manganese	0.95	0.91	0.99	0.01
Boron	2.89	1.16	10.30	0.02
Sulphur	1.14	1.02	1.30	0.03
Calcium	1.19	1.02	1.48	0.03
Cation exchange capacity	1.13	1.01	1.30	0.04
Silt %	0.93	0.84	1.01	0.08
Potassium	0.10	0.00	1.26	0.08
Sand %	1.05	0.99	1.12	0.09
Phosphorus buffer index	1.01	1.00	1.03	0.09
Adult paddock variables				
Silt %	0.86	0.74	0.96	0.004
Sand %	1.09	1.02	1.19	0.007
Manganese	0.94	0.89	0.99	0.007
Organic carbon %	2.12	1.03	4.65	0.041
Clay %	0.90	0.76	1.01	0.086

4.4 Association of management and soil risk factors with OJD infection level

4.4.1 Ordinal logistic regression analyses for cohort OJD prevalence (CPREV model)

Of the 88 variables investigated, 44 were unconditionally associated with cohort OJD prevalence at P value <0.2 (listed in Appendix A). After deletion of highly correlated variables and those with >10% of missing values, a total of 34 remained for inclusion in the multivariable model.

Final model results presented in Table 7 show that both management and soil factors were significant after adjusting for each other. The assumption of proportional odds for ordinality of the outcome was fulfilled (score test P = 0.3) and testing of spline of organic carbon % variable showed no significant departures from linearity (Fig 2). The model adequately fitted the data (deviance chi-square/df = 0.6).

4.4.2 Generalised linear mixed modelling for pool OJD status (PSTATUS model)

Of the 88 variables investigated, 45 management and 18 soil variables were unconditionally associated with pool OJD status (at P-value <0.2; shown in Appendix B). After deleting highly collinear variables and those with >10% missing values, 52 remained for inclusion in the final model. ICC was estimated to be 0.31 indicating that a substantial proportion of total variation was clustered at flock level, therefore justifying inclusion of flock as a random effect in multivariable mixed models.

Final model results are presented in Table 8. Variability in the data was appropriately modelled and there was no residual over-dispersion (generalized chi-square/DF = 0.9). Organic carbon, the continuous variable in the model, showed no significant departures from linearity.

4.4.3 General linear mixed model analyses for pool MAP number (MAPNUM model)

Of the 88 variables investigated, 46 management variables and 18 soil factors were associated with log pool MAP number in univariable linear regression analyses (at P-value <0.2; shown in Appendix C). After deleting collinear variables, 51 remained for inclusion in the final model. Similar to PSTATUS model, the ICC was 0.31 justifying the use of mixed models.

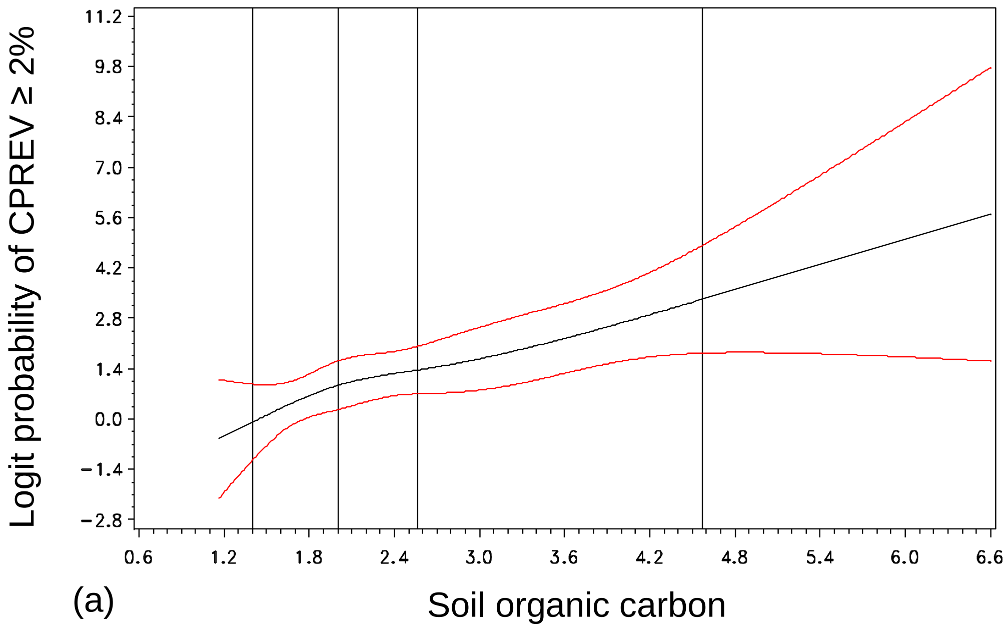
Final model results are presented in Table 9. Examination of residuals revealed no evidence of departure from normality.

Table 7
Final ordinal logistic regression model for cohort OJD prevalence categorised as low (<2%), medium (2-10%) and high (>10%), based on 95 sheep cohorts in 89 flocks in the OJD risk factor study conducted during 2004 - 05 in Australia

