

On farm

OVINE JOHNE'S DISEASE

*An update of Australian
Research*

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Animal Health and Welfare



Introduction

The OJD Harvest Year forum and workshop was the latest in a series of annual events to provide the research community and other interested parties an update of recent research in Ovine Johne's Disease.

The current national program to evaluate options for the future management of Johne's disease in sheep will cease in June 2004. The decision of industry is to adopt assurance based trading arrangements. The research and development program is also near completion. There have been important outcomes of the research already (summarised in appendix 1, page 108) and a number of significant projects will end during 2004. The outcomes of this research will be communicated to industry during 2004 and 2005. The events that will occur will be publicised in the near future.

Further copies of these proceedings can be ordered by contacting Eileen Leung on 02 9463 9288 or by email eleung@mla.com.au.

Executive Summary

The Ovine Johne's Disease (OJD) "Harvest Year" Workshop was held on the 25th March, 2004 at Rydges, North Sydney. The workshop was hosted by Meat and Livestock Australia (MLA) and was attended by researchers, advisors, veterinarians and sheep producers from around the country. The concept of an OJD "Harvest Year" was developed by MLA to begin a process of "harvesting" and collating the results and outcomes from the extensive research program, which has been conducted in Australia over the last 5 to 8 years.

At this workshop updates were presented on 14 OJD-related research projects. These projects included research in the areas of vaccination, grazing management, eradication, economics, diagnosis and cross-species transfer. Following the presentations small group workshops were held to identify the key benefits and potential implications of each study for producers, farm advisors and regional authorities. Potential areas of future research were also identified.

A summary of the research projects presented is provided below. More specific information is presented in the research reports later in this publication together with the outcomes from the workshop discussions.

Vaccination

A major field evaluation of the OJD vaccine Gudair™ conducted by NSW Agriculture is now approaching completion. The study has shown that vaccination reduces OJD-related mortalities and faecal shedding of the causative organism *Mycobacterium avium* subsp. *paratuberculosis* (*Mptb*) by an impressive 90%. This figure is considered to be conservative as vaccinated sheep were run with unvaccinated controls throughout this trial, providing a continuous high level of challenge.

However, all the dead vaccinates in the study had severe multibacillary lesions and are likely to have been excreting very high levels of *Mptb* prior to their death. Additionally vaccination appears to have less effect on reducing the prevalence of sub-clinical infections. Therefore despite vaccination proving to be a very cost effective control strategy, it is important to remember that it is not a cure and it is likely that sustained vaccination may be needed to avoid the emergency of the disease on many properties.

Researchers at the University of Sydney have now completed a four-year study investigating the effect of whole flock vaccination on mortality rates on a heavily infected property. Vaccination was found to have little effect on mortalities during the first 2 years of the study, however in the third and fourth years there was a dramatic reduction in deaths towards levels normal for this type of enterprise. Significant changes in management strategy did occur over the duration of the trial making it difficult for the researchers to separate the contribution of these changes from the effect of vaccination on the substantial decrease in mortality risk.

Vaccination with the Gudair vaccine is associated with a high proportion of injection site lesions. To determine the potential cost to producers of these lesions at slaughter the Central Tablelands Rural Lands Protection Board is currently carrying out a survey of 30 vaccinated sale lots. Preliminary results indicate that despite a high incidence of identifiable vaccination site lesions, costs due to carcass trimming and downgrading are insignificant and are likely to be of little cost to the industry.

Grazing Management

There is growing evidence that the success of vaccination in OJD control programmes is significantly influenced by other management factors which ultimately determine the long-term levels of exposure to *Mptb* experienced by young sheep in an infected flock. An extensive 3-year field trial has been conducted by the University of Sydney to investigate at what age, and from what source, lambs become infected. The study is now complete and has identified a positive relationship between the level of exposure and the incidence of OJD infection and death rate. Continuing exposure in both the pre-weaning and post-weaning phase resulted in high levels of infection. Lambs from infected dams, which grazed heavily contaminated pre-weaning pasture had higher infection rates compared to lambs from non-infected dams grazing similar pastures. The study also identified that sheep beyond weaning age remained susceptible to infection and there was no significant difference in the severity of infection or death rates when lambs were exposed to the infection in the pre-weaning or post-weaning phase only.

As a result of this study it should be possible to reduce the incidence or severity of OJD by reducing the levels of exposure in young sheep in the pre-weaning and post-weaning periods by implementing lambing, weaning and grazing management strategies.

Additional management strategies such as mineral supplementation and internal parasite control have been evaluated by a field study on Kangaroo Island however the results are inconclusive.

Eradication

The efficacy of a destocking-restocking strategy to eradicate *Mptb* from individual properties is still under evaluation in a major research project being co-ordinated by NSW Agriculture. Results are now available at two and three years after restocking. Unfortunately 24 of 41 properties tested to date have failed the eradication process. Failures are believed to have resulted from restocking with infected sheep and from local eradication failures such as incomplete pasture disinfection and/or reinfection from neighbours. The probability of successful eradication under this protocol is now considered to be low.

Cross-species Transfer

A prevalence survey for *Mptb* in macropods has been conducted on Kangaroo Island, South Australia. Positive tissue cultures for the S strain of the organism were found in 1.7% of sampled animals. In addition six animals, which had negative tissue cultures had histological lesions consistent with OJD, however attempts to strain type the organisms were unsuccessful. Faecal excretion of *Mptb* was not demonstrated in any animal and it considered unlikely, based on the results of this study, that macropods play a significant role in the epidemiology of OJD to sheep.

Economics

There has been considerable debate regarding the impact of OJD on infected farms due to a lack of information on the mortality rates attributable to the disease. This uncertainty has provided opportunity for some industry action groups to downplay the importance of the disease in the face of a national control program. Producers have also found it difficult to estimate the cost effectiveness of control strategies such as vaccination due to the paucity of information on the economic impact of the disease.

In order to address this key knowledge gap, the University of Sydney has been studying the OJD-related mortality rate in twelve NSW sheep flocks in NSW and has estimated the economic cost of the disease to producers. Results show that the average annual mortality rate was 6.2% on these properties, which is more than twice the accepted death rate (from all causes) for Australian sheep flocks. These results confirm that mortality rates due to OJD are considerable and contribute to significant economic losses on infected properties.

An estimation of OJD-related mortality has also been conducted on Flinder's Island. Although insufficient data was obtained to estimate annual OJD-related mortality rates in this study, OJD was found to be responsible for over 70% of adult sheep deaths. This figure is very similar to that found in the NSW study.

As a result of these research projects more accurate estimations of the cost-effectiveness of vaccination and other on-farm management options can now be provided to producers, for future control and management of the disease.

Diagnosis

A large amount of research is continuing to develop more effective tests for the diagnosis of OJD infection in both individual animals and flocks. Five papers were presented at this workshop in the area of diagnosis.

The CSIRO has undertaken an extensive evaluation of the gamma interferon test, which previous research had identified as a promising test for early diagnosis of infection. Unfortunately the sensitivity of this test has proved to be variable and a high number of false positives can occur. At this time the gamma interferon test cannot be recommended as a routine diagnostic tool for OJD.

Abattoir surveillance has proved to be an efficient and low cost method for diagnosing OJD infected flocks, with over 18 million sheep examined since late 1999. Less than 4% of lines have been found to be positive during this time. It may be possible to use the negative data from abattoir surveillance as an efficient and economic means of providing information for a flock assurance scheme if the sensitivity of visceral examination at an individual animal level was known. Therefore the Victorian Department of Primary Industries has conducted a study to investigate the sensitivity of individual inspectors in detecting OJD when examining viscera in real-life conditions in a meatworks. The sensitivity of inspection was found on average to be 70% (range of 52 to 87%) with a high level of specificity.

The potential use of OJD abattoir inspection for the purpose of negative flock assurance will depend on the use of a sheep identification system and on properly trained and accredited inspectors. However once these issues have been addressed, producers may benefit from the assurance information gained from this potentially highly specific methodology.

The difficulty in confirming a negative diagnosis in suspect flocks has been shown by a longitudinal case study, which investigated the spread of OJD in a cross-bred flock in south-eastern NSW. Infected flock profiling on this property identified a clustering of infection and shedding within one or two age groups only. Additionally shedding was not apparent in these mobs until the animals were over five years of age in contrast to the commonly reported 12 months incubation period. This study demonstrates how difficult effective OJD surveillance may be unless an exact history is known and the correct age groups or mobs are tested. Alternatively testing must be repeated over a long period of time as clustering of infection will reduce the confidence in a negative test outcome if sampling is random. This has significant implications for market assurance program and surveillance testing.

Most of the tests used in the OJD program are flock tests. Due to the insidious nature of the disease, in particular the very long incubation period and the delay in faecal shedding and seroconversion, antemortem individual animal testing has usually been considered impractical and unreliable. NSW Agriculture has now completed a longitudinal study evaluating the efficacy of a whole range of individual animal tests, including intestinal biopsy, for detecting OJD infection. Full analysis of the results has not yet been completed however preliminary analyses suggest that intestinal biopsy is the most sensitive technique for identification of individual infected animals, although even at 36 months only 2/3 of infected sheep can be detected. This study also revealed that around 15% of sheep recover from early *Mptb* infections, and therefore early testing, even with a highly sensitive test such as culture of biopsied tissues, may not be predictive of later disease status.

