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On farm

A Study of the Biological and Economic Impact of OJD in affected Sheep Flocks in NSW

12 Farm Mortality Study

Progress report: OJD.023

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Animal Health and Welfare

Abstract

This study provides industry with factual information about the impact of OJD on farms in southern Australia. This is needed to assess the magnitude of the OJD problem and to direct control and management programs. On 12 farms, the average OJD mortality rate based on inventory records was 6.2% (range 2.1% to 17.5%), more than twice the accepted mortality rate (from all causes) for Australian sheep flocks. OJD losses accounted on average for 70% (range 17% to 100%) of the estimated total economic loss associated with sheep deaths over this 12-month study. In addition this project confirmed that prevalence based on pooled faecal culture (PFC) could be used as an indicator of OJD mortality level and provided support for further investigation of several risk factors in a future study focused on a specific sheep cohort and involving a larger number of farms. We recommend that producers, through industry extension activities, be informed of the substantial losses associated with OJD and be encouraged to implement control measures on their farms. We also recommend the development of a model to predict on-farm economic impact of OJD.

Executive Summary

This study, conducted on 12 farms in southern New South Wales (NSW), was undertaken to provide accurate information about the impact of OJD on sheep mortality and economic status on infected farms. This research was deemed important by industry because little credible information was available about the magnitude of the OJD problem and the response required to control and manage OJD in southern Australia.

In brief, the objectives of the study were:

- To determine the mortality rate due to OJD in twelve sheep flocks
- To describe the relationship between age, sex and OJD mortality rate in affected flocks
- To investigate the relationship between OJD mortality rate and OJD prevalence and faecal excretion rate in two-year old sheep based on pooled faecal culture in affected flocks
- To investigate the relationship between OJD mortality rate and age-related seroprevalence of OJD in affected flocks
- To relate seasonal variation in OJD mortality rate to environment, management and factors and identify factors worthy of further investigation
- To provide an accurate estimate of the cost of OJD in affected flocks.

A 12-month observational study was completed on 12 OJD-infected farms in southern NSW. OJD mortality estimates were derived from farm records (livestock inventories) and quarterly farm visits (necropsy inspections). Questionnaires, climatic records and pasture samples enabled a detailed description of each farm to be made and a single collection of blood and faecal samples provided OJD prevalence information for specific age cohorts of sheep in each flock.

From the four 5-day necropsy inspections, a most likely cause of death was determined for 362 necropsied sheep on the basis of findings related to the environment, clinical signs, gross pathology and histopathology. Of these, OJD was most likely to have contributed to the death of 250 sheep, OJD was unlikely to have contributed to the death of 1 sheep and OJD did not contribute to death of 111 sheep. The distribution of necropsied sheep where OJD contributed to death across age groups and sexes showed that OJD mortality increased from 1 year of age (10.4%) to peak at 4 years of age (35.6%) and then fell at over 4 years of age (19.2%), and was very similar between wethers (49.6%) and breeding ewes (50.4%). Distribution across inspection periods showed a trend among OJD-related necropsies and total necropsies with the majority occurring in winter (31%) and spring (35%) and fewer in autumn (18%) and summer (16%).

On the 12 farms, the average OJD mortality rate based on inventory records was 6.2% (median 5.8%, range 2.1% to 17.5%) which was similar to the average OJD mortality rate based on necropsy inspections (6.7%, median 4.4%, range 1.1% to 15.0%). These average mortality rates were more than twice those considered acceptable (from all causes) in sheep flocks in southern Australia.

The OJD prevalence in 2-year old sheep based on pooled faecal culture (PFC) ranged from 0.7% to > 23% on the 12 farms and was found to be associated with OJD mortality rate ($P = 0.02$). In contrast, no significant relationship was found between faecal excretion rate of MAP in two-year old sheep based on PFC and OJD mortality rate, or between age-related OJD seroprevalence and OJD mortality rate.

The association between various environment, management and disease factors and quarterly OJD mortality rate was analysed and several factors (including flock size, stocking rate, area of improved pasture and weaning age) were identified as being important for further investigation. Definite conclusions based on statistical analysis could not be made due to the small number of farms and use of whole flock data, however, the results provide strong support for a study to identify the major risk factors for OJD that involves a large number of farms and focuses on a specific sheep cohort.

Gross margins were calculated for each of the 12 farms assuming each farm was free of OJD and then these were compared with the actual farm gross margin. The average % decrease in gross margin due to a farm being infected with OJD was 6.4% (median 5.5%, range 2.2% to 15.4%). The average gross margin/DSE for the OJD infected flocks was $20.58 (median $22.22, range $10.16 to $36.36) and when the effect of OJD was removed was $21.85 (median $22.86, range $11.77 to $37.19). The average gross

margin/ha for the OJD infected flocks was $167.75 (median $173.01, range $66.12 to $290.86) and when the effect of OJD was removed was $178.65 (median $179.69, range $70.60 to $297.53).

Using the necropsy inspection information the average estimated cost of OJD losses on the 12 farms over the 12- month study period was $64,100 (median $44,942, range $15,569 to $154,083). The average estimated cost of annual OJD losses/DSE was $7.68 (median $4.11, range $0.84 to $20.51) and of annual OJD losses/ha was $65.92 (median $25.09, range $6.75 to $244.80). The estimated cost of OJD losses accounted on average for 70.1% (median 68.5%, range 16.5% to 100%) of the estimated total loss related to sheep deaths over the 12-month study.

This study provided the first objective data on the true impact of OJD on 12 farms, and the findings are generally applicable to sheep flocks in southern Australia. Industry groups claiming that OJD does not present a threat on-farm can now be provided with accurate figures on direct losses attributable to OJD within the endemic area of NSW. There was a wide range of impacts, with some very high mortality rates. The data can be used to justify vaccination programs, other control options and the general concept of disease control and prevention.

Further, this study provided objective data that quantified the economic losses in infected flocks experiencing OJD mortalities and that indicated the need to further investigate some potential risk factors associated with OJD losses.

Recommendations that arise from this work include:

1. Development of a fact sheet on OJD mortalities and direct economic losses as reported here, for distribution through MLA mailing lists and AHA OJD communications program

2. Use of data collected over 3-4 years on the 12 farms to develop a model to predict the economic impact of OJD on individual farms

3. Further investigation of risk factors indicated in this study in the OJD risk factor study (MLA OJD.038)

4. Use of the data reported here for benchmarking, specifically for comparison against future mortality rates measured following adoption of OJD control measures that are currently being considered for inclusion in a revised NOJDP.

The challenge for industry is to use the scientific findings from this study and other recent research to prepare education and extension material to address issues of misinformation about OJD and to, in conjunction with others, develop cost effective strategies for the future control and management of OJD.

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1. Background

Ovine Johne's disease (OJD), a globally dispersed chronic enteric disease of sheep caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is a problem throughout south-eastern Australia, particularly southern New South Wales. There continues to be much debate regarding the impact of OJD on infected farms due to lack of information from any country on mortality rates attributable to OJD. The uncertainty has provided opportunity for some industry action groups to downplay the importance of the disease in the face of a national control program. This has contributed to the significant level of disagreement on appropriate control strategies. This project was established to address a key knowledge gap, with a likely outcome being provision of information for objective debate on control strategies. Establishing the biological impact of OJD will also provide an insight into the economic significance of this disease and will contribute to the development of cost effective strategies for the future control and management of OJD.

In Australia, annual mortality rates attributed to OJD in adult sheep have primarily been based on flock owners' estimates and are extremely variable. They range from less than 1% to over 10%[1] with some mortality estimates possibly as high as 25%[2]. A tendency exists for producers to attribute the majority of losses to OJD once the disease has been diagnosed on their farm despite the existence of other disease states displaying similar clinical signs. Obtaining an accurate estimate of true total annual mortality rates and the proportion of this attributable to OJD is therefore considered important.

Post-mortem examination of every dead sheep over a twelve-month period would provide the most reliable assessment of an annual mortality rate attributable to OJD. The logistical difficulties associated with such a project include locating every dead sheep and collecting suitable samples to enable an accurate diagnosis of the most likely cause of death. This would be time consuming and the expense involved would be prohibitive. A protocol was therefore developed to estimate the annual OJD mortality rate through the sampling of daily mortalities over four 5-day periods[3]. Combined with stock inventory records that document flock numbers throughout the year, this approach has been successfully applied to a single farm since 1999 [2,3] and was considered suitable for extension to a number of farms across several locations. The results of a 12-month study, conducted in 2002, involving twelve sheep flocks from southern NSW are reported in this document.

2. Project objectives

- To determine the mortality rate due to OJD in twelve sheep flocks.
- To describe the relationship between age and OJD mortality rate in affected flocks.
- To describe the relationship between sex and OJD mortality rate in affected flocks.
- To investigate the relationship between OJD mortality rate and prevalence of OJD in two-year old sheep based on pooled faecal culture in affected flocks.
- To investigate the relationship between OJD mortality rate and faecal excretion rate of MAP in two-year old sheep based on pooled faecal culture in affected flocks.
- To investigate the relationship between OJD mortality rate and age-related seroprevalence of OJD in affected flocks.
- To relate seasonal variation in OJD mortality rate to environment, management and disease factors and identify which factors are worthy of further investigation.
- To provide an accurate estimate of the cost of OJD in affected flocks.

3 Methodology

3.1 Overview

A 12-month observational study commencing in March 2002 was carried out on twelve infected farms from southern NSW to investigate the impact of OJD. Mortality estimates were derived from farm records (livestock inventories) and quarterly farm visits (necropsy inspections). Questionnaires, climatic records and pasture samples enabled a detailed description of each farm to be made and a single collection of blood and faecal samples provided OJD prevalence information for specific age cohorts of sheep in each flock.

3.2 Selection of 12 farms

Following a call for expressions of interest through advertisement in The Land newspaper and consultation with Rural Lands Protection Board (RLPB) District Veterinarians (DVs) in southern NSW, twelve OJD infected sheep flocks were enrolled in this study. Farms were selected by purposive sampling due to the need for farms to meet as many of the specified selection criteria (Table 1) as possible and for farm owners/managers to be willing co-operators over the 12-month study period.

Another issue considered during selection of farms was the proximity of farms to each other in order to facilitate visits to three farms each day during a 5-day necropsy inspection period. The 12 farms selected were grouped in four areas (Bungendore / Taralga / Gunning / Harden). These offered a range of topography, soil type, climate and land use, including farms predominately grazing sheep, some grazing both sheep and cattle, and several where grazing was accompanied by cropping.

Table 1
Criteria used to select farms for inclusion in the study

Selection criteria	Number of farms that met criteria
OJD positively diagnosed	12
OJD present for 4 years or more	11
Farmer estimated annual mortalities due to OJD ≥ 5%	12
Self-replacing Merino sheep operation	11
Sheep numbers > 4000 head	11
All animals > 1-year of age not vaccinated with Gudair®	12

3.3 Data collection

3.3.1 Questionnaires and records

Questionnaires

An extensive questionnaire was delivered during the first visit to each farm in March 2002 to collect information on current flock management practices as well as a detailed farm and flock history. In January 2003 each farm received an additional survey to document the effects of the dry seasonal conditions,

experienced during the 12 months of the study, on flock management.

Rainfall, temperature and evaporation

The monthly distribution of rainfall was recorded using a daily rainfall chart while temperature and evaporation data were gained from the official meteorological station[4] closest to each property.

Flock inventory

To provide accurate estimates of mortality rate, each farm maintained a flock inventory throughout 2002, with flock numbers recorded on each occasion the sheep were handled, and all sales, purchases and slaughterings documented.

3.3.2 Necropsy inspection periods

The necropsy inspection periods were 5-days in length (Monday to Friday) and were conducted to coincide with each season: autumn (March/April), winter (June), spring (September) and summer (November / December).

Pasture samples

To estimate available pasture and nutritional status at each necropsy inspection period, pasture samples representing a cross section of paddocks at each farm were collected using a $0.1m^2$ quadrat, one sample per site. The sites chosen during the first visit were sampled at each subsequent necropsy inspection period. These samples were dried for 72 hours at 65^oC to determine the pasture availability (quantity – DM/m^2). The pasture quality was then determined by assessing the crude protein (nitrogen) and acid detergent fibre (ADF) for each sample, by the Kjeldahl technique[5,6], in order to predict the digestible dry matter % (DDM)[7]. Metabolisable energy (ME)[8] levels were then estimated using the empirical formula:

ME content MJ/kg DM = 0.17DDM% - 2.0 [where DDM% = 83.58 - 0.824ADF% + 2.626N%]

This information was used to report on the seasonal variation in pasture quantity and quality throughout 2002 and to investigate the association between nutrition and OJD mortality rate.

Necropsies

On the 12 farms all mobs of sheep over 6 months of age were inspected each day during each necropsy inspection period to collect dead and moribund sheep for necropsy examination. The inspection of flocks and collection of sheep for necropsy was performed according to the method of McGregor et al.[3] Briefly, during each of the four necropsy inspection periods each farm was visited daily for five consecutive days and all mobs of sheep over 6 months of age were inspected. Necropsies were performed on all sheep that were found dead or moribund between midnight Sunday and midnight Friday. A sheep was considered to be moribund if it was found down in a paddock and could not get up or if it fell over when approached and could not get up. A standard protocol was followed for the gross examination of all organ systems except the brain. During the post mortem inspections, thickening of the bowel was scored on a scale of 0 (nil) to 5 (extreme). Tissues were collected from the bowel and mesentery as well as any organ showing abnormalities. These were fixed in formal saline for the histopathological diagnosis of OJD and other disorders. Abomasal and proximal small intestinal washings were collected to allow nematode identification and counts. Each organ was washed in 2 litres of water before removing a 200 ml aliquot. Formalin was added as a preservative. The protocol used during each necropsy is presented in Appendix A.

Nematode counts

Separate counts of *Ostertagia*, *Trichostrongylus* and *Haemonchus* species were performed using abomasal washings and of *Nematodirus, Trichostrongylus* and *Cooperia* using small intestinal washings. To determine the worm burden of necropsied sheep, the nematode counts were categorised as low, medium, high or fatal according to a protocol based on Skerman and Hillard[9] (Table 2).

