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Risk factors for ovine Johne's disease in infected sheep flocks in Australia

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Abstract

We conducted a cross-sectional study in 2004-05 to investigate risk factors for ovine Johne's disease (OJD) involving 92 infected Merino sheep flocks in Australia. In each enrolled flock we collected pooled faecal-samples from 3- to 5-year-old sheep and cultured them for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) to determine their OJD status. Based on pooled faecal-culture (PFC) results, three outcome variables representing different facets of disease biology were derived: pool OJD status (binomial: positive or negative), log pool MAP number (continuous) and cohort OJD prevalence level (ordinal: low (<2%), medium (2-10%) and high (>10%) prevalence). We used these outcomes in three separate multivariable analyses to identify risk factors, which were based on a questionnaire administered during a face-to-face interview with the farmer.

We found higher OJD infection in sheep whose dams had been in poor condition and kept at a high stocking rate during lambing and in sheep which had experienced a longer period of growth retardation during their lifetime. Flocks that had vaccinated for >2 years (rather than only 1 to 2 years) with a killed MAP vaccine had significantly lower OJD infection. In addition, practices including culling low body-weight sheep or selling sub-flocks experiencing high losses, sharing of roads between neighbouring farms, and greater frequency of application of super-phosphate fertilizers were associated with higher OJD. Of the confounders investigated, infection was higher in flocks experiencing high mortalities; in wethers compared to ewes; and in 3-year-old sheep compared to 4-year-old sheep.

Keywords: risk factors, paratuberculosis, ovine Johne's disease, Australia, Mycobacterium, epidemiology, cross-sectional study

1. Introduction

Ovine Johne's disease (OJD), a chronic enteric disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is characterised by progressive emaciation and intermittent diarrhoea in some animals. It was first reported among sheep in Australia in the early 1980s (Seaman et al., 1981). By December 2005, 1840 flocks were reported infected, mostly in the state of New South Wales (NSW) (Kennedy and Sergeant, 2005). In the southern tablelands of NSW, OJD mortality ranged from 2.1% to 17.5% on 12 infected farms in 2002, resulting in a decrease in farm gross margin ranging from 2.2% to 15.4% (Bush et al., 2006). Mortalities and the subsequent economic losses vary considerably among infected sheep farms, even between flocks with apparently similar characteristics. Some inter-flock variation might be related to differences in the stage of the disease epidemic between flocks; however, there appear to be other risk factors that are capable of affecting OJD prevalence on-farm.

Limited information about the risk factors for OJD is reported in the literature. Two Spanish studies identified practices related to intensive management such as herd size, foreign breeds and high replacement rate as risk factors for OJD (Mainar-Jaime and Vazquez-Boland, 1998; Reviriego et al., 2000). A postal survey of affected farmers in Australia reported associations with factors such as time since flock infection, altitude, breed, culling practice, proportion of quality pasture and soil texture (Lugton, 2004). However, these findings were not conclusive due to limitations of the study design (particularly the subjective nature of outcome variables). An opportunistic investigation of risk factors for OJD mortality rate on 12 infected farms in southern NSW found associations with flock size, proportion of improved pasture, stocking rate and lamb weaning age but interpretation was constrained by the small sample size (Toribio et al., 2004). A recent field trial, also conducted in southern NSW, identified high levels of pasture contamination and exposure of young sheep as the main factors responsible for presence of severe histological lesions or occurrence of mortality due to OJD (Whittington and McGregor, 2005) but that study was not designed to investigate other risk factors.

Currently, vaccination with killed MAP vaccine (Gudair™) is the only practical method of OJD control. Though effective, it is expensive and there might be circumstances, such as low prevalence areas or flocks, where control without vaccination would be desirable. On-farm control strategies such as purchasing sheep from only known uninfected traders, decontaminating lambing or weaning paddocks by destocking, and culling infected sheep are recommended to farmers but little is known about their impacts. Eradication by destocking sheep has had limited success in Australia with the disease reappearing in flocks when restocked (Taylor, 2004).

We conducted this study to identify risk factors for OJD that could be manipulated by sheep farmers to improve on-farm disease control in the absence of vaccination, or as an adjunct to vaccination on some farms.

2. Methods

2.1. Study design and sampling methodology

2.1.1. Selection of sheep flocks

The reference population for this cross-sectional study was OJD-infected sheep flocks in Australia and the study population was flocks that met the following selection criteria:

1. Self-replacing Merino flocks infected with MAP for ≥ 3 years;
2. Flocks that included ≥ 210 non-vaccinated sheep in 3-year-old, 3- and 4-year-old, or 4- and 5-year-old age groups.

We screened official data to identify known OJD infected flocks and then contacted their owners or managers by phone to gain approval for their participation and to determine whether their flock met the selection criteria. A sample size of 80 to 100 flocks was required to provide 90% or 95% confidence of detecting a significant difference of odds ratio of 5, assuming 10% of flocks in the low-prevalence group have the factor of interest and there are equal numbers of low- and high-prevalence flocks.

2.1.2. Selection of sheep cohorts within flocks

A cohort represented sheep of a specific age and sex group in a flock and was enrolled at the time of faecal-sample collection. We designated the size of a cohort to be 210 sheep per flock because MAP culture results for 7 pools of 30 sheep were considered to be adequate to classify each cohort as high, medium or low prevalence. We sought to collect all 7 faecal pools (of 30 sheep each) from one specific age and sex group (preferably 3-year-olds of the sex with the largest number of animals on the farm) in a flock, so that one cohort was enrolled per flock. However, two or more cohorts per flock were enrolled in two circumstances:

- In flocks where ≥ 210 animals of each sex in one age group were available, we collected 7 faecal pools from each sex, thus enrolling 2 cohorts in these flocks.
- Secondly, when 210 sheep of one specific age and sex group were not available in a flock on the day of sampling, we collected additional samples to make a total of 7 pools from either an older age group or the other sex or both, thereby selecting 2 or more cohorts from the same flock.

For 12 flocks enrolled in this study, we also used pooled faecal-samples previously collected (February 2003 to April 2004) from 3- and 4-year-old sheep (usually 7 pools of 50 sheep per flock).

2.1.3. Pooled faecal-sample collection and culture

Faecal-sample collection from cohort sheep was performed by the district veterinarian located in the district of each enrolled flock. Individual animals were selected using systematic sampling and one faecal pellet was collected per rectum from each selected sheep. The pellets collected from 30 consecutive sheep were pooled in a sterile labelled jar; the collectors changed gloves between pools. The pooled samples were stored in a refrigerator at 4 ^{O}C until dispatched to the laboratory and at -80 ^{O}C in the laboratory, until cultured.