Recent advances in the fields of molecular biology, genomics and proteomics have provided new tools to evaluate *M.ptb* and how it may cause disease. A PhD project currently underway at the University of Sydney has identified a number of genetic differences between the sheep (S) and cattle (C) strains of this organism. It is hoped that further characterisation of these genetic differences may explain the different host specificities of the two strains and enhance our understanding of how this organism causes disease.

OJD Forum Program –

Jacaranda Room at Rydges North Sydney Thursday, 25th March 2004

- 8.30am** Tea & Coffee on Arrival
- 9.00am** **Welcome** (Peter Rolfe)
- 9.00am** **PLENARY SESSION 1**
- **Vaccination**
 - Progress Report – OJD.015 Whole Flock Vaccination (Helen McGregor)
 - Intensive Vaccination Trial – OJD.009 Field Evaluation of OJD Control using Gudair™ (Leslie Reddacliff)
 - Vaccination Site Lesions – OJD.032 Surveying the Impact of Vaccination Site Lesions (Jeff Eppleston)
 - **Grazing Management**
 - Progress Report – OJD.002A Pasture Management and OJD (Helen McGregor)
 - Best Practice Management of OJD on Kangaroo Island (Deb Lehman)
 - **Eradication**
 - NOJDP Research Progress Report – OJD.001 Evaluation of Eradication Strategies for OJD (Pat Taylor)
 - **Questions**
- 10.15am** Morning Tea
- 10.45am** **Workshop 1**
- Vaccination
 - Grazing Management
 - Eradication
- 11.30am** **PRESENTATIONS FROM WORKSHOP 1**
- 12.00pm** **PLENARY SESSION 2**
- **Cross Species**
 - South Australia - Cross Species Transfer (KI) – Prevalence Survey of *M. ptb* in Kangaroo Island Macropods (Paul Cleland)
 - **Economics**
 - NOJDP Research Progress Report – OJD.023 Biological and Economic Impact of OJD in Affected Sheep Flocks in NSW (Russell Bush)
 - NOJDP Research Progress Report – Estimation of OJD-Related Mortality on Flinders Island (Cameron Bell)
 - **Questions**
- 12.30pm** **Brief overview on Contracts** (Skye Voveris)
- 12.35pm** Lunch – served in Gnomes Restaurant
- 1.15pm** **PLENARY SESSION 3**
- **Diagnosis**
 - Gamma Interferon to Diagnose OJD (David Stewart)
 - Abattoir Surveillance – OJD.029 Determination of Individual Animal Level Sensitivity of Abattoir Surveillance for OJD (Tracey Bradley)
 - Infected Flock Profiling – Longitudinal Study of OJD Spread in a Sheep Flock (Luzia Rast)
 - Biopsy Trials – OJD.020 Individual Animal Tests to Diagnose OJD (Leslie Reddacliff)
 - Molecular Biology of OJD (Ian Marsh)
 - **Questions**
- 2.15pm** **WORKSHOP 2**
- Cross Species
 - Economics
 - Diagnosis
- 3.00pm** Afternoon Tea
- 3.15pm** **WESTERN AUSTRALIA**
- Ovine Johne's Disease in Western Australia (Fiona Sunderman)
- 3.25pm** **PRESENTATIONS FROM WORKSHOP 2**
- 5.00pm** **What's going to happen next?** (Peter Rolfe)

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VACCINATION

A LONGITUDINAL STUDY OF OJD AND THE EFFECTS OF WHOLE FLOCK VACCINATION WITH GUDAIR™

PROGRESS REPORT – WHOLE FLOCK VACCINATION

PROJECT NUMBER: OJD.015

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Background and Objectives

Mortality rate estimations are used to assess the on-farm economic significance of OJD and the economic viability of management and control programs, including use of vaccine, which is a significant management tool for control of OJD in infected flocks. Accurate estimation of mortality rate in OJD infected flocks in Australia has proven difficult, with estimations reliant on producer observation and inventory records. This study proposed a structured approach to investigating mortality rate estimation and the effect of whole flock vaccination in a heavily OJD infected commercial flock. Mortality rates, seroprevalence and faecal excretion of *Mptb.* were used as indicators of the effect of whole flock vaccination.

Methods and Results

Methodology

The study was conducted over a four-year period. This time period was divided into study years with each study year commencing and finishing at shearing to facilitate the comparison of post-mortem and inventory data. The property was visited four times in each study year and postmortem was conducted on all sheep dying within a five-day period. The initial sampling period was conducted pre vaccination. Vaccination of all animals commenced following this sample period and at lamb marking in each subsequent year. All animals in the flock were vaccinated in year 0, except for a small group of ear tagged, numerically identified animals in each age group which remained non-vaccinates for the duration of the trial. Non-vaccinates always run as part of the main flock, with their vaccinated cohorts and comprise about 7% of each age group born after 1996. Blood for serology and individual faecal culture was collected from vaccinated and non-vaccinated cohorts each year. Humoral responses to *Mptb* were measured using ELISA (Paracheck™, CSL Ltd). Faecal culture was by modified BACTEC 12B radiometric culture and direct confirmation by IS900 PCR.

Within each 5-day post-mortem visit, all sheep that died within this period were submitted for postmortem. Clinical, anti-mortem and gross postmortem information was recorded and samples taken for histopathology, for identification of OJD infection. Samples were also taken from stomach and intestinal washings for total worm count estimations.

The most likely causes of death were determined from postmortem data and material collected at the time of necropsy. The significance of the presence of OJD to the animal's death was derived from histopathological examination and grading of lesions in tissues by the modified Perez system (refer to description in OJD 023 report). Sheep were classified as OJD having contributed to their death when

there was histopathological evidence of severe OJD infection, indicated by the presence of an advanced, diffuse, granulomatous enteritis. Sheep with less severe OJD lesions on histopathology were classified as OJD having not contributed to their death. Sheep with no evidence of OJD infection on histopathology were classified as having died from other causes.

Mortality risk estimations from the inventory were used along with the postmortem classification to calculate the risk of mortality faced by each sheep in each year of the study. In all years the data represented refers to sheep aged 12 months or more at the start of each study year.

Results

Mortality risk estimations

Table 1. Postmortem figures, mortality risk estimations from inventory and mortality risk estimations due to the contribution of OJD. "Other mortality risk" includes animals where it was considered OJD did not contribute to death, plus deaths from other causes.

Study year	Total adults	OJD contributed to death %	Mortality risk	OJD extrapolated mortality risk	Other mortality risk
0	42	78.7	24.2	19.0	5.2
1	24	87.5	21.3	18.6	2.7
2	14	78.6	15.5	12.2	3.3
3	2	50.0	2.8	1.4	1.4

Serology

Table 2. Seroprevalence (Paracheck™ ELISA) of non-vaccinated sheep by age (both sexes)

	Year 0		Year 1		Year 2		Year 3	
Age at sampling (yrs)	Number sampled	% Positive	Number sampled	% Positive	Number sampled	% Positive	Number sampled	% Positive
0.6	150	2.67	146	0.68	142	0.00	102	4.9
1.6	148	8.11	107	8.41	123	16.26	90	6.67
2.6	104	13.46	61	19.67	83	22.89	70	20.00
3.6	110	9.09	59	11.86	43	11.63	17	17.65
4.6	101	12.87			49	16.33	16	6.25
5.6	98	10.20					7	14.29

Despite a reduction in mortalities in years 2 and 3 (table 1), seroprevalence in all age groups over this

period, has remained comparable with years 0 and 1 of the study. The 2.6 year old cohort has the highest rate of seroprevalence in all years

Faecal culture

Table 3. Total percentage shedders, 2 year old vaccinates and non-vaccinates, both sexes.


Vaccination status	Year 0	Year 1	Year 2	Year 3
Vaccinates	50.00	5.26	2.56	0.00
Non-vaccinates	49.02	17.50	15.38	20.93

All isolates were of the S strain of *Mptb*. Sampling in year 0 took place pre-vaccination. Animals had been identified and allocated to either non-vaccinate or vaccinate groups but vaccination did not take place until after collection. Both groups of animals in this year have a similar status, in comparison to significant differences between vaccinates and non-vaccinates in all other years.

Conclusions to Date


The mortality risk figures for this flock for years 0 to 2 of the study are considerably higher than those expected for well-managed OJD-free Merino enterprises in this part of NSW, where figures of 4-6% of adult sheep would be more common. The mortality risk from other causes is within this range in all years. The presence of OJD on this property has increased the mortality risk by greater than 4 times for years 0 to 2 and doubled it in year 3.