Table 2
Categorisation of nematode counts by species

Site / Species	Nematode count categories			
	Low	Medium	High	Fatal
Abomasum				
Ostertagia	<1,000	1,000 to 10,000	10,000 to 20,000	>20,000
Trichostrongylus	<1,000	1,000 to 10,000	10,000 to 20,000	>20,000
Haemonchus	<500	500 to 1,500	1,500 to 3,000	>3,000
Small intestine				
Nematodirus	<3,000	3,000 to 10,000	10,000 to 15,000	>15,000
Trichostrongylus	<1,000	1,000 to 10,000	10,000 to 20,000	>20,000
Cooperia	<10,000	10,000 to 20,000	20,000 to 25,000	>25,000

Histopathology

To determine the contribution of OJD in an animal's death, a histopathological diagnosis of OJD was made using tissues taken from the terminal ileum, ileo-caecal junction, caudal jejunal lymph node and ileo-caecal lymph node. The haematoxylin-eosin and Ziehl-Neelson methods were used to stain tissue sections. OJD lesions were classified using a system modified from Perez *et al.*[10] Briefly, lesions were divided into three main categories Type 1, Type 2, and Type 3 with further classification of Type 3 lesions into three subtypes: Type 3a, Type 3b (multibacillary) and Type 3c (paucibacillary). The histopathology slide reading protocol used to classify lesions associated with OJD is contained in Appendix B.

Determination of most likely cause of death

Determination of the most likely cause of death of necropsied sheep was based on consideration of several observations: the environment in which the animal was found, clinical signs, gross pathology and histopathology. The definitive diagnosis of the most likely cause of death for each animal in this study was based on histopathology (Figure 1).

Sheep were classified as "OJD most likely to have contributed to death" when there was histological evidence of 3b or 3c lesions, indicating advanced granulomatous enteritis (regardless of other findings that may have led to death e.g. drowned in dam, pneumonia). This category will be referred to by the term 'OJD contributed to death' for the remainder of this document. Sheep were classified as "OJD unlikely to have contributed to death" when there was histological evidence of 1, 2 or 3a score lesions, indicating mild granulomatous enteritis (in the presence or absence of clinical and/or pathological evidence of other disease state/s e.g. flystrike, parasitism, cancer). Sheep were classified as "OJD not contributing to death" when there was no histological evidence consistent with paratuberculosis.

A further category termed "malnutrition" was used for sheep where OJD did not contribute to death but there was a very low condition score, signs of weakness or death, depletion or serous atrophy of fat reserves, and in a number of cases, oedematous thickening of the mesentery and serosa of the bowel.

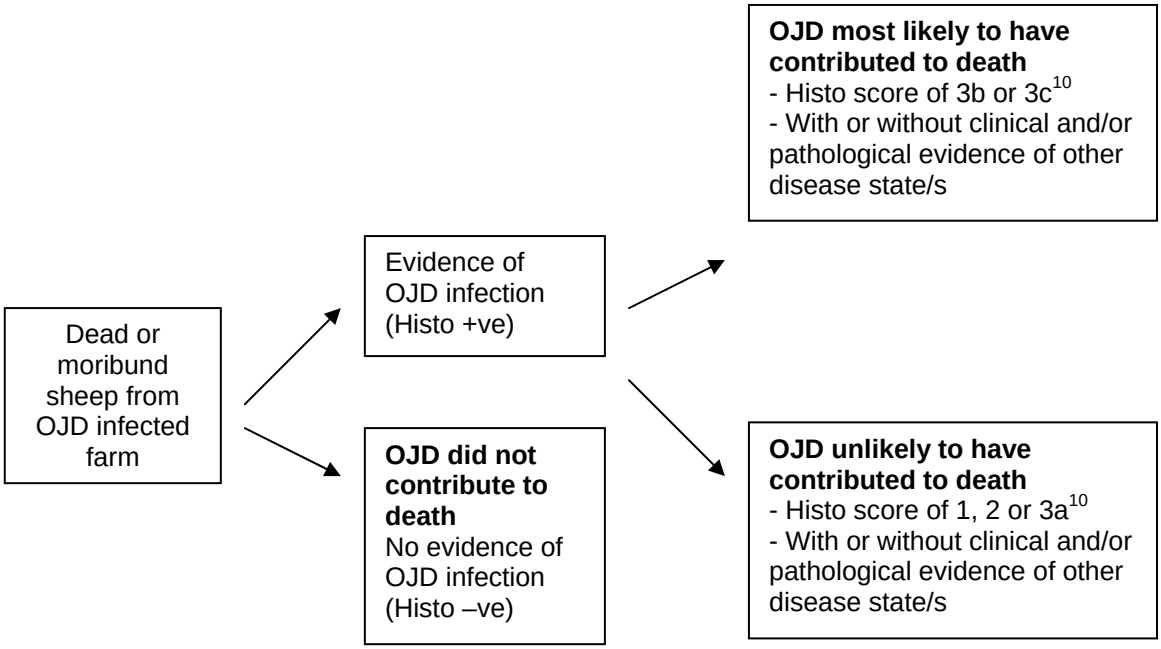


Figure 1. Classification of the "most likely cause of death" following post mortem examination

3.3.3 Serology and bacteriology of specific age cohorts of sheep

An additional visit was made to each farm to collect blood and faecal samples from a random sample of ewes and wethers in specific age cohorts (where both sexes were available). Blood samples were collected from 100 two-year old, 100 three-year old and 100 four-year old sheep for serological testing. Faecal samples were collected from 200 two-year old sheep for bacteriological culture, except for one farm where only 140 sheep of this age group were available.

Seroprevalence based on agar gel immunodiffusion

The age related seroprevalence of OJD in each flock was established using the agar gel immunodiffusion (AGID) method as described by Whittington *et al*.[11], a method based on those of Merkal *et al*.[12] and Goudswaard and Terporten-Pastoors.[13] Briefly, 28 μl of undiluted serum was tested against 28μl French pressed antigen of *M paratuberculosis*, supplied by Elizabeth Macarthur Agricultural Institute (EMAI). Tests were carried out on borate agar plates that were incubated in a humid chamber at 37^{o}C overnight. Positive control sera were included. Precipitin lines were graded as trace, 1+, 2+ or 3+ (strong positive). Positive and non-specific reactions were confirmed by a subsequent test with an adjacent positive control.

Prevalence and excretion rate based on pooled faecal culture (PFC)

The MAP faecal excretion rates and OJD infection prevalence of two-year old sheep on each farm were measured by culturing faeces in pools of 10 sheep. A total of 20 pools from each farm were cultured according to the method of Whittington *et al.*[14] Briefly, following a double incubation preparation, a small amount of the pooled faeces was cultured in a radiometric medium consisting of BACTEC 12B with PANTA PLUS, mycobactin J and egg yolk. The growth of *M paratuberculosis* was confirmed using a PCR test to identify the presence of IS*900* in positive cultures. OJD infection prevalence for each farm based on PFC was estimated using method 6 as defined by Cowling *et al*.[15] Briefly, this approach generates point and confidence-interval estimates of disease prevalence for individual animals based on pooled tests when sensitivity and specificity are unknown. Estimates for sensitivity and specificity were set at 0.75 and 1.0, respectively, based on sensitivity estimates for multibacillary and paucibacillary cases in

pools of 50 of 100% and 50%[16]. The sensitivity estimate of 75% was based on an assumption of 1:1 ratio of multibacillary:paucibacillary sheep in the 2 year sheep sampled.

The daily MAP excretion levels for each farm were estimated using two methods. Method A estimated the number of MAP excreted per flock per day by multiplying the number of OJD infected sheep in each flock, using the OJD infection prevalence information, by the expected number of MAP excreted from the multibacillary cases[17] in each flock. Method B used an evaluation of the number of MAP per gram of faeces for each pool determined by incubation time required to reach a cumulative growth index of 1000 as outlined by Reddacliff *et al*.[18]. MAP numbers excreted per flock per day were then estimated by multiplying the total flock size for each farm by the estimated number of MAP excreted per sheep per day using the information from both positive and negative pools. Method B assumes random selection of sheep for pooling with each sheep sampled producing 760 grams[5,17] of faeces per day. The correlation between methods A and B was measured using GenStat®[19] (Correlations Function).

3.4 Data analysis

3.4.1 Annual mortality rates based on inventory records

Information recorded on the inventory records for each farm was used to calculate the crude mortality rate (inventory) and adjusted mortality rate (inventory) for each age group per farm.

Crude mortality rate (inventory)

The crude mortality rate (inventory) was calculated using the formula:

$$\text{Crude mortality rate (inventory)} = \frac{\text{Total Mortalities}}{\text{Opening Number}} \times 100$$

Definitions for terms in this formula were:

Total mortalities	The total number of sheep that were unaccounted for and presumed to have died on each farm during the study period.
Opening number	The sheep present at the beginning of the study period.

For situations when the opening number for a specific age group was zero but sheep of this age were introduced during the study year, an adjusted opening number was calculated and used as the denominator in this formula. The definition for the adjusted opening number was:

Opening number (adjusted)	The sheep present at the beginning of the study period accounting for sheep introduced during the study year.

Adjusted mortality rate (inventory)

The adjusted mortality rate (inventory) was calculated using the formula:

$$\text{Adjusted mortality rate (inventory)} = \frac{\text{Total Mortalities}}{\text{Sheep Years at Risk}} \times 100$$

Definitions for terms in this formula were:

Total mortalities	The total number of sheep that were unaccounted for and presumed to have died on each farm during the study period.
Sheep years at risk	Represents the population present for the entire study period and takes into account changes in mob composition for each month. This figure was calculated by dividing the summed monthly averages by the total number of months sheep were present during the study period.

In order to calculate sheep years at risk, monthly average numbers for each age group were required. The definition used for monthly average was:

Monthly average number	The average number of sheep that are present on a farm each month. This accounts for all purchases, sales and deaths and the time within each month when the transaction took place. Changes in animal numbers during a month were weighted to account for animals being present for most or only a small part of that month. If no transaction date was recorded it was assumed transactions occurred on day 15 (to minimise the impact of actual dates being at the beginning or end of a month).

3.4.2 Annual mortality rate where OJD contributed to death

The annual mortality rate where OJD contributed to death (that is, the OJD mortality rate) on each farm was estimated by two methods. The first used information from the necropsy study and flock inventory records to calculate the adjusted OJD mortality rate (inventory). The second extrapolated from the number of OJD contributed deaths from the necropsy study by multiplication to provide an estimate of the number of OJD contributed deaths over the 365-day study period.

Adjusted OJD mortality rate (inventory)

The adjusted OJD mortality rate (inventory) was calculated using the formula:

Adjusted OJD mortality rate (inventory) = Proportion of OJD contributed deaths from the necropsy study X Adjusted mortality rate (inventory)

Extrapolated OJD mortality rate (necropsy study)

The extrapolated OJD mortality rate (necropsy study) was calculated using the formula:

Extrapolated OJD mortality rate (necropsy study) =

$$\frac{\text{Number of OJD contributed deaths from the necropsy X } 18.25}{\text{Opening number}} \text{ X } 100$$

The correlation between the adjusted OJD mortality rate (inventory) and the extrapolated OJD mortality rate (necropsy study) was measured using GenStat®[19] (Correlations Function).

3.4.3 Necropsied sheep where OJD contributed to death

Descriptive analyses were performed to describe the distribution of necropsied sheep where OJD contributed to death between age groups, sex groups and necropsy inspection periods on the 12 farms. These analyses were conducted using GenStat®[19].

3.4.4 Predicting OJD mortality rate from OJD prevalence based on pooled faecal culture, MAP faecal excretion and OJD seroprevalence information

This analysis was carried out to establish if OJD mortality rate could be predicted from either faecal excretion, OJD prevalence based on PFC or seroprevalence information. Separate logistic-regression models (using GenStat®[19] (Generalized Linear Model - modelling of binomial proportions)) were constructed to assess the unconditional association between OJD mortality rate and OJD prevalence based on PFC (prevalence point estimates), MAP faecal excretion and age related seroprevalence of OJD. The dependent variable was OJD mortality rate and consisted of the adjusted OJD mortality rate (inventory) as the numerator and the opening number as the denominator for each farm. The use of individual mortality and flock numbers, which account for differences in farm and flock sizes, was preferred compared to using mortality percentages from each farm. The dispersion parameter was estimated by GenStat®[19] to correct for overdispersion and account for variation between farms. An association was considered significant at $P < 0.05$.

OJD prevalence based on PFC (prevalence point estimates) was weighted to account for wide confidence intervals for some farms. This gave greater weight to prevalence estimates with narrow confidence intervals. The weighting was based on $1/[Se(\text{PFC prevalence})]^2$ for each farm's prevalence point estimate.

3.4.5 Association between quarterly OJD mortality rate and various environment, management and disease factors

Analyses were performed to investigate the association between various environment, management and disease factors and quarterly OJD mortality rate. Twenty-six farm-level independent variables, covering 19 areas relating to environment, management and disease were investigated (Appendix C). These independent variables were examined using screening methods described by Erb[20]. Variables with missing values (for greater than 25% of flocks) or with very little variation were not considered further. Correlation between the remaining 24 independent factors was assessed to identify moderate (r = 0.3 to 0.5) to high (r > 0.5) dependencies among these factors that would need to be considered during model construction,

The dependent variable was the quarterly OJD mortality rate and consisted of the number of OJD mortalities per quarter as the numerator and flock size (opening number for each farm) as the denominator.

The generalised linear mixed model (GLMM) approach for binomial data using the logistic link function (performed by ASReml[21]) was used for model construction. First, the factors region, season, farm and flock size were introduced to the model and an assessment made of the remaining variation in OJD mortality rates – whether there was sufficient variation to warrant investigation of further independent variables. Second, independent variables were added to the basic model (individually and selected sets of correlated variables) and assessed. Third, 8 variables considered worthy of further assessment were added to the model and stepwise backward elimination used to establish a final model. Variables in the final model were designated as likely to be detrimental (that is, associated with higher OJD mortality rates on the basis of a positive co-efficient in the final model) or likely to be protective (that is, associated with lower OJD mortality rates on the basis of a negative co-efficient in the final model).

3.4.6 Economic impact of OJD

Two approaches were used to estimate the economic impact of OJD. The first approach used a gross margin analysis over a one-year time period and the second, placed an economic value on the mortalities inspected during the four-necropsy inspection periods associated with this study.

Gross margin comparison of flocks with and without OJD over a 1-year period

A gross margin for each farm was constructed in MS Excel using information from flock inventories, questionnaires and the necropsy study combined with parameters and prices compiled by NSW Agriculture[22] for their August 2002 gross margins for 19 micron merino wool production and 2nd cross

lamb production. Flock inventory information was used to model each farm's flock structure over a 12-month period and establish the number of sheep present at each point in the annual calendar of operations (e.g. crutching, shearing, drenching). Flock inventory and necropsy study information provided mortality rates and an estimate of the contribution of OJD to the mortality rate for each farm. Income information was compiled using wool (number of sheep alive at shearing) and sheep sales (number of culls/surplus sheep) data provided from each farm and values for quantities and prices provided by NSW Agriculture. The variable costs for each flock (sheep health, selling wool & sheep, fodder and replacements) were determined using questionnaire information about the husbandry procedures performed on each farm and the respective operation cost provided by NSW Agriculture. The number of replacements required was established using questionnaire information (lambing percentages, ram purchases) from each farm.