Each pooled faecal sample was cultured using a modified BACTEC radiometric method (Whittington et al., 2000). Briefly, the sample was decontaminated by the double-incubation method using vancomycin, nalidixic acid and amphotericin B (VAN) prior to culture (Whitlock and Rosenberger, 1990). Radiometric BACTEC 12B media supplemented with PANTA PLUS, mycobactin J and egg yolk was used for culturing. Confirmation of MAP in the broth culture was done using polymerase chain reaction (PCR) and restriction endonuclease analysis (REA) by demonstrating the presence of IS*900* (Cousins et al., 1999; Whittington et al., 1998). For pools which exhibited growth in BACTEC medium but were PCR negative, DNA was purified from the broth by column chromatography on silica (Wizard PCR preparations, Promega) and PCR was re-performed.

Sensitivity of PFC varies with pool size and disease pathology. At pool sizes varying from 10 to 100, the PFC sensitivity ranged from 0.9 to 1 in multibacillary sheep, and from 0.45 to 0.6 in paucibacillary sheep, considering only one infected pellet per pool (Whittington et al., 2000; Whittington et al., 1998). In contrast, the specificity of PFC is considered to be near perfect, partly because of the nature of the test (because it identifies MAP) and partly because samples detected positive in culture were further verified by PCR and REA.

2.2. Farmer interview

A questionnaire to collect information about risk factors from each enrolled flock was designed following standard guidelines (Cameron et al., 2004 pp. 15-54; Dohoo et al., 2004 pp. 53-64). It had three sections to gather information about the farm environment and management, OJD infection history and management, and cohort history and management. It consisted of open, closed and semi-closed questions (checklist, two-choice, multiple choice and rating questions) and was piloted with four sheep producers to improve design and aid producer response. A complete copy of the questionnaire (in English) is available from the corresponding author on request.

The questionnaire was administered in a face-to-face interview with the owner or manager of each enrolled flock. Two animal-health professionals with experience in sheep husbandry conducted interviews over 4 months from August to December 2004, each lasting for approximately 1½ hours (range 1 to 2 hours). Both interviewers participated in pilot interviews to reduce inter-observer variation in questionnaire administration. The farmers were compensated for their time.

The method for selection of farmers, structure and implementation of questionnaire and compensation for farmer time was approved by the human ethics committee at The University of Sydney.

2.3. Data management

A relational database created in Microsoft Access was used to manage questionnaire and laboratory data, but for statistical analyses we imported all the tables from this database into SAS statistical software (release 9.1, © 2002-03, SAS Institute Inc., Cary, NC, USA). The data set for all analytical analyses presented in this paper 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).

2.4. Statistical analysis

2.4.1. Outcome variables

We used three outcome variables: cohort OJD prevalence level (CPREV), positive or negative status of faecal pools (PSTATUS) and the log pool MAP number (MAPNUM) for three separate analyses.

2.4.1.1. Cohort OJD prevalence level (CPREV)

Individual-animal OJD prevalence for each cohort was calculated based on the PFC results employing the variable pool size method of William and Moffit (2001). Pooled Prevalence Calculator, an online program (http://www.ausvet.com.au/pprev), facilitated the application of this method (Sergeant, 2004). Although this method assumed perfect test sensitivity and specificity, it was the only method available that could incorporate variable pool size in prevalence calculation. It was therefore the most appropriate method for the pool data in this study, which due to logistics of sample collection, contained pools of varying size.

The resulting cohort OJD prevalence was categorised to designate each sheep cohort as either a low-(<2%), medium-(2-10%) or high-(>10%) prevalence cohort, based on expert advice about OJD biology and dynamics under Australian circumstances. We used this outcome variable, cohort OJD prevalence level (CPREV), in univariable and multivariable ordinal logistic regression analyses to identify factors statistically associated with prevalence level and to quantify the magnitude of these associations.

2.4.1.2. Pool OJD status (PSTATUS)

The PFC result for each faecal pool was used to create this binary outcome variable representing the positive or negative OJD status of each pool. We used the binary variable in binomial logistic regression analyses at the univariable level and in generalised linear mixed-model analyses to identify factors statistically associated with positive pool status.

2.4.1.3. Log pool MAP number (MAPNUM)

The log of the viable number of MAP per gram of faeces was calculated for each faecal pool based on the number of days taken by the sample to reach a cumulative growth index of 1000 (dcgi1000) in the BACTEC media. We used the model: Log_{10} inoculum size = 9.25 – (0.185*dcgi1000) (Reddacliff et al., 2003). We exponentiated log_{10} inoculum size to obtain absolute MAP numbers and assigned zero MAP numbers to all negative samples. To avoid negative infinity for the log of zero, all MAP numbers were increased by 1 before calculating log_{10} to create the continuous outcome variable, MAPNUM. We used this continuous outcome variable in linear regression at the univariable level and in general linear mixed model analyses to identify factors statistically associated with MAPNUM.

2.4.2. Explanatory variables

The explanatory variables investigated in this study are listed in Table 1. All 68 of these explanatory variables were categorical with discrete data collected for 18 and continuous data collected for 22 and then categorised based on median (or quartiles where appropriate) or on biological plausibility. Further, 28 were categorical composite variables created using information from two or more questions in the questionnaire.

Table 1: 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	Variables
Flock-level variables	
Potential Confounders	5-year mean OJD mortality; Age of youngest mortality; Current OJD mortality; Interviewer's assessment of duration of OJD infection; Level of infection (based on trend in mortalities); Peak OJD mortality; Signs of OJD observed by farmer.
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; Interviewer's assessment of effectiveness of worm-control program; Interviewer's 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 1 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 being 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 paddock 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-paddock 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-paddock 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-paddock 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

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.

All mortality variables (peak, current, and mean mortality) had 3 categories (nil, low and high) based on 2% cut-off between low and high, because mortalities are readily discerned by producers at ≥ 2% level. Similarly the variable 'Years since commencement of OJD vaccination' had three categories (nil, 1-2 years and >2 years) because a previous study indicated that it takes about 2 years for vaccination to show its effects (McGregor, 2004). Lambing season was categorised based on expert advice about cut-off dates for various seasons based on farm altitude.

2.4.3. Descriptive analyses

We calculated percentages for categorical variables and median, percentiles and range for continuous variables to provide a detailed description of each variable using all available data on explanatory variables and outcome variables.

2.4.4. Univariable analyses

We performed univariable analyses to investigate the association between each outcome variable and each explanatory variable on an individual basis using the likelihood-ratio chi-square test for CPREV and PSTATUS and F test for MAPNUM. Explanatory variables unconditionally associated with each outcome variable at $P < 0.25$ were selected for further analyses (all P-values mentioned in this paper are two sided). All selected variables were tested for collinearity in pairs using Spearman rank correlation (all selected variables had some inherent order or were created by categorisation of continuous data) and highly correlated variables (coefficient > $|0.70|$) 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 detected, only one out of the pair of variables was retained for further multivariable analysis based on our opinion of biological plausibility. 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.