Parameters	*b*	*SE(b)*	OR	OR 95% CL[a]	*P*[b]
Constant					
>10% versus 2-10% and <2%	-6.41	1.38	-	-	-
>10% and 2-10% versus <2%	-1.57	1.13	-	-	-
Confounders	-	-	-	-	-
Cohort age	-	-	-	-	0.9
3 years	0.00	-	1.0	-	-
4 years	-0.21	0.61	0.81	0.24, 2.7	0.7
Cohort sex	-	-	-	-	0.002
Ewes	0.00	-	1.0	-	-
Wethers	1.86	0.62	6.41	1.9, 23.5	-
Current OJD mortality [c]	-	-	-	-	<0.001
No mortalities	0.00	-	-	-	-
<2% mortalities	1.58	1.09	-	-	-
≥ 2% mortalities	5.48	1.28	-	-	-
Fixed Effects	-	-	-	-	-
Application of fertilizers other than super phosphate and lime	-	-	-	-	0.002
No	0.00	-	1.0	-	-
Yes	-2.65	0.96	0.07	0.01, 0.4	-
Lambing paddock stocking rate [d]	-	-	-	-	0.005
< 14 dse /hectare	0.00	-	-	-	-
≥ 14 dse /hectare	3.05	1.16	-	-	-
Organic Carbon %	0.75	0.34	2.11	1.1, 4.3	0.023
Presence of wildlife other than kangaroos and rabbits	-	-	-	-	0.03
No	0.00	-	1.0	-	-
Yes	-1.24	0.57	0.29	0.09, 0.9	-
Years since commencement of OJD vaccination	-	-	-	-	0.015
1 or 2 years	0.00	-	1.0	-	-
>2 years	-1.76	0.84	0.17	0.03, 0.8	-
Vaccination not being done	-2.47	0.97	0.08	0.01, 0.5	-
Current OJD mortality × Lambing paddock stocking rate	-	-	-	-	0.002
<2% mortalities × high SR	0.74	1.38	-	-	-
≥ 2% mortalities × high SR	-3.48	1.43	-	-	-

Deviance =107.5 , DF= 174 ; Deviance/DF = 0.62

a. Profile likelihood confidence intervals for odds ratios; b. Based on likelihood ratio chi-square test of significance; c. Farmer-reported flock-OJD mortality in adult sheep (>2 year old) for previous 12 months; d. 1 dam = 2.45 dry sheep equivalent (dse)



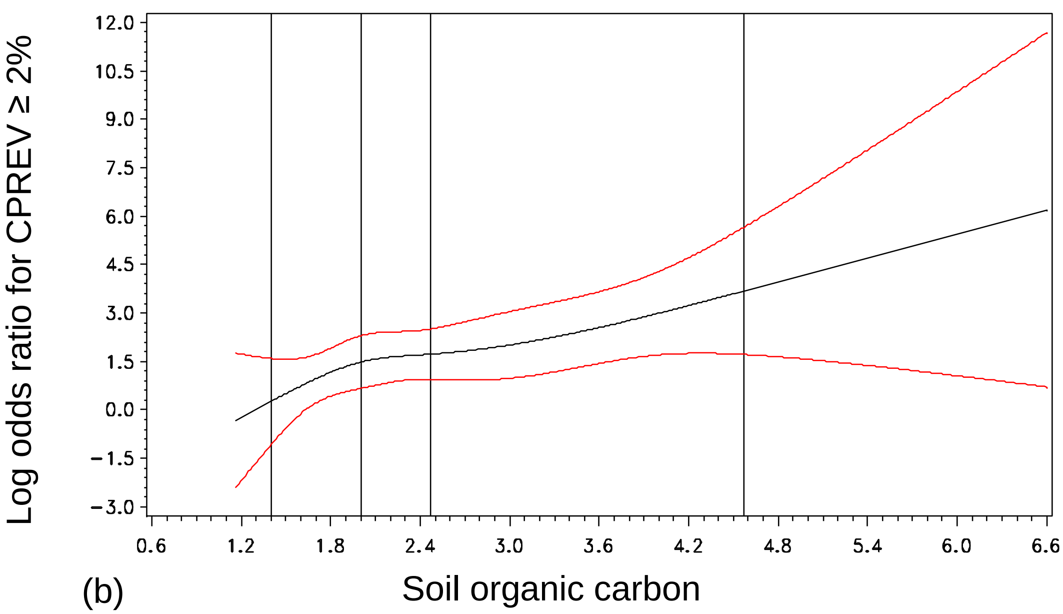


Figure 2. Estimated spline transformation and 95% confidence limits of the association of soil organic carbon % with the cohort OJD prevalence (CPREV): (a) unconditional and (b) after adjusting for the variables in the final model (cohort age, cohort sex, current OJD mortality, application of fertilizers other than super phosphate and lime, presence of wildlife other than kangaroos and rabbits, years since commencement of OJD vaccination and the interaction term between current OJD mortality and lambing paddock stocking rate)

Table 8

Final generalised linear mixed model for pool OJD status (PSTATUS), based on culture results of 637 faecal pools (positive or negative) collected from 84 flocks in the OJD risk factor study conducted in Australia in 2004-05.

Parameters	*b*	*SE(b)*	OR	OR 95% CL[a]	*P*[b]

Flock-level random effects	0.5	0.22	-	-	-
Constant	-2.8	2.4	-	-	-
Confounders	-	-	-	-	-
Cohort age	-	-	-	-	0.002
3 years	0.00	-	1.0	-	-
4 years	-0.18	0.31	0.8	0.5, 1.5	-
Cohort sex	-	-	-	-	0.2
Ewes	0.00	-	1.0	-	-
Wethers	1.68	0.82	5.4	1.1, 26.8	-
Current OJD mortality[a]	-	-	-	-	0.9
No mortalities	-	-	1.0	-	-
<2% mortalities	0.48	0.36	1.6	0.8, 3.3	-
≥ 2% mortalities	1.7	0.37	5.6	2.7, 11.7	-
Log of faecal pool size	0.48	0.69	1.6	0.4, 6.3	0.5
Fixed Effects					
Application of fertilizers other than super phosphate and lime	-	-	-	-	0.0008
No	0.00	-	1.0	-	-
Yes	-1.45	0.43	0.2	0.1, 0.6	-
Condition score of weaners [b]	-	-	-	-	0.08
< 3	0.00	-	1.0	-	-
≥ 3	-0.56	0.32	0.6	0.3, 1.1	-
Cull low body weight sheep or sell sub-flocks experiencing high losses	-	-	-	-	0.001
No	0.00	-	1.0	-	-
Yes	0.31	0.31	1.4	0.7, 2.5	-
Lambing paddock stocking rate [c]	-	-	-	-	0.01
< 14 dse/hectare	0.00	-	1.0	-	-
≥ 14 dse/hectare	0.74	0.30	2.1	1.2, 3.8	-
Organic Carbon %	0.5	0.18	1.7	1.2 2.4	0.004
Presence of wildlife other than kangaroos and rabbits	-	-	-	-	0.002
No	0.00	-	1.0	-	-
Yes	-0.92	0.30	0.4	0.2, 0.7	-
Sharing of roads with neighbours	-	-	-	-	0.06
No	0.00	-	1.0	-	-
Yes	0.55	0.30	1.7	1.0, 3.1	-
Interaction terms[d]					
Cohort sex × Cohort age group	-	-	-	-	0.006
Wethers × 4-years	-2.19	0.79	0.11	0.02, 0.5	-
Cohort sex × Cull low body weight sheep or sell sub-flocks experiencing high losses	-	-	-	-	0.004
Wethers × Cull or sell	2.5	0.88	12.7	2.3, 71.4	-
Cohort sex × Current OJD mortality					0.007
Wethers × <2% mortalities	-0.97	0.96	0.38	0.06, 2.5	
Wethers × ≥ 2% mortalities	-3.16	1.09	0.04	0.0, 0.36	