To establish the economic impact of OJD gross margins were constructed for the 12 farms assuming each farm had no OJD infection and were compared with the actual gross margins for the same farms, which accounted for an increase in mortalities due to OJD. This comparison also considered the effect on the price of sale sheep due to zoning, with Gudair® vaccination and any additional labour/infrastructure costs associated with managing OJD. An example of the gross margin used to calculate the economic impact of OJD is contained in Appendix D.

Economic value of necropsied sheep

Information relating to sheep age, sex, body condition score, reproductive status, wool fibre diameter and wool length was collected for each sheep necropsied during the inspection periods. All mortalities were given an economic value according to data compiled by NSW Agriculture[22] in the above gross margin analysis. To enable a comparison between farms the following assumptions were made:

- All sheep come from either 19 micron merino wool producing flocks or 2nd cross lamb producing flocks
- All sheep reach 5 years of age before culling and therefore produce a fleece (and if an adult female possibly a lamb) each year until culled
- A value for lost reproduction is based on multiplying the 2002 weaning % of each farm by the number of years a ewe could remain in the flock before she reaches 5 years of age and is culled.

Economic values were established for hogget wethers, hogget ewes, adult wethers and adult ewes for the 20-day necropsy study period to estimate an overall value for the cost of total losses. An estimate of the economic value for losses where OJD contributed to death was then calculated for the 12-month study period by multiplying the economic values obtained from the 20-day necropsy study period by 18.25 to estimate values for the full 365-day year. An example of the MS Excel spreadsheet used to calculate the economic impact of OJD using necropsy information is contained in Appendix E.

4. Results

4.1 Annual mortality rates based on inventory records

Based on inventory records, the average crude mortality rate (inventory) was 7.8% (median 7.7%, range 2.7% to 16.7%) and the average adjusted mortality rate (inventory) was 8.6% (median 8.2%, range 3.1% to 18.2%) for the twelve flocks. The mortality rates for each farm are presented in Table 3.

Table 3
Estimated annual mortality rates (crude and adjusted) based on inventory records for the 12 farms in 2002

Area/Farm	Crude Mortality Rate%	Adjusted Mortality Rate %
1/1	6.3	7.5
1/2	9.1	9.6
1/3	2.7	3.1
2/1	7.7	8.2
2/2	8.2	8.9
2/3	16.7	18.2
3/1	10.2	10.8
3/2	6.3	7.5
3/3	7.7	8.0
4/1	5.5	5.5
4/2	8.4	10.9
4/3	5.0	5.4
Average	**7.8**	**8.6**
SD	**3.5**	**3.8**
Median	**7.7**	**8.2**

4.2 Most likely cause of death for necropsied sheep

A total of 399 sheep were examined over the four-necropsy inspection periods, with 392 eligible to remain in the study. Seven sheep were excluded, three lambs due to age (< 6 months old) and 4 adults to avoid inclusion of animals that may have died outside the specified 5-day necropsy inspection period. These animals had either been dead for over 12 hours on the first day of each study period or were moribund and may have survived beyond midnight on the last day of the inspection period.

A most likely cause of death was determined for 362 of the necropsied sheep. Of these, OJD was most likely to have contributed to the death of 250 sheep, OJD was unlikely to have contributed to the death of 1 sheep and OJD did not contribute to death of 111 sheep. For the remaining 30 necropsied sheep, the most likely cause of death could not be confirmed due to post mortem autolysis or post mortem predation preventing the collection of suitable samples. A summary of the most likely cause of death for each of the four-necropsy inspection periods is shown in Table 4. OJD was the most likely cause of death of 52% to 76% of sheep depending on inspection period.

Table 4
Most likely cause of death for 392 sheep necropsied over four inspection periods on 12 farms during 2002

Inspection period	No. of sheep examined	Cause of death (%)			
		OJD most likely (n = 250)	OJD unlikely (n = 1)	Not OJD (n = 111)	Unknown (n = 30)
Autumn	64	69	0	20	11
Winter	149	52	1	38	9
Spring	126	70	0	26	4
Summer	53	76	0	17	7
Total	392				

The most likely cause of death in 111 sheep was attributed to causes other than OJD (Table 5). These causes included malnutrition (22.5%), malnutrition plus pregnancy related disorders (33%), malnutrition plus pregnancy and internal parasites (7%), pregnancy plus lambing related disorders (14.5%), internal parasites (3.5%), blowfly strike (3%) and post shearing stress/pneumonia (5.5%). Other causes contributing to a smaller proportion of deaths included drench capsules being lodged in the oesophagus, sheath rot, peritonitis, enteritis, cancer, photosensitisation, pulpy kidney and misadventure (eg being stuck in the dam).

Table 5
Most likely cause of death for 111 necropsied sheep where death was not related to OJD over four inspection periods on 12 farms during 2002

Most Likely Cause of Death	Necropsy inspection period			
	Autumn	Winter	Spring	Summer
Malnutrition	5	8	8	4
Malnutrition + pregnancy related disorders	0	32	5	0
Malnutrition + pregnancy + parasites	0	7	1	0
Pregnancy + lambing related disorders	0	9	7	0
Post shearing stress / pneumonia	0	0	5	1
Internal parasites	0	0	4	0
Blowfly strike	1	0	0	2
Chronic peritonitis / nephritis / enteritis	0	0	2	1
Sheath rot	2	0	0	1
Lodged drench capsule	0	0	1	0
Misadventure	2	0	0	0
Eye cancer	1	0	0	0
Photosensitisation	1	0	0	0
Pulpy kidney	1	0	0	0
Total	13	56	33	9

The “malnutrition” category applied to 70 (63%) of the 111 sheep where the most likely cause of death was attributed to causes other than OJD. Table 6 contains a summary of the most likely cause of death for each of the four-necropsy inspection periods with the sheep from the “malnutrition” category removed to reflect the distribution of deaths unrelated to OJD in a non-drought year where nutrition was adequate. Note that in this case OJD accounts for 75 to 82% of mortalities.

Table 6
Most likely cause of death for 322 sheep necropsied over four inspection periods on 12 farms during 2002 following removal of sheep whose death was related to "malnutrition"

Inspection period	No. of sheep examined	Cause of death (%)			
		OJD most likely (n = 250)	OJD unlikely (n = 1)	Not OJD (n = 41)	Unknown (n = 30)
Autumn	59	75	0	13	12
Winter	104	75	1	11	13
Spring	112	81	0	15	4
Summer	49	82	0	10	8
Total	322				

During the four inspection periods, thickening of the bowel was recorded in 370 necropsied sheep. Of these, 17 scored zero, 55 scored 1 (slight) and 298 scored 2 (mild) or greater. Twenty-two animals were not scored due to post mortem predation. Based on histopathology results, the percentage of OJD negative sheep among the sheep with a score of zero was 100% (17/17) and with a score $\geq$ 2 was 18% (54/298). Microscopically, all of these 54 sheep with putative gross OJD lesions had serosal thickening attributed mostly to oedema and 44 of these animals had a body condition score of 1.5 or less.

4.3 Annual mortality rate where OJD contributed to death

Based on information from the necropsy study and inventory records, the average adjusted OJD mortality rate (inventory) for the 12 farms was 6.2% (median 5.8%, range 2.1% to 17.5%). Further information from the necropsy study was used to extrapolate the OJD mortality rate for each flock. The average extrapolated OJD mortality rate (necropsy study) was 6.7% (median 4.4%, range 1.1% to 15.0%). The OJD mortality rates (adjusted and extrapolated) for each farm are presented in Table 7. The correlation between the adjusted and extrapolated OJD mortality rates was high (r = 0.81, P = 0.001).

Table 7
Adjusted OJD mortality rate (inventory) and extrapolated OJD mortality rate (necropsy study) for the 12 farms in 2002

Area/Farm	Adjusted OJD mortality rate (inventory) %	Extrapolated OJD mortality rate (necropsy study) %
1/1	3.8	1.5
1/2	6.8	8.2
1/3	2.5	4.1
2/1	8.2	12.8
2/2	5.4	3.1
2/3	17.5	13.9
3/1	8.8	15.0
3/2	5.8	6.1
3/3	7.6	12.0
4/1	2.1	0.9
4/2	2.9	1.6
4/3	3.4	1.1
Average	**6.2**	**6.7**
SD	**4.2**	**5.4**
Median	**5.8**	**4.4**

4.4 Necropsied sheep where OJD contributed to death

4.4.1 Distribution by age and sex

The distribution of mortalities between age and sex groups among the 250 necropsied sheep where OJD contributed to death is presented in Table 8. The distribution across age groups indicated that mortalities where OJD contributed to death increased from the 1 year-old age group (10.4%) to peak at the 4 year-old age group (35.6%) and then decreased for the over 4 year-old age group (19.2%). The distribution of mortalities where OJD contributed to death between sexes was very similar with 49.6% among wethers and 50.4% among breeding ewes.

Table 8
Distribution of mortalities due to OJD based on necropsy information for the 12 farms in 2002

Age	Total Number (%)	Sex	Total number
1Yr	26 (10.4)	F	15
		W	11
2Yr	33 (13.2)	F	19
		W	14
3Yr	54 (21.6)	F	22
		W	32
4Yr	89 (35.6)	F	38
		W	51
4+Yr	48 (19.2)	F	32
		W	16
Total	250		250

4.4.2 Distribution by necropsy inspection period

The majority of necropsied sheep died during winter (37.5%) and spring (33%) with a reduction in numbers during autumn (16%) and summer (13.5%). There was a similar trend where OJD contributed to death with the majority in winter (31%) and spring (35%) and fewer in autumn (18%) and summer (16%). A full description of the seasonal variation in both total mortalities and mortalities where OJD contributed to death for each farm is presented in Table 9.

Table 9
Seasonal variation in total mortalities and where OJD was most likely to have contributed to death for the 12 farms in 2002

	Season									
	Autumn		*Winter*		*Spring*		*Summer*		*Total*	
Area/Farm	Total	OJD most likely	Total	OJD most likely	Total	OJD most likely	Total	OJD most likely	Total	OJD most likely
1/1	6	1	4	3	11	2	5	4	26	10
1/2	8	7	11	10	19	8	8	6	46	31
1/3	1	1	4	4	6	4	0	0	11	9
2/1	4	4	12	11	11	11	3	2	30	28
2/2	8	7	4	2	7	2	1	0	20	11
2/3	4	3	25	24	13	12	12	11	54	50
3/1	2	2	8	6	13	12	5	3	28	23
3/2	10	8	3	2	9	6	2	1	24	17
3/3	7	7	9	8	16	15	7	7	39	37
4/1	6	1	6	2	4	1	6	3	22	7
4/2	5	3	53	1	13	13	2	1	73	18
4/3	3	0	10	5	4	2	2	2	19	9
Total	64	44	149	78	126	88	53	40	392	250
Seasonal Distribution (%)	**16**	**18**	**37.5**	**31**	**33.5**	**35**	**13**	**16**	**100**	**100**

4.5 Predicting OJD mortality rate from OJD prevalence based on pooled faecal culture, MAP faecal excretion and OJD seroprevalence information

4.5.1 OJD prevalence based on pool faecal culture

Point estimates and confidence intervals of OJD prevalence based on PFC results for the 2 year-old sheep on each farm are presented in Table 10. OJD prevalence was < 5% on 3 farms, between 5 to 10% on 1 farm, between 10 to 20% on 3 farms and > 20% on 5 farms. Further, on eight of nine farms, where faecal samples were collected separately from both ewes and wethers, more wether cultures tested positive for MAP than ewe cultures (Table 10).

A significant relationship ($P < 0.001$) was identified between OJD prevalence and OJD mortality rate (Figure 2).

Table 10
Point and confidence interval estimates of OJD prevalence for 2 year-old sheep based on pooled faecal culture tests for the 12 farms in 2002

Area/Farm	Number of Positive Pooled Cultures (out of 20 pools)			CI_L (%)	Prevalence (%)	CI_U (%)
	Wethers	Ewes	Total			
1/1	5	2	7	0.76	6.09	11.42
1/2	8	4	12	-0.06	14.87	29.79
1/3	- [a]	3	3	-0.42	2.21	4.83
2/1	10	7	17	<-17.13	>23.72	>64.58
2/2	12	- [b]	12	-0.06	14.87	29.79
2/3	10	9	19	<-17.13	>23.72	>64.58
3/1	- [a]	9 / 14 [c]	9 / 14 [c]	-5.30	17.68	40.66
3/2	6	8	14	-17.13	23.72	64.58
3/3	10	4	14	-17.13	23.72	64.58
4/1	1	0	1	-0.68	0.69	2.05
4/2	10	4	14	-17.13	23.72	64.58
4/3	3	2	5	0.13	3.97	7.82

[a] faecal samples only collected from ewes
[b] faecal samples only collected from wethers
[c] 14 pools of 10 samples instead of 20 pools of 10 samples

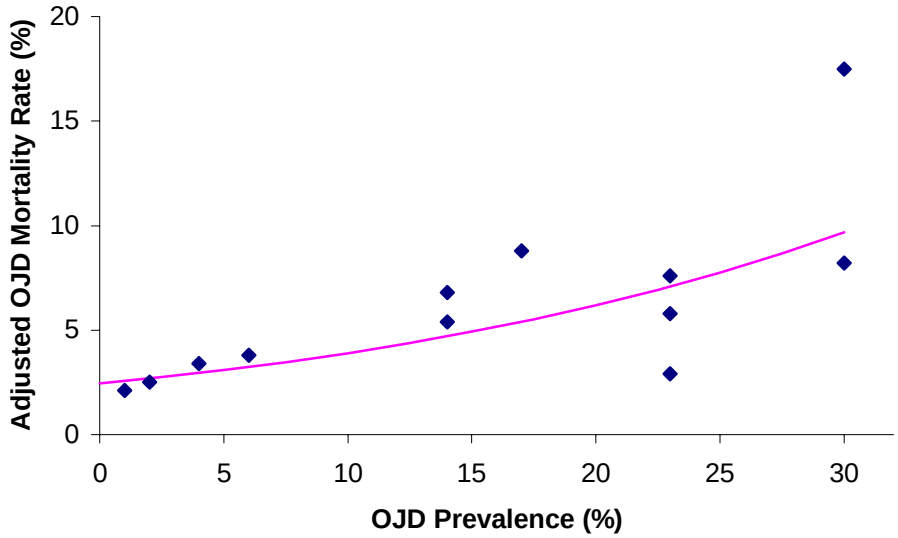


Figure 2. Relationship between OJD prevalence based on PFC and OJD mortality rate

4.5.2 Faecal excretion of MAP

The estimated daily faecal excretion of MAP for 2 year-old sheep on each farm is presented in Table 11. High correlation was measured between the results produced by Method A and Method B (r = 0.83, P = 0.001).