2.4.5. Multivariable analyses

2.4.5.1. Model building for cohort OJD prevalence level (CPREV)

A manual stepwise procedure was used during the construction of ordinal models. Forward variable selection was based on changes in log likelihood (retaining variables with P < 0.10), with further 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) using the SAS LOGISTIC procedure (Stokes et al., 2000). We exported the results of log likelihood for each model to Microsoft Excel as suggested by Dhand and Toribio (2006), and calculated likelihood-ratio chi-square tests and the respective P values in Excel. Three variables (cohort age, cohort sex and current flock OJD mortality) were forced into each model as fixed effects. First-order interaction terms were then added to the final model and retained when significant at P < 0.05. The proportional odds assumption of the model was tested by the score test (Clark, 2005).

2.4.5.2. Model building for pool OJD status (PSTATUS)

Generalised linear mixed models for pool OJD status were constructed using the SAS GLIMMIX procedure (Anonymous, 2005; Schabenberger, 2005). The technique for model building was similar to that described above for CPREV except for the addition of a random-effects flock variable in the model to account for clustering of pools within flock. Log of pool size was forced into the model as a fixed effect to account for variable number of animals constituting different pools, in addition to cohort age, cohort sex and current OJD mortality. We estimated the intra-cluster correlation (ICC) for PSTATUS at flock level by considering PSTATUS as a continuous variable and calculating the proportion of total variance explained by flock using the SAS MIXED procedure. Although this method was not ideal, it provided an approximation of ICC and we were unaware of an alternate method using available statistical software.

2.4.5.3. Model building for pool MAP number (MAPNUM)

General linear mixed models for MAPNUM were constructed using the SAS MIXED procedure (Brown and Prescott, 2000) following a procedure similar to that for PSTATUS. We added random effects to allow for clustering at flock level and forced log of pool size into the model in addition to other confounders, similar to the PSTATUS model. ICC at flock level was calculated using MAPNUM as the outcome variable (Dohoo et al., 2004 pp. 475-479). Model assumptions and influential diagnostics were checked by examining residuals.

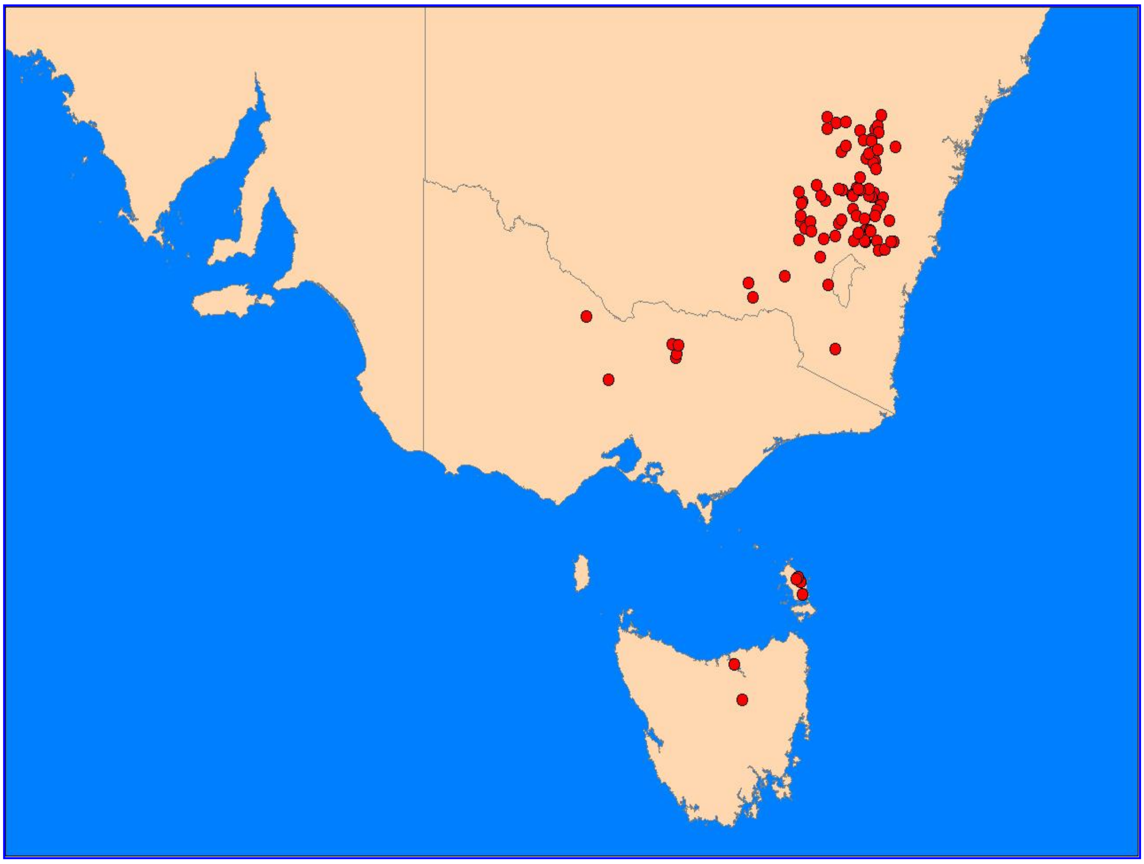
3. Results

3.1. Study flocks

We investigated a total of 233 known OJD-infected flocks to identify eligible flocks, of which the eligibility of 32 (13.7%) could not be determined because the farmer refused to participate for various reasons (lack of interest (6), old age or health problems (4), inability to muster sheep (2), anger about past surveys (1) and no reason given (19)). Of the remainder, 92 flocks met the study selection criteria and all these were enrolled. Farmer interviews were conducted from 18th August to 21st December 2004. Most of the enrolled flocks were located in New South Wales (77) of which 26 were in Central Tablelands, 16 in Goulburn, 12 in Young, 10 in Yass, 7 in Gundagai, 4 in Molong and 2 in Hume Rural Land Protection Board (RLPB) districts. In addition, 15 flocks were enrolled from other states of Australia (7 from Victoria, 6 from Tasmania and 2 from Western Australia) (Figure 1).

These flocks were run on a median farm size of 1032 hectares (range 81 to 8100) of which 40 to 100% was being grazed by sheep. On these farms, median numbers of sheep per flock were 1705 (range 0 to 12,324) for ewes and 600 (0 to 11,808) for wethers. Wool production was the sole enterprise on 19 farms and was combined with only either cattle production or cropping on 27 and 8 farms, respectively. For the remaining 38 farms, wool was one of three or more enterprises.

Figure 1: Distribution of flocks included in the ovine Johne's disease risk factor study conducted in 2004-05 in Australia. Inset shows the location of all 92 study flocks whereas the main map depicts south-eastern region of Australia where 90/92 flocks were located. Numbers of flocks enrolled in each state are given in parenthesis.