Generalised chi-square = 579.4; Generalised chi-square/d.f. = 0.9; a. Farmer-reported flock-OJD mortality in adult sheep (>2 year old) for previous 12 months; b. body-condition scores: 1-emaciated, 2-thin, 3-average, 4-fat, 5-obese; c. 1 dam = 2.45 dse (dry sheep equivalent); d. The terms present in interactions with zero *b* are not shown.

Table 9

Final linear mixed model for log pool MAP number (MAPNUM) based on 637 pools collected from 84 flocks in the ovine Johne's disease risk factor study conducted in Australia in 2004-05

Parameters	*b*	*SE(b)*	95% CL of *b*	*P*
Flock-level random effects	0.15	0.08		0.02
Residual	2.33	0.14		<0.001
Constant	0.88	1.50	-2.11, 3.9	-
Confounders	-	-	-	-

Cohort age	-	-	-	0.05
3 years	0.00	-	-	-
4 years	0.14	0.20	-0.2, 0.5	-
Cohort sex	-	-	-	0.1
Ewes	0.00	-	-	-
Wethers	0.27	0.28	-0.28, 0.8	-
Current OJD mortality[a]	-	-	-	<0.001
No mortalities	0.00	-	-	-
<2% mortalities	0.50	0.22	0.07, 0.9	-
≥ 2% mortalities	1.19	0.22	0.8, 1.6	-
Log of faecal pool size	-0.02	0.43	-0.9, 0.8	0.9
Fixed Effects	-	-	-	-
Application of fertilizers other than super phosphate and lime	-			<0.001
No	0.00	-	-	-
Yes	-0.99	0.26	-1.5, -0.5	-
Organic carbon %	0.31	0.09	0.1, 0.5	<0.001
Condition score of ewes at the start of lambing [b]	-			0.01
≤ 3	0.00	-	-	-
> 3	-0.40	0.16	-0.7, 0.08	-
Cull low body weight sheep or sell sub-flocks experiencing high losses	-	-	-	<0.001
No	0.00	-	-	-
Yes	0.20	0.19	-0.2, 0.6	-
Lambing paddock stocking rate [c]	-	-	-	0.02
< 14 dse/hectare	0.00	-	-	-
≥ 14 dse/hectare	0.44	0.18	0.1, 0.8	-
Presence of wild animals other than kangaroos and rabbits				0.002
No	0.00	-	-	-
Yes	-0.54	0.17	-0.9, -0.2	-
Proportion of farm boundary receiving run off water	-	-	-	0.06
≤ 10%	0.00	-	-	-
>10% to ≤ 30%	-0.45	0.23	-0.9, 0.0	-
>30% to ≤ 60%	-0.45	0.22	-0.9, - 0.03	-
> 60%	-0.50	0.23	-1, -0.04	-
Sharing of roads with neighbours	-	-	-	0.01
No	0.00	-	-	-
Yes	0.44	0.17	0.1, 0.8	
Interaction terms[d]				
Cohort sex × Cohort age	-	-	-	0.003
Wethers × 4 years	-1.02	0.34	-1.7, -0.4	-
Cohort sex × Cull low body weight sheep or sell sub-flocks experiencing high losses	-			0.003
Wethers × Yes	0.97	0.32	0.3, 1.6	-

a. Farmer-reported flock-OJD mortality in adult sheep (>2 year old) for previous 12 months; b. Body-condition scores: 1-emaciated, 2-thin, 3-average, 4-fat, 5-obese; c. 1 dam = 2.45 dse (dry sheep equivalent); d. The terms present in interactions with zero *b* are not shown.

5 Discussion

In 2004 we conducted a cross-sectional study to identify management and soil risk factors associated with OJD expression in infected flocks. The aim was to use our findings to refine recommendations for on-farm OJD control measures. This report presents the findings of extended analysis of the data related particularly to soil risk factors from this study.

The Final Report for OJD.038 discussed in detail the strengths and limitations of the cross-sectional study. This study, whilst obviously constrained by the nature of the cross-sectional study design, had notable strengths in relation to objective measurement of the disease outcome (at cohort and pool level) and of the soil explanatory variables based on soil samples from paddocks grazed by cohort sheep. For this report the soil variables were analysed as continuous variables further enhancing our ability to detect and quantify relationships. We believe use of property specific information based on collected soil samples was more accurate than data based on regional soil surveys and that changes in climate and management from time of cohort grazing to sample collection would have minimal impact on soil composition. Consequently concern about information bias relevant to this report is limited to the cohort-level management risk factors in the composite models which are likely to have been impacted by recall bias (often reported to be differential in nature). Strategies used to minimise recall bias included questionnaire administration by personal interview, focusing on the management of specific sheep age group/cohort within each flock, and use of a series of questions by the interviewer to determine flock status for duration of flock OJD infection, mineral deficiency and worm control.