No significant relationship was demonstrated between total estimated daily MAP excretion in 2 year olds and OJD mortality rate (“Method A” P = 0.87 and “Method B” P = 0.29).

Table 11
Daily MAP faecal excretion rates for 2 year-old sheep based on pooled faecal culture tests for the 12 farms in 2002

Area/Farm	Estimated MAP numbers excreted per flock/day	
	Method A	**Method B**
1/1	5.3×10^{12}	4.2×10^{9}
1/2	7.2×10^{12}	1.7×10^{12}
1/3	6.2×10^{11}	2.8×10^{9}
2/1	8.4×10^{12}	5.0×10^{10}
2/2	1.4×10^{13}	1.3×10^{11}
2/3	1.5×10^{13}	2.8×10^{11}
3/1	3.5×10^{12}	3.3×10^{11}
3/2	8.4×10^{12}	1.4×10^{11}
3/3	9.3×10^{12}	6.9×10^{10}
4/1	6.9×10^{11}	2.5×10^{7}
4/2	3.4×10^{13}	5.6×10^{12}
4/3	4.0×10^{12}	7.6×10^{11}

4.5.3 OJD seroprevalence

The age related seroprevalence of OJD for each farm is presented in Table 12. The OJD seroprevalence for the 2 year-old age group was < 5% on 7 farms, between 5 to 10% on 2 farms, between 10 to 20% on 2 farms and > 20% on 1 farm. The OJD seroprevalence for the 3 year-old age group was < 5% on 3 farms, between 5 to 10% on 5 farms, between 10 to 20% on 3 farms and > 20% on 1 farm. The OJD seroprevalence for the 4 year-old age group was < 5% on 6 farms, between 5 to 10% on 3 farms and between 10 to 20% on 2 farms.

No significant relationship was demonstrated between age-related OJD seroprevalence and OJD mortality rate (2 Yr-old P = 0.24, 3 Yr-old P = 0.39 and 4 Yr-old P = 0.52).

Table 12
Age related seroprevalence of OJD for the 12 farms in 2002

Area/Farm	% Positive		
	2 Yr-old	**3 Yr-old**	**4 Yr-old**
1/1	1.0	5.0	2.0
1/2	24.0	9.0	-[a]
1/3	3.0	8.0	4.4
2/1	18.0	13.0	11.0
2/2	2.0	9.1	2.0
2/3	5.0	10.0	3.1
3/1	9.0	3.0	5.0
3/2	12.0	11.0	6.1
3/3	4.0	8.0	6.0
4/1	0.0	1.0	3.1
4/2	3.0	1.0	11.7
4/3	1.0	20.2	4.0

[a] Age group sold prior to blood collection

4.6 Association between quarterly OJD mortality rate and various environment, management and disease factors

Descriptive statistics on each of the 26 independent variables are presented in Appendix C. The basic model, including region, season and flock size as fixed effects and farm as a random effect, showed sufficient variation in OJD death rates among farms to warrant an assessment of other independent variables. Of the 24 independent variables investigated, the 8 variables identified as worthy of inclusion in a multivariable model were:

- Improved pasture area (%)
- Improved pasture metabolisable energy content (MJ/kg DM)
- Year first noticed OJD losses
- Total replacements bought annually
- Drench resistance
- Hand feeding not in drought
- Lamb age at weaning
- Stocking rate (DSE/ha).

The final model is shown in Table 13 and a detailed report of the results found during the model building process is presented in Appendix F. In the final model, the four variables significantly associated with quarterly OJD mortality rate were:

Variable	Effect on OJD mortality rate	Description of effect
Flock size	Protective	Reduction in OJD mortality proportion with larger flock sizes
Improved pasture area (%)	Detrimental	Increase in OJD mortality rate with higher proportion of improved pasture area on farm
Stocking rate (DSE/ha)	Protective	Reduction in OJD mortality with higher stocking rates
Lamb age at weaning	Detrimental	Increase in OJD mortality rate with weaning lambs at 10-14 weeks old

Table 13
Final mixed linear model for quarterly OJD mortality rate on the 12 farms in 2002

Variables	b	SE(b)	P[a]
Random effect			
Seasons within farm	-	-	-
Fixed effects			
Flock size	-0.00013	0.000034	<0.001
Improved pasture area (%)	0.021	0.0065	<0.01
Stocking rate (DSE/ha)	-0.116	0.044	<0.05
Lamb age at weaning (weeks)			<0.05
10-14 (1)	0.45	0.21	
15-16 (0)	0.0		

4.7 Economic impact of OJD

4.7.1 Gross margin comparison of flocks with and without OJD over a 1-year period

The average % decrease in gross margin due to a farm being infected with OJD was 6.4% (median 5.5%, range 2.2% to 15.4%). The average gross margin/DSE for the OJD infected flocks was $20.58 (median $22.22, range $10.16 to $36.36) and when the effect of OJD was removed was $21.85 (median $22.86, range $11.77 to $37.19). The average gross margin/ha for the OJD infected flocks was $167.75 (median $173.01, range $66.12 to $290.86) and when the effect of OJD was removed was $178.65 (median $179.69, range $70.60 to $297.53). Gross margins for the flocks with and without OJD are presented in Table 14.

Table 14
Gross margins from 12 flocks with and without OJD in 2002

	Without OJD			With OJD			
Area/Farm	**Gross Margin**	**GM / DSE**	**GM / ha**	**Gross Margin**	**GM / DSE**	**GM / ha**	**% decrease in GM due to OJD**
1/1	$349,369	$23.76	$190.08	$339,872	$23.11	$184.91	2.7
1/2	$193,998	$11.77	$70.60	$181,689	$11.02	$66.12	6.3
1/3	$276,044	$29.44	$184.03	$268,421	$28.63	$178.95	2.8
2/1	$72,087	$13.20	$171.64	$65,869	$12.06	$156.83	8.6
2/2	$315,996	$11.55	$166.31	$277,999	$10.16	$146.32	12.0
2/3	$236,073	$31.43	$251.41	$199,778	$26.59	$212.76	15.4
3/1	$81,012	$11.94	$95.53	$73,596	$10.85	$86.79	9.2
3/2	$141,158	$25.05	$175.35	$134,487	$23.87	$167.06	4.7
3/3	$150,693	$17.09	$99.14	$140,257	$15.91	$92.27	6.9
4/1	$513,927	$27.83	$222.67	$500,856	$27.13	$217.01	2.5
4/2	$868,204	$37.19	$297.53	$848,727	$36.36	$290.86	2.2
4/3	$453,237	$21.95	$219.49	$440,220	$21.32	$213.18	2.9
Average	$304,317	$21.85	$178.65	$289,314	$20.58	$167.75	6.4
Median	$256,059	$22.86	$179.69	$234,100	$22.22	$173.01	5.5

4.7.2 Economic value of necropsied sheep

The costs associated with total mortalities and mortalities where OJD contributed to death were estimated for each of the twelve OJD infected flocks over 4 inspection periods and then multiplied by 18.25 to give an annual estimate of losses. The average estimated cost of total losses was $95,251 (median $85,677, range $30,607 to $240,258) and of OJD losses was $64,100 (median $44,942, range $15,569 to $154,083). The cost of OJD losses accounted for on average 70.1% of the total estimated economic losses (median 68.5%, range 16.5% to 100%) for the year. The average estimated cost of annual OJD losses/DSE was $7.68 (median $4.11, range $0.84 to $20.51) while the average cost of annual OJD losses/ha was $65.92 (median $25.09, range $6.75 to $244.80). The economic impact of OJD using information from the necropsy study is presented in Table 15.

Table 15
Estimates of annual economic losses based on the sheep necropsied on 12 farms in 2002

Area/Farm	Cost of Total losses	Cost of OJD losses	% Cost of OJD losses	OJD losses $/DSE	OJD losses $/ha
1/1	$53,183	$24,923	46.9	$1.69	$13.56
1/2	$133,674	$80,810	60.5	$4.90	$29.41
1/3	$36,239	$31,165	86	$3.32	$20.78
2/1	$102,817	$102,817	100	$18.83	$244.80
2/2	$41,697	$27,242	65.3	$1.00	$14.34
2/3	$158,052	$154,083	97.5	$20.51	$164.09
3/1	$143,402	$124,917	87.1	$18.41	$147.31
3/2	$69,961	$50,160	71.7	$8.90	$62.31
3/3	$101,394	$98,071	96.7	$11.12	$64.52
4/1	$30,607	$15,569	50.9	$0.84	$6.75
4/2	$240,258	$39,724	16.5	$1.70	$13.61
4/3	$31,735	$19,724	62.2	$0.96	$9.55
Average	$95,251	$64,100	70.1	$7.68	$65.92
Median	$85,677	$44,942	68.5	$4.11	$25.09

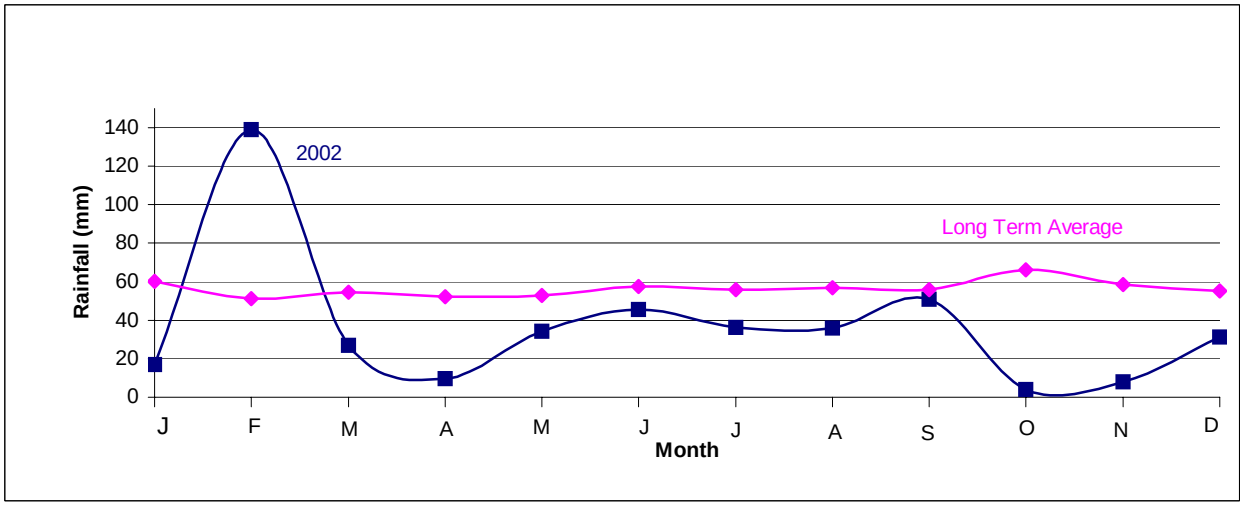
4.8 Seasonal conditions during 2002

4.8.1 Rainfall and Evaporation

Drier than normal seasonal conditions were experienced on all 12 farms during 2002 with an average 61.3% (median 61%, range 52% to 86%) of the expected average annual rainfall received across the four areas. Farmer rainfall records provided a summary of the average annual rainfall and the rainfall received on each farm during 2002 (Table 16). The distribution of rainfall throughout 2002 is shown in Figure 3. This indicates above average rainfall was received in February and below average rainfall from March to December.

Table 16
Average annual rainfall and actual rainfall received during 2002 on the 12 farms

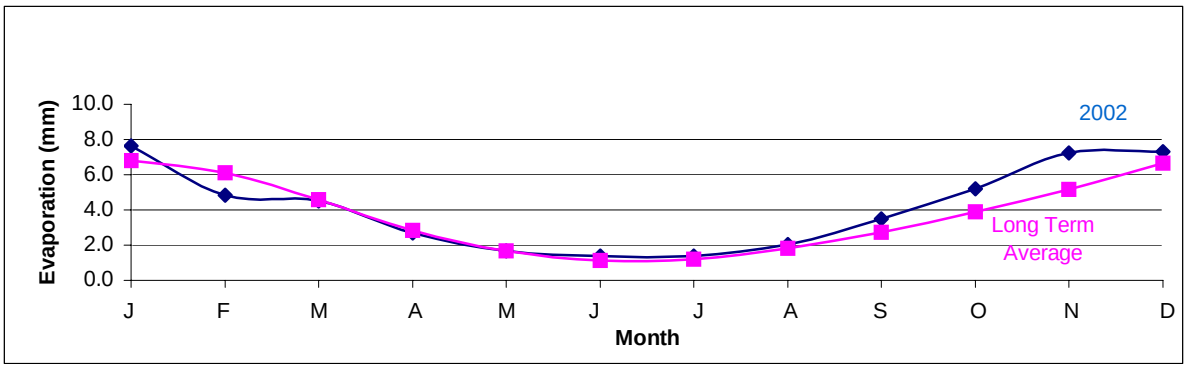
Area	Farm	Locality	Average Annual Rainfall (mm)	2002 Rainfall (mm)	% of Average Annual Rainfall received in 2002
1	1	Goulburn	735	387	53
1	2	Bungendore	702	377	54
1	3	Bungendore	702	377	54
2	1	Laggan	800	503	63
2	2	Taralga	800	510	64
2	3	Roslyn	800	521	65
3	1	Gunning	740	429	58
3	2	Gunning	650	423	65
3	3	Gunning	650	560	86
4	1	Harden	625	380	61
4	2	Harden	650	341	52
4	3	Cootamundra	675	410	61
		Average	710.8	434.8	61.3
		Median	702	423	61



Source: Farm Rainfall Records and Bureau of Meteorology

Figure 3. Long term and 2002 rainfall distribution for the 12 farms

The pan evaporation throughout 2002 is shown in Figure 4. This indicates below average evaporation was recorded in February while above average evaporation was experienced from September to December.