3.2. OJD infection history and control

All 92 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). Owners or managers of 73 flocks reported sheep deaths due to OJD with median current- and peak-flock OJD mortality in 92 study flocks being 0.9% (range 0 to 20%) and 1.3% (0 to 20%), respectively. Signs of OJD were reported to be death (73 flocks), loss of condition (72) and scouring (55) but owners or managers of 19 flocks reported seeing no signs of clinical disease.

One or more OJD-control practices were implemented by the owners or managers of 88 study flocks. Vaccination of sheep with killed MAP vaccine, Gudair™, was reported for 79 study flocks of which 21 had been vaccinating for 1 or 2 years, 53 for 3 or 4 years, 4 for 5 years and 1 for 7 years. This vaccine, available for experimental work since 1999, was not approved for general use in Australia until April 2002. Other control procedures used included 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).

3.3. Outcome variables

Pooled faeces and information about 124 sheep cohorts was collected from the 92 study flocks, of which 98 cohorts (representing 87 flocks) were included in the analytical data set (Table 2). Of these, 77 were made up of 7 pools each while the remaining 21 were made up of 4 to 6 faecal pools each. We collected 717 pools, but after excluding those from 5-year-old sheep, 673 pools representing 88 flocks remained.

Descriptive information about median OJD prevalence, MAP numbers per gram of faeces and frequency of OJD positive pools is presented in Table 2.

Table 2: Descriptive information about three outcomes in the ovine Johne's-disease (OJD) risk-factor study conducted in 2004-05 in Australia. Cohort OJD prevalence level (CPREV) is based on the cohort level data set while pool OJD status (PSTATUS) and log pool MAP number (MAPNUM) are based on the pool level data set.

	Sex groups		Age groups	
	Ewe	Wether	3-year old	4-year old
Cohort OJD prevalence level (CPREV)				
Number of cohorts	74	24	59	39
Median OJD animal level prevalence (%)	4	6	5	4
Pool OJD status (PSTATUS)				
Number of pools	501	172	409	264
Number of pool positive	318	133	286	165
% of pool positive	64	77	70	63
Pool MAP number[a]				
Median MAP number/gram faeces	71	912	254	166

a. Pool MAP number was transformed to create MAPNUM outcome variable as described in section 2.4.1.3. (Log pool MAP number)

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

Of the 68 variables investigated, 19 flock-level variables (including all 7 confounders) and 16 cohort-level variables were unconditionally associated with cohort OJD prevalence at $P < 0.25$. After deletion of highly correlated variables, a total of 29 remained for inclusion in multivariable models. Contingency tables of some important variables for CPREV are shown in Table 3; information about the remaining variables is available from the corresponding author on request. Final-model results are presented in Table 4 and Figure 2. The assumption of proportional odds for ordinality of the outcome was fulfilled (score test $P = 0.5$) and the model explained about 66% of the variation in the data. We did not use a random-effects model for cohort OJD prevalence because 76 flocks were represented by only one cohort and the remaining 11 by two cohorts each.

Table 4: Final ordinal logistic regression model for cohort OJD prevalence categorised as low (<2%), medium (2-10%) and high (>10%), based on 97 sheep cohorts in 86 flocks in the Ovine Johne's-disease risk-factor study conducted during 2004-05 in Australia.

Parameters	*b*	*SE(b)*	OR and 95% CL[a]		*P*[b]
Constant	-	-	-	-	-
>10% versus 2-10% and <2%	-4.62	1.06	-	-	-
>10% and 2-10% versus <2%	0.07	0.90	-	-	-
Confounders	-	-	-	-	-
Cohort age	-	-	-	-	0.9
3 years	0.00	-	1	-	-
4 years	-0.10	0.60	0.9	0.3, 3.0	-
Cohort sex	-	-	-	-	0.001
Ewes	0.00	-	1	-	-
Wethers	1.98	0.61	7.2	2.3, 25.9	-
Current OJD mortality [c]	-	-	-	-	<0.001
No mortalities	0.00	-	-	-	-
<2% mortalities	2.37	1.07	-	-	-
≥ 2% mortalities	6.03	1.30	-	-	-
Fixed Effects	-	-	-	-	-
Application of fertilizers other than super phosphate and lime	-	-	-	-	0.001
No	0.00	-	1	-	-
Yes	-2.89	0.92	0.06	0.01, 0.3	-
Lambing paddock stocking rate [d]	-	-	-	-	0.001
< 14 dse /hectare	0.00	-	-	-	-
≥ 14 dse /hectare	3.45	1.20	-	-	-
Presence of wildlife other than kangaroos and rabbits	-	-	-	-	0.09
No	0.00	-	1	-	-
Yes	-0.91	0.54	0.4	0.1, 1.2	-
Years since commencement of OJD vaccination	-	-	-	-	<0.001
1 or 2 years	0.00	-	1	-	-
>2 years	-2.61	0.80	0.07	0.01, 0.3	-
Vaccination not being done	-2.76	0.92	0.06	0.01, 0.4	-
Current OJD mortality × Lambing paddock stocking rate	-	-	-	-	0.002
<2% mortalities × high SR	0.18	1.34	-	-	-
≥ 2% mortalities × high SR	-3.63	1.42	-	-	-
Deviance = 83.02, DF=127; P=0.9					

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 years old) for previous 12 months; d. 1 dam = 2.45 dry-sheep equivalent (dse)

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

Of the 68 variables investigated, 29 flock-level variables (including all 7 confounders) and 19 cohort-level variables were unconditionally associated with pool OJD status. After deleting highly collinear variables, 41 remained for inclusion in the final model. Descriptive information about some important variables is shown in Table 3 and the final-model results in Table 5 and Figure 2. ICC was 0.31 indicating that a substantial proportion of total variation is clustered at the flock level.