Vaccination of this flock appears to have had little effect on mortalities through the first 2 years of the study, with the overall mortality risk and risk of mortality due to the presence of OJD on the property remaining high. In the third and fourth years of the study, there was a dramatic reduction in mortality risk towards levels normal for this type of Merino enterprise, however there are a number of factors in addition to whole flock vaccination that could have contributed to this. Observations from this study and from field experience have been that despite vaccination, adult sheep with advanced Johne's disease will progress to death. It is possible that the dramatic drop-off in mortality risk in the 3rd and 4th years of the study may be partly attributed to an effect of vaccination, with a larger proportion of the flock protected through vaccination as lambs or young hoggets, however, several management and environmental factors also need to be considered. Significant changes in management strategy occurred over the duration of the trial and most extensively in years 2 and 3. Sheep identified as suspect OJD sub-clinical or early clinical cases were drafted and removed from the flock on a more efficient and regular basis. The age of the flock was reduced through this period, with animals culled at a younger age increasing the proportion of young animals in the flock vaccinated as lambs, prior to continued exposure to high levels of infection. Stocking densities were reduced with the sale but not replacement of older sheep, including a large proportion of the whether mob, which from serological testing, faecal culture and clinical observation had been suspected to have higher infection rates than other mobs. Lambing management was also changed during the study. Lambing ewes had previously remained within the cell grazing management system throughout lambing and lactation. Set stocking was gradually introduced for the lambing and lactation period incorporating significant reductions in stocking densities. In addition, climatic differences in all years may have also had an effect on mortalities through indirectly affecting the health of the flock through available nutrition, feeding practices and behavior. This effect may be particularly significant in years 2 and 3 when drought management and feeding practices were employed extensively for all mobs of sheep. The serological and faecal culture results suggest, that a continuing high level of infection may have been present in this flock throughout the study period. However, it would appear that by instituting dramatic changes in management practices combined with increasing effect of vaccination of the younger flock, a significant reduction in mortality risk has been observed in the final two years of the study.







A Longitudinal Study of OJD and the Effects of Whole Flock Vaccination with Gudair™

OJD.015
Whole Flock Vaccination




Helen McGregor









Project Background and Methodology

- Evaluation of the effect of whole flock vaccination on a heavily infected property over time, using serology, mortality risk and faecal excretion as indicators of the level of disease
- Vaccination of all animals except for identified non vaccinate population
- On farm postmortems – 5 day period 4 times per year – histopathological diagnosis of OJD
- Collection of faecal and blood samples twice yearly
- Mortality risk estimation from inventory
- Estimation of the contribution the presence of OJD makes to mortality risk over time

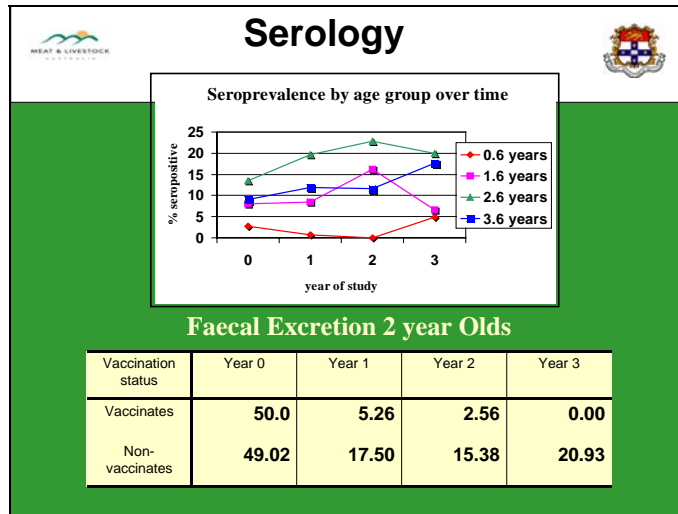




Mortality Investigations



Study Year	Mortality risk (%)	OJD extrapolated mortality risk (%)	Other mortality risk (%)
0	24.2	19.0	5.2
1	21.3	18.6	2.7
2	15.5	12.2	3.3
3	2.8	1.4	1.4



Potential factors involved in reduction in mortalities

- Changes in age and composition of flock
- Management changes
 - Reduce CFA
 - Cull older thin sheep and suspects
 - Reduce stocking density
 - Feeding/drought management
 - Identify potential high risk mobs
 - Lambing management
- Introduction of vaccine
 - Protect young animals
 - Reduce shedding and pasture contamination

INTENSIVE VACCINATION TRIAL

FIELD EVALUATION OF OJD CONTROL USING GUDAIR™

PROJECT NUMBER: OJD.009

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Background and Objectives

Paratuberculosis of sheep is known to cause significant mortality of adult sheep in infected flocks, particularly in self-replacing Merino flocks that have been infected for an extended period. In areas where the flock prevalence of the disease is known to be high, eradication by destocking was restricted because of the risks of reinfection from neighbours and from local environmental sources. Vaccination against OJD had been used in several overseas countries with encouraging results however there were few scientifically controlled reports demonstrating its effectiveness.

The objectives of the project (as revised in 2001) were:

- 1) To determine if vaccination with Gudair™ Vaccine can reduce mortality in merino sheep due to OJD under Australian conditions.
- 2) To assess any reduction in bacterial shedding of *M. paratuberculosis* that may occur in vaccinated sheep in comparison with non-vaccinated controls.
- 3) To investigate the immune responses following vaccination with Gudair™ Vaccine.
- 4) To assess the field safety of Gudair™ Vaccine in vaccinated flocks.
- 5) To determine changes in productivity parameters associated with vaccination or subclinical disease.

These objectives were also designed to facilitate the registration of Gudair™ for use in Australia. (This occurred in mid 2002 in NSW.)

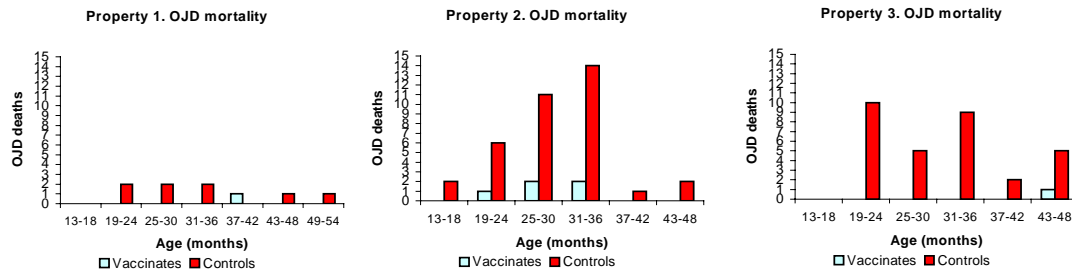
Methods and Results

Three properties in the Central Tablelands of NSW, deemed to be heavily infected with OJD, were selected. Each was losing in excess of 5% of adult sheep per annum to OJD, and lambed at least 800 Merino ewes annually to allow adequate selection of trial lambs. All had been running a self-replacing fine wool Merino flock for several decades and the level of management was deemed adequate to exclude other major causes of mortality.

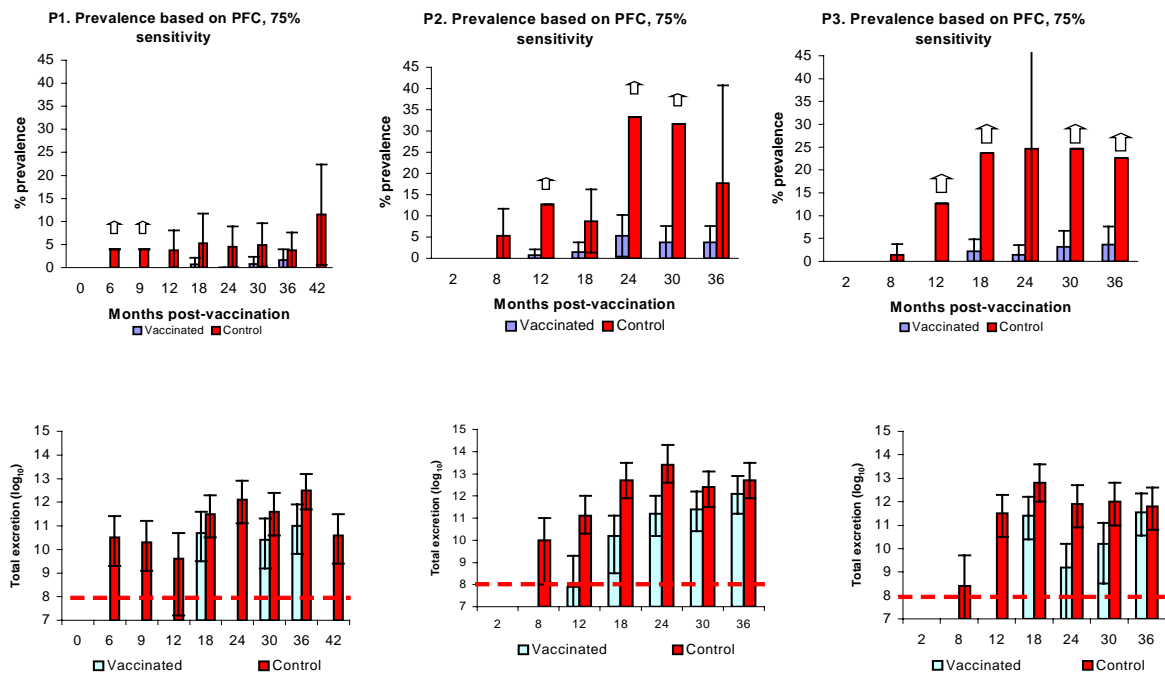
On each property, 400 predominantly female lambs were selected. At 2-3 months of age, 200 were vaccinated with Gudair™, while 200 were sham vaccinated with saline as controls. On P1, spring 1999 born lambs were treated in December 1999, while on Properties 2 and 3, autumn 2000 born lambs were treated in June 2000. Each trial property was visited approximately 2 and 6 months post vaccination then six-monthly thereafter, until the end of the trial in mid-late 2004. Faecal samples were collected for pooled faecal culture at each visit, and for individual faecal culture from December 2000. Pooling rates were progressively increased from 40 per pool to 10 per pool, to better define prevalence of excretors, and target the processing of individual faecal samples. Blood samples were taken at each visit for the determination of cell mediated immune responses to Johnin PPD using IFN- γ (Bovigam™) and plasma ELISA antibody (Parachek™). On each farm, a sub-sample of trial sheep were culled for normal flock management reasons at 18-28 months post vaccination, and were examined by histopathology at slaughter for evidence of sub-clinical OJD. Trial sheep that died or had clinical signs of OJD, were sampled for histological assessment of paratuberculosis. Production traits (condition score, liveweight,

fleece weight and fibre diameter) were measured at each sampling or shearing. Post-vaccinal lesions were also measured. A sub-sample of at least 100 sheep (50 vaccinates and 50 controls) from each property will be assessed at slaughter by culture of tissues and histopathology to determine the subclinical infection status of the animals at trial end (samples from P3 have already been collected).