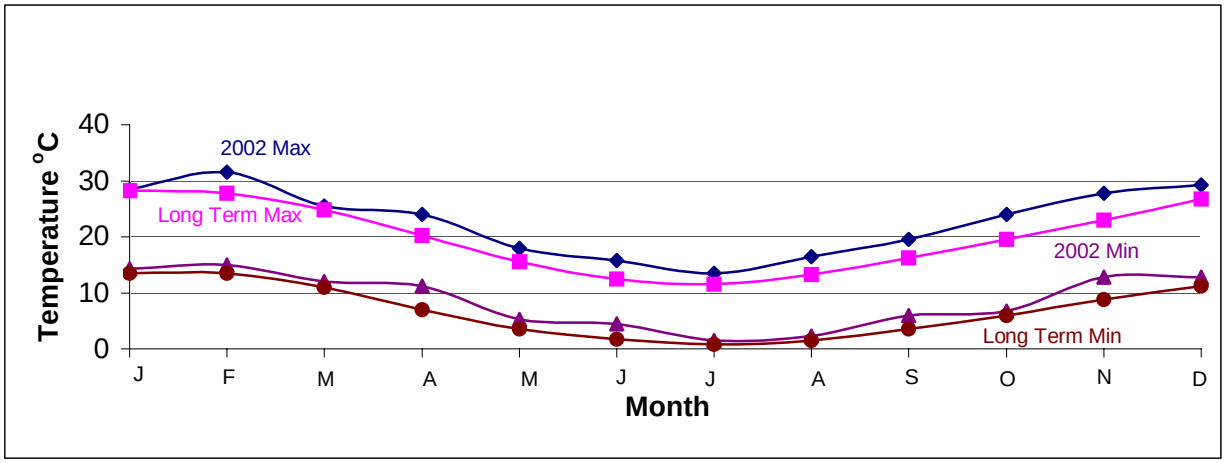


Source: Farm Rainfall Records and Bureau of Meteorology

Figure 4. Long term and 2002 pan evaporation for the 12 farms

4.8.2 Temperature

The average maximum and minimum temperatures experienced across the four areas throughout 2002 were higher than the long-term average maximum and minimum temperatures (Figure 5).



Source: Bureau of Meteorology

Figure 5. Long term and 2002 mean maximum and minimum temperatures for the 12 farms

4.8.3 Pasture

Pasture samples were collected at each necropsy inspection period to assess the seasonal variation in quantity (DM/m2) and quality (ME MJ/kg DM). Due to a large variation in the recent grazing history (grazed versus spelled) at each site during sample collection, seasonal differences in pasture quantity could not be accurately assessed. However, seasonal pasture quality was determined for improved pasture on all 12 farms as well as for native pasture and stubble on farms where this type of vegetation was present (Table 17).

Table 17
Pasture quality measurements from samples collected at each necropsy inspection on the 12 farms during 2002

	Improved Pasture (ME MJ/kg DM)				Native Pasture (ME MJ/kg DM)				Stubble (ME MJ/kg DM)	
Area/Farm	*Autumn*	*Winter*	*Spring*	*Summer*	*Autumn*	*Winter*	*Spring*	*Summer*	*Autumn*	*Summer*
1/1	7.9	8.6	8.2	8.3	9.0	8.1	9.5	7.7	NS	NS
1/2	8.0	8.4	8.6	9.5	NS	NS	NS	NS	NS	NS
1/3	6.9	7.1	7.6	8.6	8.3	8.4	10.5	8.5	NS	NS
2/1	8.4	8.7	10.0	8.6	NS	NS	NS	NS	NS	NS
2/2	10.5	10.3	11.8	8.1	NS	NS	NS	NS	NS	NS
2/3	8.0	9.7	11.3	8.3	NS	NS	NS	NS	NS	NS
3/1	7.5	11.0	11.3	9.0	6.9	6.8	10.7	8.2	NS	NS
3/2	8.3	9.2	11.8	8.4	NS	NS	NS	NS	NS	NS
3/3	8.7	7.8	10.1	7.9	9.1	8.3	11.2	6.9	NS	NS
4/1	5.0	9.2	10.4	7.3	8.5	MS	10.3	6.9	4.4	7.3
4/2	7.1	10.6	10.1	9.1	NS	NS	NS	NS	4.7	7.1
4/3	8.7	10.0	11.2	7.6	8.0	8.3	10.0	8.4	4.1	6.0
Average	7.9	9.2	10.2	8.4	8.3	8.0	10.3	7.7	4.4	6.8
Median	8.0	9.2	10.3	8.3	8.4	8.3	10.4	7.9	4.4	7.1

NS - No sample collected for native pasture and/or stubble on this farm

MS - Missing sample due to insufficient pasture quantity to enable pasture quality determination

4.9 Reduction in sheep numbers during 2002

Reduction in flock size due to the effects of drought in 2002 was recorded for the 12 farms (Table 18). The average flock size at the end of 2002 was 79.2% (median 84.2%, range 50% to 90%) of numbers normally stocked on the 12 farms.

Table 18
Reduction in flock sizes on the 12 farms due to the effects of drought in 2002

Area/Farm	Usual Sheep Numbers	Sheep Numbers at the end of 2002	% of normal flock numbers present at the end of 2002
1/1	12369	10575	85.5
1/2	8500	6900	81.2
1/3	6000	5142	85.7
2/1	4000	3500	87.5
2/2	20000	15000	75.0
2/3	8611	7175	83.3
3/1	3500	2500	71.4
3/2	4700	4000	85.1
3/3	6850	6461	94.3
4/1	18000	11000	61.1
4/2	20000	10000	50.0
4/3	15000	13500	90.0
Average	10627.5	7979.4	79.2
SD	6225.5	4020.4	12.8
Median	8555.5	7037.5	84.2

5. Discussion

This is the first multi-farm study in Australia to quantify the contribution of OJD to on-farm annual mortalities. During 2002 based on inventory records from the 12 farms, the annual mortality rates ranged from 3.1% to 18.2% and the OJD mortality rates from 2.1% to 17.5%. This range of OJD mortality rates is consistent with reported flock owner estimates of OJD mortality ranging from less than 1% to over 10%[1]. Further, and of real concern, the average OJD mortality rate was 6.2%, more than twice the accepted mortality rate of 2-3%[23] (from all causes) for Australian sheep flocks.

The protocol established by McGregor et al. (2001) to estimate annual OJD mortality rate has proven robust and reliable in this study. The necropsy study successfully determined the most likely cause of death of 362 necropsied sheep and this information was used to estimate OJD mortality rate. A significant correlation (r = 0.81, P = 0.001) was measured between the extrapolated OJD mortality rate based on necropsy study information and the adjusted OJD mortality rate obtained by combining inventory record and necropsy study information. Discrepancies between the extrapolated and adjusted OJD mortality figures can be attributed to the fact that extrapolation of necropsy data from a 20-day period will not account for any clustering of deaths that may occur throughout a 12-month period.

An average of 71.5% of mortalities from the autumn, spring and summer periods were attributed to OJD. In comparison, the contribution of OJD to mortality for the winter inspection period was 52% of all necropsies. This reduction was due to a large number of deaths associated with malnutrition and advanced pregnancy occurring on one farm where the manager had problems providing adequate nutrition to his ewes. As malnutrition was a factor in the majority of deaths attributed to other causes during the four-necropsy inspection periods, removal of these animals from the necropsy summary provided an estimate of the number of mortalities that could be expected during a year with reasonable seasonal conditions. Following removal of sheep with malnutrition, OJD mortalities increased to an average of 78% of mortalities across the four necropsy inspection periods.

The number of mortalities where OJD contributed to death increased with age, peaking with the 4 year old age group (35.6%). Farms in this study were first diagnosed over a 10-year period (1991 to 2001), with 9 of the 12 farms diagnosed since 1996, therefore it is possible that the distribution of mortalities across age groups reflects the time of the first OJD infection and the level of OJD infection on the majority of farms. The fact that most farms have been infected for less than five years would account for the majority of mortalities occurring in older sheep (3 year-old to 4+ year-old). A reduction in OJD mortalities in the 4+ year-old age group may be due to a combination of culling at an earlier age and death prior to reaching this age. Sex did not appear to influence the likelihood of OJD contributing to death, as there were an equal proportion of ewes and wethers where OJD contributed to death.

Diseases and conditions other than OJD were responsible for 31% of the deaths in this study. This highlights the fact that once OJD has been diagnosed on a farm all mortalities from that point cannot be attributed to OJD. The importance of maintaining appropriate flock health management to minimize overall mortality rates, when OJD is present in a flock, reinforces the need for a whole farm approach to flock health management. However, control of these other diseases alone is unlikely to remove the major impact on mortality rate.

Misdiagnosis of OJD based on gross pathology is a potential problem in the field. In this study 18% of necropsied sheep with gross and microscopic lesions of thickening of the bowel attributed mostly to serosal oedema had no histological evidence of OJD. In these sheep the gross thickening of the intestine with no histological evidence of OJD but with extensive oedema of serosa and/or submucosa was considered to be a result of hypoprotenaemia due to nutritional stress.

Thirty animals (7.7%) could not have a most likely cause of death determined due to autolytic changes precluding conclusive histopathological findings, or post mortem predation. Higher than normally expected ambient temperatures, during the winter and spring collections, combined with the large number of animals examined, contributed to autolysis being a factor during these inspections. Post mortem predation occurred during all inspection periods, though was less of a problem during the spring collection, probably due to predators focusing more on newborn lambs than moribund sheep. To reduce the impact of predation and autolysis on future studies following the same protocol, it is suggested that

predator control measures be implemented prior to each necropsy inspection period and additional staff be used to collect and sample animals prior to the onset of autolysis.

Point estimates of OJD prevalence for 2-year old sheep based on PFC results for the 12 farms involved in this study ranged from 0.69% to >23.72%. Effectively, 3 farms could be considered to have a low OJD prevalence (<5%), 3 farms a medium OJD prevalence (5-15%) and 6 farms a high OJD prevalence (>20%). The 3 farms with the lowest OJD prevalence either produce 2nd or 3rd cross prime lambs (0.69% and 2.21% prevalence) or were diagnosed with OJD in 2001 (3.97% prevalence). The OJD seroprevalence based on AGID results did not indicate any discernable trends between age groups or farms.

This study showed a significant association between OJD mortality rate and OJD prevalence based on PFC results (P = 0.02). However age-related seroprevalence of OJD was shown not to be useful as a predictor of OJD mortality rate. This reinforces the usefulness of PFC, not only a diagnostic tool, but also as an indicator of the potential impact of OJD at varying prevalence levels. Several methods are available to estimate OJD prevalence from PFC results but each method generates a different set of point and confidence interval results. Method 6, as defined by Cowling *et al*.[15], was chosen as the best approach to estimate the OJD prevalence from PFC in this study given the limitations on knowledge about test sensitivity and specificity at various pool sizes. However, due to the large number of positive cultures being recorded by some of the 12 farms, the very wide confidence intervals produced reduce the usefulness of these results. These concerns are also raised in another study[24] and may be addressed with the development of a Bayesian approach to more accurately estimate disease prevalence from PFC.

Faecal excretion rates of MAP provide a measure of the environmental contamination and risk of OJD infection to other sheep. This study found no significant association between MAP faecal excretion rate (estimated by two separate methods) and OJD mortality rate. However the large range in estimated daily excretion rates between sheep (e.g. 7.6×10^2 to 1.2×10^8 on farm '1/2') and total excretion rates between farms (2.5×10^7 to 5.6×10^{12} from "Method B") is likely to reflect the vast numbers of organisms that can be produced by individual sheep affected with the multibacillary form of OJD.[17] Thus it is likely to be difficult to relate MAP excretion rates to mortality rates considering the effect a few multibacillary sheep may have when determining the MAP numbers from a positive pool. It is also important to note that there is a reduction in MAP numbers during the PFC faecal decontamination procedure therefore the faecal excretion rates reported here are underestimates of the actual environmental contamination on each farm.

This study, conducted to measure annual OJD mortality rate on 12 farms, was not designed to identify risk factors for OJD mortality. However, it was able to act as a pilot for future studies on risk factors, in particular, MLA OJD.038 – the OJD risk factor study. In relation to investigation of risk factors this study was constrained from commencement by the small sample size. In contrast to this constraint, efforts to collect accurate data on dependent and independent variables and to maximise power during statistical analyses justified assessment of the association between seasonal variation in OJD mortality and various environment, management and disease factors.

Risk factor analyses in this study produced some findings worthy of consideration in future work. The fact that, with only 12 farms, variation in OJD death rates was not accounted for totally by the effects of region, season, farm and flock size demonstrates that other factors are influential. Further it strongly indicates that investigation of a larger number of farms would enable these other factors to be more closely investigated and the most significant factors identified.

Associations between eight farm-level factors relating to management practices, flock health and the environment and OJD mortality rate were found. The evidence for these associations, though not compelling, suggests that these factors be further assessed in future work. The final model clearly defined the relationship between OJD mortality, 3 of the 8 farm-level factors (stocking rate, % improved pasture, weaning age) and flock size. However, the direction of association for each factor, particularly stocking rate, was counter-intuitive (see discussion below). It is apparent that this work has not clearly defined these relationships but it has indicated these factors (or other factors closely correlated with them) should be targeted for further investigation. For future work, these results also demonstrate the limited capability of statistical analyses using whole flock OJD mortality and annual or whole farm data to identify risk factors for OJD. It is strongly suggested that future studies focus on OJD prevalence in and the

management of a specific age (or management) cohort of sheep (rather than the whole flock) and control for several confounding factors.

Counter to initial expectation, flock size was found to be protective (that is, associated with lower OJD mortality rates) and percentage of improved pasture to be detrimental (that is, associated with higher OJD mortality rates). These relationships identified by the model can be seen in the raw data for flock size, improved pasture area and OJD mortality rate for the 12 farms (Table F.1 and Figure F.1 in Appendix F). In the analyses flock size was investigated rather than farm size due to its more direct link to mortality rate. However, flock size and farm size are highly correlated (r = 0.75, P = 0.005). As farm size relates to land use, larger farms are likely to have a greater variation in topography and land use with different proportions of improved pasture, native pasture and area cropped compared to smaller farms. In fact smaller farms due to production pressure are more likely to have a higher proportion of improved pasture than larger farms. In relation to sheep exposure to OJD contaminated pasture, larger farms with more options for spelling pasture and for grazing crops pre- and post-harvest have an advantage over smaller farms where use of improved pasture, in addition to improving nutrition, results in higher stocking rates[23] and longer periods of exposure to contaminated pasture. Further improved pasture would have different pasture species and likely also different history of application of fertilisers than native pasture, which could influence microclimate and soil chemistry. Soil pH has been suggested as a risk factor for JD in cattle[25].

In this study stocking rate was found to be protective (that is, associated with lower OJD mortality rates). Stocking rate varies with available pasture[5] therefore this association may be a reflection of the options available for nutrition on a farm. Although stocking rate would be expected to vary with farm size, correlations with farm size (r = -0.11, P = 0.73) and flock size (r = 0.22, P = 0.49) were low. This indicates that stocking rate is determined more by farm management, that is, the decisions made by farmers to meet the nutritional needs of the flock than farm or flock size. More detailed information about stocking rate (such as quarterly or monthly stocking rates) should be collected in future studies to gain a better understanding of this association between stocking rate and OJD mortality rate.