At this point it important to state that this study, purposely focused on infected flocks, did not investigate the role of soil risk factors in relation to the introduction and establishment of OJD infection in a flock. Furthermore, of currently infected Australian flocks, the 92 study flocks who met specific selection criteria (reported in the OJD.038 Final Report) are a non-representative sample, so our study findings should be extrapolated with caution to other flocks.

5.1 Investigation of the association of soil risk factors with OJD infection level

For this report we re-investigated the soil explanatory variables without categorisation (except for parent soil type) and including data for additional variables (iron, boron, calcium, copper, iron, magnesium, manganese, nitrate nitrogen, organic carbon% and zinc). Analysis of these soil variables in continuous form permitted the real associations with OJD infection to be displayed including even non-linear associations for sulphur and soil pH. Given the effort taken to collect and analyse soil samples from paddocks grazed by cohort sheep, it was deemed inappropriate to collapse data into categories which might reduce ability to elucidate real associations or to exclude data from investigation which might prohibit identification of an as yet unknown influential soil risk factor.

The results summarised in Table 10 are consistent with our previous findings that higher OJD levels were associated with more fertile soil and soils with higher clay and lower sand content. The outstanding difference is the prominence of organic carbon % in the latest models. Organic carbon %, a variable not included in the previous analyses, is clearly more strongly associated with OJD infection level than the three previously identified risk factors reflecting soil fertility – carbon exchange capacity, phosphorus buffer index and phosphorus. The role of organic matter, however, was anticipated as we had recognised cation exchange capacity to be an indirect indicator of organic matter in soil.

Organic carbon % has a consistent positive linear association with OJD infection in 10 of 12 models presented in this report. Organic carbon, an indicator of soil organic matter content (Baldock and Skjemstad, 1999) might favour the survival of MAP, either directly by providing essential nutrients for its continued existence outside the biological host or indirectly by increasing pasture growth and thus the availability of shade which is reported to aid MAP survival (Whittington et al., 2004). Although organic matter also increases the water holding capacity of soil (Krull et al., 2004) and soil moisture is not known to influence MAP survival (Whittington et al., 2004), it is apparent that MAP is likely to be available in greater numbers to the animals grazing on soils with a higher organic matter content.

Apart from its effect on increased mycobacterial survival, organic matter content could also be a confounder for higher stocking rates because the owners of the farms with more fertile soil might tend to increase sheep numbers per unit area of pasture. As we found stocking rate in the lambing paddock to be detrimental for OJD (Dhand et al., 2007); there was clearly a need to investigate this association further via composite models including management and soil risk factors.

Similar to organic carbon, higher clay-content soils were associated with higher OJD prevalence. As we previously proposed clay could well adsorb MAP thus increasing its availability to sheep by retaining it in the upper soil layers rather than allowing it to be leached to the deeper layers. Clay particles are very small in size (<2µ) allowing a large number of clay particles to be packed in a specified volume of soil; as a result they offer a large surface area for bacterial attachment (Marshall, 1975). Adsorption of various bacteria and viruses of public health significance to clay particles has been reported previously (Banks et al., 2003; 2005; Moore et al., 1981; Taylor et al., 1981). This is mediated by electrostatic and van der Waals' forces or by cell surface hydrophobicity (Taylor et al., 1981). At present there is no direct evidence of mycobacaterial adsorption to clay particles, although one study reported recovery of only 3.5% of nontuberculous mycobacteria inoculated into soil samples and attributed this to adsorption (Brooks et al., 1984). Whittington et al (2003) inferred a similar phenomenon for MAP, but further studies are required to substantiate this.
In contrast, sand particles are larger in size and offer less surface area for bacterial adsorption. This might account for the lower OJD prevalence in flocks raised on soils with a higher sand %. These results are contrary to a previous study (Ward and Perez, 2004) that reported higher JD prevalence in dairy cattle on farms with sandy soils. This might reflect the variability in management between enterprises and/or countries or confounding of soil variables by other factors. It could also reflect methodological differences between studies, because soil factors in this study are based on samples collected from paddocks grazed by sheep, while in the previous study, soil type was characterized on the basis of herd location.

Similar to a previous study (Johnson-Ifearulundu and Kaneene, 1999), our results indicated an increase in OJD prevalence with an increase in soil iron content of the soil. This association might be due to increased survival of MAP in soils with high iron content. However, it is interesting to note that apart from iron, most of the cations investigated in this study (including cation exchange capacity) had a positive association with OJD at the univariable level. The association of cations with OJD observed in this study might in fact be due to their association with organic carbon or clay. The latter being negatively charged usually hold large number of cations (Marshall, 1975). Nevertheless, it is worth noting that iron content was significant in some of the multivariable models after adjusting for organic carbon whereas most other cations were not.
The association of sulphur with OJD was complex because it was detrimental for sheep at both lower and higher levels (Fig. 1d). The unfavourable effects of sulphur deficiency in the soil might be as a consequence of its ensuing deficiency in sheep, whereas the effects of higher soil sulphur levels might be due to its association with organic matter. Most sources of sulphur in soil are actually constituents of organic matter. Sulphur was positively correlated with organic carbon in this study (Pearson correlation 0.5); in fact, organic carbon was only non-significant in two models where sulphur was present.

The protective association of nitrogen might reflect better protein synthesis and hence immune protection of the sheep but could also be a confounder for other agronomic practices. For example, deep rooted perennial pastures are reported to reduce nitrogen loss and therefore help to build up nitrogen levels in the soil. The association with nitrogen could be due to such unmeasured factors and therefore might warrant further investigation.