Mortality. Across all three farms, mortality due to OJD is reduced by about 90%. So far 75 controls have died of OJD, compared to only 7 vaccinates. All dead vaccinates had severe multibacillary pathology, and would have been excreting very large amounts of *M. a. paratuberculosis* leading up to their death.

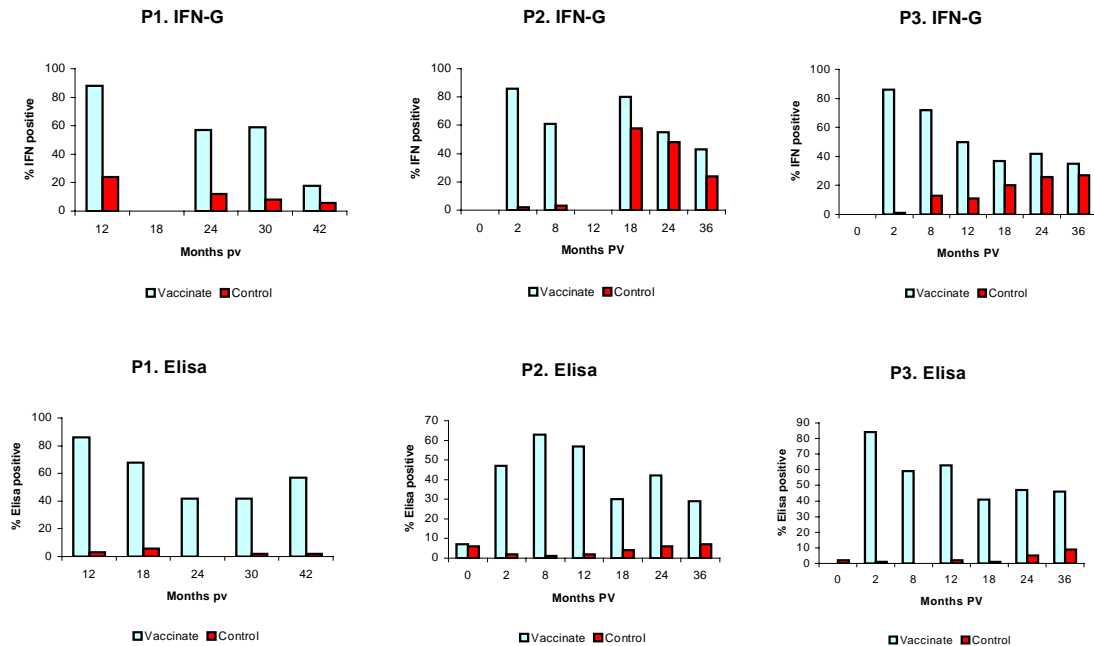


Faecal excretion. The prevalence of sheep excreting *M. a. paratuberculosis* is also reduced by about 90% by vaccination. Total numbers of organisms excreted are reduced by about a one log, which is also about a 90% reduction, although the numbers excreted by vaccinates at some samplings are still very large. Data from individual faecal cultures supports the PFC prevalence estimates.



Immunological responses. Initially, vaccination induces humoral and CMI responses in the majority of sheep. There are obvious differences between farms. The lower level of infection on P1 is reflected in the lower numbers of control sheep reacting to either immunological test. A trend for decreasing IFN- γ levels in vaccinates over time continues on all farms. This trend is most marked on the lower prevalence farm P1. Higher level exposure on P2 and P3 may be inducing anamnestic responses in vaccinates. Maternal

antibodies are reflected by the pre-vaccination Elisa results on the heavily infected farm, P2.



Vaccination site lesions. These persist, with about 20-25% of sheep having palpable lesions after 3-4 years. Detection rate is lower in longer wool sheep.

Production data. There is little difference in any parameter between vaccinates and controls. However, at several sampling times, considering only surviving sheep across all farms, there is a small but statistically significant reduction in the live-weight of vaccinates compared to controls.

Subclinical infections. Of 77 hogget culls across all farms, OJD lesions were present in 49% of controls (9% multi, 40% pauci) and 18% of vaccinates (6% multi, 12% pauci). Sheep from P3 have been examined at slaughter ahead of schedule because the farm was sold. Histological examination of a sub-sample of 100 showed OJD lesions in 40% of controls (2.5% multi, 37.5% pauci) and 11% of vaccinates (all pauci).


Conclusions to Date

Conclusions from the above results are conservative with regard to vaccine use in the field, because in this trial vaccinated sheep are running with unvaccinated controls, providing a continuous high level of challenge. Recent results highlight large differences between farms. P1 appears to have a lower amount of OJD than the other two farms, and at some samplings no excretion was detected among vaccinates.

A figure of 90% efficacy for Gudair for control of OJD is reasonable. Mortalities, prevalence of sheep excreting *M. a. paratuberculosis* and levels of excretion are all reduced by vaccination. This figure is somewhat better than the 70% guesstimates used in the modelling work to date, and underscores the incorporation of vaccination into assurance programs. Vaccination may have less effect on subclinical infections (determined by histology), reducing prevalence by about 60-70%.


Final necropsy data (when available), combined with individual faecal culture results, will allow comparison of immunological, production and lesion data with disease and vaccination status.

The multibacillary disease seen in all the vaccinates which have died, and the high level of group excretion among vaccinates at some samplings, reminds us not to be carried away by the success, that in this business 100% guarantees are unrealistic, and that we will need to emphasise sustained vaccine use to avoid recrudescence.




Intensive Vaccination Trial

OJD.009 – Field evaluation of OJD Control using Gudair™




Leslie Reddacliff



OJD.009 – Field evaluation of OJD Control using Gudair™

- Current trial set up in 1999. At that time:
- Control of OJD in Australia emphasized destocking, but a need for vaccination in high prevalence areas was apparent
- Limited overseas vaccine studies showed reduction in clinical cases and mortality, but infection persisted
- Needed local studies, with merino sheep in the Australian environment
- Especially the potential excretion of the organism by vaccinates
- Modified 2001 to facilitate local registration of Gudair

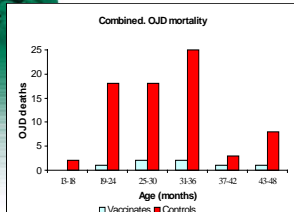


Trial design

- 3 heavily infected properties, vaccinates and controls run together
- 200 vaccinates, 200 controls each, vaccination at 2-3 mths of age
- tested at vacc, 2 mth post-vacc, then 6-monthly, to 4 years
- Faecal culture – PFC (10-40/pool), IFC (+ve pools), quantitative est.
- Immunological testing - IFN-G and Elisa (done by CSL)
- Production and vacc lesion data
- Monitor mortalities, hogget culls and at final abattoir slaughter

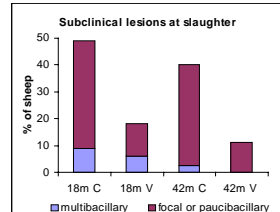
Results

Combined OJD mortality

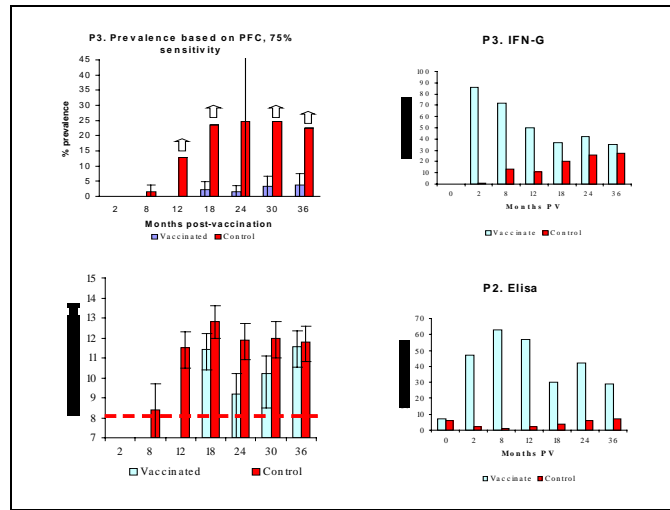



Age (months)	Vaccinates	Controls
15-18	~1	~2
19-24	~1	~18
25-30	~1	~18
31-36	~1	~25
37-42	~1	~3
43-48	~1	~8

Subclinical lesions at slaughter



Group	Multibacillary (%)	Focal or paucibacillary (%)
18m C	~10	~40
18m V	~10	~0
42m C	~10	~30
42m V	~10	~0





Conclusions

- Vaccination with GUDAIR, even in high challenge situation is 90% effective:
 - Delays and reduces mortality due to OJD
 - Delays and reduces the faecal excretion
 - Vaccination does not prevent severe multibacillary disease in some sheep
 - Stimulates both CMI and humoral immunity
 - Induces persistent vaccination lesions
- An important role in the control of OJD in Australia is justified

VACCINATION SITE LESIONS

PROGRESS IN A PRELIMINARY SURVEY OF THE COST AT SLAUGHTER OF GUDAIR™ INJECTION SITE LESIONS TO THE AUSTRALIAN SHEEP INDUSTRY

PROJECT NUMBER: OJD.032

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Background and Objectives

While the current vaccination trial (MLA.009) has demonstrated the efficacy of vaccination with Gudair™ for controlling OJD, one disadvantage following its use has been the development of persistent lesions at the site of vaccination (Eppleston et al 2003). This has been reported previously in overseas trials using both live (Neoparasac) & killed (Gudair) vaccines (Collett & West, 2001).