During our evaluation of flock size and stocking rate, we considered the fact that these protective associations could be the result of producer response to OJD prevalence rather than a cause of OJD prevalence. However the questionnaire data provided no evidence of a reduction in flock size or stocking rate being a producer response to a positive OJD diagnosis on these 12 farms. In fact the questionnaire data tends to indicate the opposite:

The reviewer states that two of the putative risk factors could in fact be the result of producer response to OJD prevalence rather than a cause of OJD prevalence. This issue was considered in our evaluation of the dataset and the model output. For stocking rate and flock size review of data collected by the questionnaire showed no evidence that reduction in stocking rate and flock size was a producer response to a positive OJD diagnosis on these 12 farms. In fact the questionnaire data tends to indicate the opposite with increased cropping area (and presumably stocking rate) on 2 farms, increases in both flock size and stocking rate on 6 farms, and no change in flock size or stocking rate on the remaining 4 farms.

The final model showed reduction in weaning age to be detrimental (that is, associated with higher OJD mortality rates). Although association of weaning age with OJD mortality is credible (supported by current knowledge of the epidemiology and clinical expression of OJD), the direction of the association was contrary to expectation and requires consideration and further investigation. One possible explanation is that weaning lambs at 15-16 weeks rather than 10-14 weeks of age means they are stronger at weaning and that the 2-weeks additional exposure to contaminated faeces of infected ewes is inconsequential for older lambs.

The economic impact of OJD was estimated to provide information about the effect of this disease on farm profit levels over a 12-month period. Although costs associated with decreased production should be considered in an economic analysis that seeks to assess the economic impact of an animal disease[26], we did not consider these in this work due to the currently limited knowledge about the effect of subclinical OJD. Parameters for income and expenditure from published sources[22] were used to enable a comparison of economic performance between the 12 flocks with and without OJD as well as focus on the economic losses associated with lost production due to increased mortalities. This study used two

different approaches to estimate the economic impact of OJD on the 12 farms.

The first approach compared gross margins for each of the 12 farms with and without OJD. Direct costs associated with OJD such as loss in production due to death and cost of the Gudair® vaccine for prevention were considered in the gross margin analysis as these costs potentially had the greatest economic impact and could be accurately determined. All 12 flocks were located within the "management zone" at the time of this study therefore the impact of zoning was consistent between all 12 farms and not considered. Also, according to questionnaire responses no additional labour/infrastructure costs associated with managing OJD had been incurred by any of the 12 farms and therefore were not considered.

The gross margin for an OJD infected flock was on average 6.4% less than the gross margin for a non-OJD infected flock. The average gross margin (GM) per dry sheep equivalent (DSE) for the 12 OJD infected flocks was $20.58 compared to $21.85 if the same flocks were non-OJD infected. The range of values for GM/DSE ($10.16 to $36.36) demonstrates the large variability between flocks and reflects variation in farm size and management. The flock with the highest OJD mortality rate (17.5%) returned a GM/DSE of $26.59. Possible explanations for the higher gross margins/DSE include economies of scale absorbing some of the variable input costs or producers failing to undertake some management procedures such as regular application of fertiliser, jetting, drenching and vaccinating. While these producers save money in the short term by not undertaking some or all of these management procedures, the cost to production in the long term may be considerable if there is an associated loss of production through either poor performance or increased mortalities. Those farms with high OJD mortality rates and above average gross margins could therefore improve their returns further by decreasing the number of OJD mortalities.

The second approach involved using the necropsy information from each of the 12 OJD infected farms to estimate the cost of OJD on each farm for the 12-month study period. The value of each necropsied sheep at death and the value of lost production through premature death were considered. Estimated cost associated with OJD mortalities ranged from $15,569 to $154,083 for the year with an average of $64,100 across the 12 farms. The estimated cost of losses associated with OJD accounted for between 16.5% and 100% of the estimated total losses associated with sheep mortalities and again highlights the wide range of economic impact between farms.

Drought conditions were experienced for between 2 to 10 months during 2002 on the 12 farms. All farms experienced severe drought conditions with higher than normal temperatures and pan evaporation levels toward the end of 2002. Limited pasture quantity across most areas, especially during the second half of the study period, required producers to supplementary feed their stock. Further, in an attempt to reduce the impact of the drought, during late 2002 producers reduced stock numbers on their farms to an average 79.2% of their normal carrying capacity and some altered their grazing practices. Although not recorded, fluctuations in the observed average body condition score of flocks across all four areas also reflected the effects of the drought.

This study provides the first objective data on the mortality rate attributable to OJD, the economic losses attributable to these deaths, and on possible risk factors for mortality where OJD is present. The findings should be relevant to all sheep producers in southern New South Wales. As mortality rates and economic losses were quite substantial, producers will need to be informed of the findings and encouraged to undertake control to prevent mortality rates reaching the levels seen on some farms in this study.

6. Success in Achieving Objectives

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

- **To determine the mortality rate due to OJD in twelve sheep flocks.**

For the twelve flocks, the average adjusted OJD mortality rate (inventory) was 6.2% (median 5.8%, range 2.1% to 17.5%) and the average extrapolated OJD mortality rate (necropsy study) was 6.7% (median 4.4%, range 1.1% to 15.0%). These average mortality rates were more than twice those considered acceptable (from all causes) in sheep flocks in southern Australia.

- **To describe the relationship between age and OJD mortality rate in affected flocks.**

For the twelve flocks, the distribution of necropsied sheep where OJD contributed to death across age groups increased from 1 year of age (10.4%) to peak at 4 years of age (35.6%) and then fall at over 4 years of age (19.2%).

- **To describe the relationship between sex and OJD mortality rate in affected flocks.**

For the twelve flocks, the distribution of necropsied sheep where OJD contributed to death between sexes was very similar between wethers (49.6%) and breeding ewes (50.4%).

- **To investigate the relationship between OJD mortality rate and prevalence of OJD in two-year old sheep based on pooled faecal culture in affected flocks.**

A significant relationship (P = 0.02) was identified between OJD prevalence in two-year old sheep based on PFC and OJD mortality rate.

- **To investigate the relationship between OJD mortality rate and faecal excretion rate of MAP in two-year old sheep based on pooled faecal culture in affected flocks.**

There was no significant relationship ("Method A" P = 0.87 and "Method B" P = 0.29) between the faecal excretion rate of MAP in two-year old sheep based on PFC and OJD mortality rate.

- **To investigate the relationship between OJD mortality rate and age-related seroprevalence of OJD in affected flocks.**

There was no significant relationship (2 Yr-old P = 0.24, 3 Yr-old P = 0.39 and 4 Yr-old P = 0.52) between age-related seroprevalence of OJD and OJD mortality rate.

- **To relate seasonal variation in OJD mortality rate to environment, management and disease factors and identify which factors are worthy of further investigation.**

Analyses indicated several factors (including flock size, stocking rate, area of improved pasture and weaning age) as worthy of further investigation. In addition the inability of this work to clearly define associations provides support for future work to involve a larger number of farms, focus on a specific cohort of sheep and control for several confounders.

- **To provide an accurate estimate of the cost of OJD in affected flocks.**

The average % decrease in gross margin due to a farm being infected with OJD was 6.4% (median 5.5%, range 2.2% to 15.4%). The average gross margin/DSE for the OJD infected flocks was $20.58 (median $22.22, range $10.16 to $36.36) compared to $21.85 (median $22.86, range $11.77 to $37.19) if the same flocks were non-OJD infected. Based on the necropsy study the average estimated cost of annual OJD losses was $64,100 (median $44,942, range $15,569 to $154,083) and these OJD losses accounted for on average 70.1% of the estimated total economic losses (median 68.5%, range 16.5% to 100%).

7. Meat and Livestock Industry Impact

For the first time objective data are available on the true levels of OJD-related mortality on 12 farms, and the findings will be generally applicable in southern Australia. Industry groups claiming that OJD does not present a threat on-farm can now be provided with accurate figures on direct losses attributable to OJD within the endemic area of NSW. There was a wide range of impacts, with some very high mortality rates. The data can be used to justify vaccination programs, other control options and the general concept of disease control and prevention. These findings expand on previous observations conducted in a single high prevalence flock[3].

Further, we now have objective data on the economic losses associated with OJD mortalities in infected flocks and we have identified several risk factors potentially related to OJD losses and study design issues that can be used to plan and inform further investigation. Designing an education and extension package that can incorporate these and other recent research findings to address issues of misinformation about OJD would appear to be the major challenge for the industry.

8. Conclusions and Recommendations

These findings quantify OJD mortalities in 12 flocks across 4 districts of south-eastern NSW, confirming that mortality rates were considerable and did contribute to significant economic loss on these farms during the 12-month study period. Examination of inventory records identified that the adjusted OJD mortality rates on the 12 farms averaged 6.2%, ranging from 2.1% to 17.5%. The necropsy study involving four 5-day inspection periods retrieved 399 sheep for necropsy of which death was determined for 362 sheep. 77% of these mortalities were attributable to OJD when adjusted for drought effects.

Estimated annual economic loss due to OJD averaged $64,100 per farm based on the necropsy inspection period information. Additional economic analysis identified an average gross margin/DSE of $20.58 for the 12 OJD infected flocks, which ranged from $10.16 to $36.36. The gross margin for an OJD infected flock was on average 6.4% less than the gross margin for a non-OJD infected flock. Therefore OJD was considered to be compromising the economic performance of some of the flocks with lower GM/DSE. Of further concern was the finding that a number of flocks achieved higher GM/DSE by reduction of variable inputs such as fertiliser and animal health treatments. The sustainability of such practices needs further examination.

A pilot examination of possible risk factors for OJD losses in these 12 flocks indicated a number of variables deserving of further investigation in a study specifically designed to identify risk factors. Moreover it clearly showed that any future risk factor study should enrol a larger number of farms, focus on OJD prevalence in and the management of a specific age cohort of sheep (rather than using whole flock data) and control for several confounding factors.

The findings of this study were presented to the participating producers and their advisors at a meeting held in Yass in December 2003. Consideration for further extension of these findings is warranted, particularly as part of an education process identified in recent reviews of the current NOJDP (e.g. Bull Report). Data from this project is of considerable importance and valuable for educating producers about the expected biological and economic on-farm outcomes of OJD infection.

Specific recommendations that arise from this project include:

1. Development of a fact sheet specifically addressing OJD mortalities and direct economic losses as reported here, for distribution through MLA mailing lists and AHA OJD communications program

2. Use of data collected over 3-4 years on the 12 farms to develop a model to predict the economic impact of OJD on individual farms

3. Further investigation of risk factors indicated in this study in the OJD risk factor study (MLA OJD.038)

4. Use of the data reported here for benchmarking, specifically for comparison against future mortality rates measured following adoption of OJD control measures that are currently being considered for inclusion in a revised NOJDP.

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Appendices

Appendix A – Necropsy Inspection Protocol

Gross PM Record Sheet

Fill out the details of each sheep eg age, sex, paddock location etc and then record the:

- Clinical signs - position of sheep when found (eg cast, down etc), fly strike (position + age of maggots eg rump/adults – identifies how long sheep has been struck) and any scouring (extent + presence of dags – indicates intermittent scouring).
- Gross appearance - wool length / estimated fibre diameter / colour.
- Chest cavity and abdominal cavity findings **–** describe the texture / colour / size (length) / location of tissues/organs (normal and abnormal) and look for ulcerations in the ilium and caecum.
- Description of extra samples collected.
- Time since death – K = killed, D = dead (estimate a time when found dead).
- Differentials/cause – OJD, other (specify if clear).

Take photos of any abnormal tissues (record roll / frame number).

Label Tissue Sample Tubes

On each collection tube label: ID number, date, sample code (ie A = illeocaecal junction, B = terminal ileum, C = lymph nodes, D = faeces, E = lung, F = kidney, G = liver) and label extra samples (eg mammary tissue, heart etc).

Label Histo/Worm Pots

On each collection pot label: ID number, date and sample - either “Histo”, ”ABOM” (abomasum) or ”SI” (small intestine).

Post Mortem

*** Always place the sheep in left recumbency

- Retract limbs - expose body surface and look for enlarged lymph nodes, fluid in peritoneal cavities and haematomas. Cut through the muscle on the hind limb to determine time since death (ie dark brown = longer since death).
- Chest cavity - open by cutting the intercostal muscle between the ribs, cut the cartilage between the ribs and sternum then break the ribs to check for indication of mineral deficiencies. Palpate the lungs, if necessary take biopsy from cranial & ventral lobe including diseased plus normal tissue. Look at the lymph track between the 2 lungs. Remove the heart and look at the fat colour and quantity, as this will indicate the metabolic state (normal = white and drawing on reserves = gelatinous/yellowy). Dissect the heart to inspect the chambers/valves. Look for fluid in the chest cavity.
- Abdominal cavity - open carefully so no organ is damaged. Scoop organs into the organ tray and work with organs in the tray. Look for fluid in the abdominal cavity and abscesses on the cavity wall when organs are removed. Look at the liver, lymph nodes, kidneys (fat = metabolic indicator), lymph chain, uterus (reproductive status) and rectum (formed faeces/scours).
- Abomasum - locate the pyloric sphincter and tie-off, separate the abomasum from the omasum, cut the junction (slightly within the omasum as forms a seal) and pour the contents of the abomasum into a bucket. Wash hands/scissors while adding water, wash the abomasum internal lining well (rub/squeeze) then ring out. Agitate washings and add additional water until bucket has 2L, put 200ml aliquot into the ABOM pot (* always take 10%).
- Small Intestine (SI) - gently separate the SI (until past the pancreatic/liver attachment) taking care not to cut the SI, pull out approx 2m (the same amount each time) and put SI section into a bucket with a little water. Dissect the length of the SI and wash the SI internal lining well (rub/squeeze) before ringing out. Agitate washings and add additional water until bucket has 2L, put 200ml aliquot into the SI pot (* always take 10%).
- Liver - feel the texture and observe the colour (look for blemishes/depth and abscesses). Look at the lymphatics and look at the gall bladder (size). Make incisions to inspect the bile ducts and

squeeze the liver tissue to exude fluid. Inspect for signs of fluke.