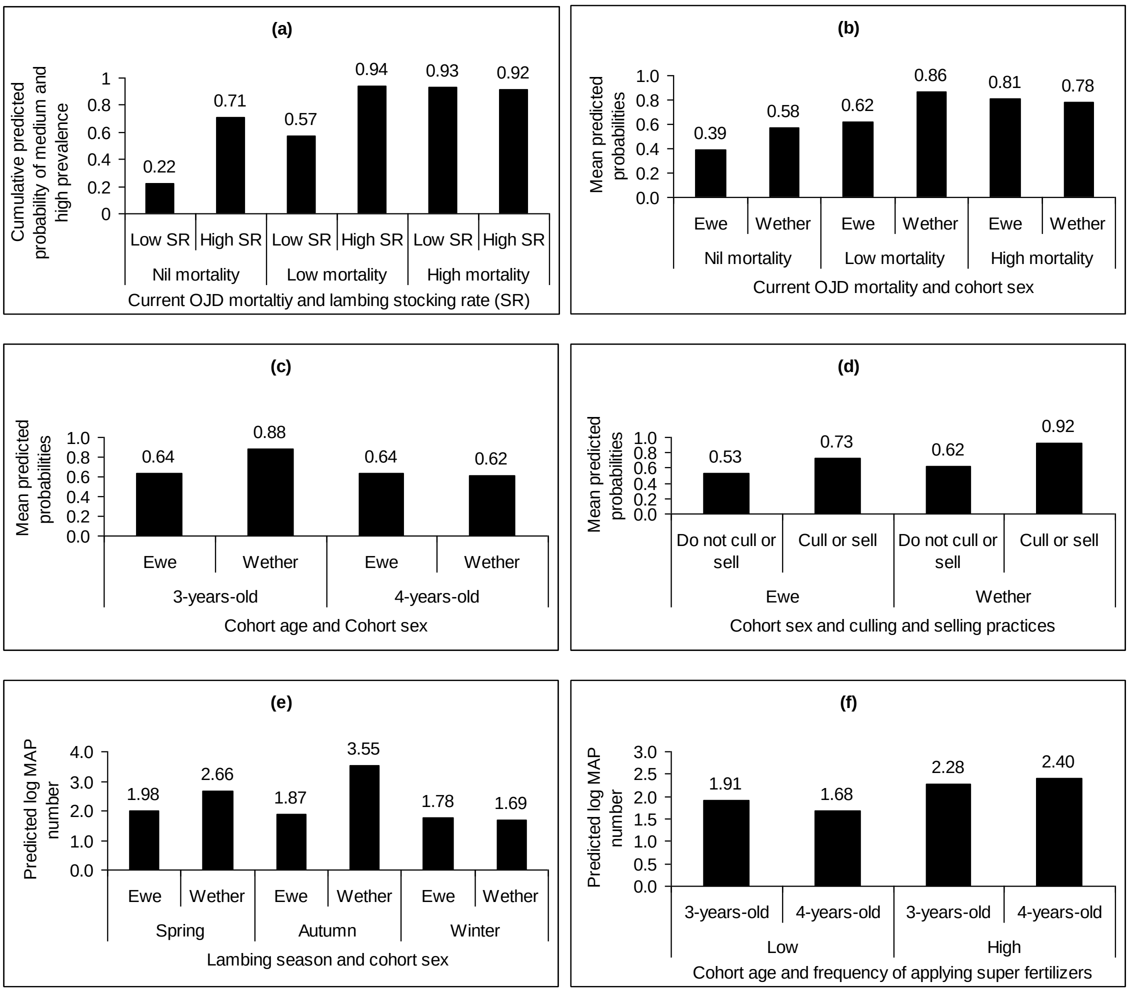
The variability in the data was appropriately modelled and there was no residual over-dispersion (ratio of the generalized chi-square and its degrees of freedom = 0.92).

Figure 2: Interaction terms present in three models implemented in ovine Johne's disease risk factor study conducted in Australia in 2004-05.

Cohort OJD prevalence (CPREV) model: Cumulative predicted probabilities of medium and high prevalence for current mortality and lambing paddock stocking rate (a)

Pool OJD status (PSTATUS) model: Modification of effect of sex by current mortality (b), age group (c), and culling low-body-weight sheep or selling sub-flocks experiencing high losses (d).

Log pool MAP number (MAPNUM) model: Effect modification between season of lambing and sex groups (e), and between age groups and frequency of application of super-phosphate fertilizers (f)



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

Of the 68 variables investigated, 27 flock-level variables (including all confounders) and 19 cohort-level variables were unconditionally associated with log pool MAP number. After deleting collinear variables, 39 remained for inclusion in the final model (Details about collinear variables are available from the corresponding author on request). Descriptive statistics of MAPNUM according to categories of important variables are shown in Table 3 and the final model results in Table 6 and

Figure 2. Similar to PSTATUS model, the ICC was 0.31 justifying the use of mixed models. Examination of residuals revealed no evidence of departure from normality and no unusual influence of any observation in the model.

Table 3: Contingency tables (for cohort OJD prevalence level: CPREV and pool OJD status: PSTATUS) and descriptive statistics (for log pool MAP number: MAPNUM) for selected explanatory variables [a] in the ovine Johne's-disease risk-factor study conducted in 2004-05 in Australia. CPREV is based on the cohort level data set (98 cohorts) while PSTATUS and MAPNUM are based on the pool-level data set (673 pools).

Variables and categories	Cohort OJD prevalence level: CPREV (Number of cohorts)			Pool OJD status: PSTATUS (Number of pools)		Log pool MAP number: MAPNUM (Descriptive statistics)		
	<2%	2-10%	>10%	Negative	Positive	Min	Median	Max
Confounders								
Cohort age								
3 years	3	13	32	59	286	0	2.4	5.9
4 years	4	11	20	39	165	0	2.2	6.1
Cohort sex								
Ewes	20	43	11	183	318	0	1.9	6.1
Wethers	4	9	11	39	133	0	3.0	5.9
Current OJD mortality [b]								
No mortalities	13	10	1	93	70	0	0.0	5.2
<2% mortalities	9	20	10	83	185	0	2.2	5.9
≥ 2% mortalities	2	22	11	46	196	0	3.2	6.1
Fixed Effects								
Application of fertilizers other than super phosphate and lime								
No	19	46	22	181	415	0	2.4	6.1
Yes	5	6	0	41	36	0	0.0	5.6
Condition score of ewes at start of lambing [c]								
≤ 3	9	31	13	103	257	0	2.8	6.1
> 3	14	21	9	110	186	0	1.7	5.9
Cull low-body-weight sheep or sell sub-flocks experiencing high losses								
No	16	21	6	134	168	0	0.4	5.9
Yes	8	31	16	88	283	0	2.8	6.1
Frequency of application of super-phosphate fertilizers								
≤ twice in 3 years or nil	14	28	8	129	213	0	1.7	6.1
> twice in 3 years	10	24	14	93	238	0	2.6	5.9
Lambing paddock stocking rate [d]								

<14 dse/ha	19	20	8	137	189	0	1.1	5.9
≥ 14 dse/ha	5	32	13	81	251	0	2.8	6.1
Period of growth check in the life of the cohort								
<12 weeks	16	19	8	121	179	0	1.2	5.9
≥ 12 weeks	8	33	14	97	266	0	2.8	6.1
Presence of rabbits on the farm								
No	8	14	8	75	140	0	2.4	5.9
Yes	16	38	14	147	311	0	2.2	6.1
Presence of wild animals other than kangaroos and rabbits								
No	14	32	20	134	323	0	2.4	6.1
Yes	10	20	2	88	128	0	1.0	5.4
Proportion of farm boundary receiving run off water								
≤ 10%	5	19	9	55	168	0	2.6	5.6
>10 to ≤ 30%	5	15	4	57	108	0	1.9	6.1
>30% to ≤ 60%	5	13	5	46	111	0	2.4	5.9
> 60%	9	5	4	64	64	0	0.0	5.9
Season of lambing								
Spring	10	28	15	103	254	0	2.4	5.4
Autumn	2	9	2	24	65	0	2.2	6.1
Winter	12	14	5	88	122	0	1.6	5.9
Sharing of roads among neighbours								
No	20	30	12	160	257	0	1.7	6.1
Yes	4	22	10	62	194	0	2.8	5.6
Years since commencement of OJD vaccination								
1 or 2 years	3	11	10	42	131	0	3.0	5.6
>2 years	16	33	11	136	268	0	2.0	6.1
Vaccination not being done	5	8	1	44	52	0	0.5	5.2

a. Only the variables significant in any of the final models are included; information about rest of the variables can be obtained from the corresponding author on request b. Farmer reported flock-OJD mortality in adult sheep (>2 year old) for previous 12 months; c. Body-condition scores: 1-emaciated, 2-thin, 3-average, 4-fat, 5-obese.; d. 1 dam = 2.45 dry sheep equivalent (dse)