In trial MLA.009, up to 50% of vaccinates had developed injection site lesions when inspected 2 months post vaccination (pv), but this proportion fell to around 25% at 30 months pv. A proportion of these residual lesions were large and ranged up to 50 mm in diameter.

Lesions could have a negative impact either on-farm or at slaughter. On-farm, anecdotal evidence suggests there are no detrimental effects of lesions even though the possibility exists for fly strike to occur in the small proportion of lesions that break open. However at slaughter additional carcass trimming and market diversion because of vaccination site lesions and the presence of enlarged pre-scapular lymph nodes has been reported in New Zealand.

Given the reliance future OJD control programs in Australia will place on vaccination, it is important that the sheep meat industry is provided with accurate estimates of the current and potential costs associated with vaccination site lesions. It is the objective of this preliminary survey to indicate whether more detailed investigations are warranted.

Methods and Results

Local sheep producers were asked to advise when they were sending lines of Gudair-vaccinated sheep to slaughter. These were kept separate during trucking and slaughter so that data on the incidence and the economic impact of injection site lesions could be recorded at the abattoir. Details on the sheep including age at vaccination and time since vaccination were obtained from the owners of the sheep.

At slaughter the incidence of injection site lesions classified by diameter was recorded by a person located at the beginning of the slaughter chain. Abattoir staff were placed at a point on the chain prior to any routine trimming of the neck region and were asked to trim & retain any lesions present. These were counted & weighed at the works. In addition abattoir workers trimming pre scapular lymph nodes were asked to retain any enlarged nodes for later inspection, weighing and sampling if required.

Selected vaccination site lesions were sent to Orange Regional Vet Lab for bacteriological (gram stain, ZN stain and routine culture) and histological examination to determine the nature of the lesion and whether secondary infection due to poor vaccination technique could be involved.

To-date 12 slaughter lots have been examined. Data on the prevalence, size and trimming of lesions are presented in Table 1.

Table 1. Incidence of vaccination site lesions and estimated value of lesion trim observed at slaughter

Product	Market	Age at vaccination	Age at slaughter	Years vaccinated	Number killed	Lesion diameter (mm)			Total with lesions	Percent with lesions	Weight of lesion trim (kg)	Value of lesion trim per carcase sold*
						<10	10-25	>25				
Mutton	Export	weaners	3-6	3	386	35	41	29	105	27%	3.6	\$0.03
		lambs	3	3	171	6	16	24	46	27%	1.9	\$0.03
		adults	2-5	1.5	226	0	1	2	3	1%	0.1	\$0.00
		lambs	3	3	149	16	14	8	38	26%	1.2	\$0.02
		adults	5	1	101	8	3	5	16	16%	0.2	\$0.00
		adults	5	3	231	22	17	10	49	21%	6.1	\$0.06
		adults	3.5	2	138	14	9	5	28	20%	1.1	\$0.01
	Domestic	weaners	3-6	3	211	8	10	17	35	17%	0.8	\$0.01
		weaners	4	3.5	159	7	10	27	44	28%	1.2	\$0.02
		lambs	1.5	1.5	148	2	13	28	43	29%	3.4	\$0.03
Lamb	Export	lambs	10mths	.5	82	22	17	15	54	66%	3.3	\$0.15
	Domestic	lambs	10mths	.5	40	16	8	1	25	63%	1.5	\$0.12

* per carcase killed (accounts for incidence of lesions)

Although this survey was not designed to identify statistical differences there are some trends that can be noted from the data presented in Table 1.

- In adult sheep vaccinated 12 months or more before slaughter, 21% of carcasses had identifiable vaccination site lesions on the slaughter chain. This proportion is similar to the 20% of live sheep with palpable lesions 30 months post vaccination in the 'intensive vaccination' trial (MLA OJD.009 – Eppleston et al 2003). The lot of 226 sheep where only 3 carcasses were recorded with lesions were the only lot killed at the Goulburn abattoir, where the head is removed early on the chain. Any lesions present may have therefore been removed with the head prior to carcase inspection.
- In the 2 sale lots killed as lambs around 64% of carcasses carried vaccination site lesions, most likely reflecting the short interval of around 6 months between vaccination and slaughter. Again this is consistent with the results from OJD.009 where up to 50% of vaccinates had palpable lesions 8 weeks post vaccination.
- A high proportion (average 37%) of the lesions identified were large, exceeding 25 mm in diameter. In MLA trial OJD.009 the diameter of residual lesions did not decrease with time pv. In the current

survey no relation between time since vaccination and lesion diameter was evident.

Losses due to lesions can be due to: value of meat trimmed, the labour cost of removing this trim, or the downgrading of the carcase to a lower value market as a result of the trimming. The total weight of trim was multiplied by the carcase value (price paid divided by average carcase weight) to estimate the value of trim removed. Abattoir management was consulted to determine the additional labour cost, if any, associated with the trimming conducted on each sale lot and the incidence and cost of carcase downgrading due to trimming.

The value of trim per trimmed carcase, calculated using the average carcase value, ranged from 3 – 28 cents for mutton, and from 19 – 23 cents for lambs. When expressed as a cost per carcase slaughtered (which accounts for the incidence of lesions), this discount range fell to 1 – 6 cents for mutton and 12 – 15 cents for lamb. On a per carcase basis this represents a discount of only 0.01 – 0.11% for mutton and 0.17 – 0.22% for lamb.

Given that these small discounts have been calculated using the average carcase value when the value of the neck region is considerably lower than the average, and the fact that the majority of the trim was lesion that would have otherwise not been present, it can be seen that the cost of trim is a minor contribution to the overall cost of using Gudair to control the on-farm impact of OJD.

Abattoir management advised that, at least in the sale lots monitored in this project, existing staff could carry out the trimming and no additional staff would be required. Hence the labour cost of trimming injection site lesions was nil. Abattoir management also advised that in the lots examined, no carcase was downgraded to a lower value market.

The value of excised enlarged prescapular lymph nodes was not calculated because of the small amount of trim recorded and because export works routinely remove these lymph nodes as part of the slaughter process.


In all, 3 injection site lesions were collected and examined histologically from one sale lot of domestic mutton that were killed around 18 months after being vaccinated as lambs. Bacteriology identified scant numbers of acid-fast bacilli, with no gram-positive organisms or bacterial growth in aerobic or anaerobic culture. This would suggest that the lesions were not the result of secondary infection of the injection site. Histology revealed that all 3 lesions examined consisted of a central area of caseous necrosis containing scant numbers of acid-fast bacilli surrounded by a thick fibrous capsule. The inner surface of the capsule contained macrophages and low numbers of giant cells surrounded by moderate numbers of lymphocytes. The central necrotic area contained holes and spaces consistent with the presence of lipid or oil. Overall the lesions were chronic and consistent with necrosis resulting from injection of an irritant material and/or the result of an immune response to Mycobacteria with no inflammation suggestive of secondary bacterial infection.

Conclusions to Date

The results to-date have been consistent in suggesting that vaccination site lesions will be of little cost to industry. However the sample size is very small and there is some risk that situations where lesions may have a greater impact have been missed. Further data gathered from a total of at least 20 sale lots will be collected to provide more confidence in the conclusions drawn.


References

- Collett MG and West DM (2001). A comparison of the injection site and draining lymph node pathology induced by an attenuated live and a killed Johne's disease vaccine in sheep. *5th Int Congr Sheep Vet S Africa*. 5; 28-29
- Eppleston J, Reddacliff L, Windsor P, Whittington R and Jones S (2003) An update on the efficacy of Gudair[™] OJD vaccine in Australia. *Proc Aust Sheep Vet Soc* (2003); 107-113.