In contrast to some previous work we found no association with soil type and little association with soil pH. No association with soil type, while different to one study in Spain which identified soil type as one of two predictor variables for flock seroprevalence (Reviriego et al., 2000), aligns with the finding of Johnson-Ifearulundu and Kanenne (1999) in Michigan. As soil type affects many soil characteristics it could well act as a surrogate variable for unmeasured factors. The association with soil pH found (with a trend toward a detrimental effect at both lower and higher pH levels) differs markedly to the Michigan study where paratuberculosis prevalence was clearly linked to low soil pH (Johnson-Ifearulundu and Kaneene, 1999). However, our result should be interpreted with caution as pH was significant only in one model, and although the study farms represented a wide range of cohort OJD prevalence (0 to 59%), all (except one) were located on predominantly acidic soil pH (range 3.7-7.5; mode and median 4.6) which might have limited detection of an association.

Considerable variability among the laboratory results for soil samples collected from 3 different paddocks on each farm necessitated development of separate models for lambing, weaning and adult paddocks to remove the confounding effect of paddock type and their associated management practices. Although the significance of variables differed slightly among the multivariable models, the subset of variables identified to have an association with OJD infection was generally consistent. However, due to multiple comparisons, caution should be observed while interpreting the associations that were significant only in one or two models such as sulphur sulphate and pH (Table 10).

Table 10
Summary of the main effect terms present in the final soil models – ordinal logistic regression model for cohort OJD prevalence level, generalised linear mixed model for pool OJD status and linear mixed model for log pool MAP number. Details of the models are presented in Tables 3, 4 and 5.

Parameters	Cohort OJD prevalence level	Pool OJD status	Log pool MAP number	Number of models where present (of 12 models)	Description of trend in effect
Organic carbon %	D	D	D	10	Greater risk of OJD in cohorts raised on paddocks with higher organic matter levels in the soil
Sand %	P	P	P	6	Less risk of OJD in cohorts raised on paddocks with higher sand content soils
Clay %		D	D	3	Greater risk of OJD in cohorts raised on paddocks with higher clay content soil
Iron			D	3	Greater risk of OJD in cohorts raised on paddocks with higher iron content soil
Nitrogen	P	P	P	3	Less risk of OJD in cohorts raised on paddocks with higher nitrogen content soil
Sulphur sulphate	M	M		2	Less risk of OJD in cohorts raised on paddocks with optimum level of sulphur; greater risk in cohorts raised at both lower and higher levels.
pH	M			1	Greater risk of OJD in cohorts raised on paddocks with soil pH <>4.5.

D = Detrimental effect; P = Protective effect; M= Mixed Effect

5.2 Investigation of the association of soil risk factors with cohort sex

On the basis of our knowledge of farmer practices, we expected soil samples from paddocks grazed by wether cohorts to be less fertile than samples from paddocks grazed by ewe cohorts. The results presented in Table 6 provide limited evidence of preferential placement of ewe cohorts on paddocks with better soil quality.

A difference, if present, should be most apparent between soil samples from paddocks grazed by the cohort sheep as adults. In support of anecdotal evidence, associations with the lowest P-values indicate that adult wethers were grazed on paddocks with lower silt % and higher sand % than ewes. However, conflicting with routine farmer practice, the data indicated that organic carbon (an indicator of organic matter) was higher for paddocks grazed by adult wether cohorts than ewe cohorts. The results for the adult paddock include only 11 wether and 48 ewe cohorts so are limited by small sample size. Nonetheless these results suggest that confounding between sex and characteristics of the soil samples particularly for the adult paddocks was not strong as anticipated.

5.3 Investigation of the association of management and soil risk factors with OJD infection level

Our results summarised in Table 11 suggest that both management and soil factors influence OJD infection level in infected flocks. As far as we are aware, this is the first time that both factors have been demonstrated to be important factors for OJD although this has been previously documented for BJD (Johnson-Ifearulundu and Kaneene, 1999).

Organic carbon %, an indicator of soil organic matter content (Baldock and Skjemstad, 1999) and thus of soil fertility, was positively linearly related with OJD infection even after adjusting for management variables, suggesting that it is a crucial factor in itself and could effect OJD infection by promoting survival of MAP. Its significance even after accounting for stocking rate of the lambing paddock indicates that the effect of organic matter is not due to its potential correlation with stocking rate. This view is further substantiated by the detection of no significant difference in mean organic carbon % between low and high stocking rate flocks (t-test P-value = 0.7). As stated previously, organic matter might support MAP survival directly by providing required nutrients or indirectly by enhancing pasture growth. The role of organic matter needs further investigation to elucidate the underlying mechanisms of enhanced MAP survival before strategies to assist disease control can be proposed.

Although organic carbon was associated with OJD in all three composite models, none of the other soil variables found to be important previously in the soil factor models (sand%, clay%, iron content, sulphate sulphur, nitrate nitrogen and soil pH) were significant in any of the final composite models. This indicates that in the soil factor models these variables were explaining some of the variability now explained by the management factors included in the final composite models. On further evaluation of the study data it was observed that most of these soil variables were associated with the variable ‘years since commencement of OJD vaccination’ which could account for their omission from the CPREV model (but not from the PSTATUS and MAPNUM models where this variable itself was not present in the final combined model). However, we are unaware of the reasons for such association/s.

Most management variables associated with OJD infection in the final composite models were included in the previously reported models of the management risk factors with similar direction of association (Dhand et al., 2007b; Toribio et al., 2005), suggesting that even after adjusting for soil variables, these variables account for significant variability in OJD infection.

The management variables which have been discussed in detail previously fall into three broad categories:

1. Associations likely to be a consequence of OJD infection (Culling of low body weight sheep or sale of high loss mobs, Years since commencement of OJD vaccination)
2. Associations related to general property features and management (Application of fertilizers other than super phosphate and lime, Presence of wild animals other then kangaroos and rabbits, Proportion of farm boundary receiving run off water)
3. Associations related to flock management (Lambing paddock stocking rate, Condition score of lambing ewes, Condition score of weaners, Movement of sheep along roads shared with neighbours).