Table 5: Final generalised linear mixed model for pool OJD status (PSTATUS), based on culture results of 644 faecal pools (positive or negative) collected from 84 flocks in the ovine Johne's-disease risk-factor study conducted in Australia in 2004-05.

Parameters	*b*	*SE(b)*	OR and 95% CI	*P*

Flock-level random effects	0.44	0.23	-	-	-
Constant	-3.87	-	-	-	-
Confounders	-	-	-	-	-
Cohort age	-	-	-	-	0.008
3 years	-	-	-	-	-
4 years	-0.05	0.33	-	-	-
Cohort sex	-	-	-	-	0.1
Ewes	-	-	-	-	-
Wethers	1.64	0.81	-	-	-
Current OJD mortality [a]	-	-	-	-	0.4
No mortalities	-	-	-	-	-
<2% mortalities	0.45	0.39	-	-	-
≥ 2% mortalities	2.19	0.37	-	-	-
Log of faecal pool size	1.32	0.68	3.7	1, 14.3	0.05
Fixed Effects					
Application of fertilizers other than super phosphate and lime	-	-	-	-	<0.001
No	-	-	1.0	-	-
Yes	-1.76	0.43	0.2	0.07, 0.4	-
Condition score of ewes at start of lambing [b]	-	-	-	-	0.01
≤ 3	-	-	1.0	-	-
> 3	-0.71	0.29	0.5	0.3, 0.9	-
Cull low body weight sheep or sell sub-flocks experiencing high losses	-	-	-	-	<0.001
No	-	-	-	-	-
Yes	0.29	0.31	-	-	-
Lambing paddock stocking rate [c]	-	-	-	-	0.006
< 14 dse/hectare	-	-	-	-	-
≥ 14 dse/hectare	0.84	0.30	2.3	1.3, 4.2	-
Season of lambing	-	-	-	-	0.05
Spring	-	-	1.0	-	-
Autumn	0.13	0.47	1.1	0.5, 2.9	-
Winter	-0.66	0.29	0.5	0.3, 0.9	-
Sharing of roads with neighbours	-	-	-	-	0.05
No	-	-	1.0	-	-
Yes	0.57	0.29	1.8	1, 3.2	-
Years since commencement of OJD vaccination	-	-	-	-	0.004
1 or 2 years	-	-	1.0	-	-
>2 years	-1.22	0.40	0.3	0.1, 0.6	-
Vaccination not being done	-1.26	0.47	0.3	0.1, 0.7	-
Interaction terms[d]					
Cohort sex × Cohort age group	-	-	-	-	0.004
wethers × 4-years	-2.27	0.80	0.1	0.02, 0.5	-
Cohort sex × Cull low body weight sheep or sell sub-flocks experiencing high losses	-	-	-	-	0.001
wethers × cull or sell	2.95	0.90	19.2	3.3, 111.9	-
Current OJD mortality × Cohort sex		-	-		0.002
<2% mortalities × wethers	-0.89	0.93	0.4	0.07, 2.6	-
≥ 2% mortalities × wethers	-3.33	1.04	0. 04	0, 0.3	-
Generalised chi-square = 574.2					
Generalised chi-square/d.f. = 0.92					

a. Farmer-reported flock-OJD mortality in adult sheep (>2 years 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 6: Final linear mixed model for log pool MAP number (MAPNUM) based on 644 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	0.13	0.08	0.05, 0.8	0.05
Residual	2.28	0.14	2.0, 2.6	<0.001
Constant	-0.42	1.51	-3.4, 2.6	-
Confounders	-	-	-	-
Cohort age	-	-	-	0.08
3 years	0.00	-	-	-
4 years	-0.09	0.24	-0.6, 0.4	-
Cohort sex	-	-	-	0.004
Ewes	0.00	-	-	-
Wethers	1.00	0.24	0.5, 1.5	-
Current OJD mortality [a]	-	-	-	<0.001
No mortalities	0.00	-	-	-
<2% mortalities	0.55	0.21	0.1, 1	-
≥ 2% mortalities	1.33	0.21	0.9, 1.8	-
Log of pool size	0.71	0.43	-0.1, 1.6	0.09
Fixed Effects	-	-	-	-
Application of fertilizers other than super phosphate and lime	-	-	-	0.001
No	0.00	-	-	-
Yes	-0.84	0.26	-1.4, -0.3	-
Condition score of ewes at start of lambing [b]	-	-	-	0.001
≤ 3	0.00	-	-	-
> 3	-0.53	0.16	-0.8, -0.2	-
Cull low body weight sheep or sell sub-flocks experiencing high losses	-	-	-	0.07
No	0.00	-	-	-
Yes	0.33	0.18	-0.03, 0.7	-
Frequency of application of super phosphate fertilizers	-	-	-	0.001
≤ twice in 3 years or nil	0.00	-	-	-
> twice in 3 years	0.15	0.21	-0.3, 0.6	-
Lambing paddock stocking rate [c]	-	-	-	0.001
< 14 dse/hectare	0.00	-	-	-
≥ 14 dse/hectare	0.61	0.19	0.2, 1	-
Period of growth check in the life of the cohort	-	-	-	0.08
<12 weeks	0.00	-	-	-
≥ 12 weeks	0.28	0.16	-0.04, 0.6	-
Presence of rabbits on the farm	-	-	-	0.06
No	0.00	-	-	-
Yes	-0.35	0.18	-0.7, 0.01	-
Presence of wild animals other than kangaroos and rabbits	-	-	-	0.01
No	0.00	-	-	-
Yes	-0.42	0.17	-0.8, -0.08	-
Proportion of farm boundary receiving run off water	-	-	-	0.001
≤ 10%	0.00	-	-	-
>10 to ≤ 30%	-0.78	0.23	-1.2, -0.3	-
>30% to ≤ 60%	-0.83	0.23	-1.3, -0.4	-
> 60%	-0.53	0.24	-1, -0.06	-

Season of lambing	-	-	-	<0.001
Spring	0.00	-	-	-
Autumn	0.14	0.30	-0.5, 0.7	-
Winter	-0.27	0.20	-0.7, 0.1	-
Years since commencement of OJD vaccination	-	-	-	0.06
1 or 2 years	0.00	-	-	-
>2 years	-0.52	0.22	-1, -0.1	-
Vaccination not being done	-0.33	0.25	-0.8, 0.2	-
Interaction terms[d]				
Cohort age × Frequency of application of super phosphate fertilizers	-	-	-	0.007
4 years × > twice in 3 years	0.87	0.32	0.2, 1.5	-
Cohort sex × Cohort age	-	-	-	<0.001
wethers × 4 years	-1.42	0.35	-2.1, -0.7	-
Cohort sex × Season of lambing	-	-	-	0.002
wethers × autumn	1.33	0.51	0.3, 2.4	-
wethers × winter	-0.56	0.36	-1.3, 0.2	-

a. Farmer-reported flock-OJD mortality in adult sheep (>2 years 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.