Vaccination Lesions



OJD.032 – Surveying the impact of vaccination site lesions




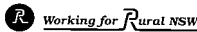
Jeff Eppleston



OJD.032 – Surveying the impact of vaccination site lesions




- Review of NZ discounting
- Survey of 30 sale lots at slaughter
- Aim: To determine
 - Incidence lesions
 - Any associated slaughter costs
 - trim lost
 - labour cost &
 - carcase downgrading
- 12 lots investigated to-date




Carcase discounting in NZ

- Industry change from live to killed vaccines
 - No difference in incidence or size in Merinos
- Much anecdotal evidence
 - Survey of NZ vets attitudes
 - Some breeders not vaccinating
 - Variable risk management by NZ works
- But little objective data
 - 1 student survey of 2 sale lots
 - \$9 per carcase downgraded but only 2-9% downgraded
 - When carcasses exported whole – decreasing



Methods

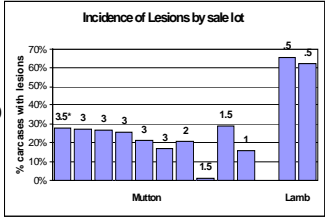
- Farmers notified their intention to sell vaccinates
- Sheep and vaccination details from farmer
- Incidence in diameter classes determined on chain
- Abattoir staff removed & retained
 - Injection site lesions & enlarged lymph nodes
 - Number & weight recorded
 - Trimming labour & downgraded carcasses
 - Valued at average carcase value
 - Bacteriology & histology on selected lesions



Results

- Incidence similar to 009
 - ~20% in adults
 - 60% in lambs
 - 40 % > 25mm
- Cost insignificant
 - Trim < 0.5% value of carcase
 - No 'additional' labour on chain
 - No carcase downgrades
- Lab analysis indicates no infectious cause
- Site of vaccination important
'extension' message

Incidence of Lesions by sale lot



Category	Lot	% carcases with lesions
Mutton	3.5	~20%
	3	~20%
	3	~20%
	3	~20%
	3	~20%
	2	~15%
	1.5	~10%
Lamb	5	~65%
	3	~60%

Vaccination – Workshop Discussion

Following these presentations a workshop of participants was held to answer the following questions:

1. *What has been achieved by the researchers?*

- Verification that Gudair vaccine is effective in a high challenge environment.
- Confirmation that shedding is delayed and reduced following vaccination.
- No negative impacts from vaccination have been identified to date. Whole flock vaccination has not accelerated death rates in animals incubating the disease.

2. *What key benefit from this research can we communicate to producers?*

- Vaccination is 90% effective even in high prevalence flocks.
- Vaccination can protect your business if you have a low prevalence flock.
- If measurement of success is mortality rate, vaccination is successful. Therefore it has big impact on farm production, but it is more difficult to ascribe “success” for trading arrangements.
- Vaccination “controls” rather than “cures”.
- Vaccination is a control measure – not an eradication technique.
- Vaccination is not a panacea.
- Don’t rely on vaccinations only - need good farm management as well.
- Self-replacing Merino flocks may need to be vaccinated indefinitely.

3. *What advice would a farm adviser now be able to provide a producer client?*

- Vaccinate before you see losses.
- Possibly vaccinate your whole flock
- Correct vaccination technique, positioning and caution is important to avoid any problems such as OH&S issues!!!
- Recommend that lambs are vaccinated as early as possible (in accordance with label directions)
- Vaccination does not affect market access- dispel the myths!

4. How could a regional authority, eg RLPB, respond to this information?

- Extension materials and press – good to have data.
- Rural press very good
- Hold forum meetings

5. What still needs to be done/researched?

- Further research on the economic impact of vaccination.
- Further research on vaccine efficacy in low prevalence flocks.
- Research on efficacy versus age of vaccination versus exposure.
- Research on the impact of vaccination on sub-clinical infections & the relationship with shedding.
- Follow up studies on vaccine “non-responders”:
 - Genetics?
 - Immune responses (type & duration)?
 - How do we identify these animals?
- Advice to producers regarding “whole flock” vaccination.
- Role/impact of vaccination on a regional basis.
- Statistics of clinical disease occurrence.
- Will long-term vaccination eventually eliminate disease? What other strategies are required?
- Age of vaccine? Can we give it even earlier?

6. Are there any other implications or warnings from this work?

- Do producers have appropriate expectations of vaccination?
- Will use continue?

7. *Have the outcomes of this work been captured in a useful form (scientific publication, advisory note etc) and what actions are required to ensure this occurs?*

- Information has already been widely distributed. Unsure of the uptake of the information. Are producers aware of vaccine usage with other management tools?

Suggestions:

- Scientific:-
 - Updates at AVA conferences & AVS important.
 - Publication of papers for scientific community
- Advisor:-
 - Tips and Tools
 - Mail out to MAP vets
 - Reminder re: MLA website and its links to other critical websites
- Producer:-
 - Tips & Tools to “engage producers”
 - Regional presentations
 - Radio
 - Ag bureau etc
 - Field days/shows
 - Local press releases.

GRAZING MANAGEMENT

EPIDEMIOLOGY 1 – EXPOSURE FACTORS LEADING TO ESTABLISHMENT OF OJD INFECTION AND CLINICAL DISEASE

PROGRESS REPORT – PASTURE MANAGEMENT AND OJD

PROJECT NUMBER: OJD.002A

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Background and Objectives

There is growing evidence that the success of vaccination in OJD control programs is significantly influenced by other management factors which ultimately determine the long-term levels of exposure to *Mptb* experienced by young sheep in an OJD infected flock. The primary aims of this study were to develop a greater understanding of the relationship between and relative importance of age at exposure, level and timing of exposure, and infectivity status of the dam. Findings from this study will facilitate the development of guidelines to provide management options for owners of OJD infected flocks taking steps to minimize the impact of OJD in their sheep.

To fulfill these objectives, an extensive field-trial was conducted over a 3 year period in a purpose-built research facility within a commercial sheep farm in a sheep-raising region of NSW. Histopathology and tissue culture were used as indicators of the presence and severity of OJD infection in a cohort of sheep raised to 3 years of age.

Methods and Results

Experimental animals

Ewes with related bloodlines were sourced from two commercial Merino enterprises in NSW. Selected ewes were tested for pregnancy and their serology status determined prior to their inclusion. Retrospective faecal culture of samples collected from the infected-flock ewes on day 39 of the trial confirmed faecal excretion rate of *Mptb* in 15% of animals from the infected ewe flock. The lambs entering the trial were born on the trial site onto pastures of high or low contamination. Lambs were raised to weaning age on pastures of 3 levels of contamination, low, medium and high, determined by dam infection status and grazing history. At weaning lambs were allocated to post weaning paddocks with high or low levels of contamination. Treatment groups were based on history of dam infection status, pre-weaning and post-weaning exposure. Treatment groups were replicated with each replicate containing 36 animals. Once allocated to a treatment group, animals remained within that group for the duration of the trial.

Pasture contamination history

Experimental pastures had been grazed by cattle, but not sheep for 18 months prior to the start of the experiment. The cattle, that were tested on 2 occasions 2 years apart, had negative serology for *Mptb*. Paddocks to be infected for the trial were grazed by infected flock ewes prior to and throughout the lambing and pre-weaning period. The number of animals grazing a paddock for a designated time created pre-determined paddock contamination rates. The contamination history of each paddock was calculated in infected sheep days per hectare. Only ewes from the tested uninfected flock grazed paddocks required to be free from infection. Post weaning exposure commenced when the lambs were aged 95 days (median). Lambs were stratified on the basis of sex and age and randomly allocate to a

post-weaning group. Pasture infectivity in post-weaning paddocks was determined in the same way as in the pre-weaning period using grazing history in infected sheep days.

Results

Results determined from histopathology and tissue culture for OJD infection rate, death rate and faecal culture results at 18 months age, are summarized below.

Exposure History	Infected dam flock	Pre-weaning exposure			Post-weaning exposure		OJD infection rate (%)	OJD death rate (%)	Faecal culture positive rate at 18 months age (%)
		Low	Medium	High	Low	High			
IHH	+			+		+	36.6	8.3	10.0
IHL	+			+	+		38.8	6.0	7.7
ILL	+			+	+		20.3	2.9	2.8
UHH			+			+	28.6	8.6	9.9
UHL			+		+		17.4	1.4	4.5
ULH		+				+	17.6	1.5	1.5
ULL		+			+		18.3	2.8	2.8

A number of factors contributed to high OJD infection rates and death rates in sheep.

There was a positive relationship identified between the level of exposure and the incidence of OJD infection and death rate. Continuing exposure in both the pre-weaning and post-weaning phase resulted in high levels of infection. The effect of dam infectivity status combined with a high exposure from heavily contaminated pre-weaning pasture produced higher infection rates compared to exposure to high levels of pasture-based contamination alone, in the pre-weaning period. From our understanding of the mechanisms for transmission of this disease in pregnant ewes, we know that it is only in clinically affected animals that we would expect significant vertical transmission of *Mptb* infection. We can postulate therefore, that the effect of the dam in this case would be most likely to provide a highly infective micro-environment, leading to contamination around the udder and belly of the ewe. This may lead to the increased exposure and therefore higher infection rates observed in lambs with infected dams and infected lambing pasture, as compared to their cohorts on infected pasture alone.

Sheep beyond weaning age remained susceptible to infection. There was no significant difference in severity of infection or death rates observed where lambs were exposed to infection in the pre-weaning or post-weaning phase only. It appears possible to avoid high rates of infection by placing previously exposed lambs onto low contamination paddocks at weaning. Here this was a significant effect only for lambs that have not been exposed to very high levels of infection in the pre-weaning period ie were exposed to infected pastures pre-weaning but were not progeny of infected dams.