- Kidney - look at external colour and internal colour (equal portions medulla/cortex). Inspect for urinalysis (enlarged center) and pulpy kidney.
- Palpate rumen for abnormalities.
- Tissue and Histo Samples - look for abnormal thickening in SI (eg corrugated effect) and look for enlarged lymph nodes in lymph chain. Place all tissue to be sampled onto a cutting board and trim any fat (be careful not to squeeze/crush tissue). Using a scalpel and tweezers, cut two small pieces of the required tissue and place one into a labeled tissue culture tube the other into a labeled Histo pot (cut into pieces of 5mm thickness so will absorb formalin). Sample the ileocaecal junction and caudal jejunum lymph nodes (Sample C), the terminal ileum (Sample B) and the ileocaecal junction (Sample A).
- Faecal sample - locate the large intestine and cut/remove a small section. Place 4-5 pellets (or equivalent) into a tissue pot (Sample D).

Sample Storage

Tissue – refrigerate at 4^{o}C until return on Friday night then store at –70^{o}C.

Histo/Worms – add formalin (2/3 fill Histo pot and add 2ml to worm pots) then store at room temperature.

Appendix B – Histopathology Slide Reading Protocol

Classification of lesions associated with OJD

Several classification systems have been used to grade lesions. The most comprehensive is that of Perez et al[10]. This system does not allow for separate grading of lymph node lesions, nor does it allow for accurate grading of AFB intensity, which are implied by the various grades. For this reason a modification developed by Whittington and Marshall (unpublished) is used at Usyd in all experiments and research trials.

Type 1 Lesions

- Location
    - only in the lymphoid tissue, never in the intestinal mucosa.
    - PPs: interfollicular spaces, in the basal zone, less often at the apex
    - MLNs: paracortex or interfollicular area, related to subcapsular or peritrabecular sinuses (MLN less often affected than PP)
- Type
    - granulomata formed by macrophage-like cells (nuclei large and clear with obvious nucleoli, abundant slightly foamy cytoplasm, lightly stained by H&E and sometimes with clear vacuoles) often with small numbers of lymphocytes and cells with elongated nuclei
    - no AFB are seen.

Type 2 Lesions

- Type
    - granulomata well delineated, round, variable in number, never enough to result in diffuse enteritis
    - AFB occasionally seen in granulomata in mucosa, but not in PPs/MLNs.
- Location
    - PPs: granulomata in a row from the most basal zone of the interfollicular area to the apex, penetrating into the lamina propria. Granulomata in the villi are always associated with granulomata in an adjacent PP
    - MLNs: similar to type 1 lesions. Less frequently seen than those in PPs and always smaller in size

Type 3 Lesions

- Granulomatous lesions affect PPs, associated mucosa and mucosa that is not associated with lymphoid tissue. There are 3 subtypes:

Subtype 3a

- Type
    - Lesions sporadic, multi-focal
    - AFB are seen in granulomata in the mucosa
- Location
    - PPs and associated mucosa:
    - lesions are similar to type 2
    - granulomata in the lamina propria are larger, extend from PPs, involve more villi, cause enlargement of villi
    - Areas of mucosa not associated with PP's:
    - granulomata are small and well delineated in lamina propria of villi and/or the basal area
    - Submucosa and serosa:
    - foci of inflammatory cells (mostly lymphocytes and macrophages) are seen around lymphatic and blood vessels
    - MLNs: granulomatous lesions

Subtype 3b (Multibacillary)

- Type
    - Diffuse granulomatous enteritis creates a mosaic formed by macrophages, epithelioid cells, a few giant cells (2-3 nuclei), small numbers of lymphocytes and other leukocytes
    - AFB in abundance, numbers in mucosa > than in lymphoid tissue.

- Location

  PPs: granulomata in the interfollicular areas, follicles and domes, with infiltrates giving a mosaic-like appearance, among lymphoid aggregates

  Mucosa:
  - villi thickened, apices flat and wide, fused, fewer crypts due to infiltration
  - in some sheep the mucosa is less thickened, epithelioid cells are seen in the villi (mostly the apex) and diffuse enteritis is due to confluence of numerous small granulomata

  Submucosa:
  - lymphocyte and plasma cell infiltrates, initially perivascular, but extending to the muscular layer, with lymphatics dilated and thrombi composed of macrophages seen within
  - Serosa:
  - Lymph-angitis/angiectasis, perivascular lymphocyte/plasma cell aggregates
  - MLN's:
  - multi-focal or diffuse granulomatous lymphadenitis
  - subcapsular sinuses usually contain macrophages
  - serosal lesions similar to those in the gut serosa

Subtype 3c (Paucibacillary)

Type
- diffuse granulomatous enteritis, but the cell types differ from type 3b
- AFB rarely seen, and then only in small numbers.

Location
- PPs: lesions similar to type 3b, but with pyknotic macrophages and giant cells
- Mucosa: diffuse granulomatous enteritis, but the predominant cells are lymphocytes in the lamina propria of the villi and the basal area; macrophages are seen among the lymphocytes either scattered or in small, well defined granulomata of up to 20-25 cells
- Submucosa: frequently oedema, with variable numbers of lymphocytes and plasma cells
  - Serosa: similar to type 3b
  - MLNs:
  - multifocal granulomata in the paracortical and interfollicular areas
  - Langhans giant cells (some with >30 nuclei) may be present
  - pyknotic macrophages and perivascular infiltrates are seen in the serosa of the LNs

Lymph Node Lesion Score

0 No lesion
1 Mild – small focal lesions
2 Moderate – larger lesions, multifocal
3 Severe – diffuse

Ziehl-Neelsen Stain Score

0 No AFB
1 Individual or small numbers, limited foci
2 Small numbers, multiple foci
3 Moderate numbers, diffuse
4 Large numbers, diffuse

Example of table for recording histopathology results

Animal no.	*Perez ileum score*	*AFB ileum score*	*Score MLN*	*AFB MLN*
Ear tag or other identifier	*1, 2, 3a, 3b, 3c*	*0 to 4*	*0 to 3*	*0 to 4*
Eg. 215	*3a*	*2*	*1*	*0*
216	*3b*	*4*	*3*	*3*

Appendix C – Farm Level Independent Variables

Variable	Description	Units	Count	Mean	Median	Range
1. Environment						
a) Rainfall (mls)	(Information gained from the Bureau of Meteorology and producer rainfall records)					
	Actual Annual Rainfall (mm)	#		711	702	625, 800
b) Pasture (%)	(Proportions of pasture/cropping areas provided from producer records)					
	i) Proportion of total farm area is improved pasture (%)	#		70	68.5	36, 100
	ii) Proportion of total farm area is cropped (%)	#		10.9	5	0, 40
c) Nutrition	(Pasture samples were collected from each farm at the four necropsy inspection periods and analysed for ADF and N which enabled ME to be estimated using: ME content MJ/kg DM = 0.17DMD% - 2.0 [where DMD% = 83.58 - 0.824ADF% + 2.626N%]) (Seasonal pasture data was recorded quarterly and combined for analysis)					
	ME - Improved Pasture - Autumn	1,0				
	- Low to Very Low (<8 MJ/kg DM)		7			
	- Medium to High (>8 MJ/kg DM)		5			
	ME - Improved Pasture - Winter	1,0				
	- Low to Very Low (<8 MJ/kg DM)		2			
	- Medium to High (>8 MJ/kg DM)		10			
	ME - Improved Pasture - Spring	1,0				
	- Low to Very Low (<8 MJ/kg DM)		1			
	- Medium to High (>8 MJ/kg DM)		11			
	ME - Improved Pasture - Summer	1,0				
	- Low to Very Low (<8 MJ/kg DM)		3			
	- Medium to High (>8 MJ/kg DM)		9			
d) Months in Drought During 2002	(Farmer observations from each farm provided by questionnaire)					
	Farmer Observations (months)	#		7.7	8.5	3, 12

Variable	Description	Units	Count	Mean	Median	Range
e) Soil	(Soil type/deficiency information provided from producer records, soil pH from producer instigated soil tests)					
	i) Major Soil Type/s	1,2,3,4,5				
	- Granite		1			
	- Granite/Clay		5			
	- Granite/Basalt		3			
	- Basalt/Shale		1			
	- Sand/Clay		2			
	ii) pH ($CaCl_2$)	1,0				
	- Highly Acidic (pH 4-5)		8			
	- Moderately Acidic (pH 5.1-6)		4			
	iii) Owner Reported Mineral/Trace Element Deficiencies	1,0				
	- Yes		7			
	- No		5			
2. Management						
a) Supplement and fertiliser application	(Supplement information and fertiliser history provided from producer records)					
	i) Mineral Supplement Used	1,0			Descriptive only	
	- None		12			
	- Blocks/Licks		0			
	ii) Fertiliser Applied	1,0				
	- Superphosphate +/- Mo Superphosphate		11			
	- None		1			
	iii) Frequency of Fertiliser Application	1,0				
	- Annually to every 2/3 years		10			
	- Infrequently/Nil		2			
b) Flock size	(Flock sizes provided from producer records)	#		9193.6	7065	3976, 20562

Variable	Description	Units	Count	Mean	Median	Range
c) Flock Age Structure	(Information based on livestock inventory from each farm)					
	Proportion of Young to Adult Sheep	1,0				
	- less than or equal to 1:4		2			
	- greater than or equal to 2:3		10			
d) OJD Clinicals Removed	(Information for each farm provided from questionnaire)	1,0				
	- Yes		8			
	- No		4			
e) Number of Sheep Introduced to farm	(Information based on livestock inventory from each farm)					
	Combined Number Bought annually	1,0				
	- < 40		9			
	- > 500		3			
f) Lambing	(Information for each farm provided from questionnaire)					
	Lambing Season	1,2,3				
	- Winter		3			
	- Spring		8			
	- Spring + Autumn		1			
g) Internal Parasites	(Drench resistance information provided by each farm).					
	Drench Resistance	1,0				
	- Yes		9			
	- No		3			
h) Supplementary Feeding	(Information from each farm provided by questionnaire)					
	Supplementary Feeding Conducted Other Than During Drought	1,0				
	- Yes		10			
	- No		2			

Variable	Description	Units	Count	Mean	Median	Range
i) Lamb Weaning Management	(Information from each farm provided by questionnaire)					
	i) Age of Lambs at Weaning	1,0				
	- 10-14 wks		7			
	- 15-16 wks		5			
	ii) Additional Nutrition/Clean Pastures Provided	1,0			Descriptive only	
	- Yes		12			
	- No		0			
j) Grazing System	(Information from each farm provided by questionnaire)					
	Usual Grazing System	1,2,3,4,5				
	- Cell		1			
	- Rotational		4			
	- Rotational/Cell		1			
	- Set Stock		1			
	- Set/Rotational		5			
k) Stocking Rate (DSE/ha)	(Information from each farm provided by questionnaire)					
	Actual Usual Stocking Rate	#		11	10	8, 18
l) Shearing Date	(Information from each farm provided by questionnaire)	1,2,3,4,5				
	- Autumn		4			
	- Winter		1			
	- Spring		1			
	- Summer		3			
	- Autumn + Spring		3			
3. Disease						
a) OJD Death Rates	(Information based on livestock inventory and necropsy information from each farm)					
	Adjusted OJD Mortalities (OJD deaths per quarter ÷ flock size) (%)	#		6.2	5.6	2.1, 17.5

Variable	Description	Units	Count	Mean	Median	Range
b) OJD Infection History	(Information from each farm provided by questionnaire)					
	i) Year First Noticed OJD Losses (year)	#		1997	1998	1987, 2001
	ii) Source of First OJD Infection	1,2,3,4				
	- Neighbour		4			
	- Purchased Ewes/Wethers		1			
	- Purchased Rams		4			
	- Unknown		3			

Appendix D – OJD Economic Impact: Gross Margin Example

Farm: "1/1"

Farm Size (ha):	1838				
Income					
Wool	**Number**	**Class**	**kg/hd**	**$/kg**	**Total ($)**
Shear	5120	ewes	4.3	$6.62	$145,833.98
	3691	wethers	4.6	$6.62	$112,466.25
	3341	hoggets	3.6	$6.62	$79,670.82
	60	rams	5.5	$6.20	$2,046.00
Crutch	9095	adults	0.35	$2.89	$9,199.59
Sheep Sales	**Number**	**Class**	**$/hd**		
	1322	CFA ewes	41.95		$55,457.90
	1471	CFA wethers	50.80		$74,726.80
	616	hoggets	60.00		$36,960.00
	12	CFA rams	50.40		$604.80
				A. Total Income:	$516,966.15
Variable Costs					
Sheep Health	**Number**	**Class**	**Cost ($)**	**Reps**	
Drenching					
-broadsprectrum	35329	adults/hoggets	0.19		$6,712.51
	3254	lambs	0.13		$423.02
- narrowspectrum	0	adults/hoggets	0.21		$0.00
	0	lambs	0.14		$0.00
Dipping	10039	adults/hoggets	0.32	1	$3,212.48
Jetting	9454	adults/hoggets	0.21	1	$1,985.34
	3254	weaners	0.11	1	$357.94
Vaccination (6 in 1)	3441	adults/hoggets	0.34	1	$1,169.94
	3386	lambs	0.34	2	$2,302.48

Vaccination (Gudair®)	3386	lambs	1.65	1	$5,586.90
Mules + Mark	3386	lambs	0.90	1	$3,047.40
Scanning	3854	ewes	0.80	1	$3,083.20
Wool Selling Costs					
Shearing	12152	wethers/ewes/hoggets	3.52		$42,775.04
	60	rams	5.02		$301.20
Crutching	4593	wethers/hoggets	0.59		$2,709.87
	4442	ewes	0.56		$2,487.52
	60	rams	1.12		$67.20
Wool tax			2.00%		$6,984.33
Commission, warehouse, testing charges			$32.41	/bale	$9,819.41
Wool - cartage	303		10.08		$3,053.99
- packs	303		10.46		$3,169.12
Livestock Selling Costs					
Livestock Cartage	3421	sale sheep	1.50		$5,131.50
Commission on sheep sales			4.50%		$7,548.73
Fodder					
Supplementary Feeding - 1kgs of oats/hd/week @ $120/tonne				**Weeks**	
	3341	young sheep	$0.12	12	$4,811.04
	6695	adults	$0.12	4	$3,213.60
Pasture Maintenance - single super applied at 100kg/ha every year				**Appl. Freq/Year**	
	1838	hectares @	$15.00	2	$55,140.00
				B. Total Variable Costs:	$175,093.75
Replacements					
	Number	**Class**	**$ / hd**		
	0	wethers	$55.00		$0.00
	0	ewes	$60.00		$0.00
	2	rams	$1,000.00		$2,000.00
				C. Total Replacements:	$2,000.00

	With OJD
Gross Margin (A-B-C)	$339,872.39
Gross Margin / DSE	$23.11
Gross Margin / ha	$184.91

1. Flock Parameters

Productive life (yrs)	5	Total mortality %	6.3
Replacement age (yrs)	5	Mortality minus OJD %	3.9
Ewe body weight (kg)	45	Marking %	87.9
Wether body weight (kg)	45	Weaning %	84.4
Stocking rate/ha (dse)	8	Ram %	1.5