In relation to flock management, our results consistently highlight the importance of the lamb/weaner lifestage. Among the infected study flocks, high stocking in the lambing paddock resulted in higher OJD infection in the cohort sheep for the 3 measures of flock infection investigated. Higher condition score among the dams and the cohort sheep as weaners was associated with lower levels of pool infection. The impact on subsequent OJD status of ensuring that sheep as lambs and weaners are not exposed to high levels of MAP pasture contamination and to nutritional stress is clear. This finding, along with biosecurity practices such as not exposing sheep to facilities utilised by other flocks, provides a basis for on-farm disease control recommendations.

Our composite model results are broadly consistent with the only similar previous study (Johnson-Ifearulundu and Kaneene, 1999), because both studies determined soil and management factors to be significant factors influencing paratuberculosis prevalence. However, we could not detect any association between iron content, soil pH or lime application with JD as suggested by previous investigators. This might indicate differences in management practices in United States and Australia where the studies were conducted, or between disease in cattle and sheep.

Our investigation also did not confirm the association between light infertile soils and OJD suggested by Lugton (2004) based on a postal survey of infected producers. On the contrary, we detected positive linear association between soil organic carbon (indicator of soil fertility) and OJD even after adjusting for management and environmental factors.

For this aspect of the study in particular we sought to investigate a large number of explanatory variables far exceeding the standard recommendation of 1 explanatory variable per 10 cases (Bagley et al., 2001). Although not uncommon among cross-sectional studies of JD (Daniels et al., 2002; Johnson-Ifearulundu and Kaneene, 1998; Lugton, 2004; Obasanjo et al., 1997), this introduces potential for the identification of spurious associations. Throughout all reported analyses we consistently adopted several strategies to reduce the number of explanatory variables investigated such as limiting the explanatory variables to factors with credible links to OJD infection or disease progression, and exclusion of variables with substantial missing values, high correlation or poor unconditional association from all multivariable analyses.

Table 11
Summary of the terms present in the three final composite models – ordinal logistic regression model for cohort OJD prevalence level, generalised linear mixed model for pool OJD status and linear mixed model for log pool MAP number. Details of the models are presented in Tables 7, 8 and 9

Parameters	Categories	Cohort OJD prevalence level	Pool OJD status	Log pool MAP number	Description of trend in effect
Confounders forced into each model					
Current OJD mortality	High and low versus nil	D	D	D	Greater risk of OJD in cohorts raised in flocks with OJD mortalities
Cohort sex	Wethers versus ewes	D	D	D	Greater risk of OJD in wether cohorts than ewe cohorts
Cohort age	4-year old versus 3-year old	P	P	D	Greater risk of OJD in 3-year old cohorts than 4-year old cohorts
Main effects					
Organic carbon %		D	D	D	Greater risk of OJD in cohorts raised on paddocks with higher organic matter levels in the soil
Lambing paddock stocking rate	High versus low	D	D	D	Greater risk of OJD in cohorts when the cohort stocking rate in the lambing paddock/s was ≥14 dse/hectare
Application of fertilizers other than super phosphate and lime	Yes versus no	P	P	P	Less risk of OJD in cohorts raised in flocks with history of applying fertilisers other than single super, molybdenum super and lime on the property
Presence of wild animals other than kangaroos and rabbits	Yes versus no	P	P	P	Less risk of OJD in cohorts raised in flocks where wild animals other than kangaroos and rabbits are present on the property
Cull low body weight sheep or sell mob experiencing high losses	Yes versus no		D	D	Greater risk of OJD in cohorts raised in flocks where producers cull low body weight sheep or sell mobs experiencing high losses
Sharing of roads among neighbours	Yes versus no		D	D	Greater risk of OJD in cohorts raised in flocks that move sheep along roads shared by neighbours
Years since commencement of OJD vaccination	>2 years and nil versus 1-2 years	P			Greater risk of OJD in cohorts raised in flocks that have vaccinated for 1-2 years with the Gudair® vaccine

Condition score of ewes at start of lambing	High versus low		P	Less risk of OJD in cohorts where the dams had a condition score >3 at start of lambing
Condition score of weaners	High versus low	P		Less risk of OJD in cohorts where the cohort sheep had a condition score ≥3 at weaning
Proportion of farm boundary receiving run off water	>60%, >30%-≤60% and >10%-≤30% versus ≤10%		P	Less risk of OJD in cohorts raised in flocks where the property receives run off water along >10% of the property boundary

D = Detrimental effect; P = Protective effect; M = Mixed effect

6 Success in Achieving Objectives

The objective of this project, to quantify the respective contributions of management and soil risk factors to the level of OJD in infected flocks investigated in OJD.038, was achieved.

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

The main benefit to industry from this project is the clarification of the respective contribution of identified management and soil risk factors to the level of OJD-infection on infected properties.

There is solid evidence from this study that aligns with other research (such as OJD.002A) that the management of sheep as lambs and weaners is critical. Practices related to the management of lambing ewes and weaners can be altered by farmers to lower lamb exposure to infected pasture and to improve lamb/weaner nutrition. This approach to on-farm disease control can be implemented either with or without vaccination. Implementation of this approach to on-farm disease control along with biosecurity practices, with or without vaccination, will lead to reduced infection levels and related losses in infected flocks.

Given similarity in the management and structure of self-replacing Merino flocks across the known OJD infected districts of Australia, inclusion 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 management recommendations from this study will continue to be relevant to newly diagnosed flocks over the coming years.

This work provides evidence that the level of organic matter in soil influences OJD infection level in sheep. It does not provide evidence of association with any other soil characteristic that is not accounted for by management. This should direct research in relation to the influence of soil upon infection level within infected flocks toward understanding of the interaction between organic matter +/-clay and MAP in soil. Focused research over the next few years on this credible association will help to further clarify speculation surrounding the role of soil characteristics on expression of OJD in infected flocks.