Faecal culture as a measure of disease at 18 months of age remains a good indicator of death rate by 3 years of age. Sheep shed *Mptb* in faeces from as early as 12 months of age. Deaths commenced at 18 months of age and the rate of mortality peaked at approximately 30 months. Most sheep which


developed patent infections died in the subsequent 18 months. Some sheep can contain the infection for at least 18 months with little development of damage to the intestine. It is possible that some sheep may recover from infection entirely.

Conclusions to Date

This study suggests that there is a strong relationship between the level of natural exposure to *Mptb* and the subsequent incidence of OJD infection. It should be possible to reduce the incidence or severity of OJD by reducing the levels of exposure in young sheep in the pre-weaning and immediate post-weaning periods, and that the infection status and stage of infection of the dam plays an important role in the pre-weaning period. Based on these findings the following recommendations form the guidelines for grazing management strategies for OJD infected flocks.

1. Produce low contamination pasture by removing *Mptb* shedding sheep for at least 3 months, preferably over summer. Pastures can be left un-grazed or grazed by adult cattle or unaffected sheep destined for slaughter.
2. Join ewes over as short a period as possible to facilitate an early weaning. Draft and remove from the lambing mob, as often as practical, ewes suspected as infected with OJD. Move the lambing flock onto the prepared low-contamination pasture just prior to the start of lambing and employ lowest possible stocking densities.
3. Wean lambs as young as possible to remove them from the greatest source of infection, their dams. Weaning at 7 weeks is the minimum recommended age, provided these lambs are moved onto highly nutritious pasture.
4. If low-contamination pasture is scarce, use it strategically post-weaning rather than for lambing ewes.
5. Leave the weaned lambs in the low challenge environment for as long as possible (six months), the paddock could then be prepared for the next year's weaners. During their first nine months of life the weaner sheep are expected to have shed very few *Mptb* onto the pasture.



Endoparasite control on prepared, low infectivity pastures needs to be considered to avoid a conflicting effect from OJD control and management practices. Extra vigilance with endoparasite control regimes may be necessary where weaner animals are being put at risk of exposure to potentially high level of endoparasite infection on prepared pastures. If adult animals have been used to reduce *Mptb* levels on pasture by pre-grazing pasture prior to weaning, the susceptibility of these animals to endoparasite infection and subsequent re-infestation of paddocks should also be considered.




Epidemiology 1 - Exposure Factors Leading to the Establishment of OJD Infection and Clinical Disease

OJD.0002A Pasture Management and OJD

Helen McGregor






Main Objectives and Methodology Overview


- Identify factors which contribute to risk of infection
 - Dam status
 - Pre-weaning exposure vs post-weaning exposure
- Provide recommendations for development of grazing management strategies for producers of infected flocks
- Extensive 3 year field study – 500 animals
- Progeny born into trial to infected or “clean” dam
- Nominated to treatment groups - pre and post weaning exposure
- Faecal and blood collection
- Post-mortem, histopathology and tissue culture at 3 years






Results – OJD Infection Rate and Death Rate

Exposure history	Infected dam	Pre-weaning exposure			Post-weaning exposure		OJD infection rate (%)	OJD death rate (%)
		Low	Med	High	Low	High		
IHH	X			X		X	36.6	8.3
IHL	X			X	X		38.8	6.0
ILL	X			X	X		20.3	2.9
UHH			X			X	28.6	8.6
UHL			X		X		17.4	1.4
ULH		X				X	17.6	1.5
ULL		X			X		18.3	2.8



Results Summary

- Positive relationship between level of exposure and incidence of OJD
- Continuing exposure in pre and post-weaning periods results in high levels of infection
- Effect of dam infectivity status plus pre-weaning exposure produced higher infection rates than pre-weaning pasture based exposure alone
- Sheep beyond weaning age remain susceptible to infection
- Avoid high rates of infection by placing previously exposed lambs onto clean pasture at weaning



Recommendations

- Produce low contamination pasture
 - Remove sheep 3 months (summer)
 - Adult cattle
 - Unaffected sheep destined for slaughter
- Join ewes over short period to facilitate early weaning
- Draft and remove suspect ewes from lambing mobs often
- Move lambing flock onto low contamination pasture close to lambing at low stocking densities
- Wean as early as possible
- Use prepared pasture preferentially in post weaning period
- Leave lambs in the low challenge environment for as long as possible

BEST PRACTICE MANAGEMENT OF OJD ON KANGAROO ISLAND

THE EFFECT OF IMPROVED HEALTH AND CREEP FEEDING OF MERINO LAMBS ON THEIR MORTALITY RATE TO 4.5YO.

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Background and Objectives

There had been suggestions by farmers, advisors and researchers that internal parasites and trace element deficiencies might play a role in the establishment of JD infection in sheep. Kangaroo Island sheep farmers often don't prevent internal parasitism or trace element deficiencies in very young lambs either because ewes are not treated pre-lambing or trace elements are not administered to lambs at marking or if they are marking is often delayed. Internal parasitism and Vitamin B12 and selenium deficiencies are a very common cause of ill-thrift in young KI lambs.

The objective of this study is to monitor the lifetime development of OJD in the 1999 progeny of Merino ewes which had various management strategies imposed upon them from pre-birth until weaning but were subsequently managed as usual for the property.

Methods and Results

A Kangaroo Island property with high within flock prevalence of OJD was selected for the study. In 1999 23% of wethers and 11% of ewes were AGID positive. The flock was thought to have been infected for at least 10 years and pastures in different paddocks grazed by the sheep were assumed to be equally contaminated with Mptb.

The property is in an area that has acidic lateritic soils with inherent low phosphorous levels, high iron content and deficiencies of the trace elements selenium, cobalt and copper.

The property had been top-dressed with 500gm per hectare of Agsel Selenium chips and 100kg per hectare of DAPS in the autumn of 1998 and copper had been applied some years ago. The lambs were thought to be predisposed to Vitamin B12 deficiency prior to marking and after weaning and selenium deficiency also after weaning.

In February 1999 the 1.5 to 4.5yo merino ewes were assigned randomly to 2 groups of approximately 500 ewes. They were joined to merino rams. A third group of ewes with poor wool types were joined to Corriedale rams to breed Comeback ewes as prime-lamb dams. All ewes were then run as a large group until 2 weeks prior to lambing.

The treatment group ewes were managed to optimise their health and that of their lambs. The ewes were treated with Cydectin plus 6in1 vaccine (Eweguard) and Vitamin B12 injection 2 weeks pre-lambing. These ewes were split for lambing into 3 paddocks. The treatment group lambs were given access to creep feeders with crushed lupins from 2 weeks of age to stimulate early rumen development.

The control group ewes were not given any treatments pre-lambing as is usual practice for the property. They were also split into 3 paddocks for lambing. The Corriedale joined ewes were split and co-grazed with the Merino joined ewes in 2 of these paddocks.

Udders of the ewes were painted with a different colour for each dam age the day before their lambs were marked. The lambs were then ear tagged and age of their dam recorded along with treatment group and birth paddock.

All lambs were treated with Glanvac 3 B12 injection at marking when 1mth old and at weaning when 3mths old. They were all drenched with Cydectin at weaning. Treatment group progeny were also given Selenium and Cobalt intra-ruminal pellets at weaning before all lambs went on to a fodder crop.

All 1400 progeny were run as one group for the next 14 months. After shearing in February 2001 merino ewes culled for wool quality and merino wether progeny were moved to the wether block where they were co-grazed until 4.5 years of age. Merino and comeback ewes were co-grazed except for joining and lambing for the next 3 years. After their third lambing in 2003, ewes dry at this and the previous one or two lambings were sold.

Comeback wethers were sold to slaughter at 1 year of age (August 2000). They were inspected at the abattoir but no visible lesions were detected. Faeces were collected on line for pooled faecal culture and were positive.

Minimal data was collected 2 or 3 times a year from all of the sheep in the other progeny groups for the next 3 years but no biological specimens were collected for OJD tests from living or dying sheep. Data recorded was bodyweight, condition score, dag score, lactation status, and evidence of other disease.

At marking, blood samples from lambs from the treatment group ewes had adequate Vitamin B12 levels whereas those from lambs from the control group ewes were deficient. Faecal egg counts were very low in treatment and control group lambs with an average of 70 epg for each group. Faecal egg counts of progeny at weaning were high but not significantly different between paddocks and averaged 900 epg.

Results are presented for the various progeny groups to 4 years of age in Tables 1 & 2.

Conclusions to Date


Progeny of maidens in all groups had high mortalities as weaners (>15% for merinos, >25% for comebacks). The mortality rate was less than half for the progeny of adult ewes. More merino lambs born to treatment group adult ewes died compared to those born to control group adult ewes. The reason is unclear. Most deaths occurred around weaning due to the combined effects of internal parasitism and flystrike from the scouring.

Merino wethers and ewes that were the progeny of maiden ewes had higher mortality rates than those that were the progeny of adult ewes in their first and third years of life. Mortality rates were very low in merino wethers and cull ewes in their second year.

Mortalities were high in comeback ewes during their first and second lambings due to dystocia so they can not be compared to merino ewe deaths in the second and third years. The mortality rate of comeback ewes in their fourth year was lower than merino ewes born and subsequently run with them.