2. Flock Structure

Activity	Ewes	Wethers	Hoggets	Lambs	Rams
Shearing	5120	3691	3341		60
Dipping	4715	2949	2315		60
Crutching	4442	2406	2187		60
Jetting	6140			3254	60
Broad Spec Drenching 1	6149	3904	2422		60
Broad Spec Drenching 2			2362		
Broad Spec Drenching 3			2328		
Broad Spec Drenching 4	4643	2875	2223		60
Broad Spec Drenching 5	4442	2167	1634	3254	60
Narrow Spec Drenching 1					
Narrow Spec Drenching 2					
Vaccination (Adult)	3441			3386	
Vaccination (Lamb)				3386	
Mules + Marking				3386	
Ewe Scanning	3854				
Supplementary Feeding	3441		3341	3254	
CFA's sold	1322	1471	616		12
Replacements bought					2

3. Wool Prices

Merino	Micron	AWEX Type	Clean Price	Yield	Greasy Price	Specifications at 35n/ktex	Proportion of Clip
Adult	19	MF4B.	$10.60	71%	$7.53	1.0% VME, 90mm	69%
Skirtings/bellies	18	MP5B.	$10.12	58%	$5.85	5.0% VMB, 80mm	22%
Cardings	19	MZ5B.	$2.64	59%	$1.57	2.0% VMB	9%
					$6.62		
Crutchings	19	MC5E.	$4.90	59%	$2.89	2.0% VMB	

Appendix E – OJD Economic Impact: Necropsy Information Example

Property: **"1/1"**

Age	Sex	Condition Score	Reprod. Status	Estimated FD (mm)	Wool Length (cm)	Death Category	Sheep Value	Lost Reprod. Value	Wool Value	Lost Wool Value	Total Value
1	W	3	-	19	9	other	$60.00	$0.00	$21.05	$121.81	$202.85
2	E	<1	Dry	20	2	other	$41.95	$151.92	$28.47	$85.40	$307.73
2	E	<1	Dry	19	1	OJD	$41.95	$151.92	$28.47	$85.40	$307.73
3	W	1.5	-	18	5	OJD	$50.80	$0.00	$30.45	$60.90	$142.16
3	E	<1	Joined	19	8	OJD	$41.95	$101.28	$28.47	$56.93	$228.63
4	W	1	-	Bare	Bare	OJD	$50.80	$0.00	$30.45	$30.45	$111.70
4	W	1	-	20	6	OJD	$50.80	$0.00	$30.45	$30.45	$111.70
4	E	<1	Dry	Doggy	5	OJD	$41.95	$50.64	$28.47	$28.47	$149.52
4	E	1.5	Dry	Doggy	2	other	$41.95	$50.64	$28.47	$28.47	$149.52
4	E	1.5	Dry	19	2	other	$41.95	$50.64	$28.47	$28.47	$149.52
4	E	2	Dry	20	2	other	$41.95	$50.64	$28.47	$28.47	$149.52
4	E	2	Dry	20	3	other	$41.95	$50.64	$28.47	$28.47	$149.52
4	E	2	Preg Empty	20	3	other	$41.95	$50.64	$28.47	$28.47	$149.52
4	E	2	Preg Empty	21	6	other	$41.95	$50.64	$28.47	$28.47	$149.52
4+	W	<1	-	20	8	OJD	$50.80	$0.00	$30.45	$0.00	$81.25
4+	W	1.5	-	20	6	OJD	$50.80	$0.00	$30.45	$0.00	$81.25
4+	W	<1	-	19	5	OJD	$50.80	$0.00	$30.45	$0.00	$81.25
4+	E	1.5	Preg Empty	19	2	other	$41.95	$0.00	$28.47	$0.00	$70.42
4+	E	1.5	Preg Empty	21	2	other	$41.95	$0.00	$28.47	$0.00	$70.42
4+	E	<1	Preg Empty	19	1	OJD	$41.95	$0.00	$28.47	$0.00	$70.42

Values

Class	Sheep Value	Wool Value	Weaning %
Hogget Ewe	$60.00	3.40 kg x $6.19 = $21.05	84.4
Hog. Wether	$60.00	3.40 kg x $6.19 = $21.05	
Adult Ewe	$41.95	4.30 kg x $6.62 = $28.47	

Cost of OJD losses: $1,365.62

Adult Wether	$50.80	4.60 kg x $6.62 = $30.45

Appendix F – Report on statistical analyses prepared by Paul Nicholls, Biometrician, EMAI, NSW Agriculture

Design

A census of OJD deaths for a 12 month period was undertaken on 12 farms in southern NSW. The 12 farms comprised 3 farms within each of 4 regions and were selected on the basis of a history of OJD. Quarterly totals of OJD deaths were recorded for each farm. A number of variables thought to be possible risk factors for OJD were recorded and a subset of 24 were statistically assessed. One of the 24 (seasonal pasture ME-MJ/kg DM improved - dichotomised) was recorded quarterly and the rest were annual or annualised continuous or discrete data, with a number of the continuous variables dichotomised.

Statistical analyses

In a first analysis of quarterly OJD death rates (OJD deaths per quarter/flock size), the method of generalised linear mixed models (GLMMs) for binomial data using the logistic link function was applied. The fixed effects were region, season and the region x season interaction while the random effects were farm and the farm x season interaction. The model indicated there were appreciable effects for each term except the region x season interaction. When flock size was added as a fixed effect to this model its effect was strong and it almost entirely accounted for the observed region effect, but the random effects of farms and the farm x season interaction remained appreciable in size. There was no overdispersion present in the model. It was concluded that there was sufficient variation in OJD death rates among farms after adjusting for flock size to warrant an assessment of the other risk factors.

In the assessment of the risk factors, the first step was to obtain the correlations/associations among the factors. This revealed a number of moderate to high dependencies among the factors which would need to be considered during the modelling, for which the above GLMM method was used. The basic model terms comprised the fixed effect of flock size and the random effects of farms and seasons within farms. A number of different sets of factors were added to the basic model and assessed: each individual factor, then selected sets of 2, 4 or 6 factors chosen with the sample correlations/associations taken into account (to avoid problems with high multicollinearities in the model). An assessment of the pattern of results from these models indicated that 8 factors might be worth modelling formally in addition to flock size:

- AIP - actual improved pasture area (%)
- SPI - seasonal pasture ME-MJ/kg DM improved (0/1)
- YFL - actual year first noticed losses
- TBA - total replacements bought annually (0/1)
- DR - drench resistance (0/1)
- HF - hand feeding not in drought (0/1)
- LAW - lamb age at weaning (0/1)
- SR - actual stocking rate DSE/ha

Stepwise backward elimination of terms was used to find a final model in which each fixed effect present had a nominal significance level of $P<0.05$ at least, with all non-zero random effects retained.

Table F.1 presents a summary of the raw data for the four factors present as fixed effects in the final model and Figure F.1 illustrates the quarterly OJD mortality rate for each of the 12 farms.

Results

The final model fixed effects were flock size (P<0.001), AIP (P<0.01), SR (P<0.05) and LAW (P<0.05); there were no random farm effects but effects of seasons within farms were present (P<0.01). The coefficients on the logistic scale for the four nominally significant effects (± SE) were:

Flock size	-0.000130 ± 0.000034 per sheep
AIP	0.0210 ± 0.0065 per %
SR	-0.116 ± 0.044 per DSE/ha
LAW	0.45 ± 0.21 [Quarterly mean rates: 1 0.063%; 0 0.040%; Average mean rate: 0.052%]
	[Annual mean rates: 1 0.253%; 0 0.162%; Average mean rate: 0.210%]

The only high correlation between the coefficient estimates was for flock size and AIP (0.70). With AIP deleted from the final model the flock coefficient was -0.000223 ± 0.000025 (cf -0.000130) and with flock size deleted the AIP coefficient was 0.0392 ± 0.0056 (cf 0.0210). The final model estimates of effects are adjusted for each other and their SEs are inflated due to the presence of random seasonal effects; for both reasons they are the most appropriate estimates to use for an interpretation of the data.

While variable selection issues may mean the nominal significance levels of the effects are overstated, this does not affect interpretation of the effects for the four terms included in the final model.

Further information about the four risk factors present as fixed effects in the final model is provided due to the unexpected direction of the coefficients for Flock size and Stocking rate.

The coefficients on the logistic scale for each of the four factors (± SE) when introduced individually as single fixed effects to the model with the non-zero random effect of seasons within farms were:

Flock size	-0.000227 +/- 0.000026
AIP	0.0364 +/- 0.0085
SR	-0.127 +/- 0.117 (NS)
LAW	-0.56 +/- 0.66 (NS)

Further when pairs of factors were introduced as fixed effects to this model with the non-zero random effect of seasons within farms the coefficients on the logistic scale (± SE) were:

Flock size (-0.000144 +/- 0.000037) + AIP (0.0172 +/- 0.0074)
Flock size + SR (-0.059 +/- 0.045 (NS))
AIP + SR (-0.165 +/- 0.058 (P<0.05))
Flock size + LAW (0.46 +/- 0.22 (P<0.05))

For this model using data from the 12 farms these results show the following:

The effects of Flock size, AIP and SR do not change in sign as the number of fixed effects increase.

The effect of LAW changes from negative (but NS) to positive (P<0.05) when adjusted for Flock size and stays significant and positive when AIP and SR are added to the model.

The effect of SR is NS when adjusted for Flock size, but is significant (P<0.05) when adjusted for AIP, and stays significant and negative when Flock size and LAW are added to the model.

The effects of Flock size and AIP are reduced in size when each is adjusted for the other.

Final points

The sample correlation between SR and AIP was only 0.07 and the correlation between their coefficients was -0.32, so there is no concern that the SR coefficient was biased by the presence of AIP in the model.

If a formal inference that the effect of SR adjusted for the other 3 fixed effects is negative is not acceptable, then you must assume that the negative effect is an artefact of the results from those 12 farms in those 12 months.

Note that when SR was removed from the final model some moderate random farm effects were present, whereas there were no farm effects with SR included.

Table F.1
Summary of the factors present as fixed effects in the final model

Farm ID	Flock size	Actual area improved pasture (%)	Stocking rate (dse/ha)	Lamb age at weaning[a]
1 / 1	12475	40	9.5	0
1 / 2	6870	66	9	1
1 / 3	3976	47	9	0
2 / 1	3999	100	15	1
2 / 2	12989	70	18	1
2 / 3	7260	100	10	0
3 / 1	2797	95	10	0
3 / 2	5079	95	10	0
3 / 3	5609	80	8	1
4 / 1	14318	43	12	1
4 / 2	20562	67	10	1
4 / 3	14389	36	12	1

a Lambing age at weaning is coded as 1 (weaned at 10-14 weeks of age) and 0 (weaned at 15-16 weeks of age).

Table F.2
Data for two factors reviewed during evaluation of the final model

Farm ID	Farm size (ha)	Actual year first noticed losses
1 / 1	1838	1998
1 / 2	2748	2000
1 / 3	1500	2000
2 / 1	420	1997
2 / 2	1900	1995
2 / 3	939	1997
3 / 1	848	1987
3 / 2	805	1997
3 / 3	1520	2001
4 / 1	2308	1998
4 / 2	2918	1998
4 / 3	2065	2000

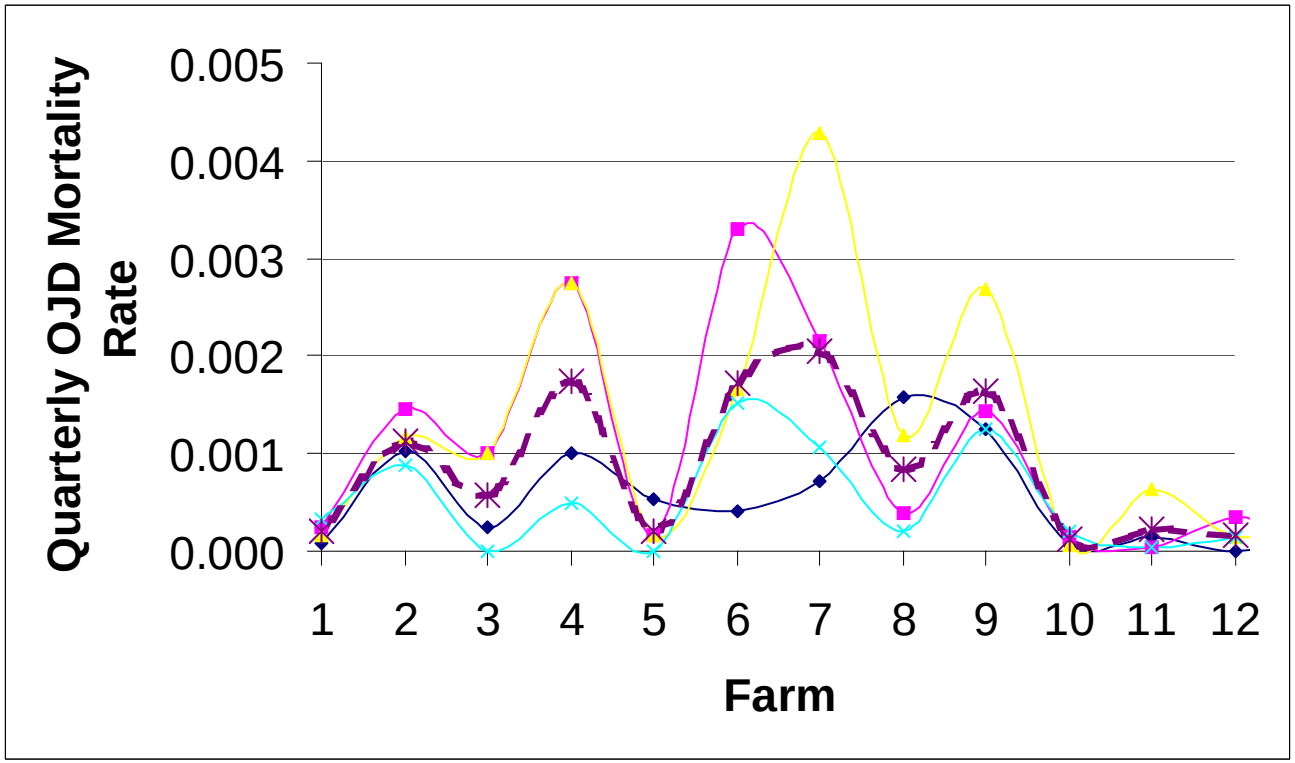

Figure F.1

Quarterly OJD mortality rate on the 12 farms in 2002