4. Discussion

In this study we contacted all farmers with flock known to be OJD infected in 2004. Despite continuing angst amongst the farmer community over OJD, we achieved a high response rate because of our good working relations with the farmers, administration of questionnaire in person, payment to the farmers for their time and labour (participation in interview and mustering sheep for faecal collection), provision of faecal-culture results free of cost, and relevance of study outcomes to the farmers. High response helped reduce non-response bias in the study which can be differential in nature (Dohoo et al., 2004). Nevertheless, due to our strict selection criteria, we actually enrolled only 39.5% of 233 known infected flocks. Though selection criteria were necessary to control confounding and improve internal validity in the study, we acknowledge that as a result we enrolled a non-representative sample of Australian OJD-infected sheep flocks. Consequently the findings of this study should be extrapolated to other types of flocks with caution.

4.1. Lamb management and nutritional factors

Association of high stocking rate in the lambing paddock with higher OJD infection highlights the importance of MAP pasture contamination and age of exposure in the epidemiology of this disease. High stocking rate leads to greater MAP pasture contamination due to greater numbers of animals per unit area and increased MAP shedding by dams as a result of nutritional stress. Consequently, lambs, known to be highly susceptible to infection, are exposed to higher doses of MAP which results in higher prevalence of disease among them over time. This study complements the results of other studies that young age and environmental contamination were risk factors for paratuberculosis (Beaudeau et al., 2005; Obasanjo et al., 1997; Whittington and McGregor, 2005). Notably, this is the first study to identify an association between paratuberculosis and higher stocking rate although stocking rate was investigated in several earlier studies (Daniels et al., 2002; Lugton, 2004; Reviriego et al., 2000).

There was higher OJD infection prevalence in sheep whose dams had lower condition scores, which are indicative of poor dam nutrition and/or greater stress (resulting in higher MAP shedding and pasture contamination). Alternatively poor condition might indicate higher OJD infection in dams increasing the likelihood of infection transmission to their progeny by both horizontal and vertical routes (Abbott et al., 2004).

Influence of dam nutrition and stress was also apparent from higher OJD infection prevalence among cohorts born in autumn (than those born in spring) when there is usually lower availability of pasture. However, lower OJD infection prevalence in cohorts born in winter (than in spring) was contrary to our expectations and could reflect the importance of weather and pasture conditions at weaning rather than during lambing. Cohorts born in winter are usually weaned in spring when pasture is available in greater quantity (implying better nutrition and less ingestion of contaminated soil). Further evidence of the impact of inadequate nutrition and stress on disease is provided by higher OJD infection prevalence in sheep that faced ≥ 12 weeks of growth retardation (or weight loss).

4.2. Fertilizer application on the farm

In contrast to the finding of lower OJD infection with better nutrition, more frequent application of super-phosphate fertilizers (which should improve pasture growth and nutrition) was associated with higher concentration of MAP in pools. This result was counterintuitive, although it might be due to correlation between frequent fertilizer application and high stocking rates, which in turn were associated with high OJD infection prevalence. However, this needs further investigation particularly because two previous studies found no link between OJD mortality or herd partuberculosis status and fertilizer application (Johnson-Ifearulundu and Kaneene, 1998; Lugton, 2004).

On the contrary, lower OJD infection in flocks applying less common fertilizers (bio-soil, pasture gold, organic manure, reactive phosphorus rock, mono-ammonium phosphate, di-ammonium phosphate, sewage ash, super potash and pasture special) might be because this variable is acting as a surrogate for cropping. Crop production (the most common reason for application of these fertilizers) required a paddock to be free of sheep during the cropping phase; this would reduce MAP pasture contamination level. Although we did not collect data on the cropping status of specific paddocks, 91% of the farms applying these fertilizers did raise crops compared to only 39% of the farms not applying these fertilizers.

4.3. OJD-control methods

Adoption of OJD control methods will, over time, reduce OJD prevalence in an infected flock. This view was corroborated by lower OJD infection prevalence in flocks that had vaccinated sheep against OJD for > 2 years (compared to 1-2 years). McGregor (2004) reported a similar reduction with lower mortality risk in the 3rd and 4th years after commencement of vaccination. On the other hand, we also observed lower OJD infection prevalence in flocks not vaccinating at all (which could reflect lower OJD mortalities in these flocks and thus little incentive to commence vaccination). In our study only 21% (3 of 14) of cohorts from unvaccinated flocks reported high current OJD mortalities in comparison to 38% of cohorts (32 of 84) from vaccinated flocks.

Similarly, we consider the high OJD infection in flocks culling low-body-weight sheep or selling sub-flocks experiencing high losses to be a management response of the farmer to higher OJD mortalities. Two previous studies also identified a significant positive association between culling of clinical or potentially clinical animals and herd/flock status and concluded that this practice was a consequence of higher losses, not a cause (Lugton, 2004; Muskens et al., 2003).

4.4. Lateral spread of OJD

Our finding of higher OJD infection on farms that move sheep along roads shared with neighbours suggests that biosecurity should be part of the OJD control plan for all infected flocks. However, wildlife might not play a major role in infection spread as advocated previously (Daniels et al., 2003), especially in already infected flocks, because we observed lower OJD infection in flocks where rabbits or other wildlife were present. We think that this protective association might reflect better water and food availability on these farms but this requires further investigation. In support of our finding, Corn et al. (2005) observed that wildlife might not contribute importantly to MAP pasture contamination in already infected flocks.

Lower OJD on farms receiving run-off water along >10% of the farm boundary was unexpected because run-off from neighbouring infected farms could bring MAP organisms onto study farms and thus increase farm MAP contamination. However, it is also probable that the additional water fosters pasture growth and is therefore advantageous to the sheep (particularly during drought conditions).

4.5. Control of confounding

To minimise confounding bias, we forced known potential confounders into the multivariable models. We accounted for flock OJD infection level by forcing current OJD mortality as a fixed effect in all multivariable models. This confounder, based on farmer-reported OJD mortality in sheep > 2-years-old during 2004, was selected from the 7 highly correlated flock-level confounders, because compared to other variables it was less likely to be affected by owner recall bias.