8 Conclusions and Recommendations

The principle finding of this work is that both management and soil factors influence OJD infection level in infected flocks. To our knowledge this is the first time that the importance of both factors has been demonstrated for OJD.

The organic matter content of soil has a strong association with OJD infection level in infected Australian flocks. Among the soil risk factors investigated, the association with organic carbon % was the only soil factor to remain in the composite model. It was retained even in the presence of stocking rate proving that it has influence on OJD infection that is beyond a purely confounded relationship with stocking rate. Organic carbon % is an indicator of soil organic matter content but the mechanism by which organic matter aids MAP survival is as yet unknown. It may be direct by providing nutrients that assist MAP survival outside a biological host or indirect by enhancing pasture growth which provides shaded conditions conducive to MAP survival or a combination of the two.

Soil clay content also has an influence on OJD based on the consistent presence of this risk factor in the soil models and on the protective effect of higher sand content indicated by some soil models. Although clay was not retained in the composite models, and so is not as influential as management and organic carbon, knowledge that some bacteria adsorp to clay particles raises a hypothesis for investigation that could contribute to our understanding of MAP retention in soil.

This study provides no evidence of association with soil type and little evidence of association with pH and iron. Although this cross-section study was not designed to address questions regarding the role of soil characteristics on the establishment or not of OJD infection in sheep flocks, by the inclusion of a spectrum of infected flocks and the collection of soil samples from paddocks grazed by the cohort sheep it provides sound findings in relation to soil factors that influence OJD infection level. These indicate that focused research on interactions between MAP, organic matter and clay will provide new insight that could direct thinking about OJD control on infected properties and possibly the potential for OJD spread into new regions in Australia.

We found limited evidence that farmers preferentially place adult ewes on paddocks with better quality soil than adult wethers. However the wether cohorts had higher OJD infection levels than the ewes and this may be explained to some extent by wethers being grazed on paddocks with higher organic carbon %.

Of the management risk factors investigated, several factors that can be manipulated by farmers were included in the final composite models. Most prominent among these are aspects of lamb and weaner management that can be modified to lower lamb exposure to infected pasture and to improve lamb/weaner nutrition. Risk factors related to lamb and weaner management were present in all final models indicating the strong association with cohort adult infection status. In addition biosecurity was emphasised by identification of a strong association with movement of sheep along roads shared with neighbours and vaccination by the reduction in flock infection achieved in flocks experiencing OJD losses through use of the Guidair vaccine over several years. Other identified management factors are less amenable to change (such as proportion of farm boundary receiving run off water) or the association remains unclear (such as application of fertilizers other than super phosphate and lime and presence of wild animals other than kangaroos and rabbits).

Although not specifically investigated in this study, the absence of significant rates of OJD infection in sheep in some regions, for example, the semi arid western division of NSW, might be explained by low soil fertility, specifically organic matter content. Soil characteristics in regions with very low prevalence of OJD should be investigated.

Recommendations:

1. **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:**

- Optimise lamb to weaner management
    - Maintain adequate ewe nutrition to avoid nutritional stress to ewes and the subsequent higher shedding among pregnant and lactating dams
    - Avoid higher stocking rates in lambing paddocks
    - Maintain sheep with condition score ≥ 3 at weaning
- Nutritional stress and higher stocking rates could expose young sheep to higher MAP levels through grazing short pasture, leading to consumption of more contaminated soil and accelerated disease progression by impeding immune function. Further research is required to investigate effects of reduction in stocking rate on economic returns.

- 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.
- Implement vaccination in infected flocks experiencing losses for at least over 3 years.

2. It is recommended that further research be undertaken to explain two soil risk factors:

- Higher OJD prevalence was linked to an increase in organic carbon % and soils having higher proportions of clay and lower proportions of sand. This suggests a detrimental affect of soil fertility on OJD level specifically related to organic matter content for which the mechanism/s is unknown.
- Further the ability of negatively charged clay particles to absorp MAP needs investigation. Potentially clay particles by retaining MAP in the top soil could act to increase the availability of the organism to sheep. In contrast in sandy soils MAP 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 and organic content, 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
    - Soil characteristics (particularly organic matter) of regions in NSW with very low prevalence of OJD.

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10 Appendices

10.1 Appendix A - Management and soil variables unconditionally associated with cohort OJD prevalence level at P-value <0.2

Parameters (Sorted by P-value)	Description	P-value
INFLEVEL	Level of infection (based on trend in mortalities)	<0.001
CARBON_MEAN	Organic carbon%	<0.001
OJDSIGNS	Signs of OJD observed by farmer	<0.001
PEAKMORT	Peak OJD mortality	<0.001
CURRMORT	Current OJD mortality	<0.001
MEANMORT	5-year mean OJD mortality	<0.001
PBI_COL_MEAN	Phosphorus buffer index	<0.001
YNGAGEMORT	Age of youngest mortality	<0.001
DROUGHT	Average difference of annual total rainfall from district long-term average over the lifetime of the cohort	0.001
SULFUR_MEAN	Sulphate Sulphur (mg/Kg)	0.002
DAMSR	Lambing-paddock stocking rate	0.003
MG_MEAN	Magnesium (Meq/100g)	0.005
CULLSELL	Cull low-body-weight sheep or sell sub-flocks experiencing high losses	0.006
CLINICALMGT	Management of OJD clinical sheep	0.007
CLAYPCNT_MEAN	Clay %	0.007
CATIONEX_MEAN	Cation exchange capacity (Meq/100g)	0.007
SEX	Cohort sex	0.008
OJD_DURN	Interviewer's assessment of duration of OJD infection	0.011
IRON_MEAN	Iron (mg/Kg)	0.013
SANDPCNT_MEAN	Sand %	0.013
PROPSR	Flock stocking rate	0.016
SUPPLFEED	Period of any supplementary feed	0.017
DROPSVACC	Years since commencement of OJD vaccination	0.020
OTHERWILDLIFE	Presence of wild animals other than kangaroos and rabbits	0.023
HGTCS	Condition-score of sheep at 1 year of age	0.028
OTHERFERT	Application of fertilizers other than super phosphate and lime	0.028
GROWTHCHK	Period of growth-check in the life of the cohort	0.030
BORON_MEAN	Boron (mg/Kg)	0.036
SHARING_ROAD	Sharing of roads among neighbours	0.039
HGTSR	Yearling-paddock stocking rate	0.047
CALCIUM_MEAN	Calcium (Meq/100g)	0.049
PSTYPE	Parent soil type	0.051

WNRSR	Weaning-paddock stocking rate	0.060
SVC_CS	Condition-score of cohort at the time of faecal sample collection	0.066
DROUGHT_YRLAMBING	Average difference in total annual rainfall from district long-term average in the year of birth of the cohort	0.070
SILTPCNT_MEAN	Silt %	0.104
LBGSSN	Season of lambing	0.108
N_MEAN	Nitrate Nitrogen (mg/kg)	0.118
YNGMGT	Separate young sheep or handle young sheep first	0.140
PCREEK	Permanent creek flowing onto the farm	0.141
SUPERFREQ	Frequency of application of super-phosphate fertilizers	0.142
WNRGMGT	Grazing management for weaners	0.146
DAMCS	Condition score of ewes at start of lambing	0.169
ADSR	Adult-paddock stocking rate	0.169

10.2 Appendix B - Management and soil variables unconditionally associated with pool OJD status at P-value <0.2

Parameters (sorted by P-value)	P- value
INFLEVEL	<0.001
PEAKMORT	<0.001
OJDSIGNS	<0.001
CURRMORT	<0.001
YNGAGEMORT	<0.001
MEANMORT	<0.001
CARBON_MEAN	<0.001
PBI_COL_MEAN	<0.001
CULLSELL	<0.001
CLINICALMGT	<0.001
DAMSR	<0.001
DROUGHT	<0.001
IRON_MEAN	<0.001
RUNOFFWATER	<0.001
CATIONEX_MEAN	<0.001
SULFUR_MEAN	<0.001
MG_MEAN	<0.001
OTHERFERT	<0.001
SHARING_ROAD	<0.001
PSTYPE	<0.001
GROWTHCHK	<0.001
DROUGHT_YRLAMBING	<0.001
OJD_DURN	<0.001
BORON_MEAN	<0.001
WNRSR	<0.001
PROPSR	0.001
CLAYPCNT_MEAN	0.001
SEX	0.001
DROPSVACC	0.001
CALCIUM_MEAN	0.003
HGTSR	0.003
LBGSSN	0.003
PCREEK	0.003
OTHERWILDLIFE	0.004
SUPPLFEED	0.004
HGTCS	0.005
WNRCS	0.006
SUPERFREQ	0.008
WORMCONTROL	0.012
SANDPCNT_MEAN	0.014
DAMCS	0.020
PHOSPHORUS_MEAN	0.021
N_MEAN	0.029
RAMRISK	0.039
WNGAGE	0.040
PINRUSH	0.042

AGEGP	0.046
WNGPCNT	0.047
MANGANESE_MEAN	0.048
ADSR	0.049
LIME	0.052
ICREEK	0.054
AL_MEAN	0.065
KANGAROO	0.088
FODSTUB	0.093
YNGMGT	0.094
SVC_CS	0.097
SHARING_SHED	0.114
PHCACL2_MEAN	0.161
ZINC_MEAN	0.174
DESTOCK	0.178
HGTHLTH	0.191
COPPER_MEAN	0.193

10.3 Appendix C - Management and soil variables unconditionally associated with log pool MAP number at P-value <0.2

Parameter (sorted by P-value)	P-value
INFLEVEL	<0.001
YNGAGEMORT	<0.001
PEAKMORT	<0.001
CURRMORT	<0.001
OJDSIGNS	<0.001
MEANMORT	<0.001
CARBON_MEAN	<0.001
PBI_COL_MEAN	<0.001
CLINICALMGT	<0.001
IRON_MEAN	<0.001
CULLSELL	<0.001
MG_MEAN	<0.001
PSTYPE	<0.001
CLAYPCNT_MEAN	<0.001
DROUGHT	<0.001
OTHERFERT	<0.001
CATIONEX_MEAN	<0.001
OJD_DURN	<0.001
DAMSR	<0.001
GROWTHCHK	<0.001
PROPSR	<0.001
BORON_MEAN	<0.001
SEX	<0.001
RUNOFFWATER	<0.001
SULFUR_MEAN	<0.001
N_MEAN	<0.001
SUPERFREQ	<0.001
OTHERWILDLIFE	<0.001
SHARING_ROAD	<0.001
DAMCS	0.001
SANDPCNT_MEAN	0.001
PINRUSH	0.001
DROPSVACC	0.001
DROUGHT_YRLAMBING	0.001
PCREEK	0.002
SUPPLFEED	0.002
HGTSR	0.003
YNGMGT	0.003
CALCIUM_MEAN	0.004
WNRSR	0.004
PHOSPHORUS_MEAN	0.005
AL_MEAN	0.005
INFNBRS	0.009
WNRCS	0.009
DESTOCK	0.011
COPPER_MEAN	0.017

WNGAGE	0.020
FODSTUB	0.023
HGTCS	0.024
KANGAROO	0.026
MANGANESE_MEAN	0.028
LBGSSN	0.039
WNRGMGT	0.042
SVC_CS	0.046
ICREEK	0.047
WNRHLTH	0.065
WNGPCNT	0.077
ADSR	0.096
ZINC_MEAN	0.105
WORMCONTROL	0.111
SHARING_SHED	0.125
RABBIT	0.193