At the cohort level, sex and age were similarly forced into all models. As expected, we found age (a well documented confounder in JD studies) to be associated with OJD infection. The trend of higher OJD in 3-year-olds compared to 4-year-olds reflects losses of some infected animals born in 2000 prior to our faecal collection. In contrast, this is the first study to identify an association between sex and OJD infection (Table 2), thus confirming the anecdotal reports of higher losses in wethers compared to ewes. This gender difference is most likely the consequence of farmers giving the nutrition and health of ewes priority over that of wethers.

4.6. Study validity, strengths and limitations

A distinguishing feature of our study was the objective measurement of the disease outcome using PFC which has considerably better sensitivity than serology and almost perfect specificity (Sergeant et al., 2001). Thus there was little likelihood of false-positive results, though some infected pools could have been declared false negatives due to imperfect sensitivity. We also acknowledge that the determination of MAPNUM is influenced by co-growth of irrelevant microbes, clumping of MAP in culture and the likelihood of the organisms to be present in a dormant stage (Reddacliff et al., 2003). Bias due to imperfect sensitivity and specificity is generally non-differential in nature, which steers the odds-ratios towards null and hence the associations reported here are likely to be conservative.

In contrast to the objective nature of the outcome variables, many of the explanatory variables measured in this study were potentially affected by differential misclassification (such as owner recall) bias to varying degrees. Though unavoidable in a cross-sectional study, we used various methods to reduce this bias. First, administration of the questionnaire by personal interview helped to avoid misinterpretation of questions by farmers. Second, to reduce bias in some variables, interviewers asked a series of questions and then used criteria to nominate a flock into one of several categories. Third, to reduce the potential impact of misclassification error on continuous variables, most were categorised prior to analysis.

Similar to other cross-sectional studies investigating chronic diseases, the potential for change in management practices from the time of flock infection to the time of survey complicated investigation of management factors in this study. To negate this, we collected information about the management of a specific cohort of sheep over their lifetime. This enabled investigation of the impact of management during specific life stages on subsequent infection status. However, for flock management, we collected information about current practices at the time of interview and assumed that these practices had not changed over time; however, if they had changed, this might have biased the results.

5. Conclusions

Detrimental effects identified in this study were high stocking rate and factors leading to poor body condition of lambing ewes or their progeny, and moving sheep along roads also frequented by sheep from neighbouring flocks. Vaccination with Gudair™ was associated with lower OJD prevalence. We confirmed the anecdotal observation of higher OJD prevalence in wethers than in ewes. Our results indicate that OJD prevalence in infected flocks is a function of the pasture contamination level, nutritional stress and infection at an early age.

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References

Abbott, K., Whittington, R., McGregor, H. 2004. Exposure factors leading to establishment of OJD infection and clinical disease (Sydney, Faculty of Veterinary Science, University of Sydney).

Anonymous 2005. The GLIMMIX Procedure (SAS Institute Inc., Cary, NC) http://support.sas.com/rnd/app/papers/glimmix.pdf.

Beaudeau, F., Le Doux, D., Poumerol, N., Joly, A., Seegers, H., 2005. Risk factors for *Mycobacterium avium* subspecies *paratuberculosis* infection in dairy herds located in Brittany (Western France). In: 8th International Colloquium on Paratuberculosis, Copenhagen, Denmark, p. 255.

Brown, H., Prescott, R., 2000, Applied Mixed Models in Medicine. John Wiley and Sons.

Bush, R.D., Windsor, P.A., Toribio, J.A.L.M.L., 2006, Losses of adult sheep due to ovine Johne's disease in 12 infected flocks over a 3-year period. Australian Veterinary Journal*, 84: 246-54*.

Cameron, A., Sergeant, E., Baldock, C., 2004, Data Management for Animal Health, Vol 1, First Edition. AusVet Animal Health Services, Brisbane, Australia, 185 p.

Clark, V. 2005. SAS/STAT User's Guide (SAS Institute Inc., Cary, NC, USA.).

Corn, J.L., Manning, E.J., Sreevatsan, S., Fischer, J.R., 2005, Isolation of Mycobacterium avium subsp. paratuberculosis from free-ranging birds and mammals on livestock premises. Appl Environ Microbiol 71, 6963-6967.

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.

Daniels, M.J., Henderson, D., Greig, A., Stevenson, K., Sharp, J.M., Hutchings, M.R., 2003, The potential role of wild rabbits Oryctolagus cuniculus in the epidemiology of paratuberculosis in domestic ruminants. Epidemiol Infect 130, 553-559.

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.

Dhand, N.K., Toribio, J.A.L.M.L., 2006. Combining the Power of ODS, Data Set Concatenation, and DDE to Output Customized Statistical Results from SAS® to Microsoft Excel. In: Proceedings of the Thirty-first Annual SAS® Users Group International Conference, San Francisco, CA, March 26-29, 2006, http://www2.sas.com/proceedings/sugi31/135-31.pdf.

Dohoo, I., Martin, W., Stryhn, H., 2004, Veterinary Epidemiologic Research, First Edition. AVC Inc., Charlottetown, Prince Edward Island, Canada.

Johnson-Ifearulundu, 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.

Kennedy, D., Sergeant, E. 2005. National Johne's Disease Control Program: National Technical Advisers' Quarterly Report October-December 2005 (Animal Health Australia), pp. 5-6.

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.

McGregor, H., 2004. A longitudinal study of OJD and the effects of whole flock vaccination with Gudair(TM). In: Ovine Johne's Disease: An update of Australian and International Research, Sydney, Australia, March 2004, pp. 2-4.

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.

Schabenberger, O., 2005. Introducing the GLIMMIX Procedure for Generalized Linear Mixed Models. In: SAS® USERS GROUP INTERNATIONAL, Philadelphia, April 10-13, 2005.

Seaman, J.T., Gardner, I.A., Dent, C.H.R., 1981, Johne's Disease in sheep. Australian Veterinary Journal 57, 102-103.

Sergeant, E. 2004. Pooled Prevalence Calculator (Aus Vet Animal Health Services) http://www.ausvet.com.au/pprev/.

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.

Taylor, P., 2004. Evaluation of eradication strategies for ovine Johne's disease. In: Ovine Johne's Disease: An update of Australian and International Research, Sydney, Australia, March 2004, pp. 29-32.

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, Meat and Livestock Australia).

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., McGregor, H. 2005. Epidemiology of ovine Johne's disease 2 – pasture contamination level and age susceptibility (Sydney, Meat and Livestock Australia (MLA)).

Whittington, R.J., Fell, S., Walker, D., McAllister, S., Marsh, I., Sergeant, E., Taragel, C.A., Marshall, D.J., Links, I.J., 2000, 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., 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.

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.