The mortality rate of cull ewes in their third year and merino ewes and wethers in their fourth year of life was lower for those that were the progeny of ewes from the improved management group. Their body weights were higher in the 6 months prior to death.

Body weights of sheep that survived each year were not different between treatment and control progeny. The majority of sheep that died had bodyweights less than 50kg (or 72% of mature weight) and condition scores less than 2 on a scale of 1 to 5 at their previous sampling. Sheep over 50kg with condition scores less than or equal to 2 also died. The mature body weight for sheep on this farm is 68kg (4yo dry sheep in 3 score condition). This target could possibly be used on this property to salvage sheep



for slaughter while still marketable.

The mortality rate of the cull ewes run under the same conditions as the wethers was higher than that of the wethers in the third and fourth years.

The total deaths for years 2+3+4 that might be attributable to OJD were 24% for merino ewes, 36.7% for comeback ewes, 41.1% for merino wethers and 55.0% for cull ewes.

Wethers	Treatment	Control	Total	Cull Ewes	Treatment	Control	Total
n total progeny	227	279	506		62	69	131
n maiden prog	77	103	180		25	25	50
n adult prog	150	176	326		37	44	81
First Year							
% Total died	11.0	9.7	10.3		10.0	9.1	9.5
% M died	15.6	16.5	16.1		14.9	15.2	15.0
% A died	8.7	5.7	7.1		7.2	4.5	5.8
B Wt Survivors	41.0(5.0)	40.5(5.5)	40.5(5.0)		37.0(5.0)	38.5(4.5)	37.5(4.5)
Condition Score	2.5	2.5	2.5		2.5	2.5	2.5
B Wt Died							
Condition Score							
Second year							
% Total died	3.5	2.9	3.2		1.6	1.4	1.5
% M died	2.3	1.0	1.7		0.0	0.0	0.0
% A died	4.0	4.0	4.0		2.7	2.3	2.5
B Wt Survivors	47.0(5.5)	46.5(5.5)	46.5(5.5)		43.5(5.0)	43.0(4.5)	43.0(5.0)
Condition Score	3.0	3.0	3.0		2.5	2.5	2.5
B Wt Died	39.5(6.0)	36.0(4.5)	36.0(4.5)		38.0	51.5	45.0(9.5)
Condition Score	2.0	2.0	2.0		1.0	2.5	2.0
Third Year							
% Total died	13.7	12.5	13.0		19.4	24.6	22.0
% M died	19.5	12.6	15.6		28.0	28.0	28.0
% A died	10.7	12.5	11.7		13.5	22.7	18.5
B Wt Survivors	55.5(6.0)	55.0(6.0)	55.0(6.0)		49.0(5.0)	50.5(5.5)	49.5(5.5)
Condition Score	2.5	2.5	2.5		2.5	3.0	2.5
B Wt Died	42.5(5.5)	42.5(5.5)	42.5(5.5)		33.0(9.5)	36.0(3.0)	35.0(4.5)
Condition Score	1.5	1.5	1.5		1.5	1.5	1.5
Fourth Year							
% Total died	18.1	21.9	20.2		30.6	31.9	31.3
% M died	16.9	21.4	19.4		28.0	36.0	32.0
% A died	18.7	22.2	20.5		32.4	29.5	30.9
B Wt Survivors	60.0(7.5)	60.0(6.0)	60.0(6.5)		64.0(8.5)	63.0(9.5)	63.5(8.5)
Condition Score	3.0	3.0	3.0		3.5	3.5	3.5
B Wt Died	43.0(5.5)	39.5(6.5)	41.0(6.5)		45.0(4.0)	47.5(5.5)	46.5(5.0)
Condition Score	1.5	1.5	1.5		2.0	2.5	2.5
Year 2+3+4							
% Total died	40.5	41.6	41.1		51.6	58.0	55.0
% M died	45.5	37.9	41.1		56.0	64.0	60.0
% A died	38.0	43.8	41.1		48.6	54.5	51.9

Table 1: Comparative mortalities, body weights (standard deviation) and condition scores for merino wether and cull ewe progeny of maiden (M) and adult (A) ewes.

Merino Ewes	Treatment	Control	Total	Cbk Ewes	Total
n total progeny	198	239	437		161
n maiden prog	69	107	176		35
n adult prog	129	132	261		131
First Year					
% Total died	10.0	9.1	9.5		8.4
% M died	14.9	15.2	15.0		25.7
% A died	7.2	4.5	5.8		3.8
B Wt Survivors	38.5(5.0)	39.0(5.0)	39.0(5.0)		40.0(4.5)
Condition Score	2.5	2.5	2.5		3.0
B Wt Died					
Condition Score					
Second year					
% Total died	6.6	5.9	6.2		16.9
% M died	5.8	3.7	4.5		17.1
% A died	7	7.6	7.3		16.8
B Wt Survivors	54.5(8.0)	53.0(7.5)	53.5(7.5)		54.0(8.0)
Condition Score	2.5	2.5	2.5		3
B Wt Died	36.5(9.0)	39.5(6.5)	38.5(7.0)		46.0(5.0)
Condition Score	2.0	2.0	2.0		2.5
Third Year					
% Total died	8.1	5.4	6.6		12.0
% M died	5.8	4.7	5.1		11.4
% A died	9.3	6.1	7.7		12.2
B Wt Survivors	58.0(5.5)	57.5(6.0)	57.5(6.0)		55.5(7.0)
Condition Score	2.5	2.5	2.5		2.5
B Wt Died	37.5(6.0)	41.5(9.0)	38.0(6.5)		50.5(6.5)
Condition Score	1.5	2.0	1.5		3.0
Fourth Year					
% Total died	9.6	12.6	11.2		7.8
% M died	7.2	14.0	11.4		8.6
% A died	10.9	11.4	11.1		7.6
B Wt Survivors	68.0(7.0)	67.0(10.5)	67.0(9.0)		55.5(8.0)
Condition Score	3.5	3.5	3.5		3.0
B Wt Died	45.0(5.5)	39.5(5.5)	43.0(5.0)		47.5(6.5)
Condition Score	2.5	1.5	2.0		2.0
Year 2+3+4					
% Total died	24.2	23.8	24.0		36.7
% M died	18.8	22.4	21.0		37.1
% A died	27.1	25.0	26.0		36.6

Table 2: Comparative mortalities, body weights (standard deviation) and condition scores for merino ewe and comeback ewe progeny of maiden (M) and adult (A) ewes.

Best Practice Management of OJD on Kangaroo Island

Deb Lehmann

Property Summary

- **Climate**
 - Mediterranean
 - 540mm Rainfall over months April to October
 - 60 - 70% relative humidity
- **Soil**
 - Acidic
 - Laterite or ironstone
 - Low phosphorous
 - Deficient in Cobalt, Selenium & Copper

Property Summary cont.

- **Flock**
 - Self replacing Merino breeding own rams
 - Corriedale stud breeding rams for own use
 - Comeback ewes as 1st cross dams to W Suffolk
 - Cull merino ewes run dry with wethers
 - Practices cell grazing
- **OJD**
 - Diagnosed Spring 1998
 - High within flock prevalence => on prop 10yrs +
 - Thin wethers - 23% AGID positive
 - Thin ewes - 11% AGID positive
 - Thin comeback ewes - 4% AGID positive

Improved Treatment/Control

	Improved Treatment			Control		
Paddock	1+	2+	3+	4-	5-	6-
DAPS kg/ha '98 kg/ha '99	125 100	125 -	- 100	- -	- 100	100 -
AGSEL 500 gm/ha '98 gm/ha '99	Au Au	Au Sp	Sp Au	Sp Sp	Sp Au	Au Sp
Prelambing Ewe Tx	Eweguard + Vitamin B12			Nil		
Lambing (Aug – Sep)	Crushed lupins in creep feeders			Nil		
Plasma B12 Lambs	Adequate			Deficient		
Marking 4 wks	Glanvac – 3 B12			Glanvac – 3 B12		
Weaning 12 - 16wks	Selenium + Cobalt Intraruminal pellets			Nil		
Next 14mths	Co-grazed			Co-grazed		

Results 1

	Wethers		Cull Ewes	
	+	-	+	-
n Total Progeny	227	229	62	69
n Maiden Progeny	77	103	25	25
n Adult Progeny	150	176	37	44
1 st Year BWt. M + A	41.0(5.0)	40.5(5.5)	37.0(5.0)	38.5(4.5)
Cond Score	2.5	2.5	2.5	2.5
4 th Year BWt. Surv.	60.0(7.5)	60.0(6.0)	64.0(8.5)	63.0(9.5)
Cond Score	3.0	3.0	3.5	3.5
BWt. Died	43.0(5.5)	39.5(6.5)	45.0(4.0)	47.5(5.5)
Cond Score	1.5	1.5	2.0	2.5
Year 2 + 3 + 4				
% Total died	35.2	37.3	51.6	58.0
% M prog died	39.0	35.0	56.0	64.0
% A prog died	33.3	38.6	48.6	54.5

Results 2

	Ewes		M Prog	M Prog -	
	+	-	+	B12 +	B12 -
n Total Progeny	198	239			
n Maiden Progeny	69	107	69	34	25
n Adult Progeny	129	132			
1 st Year BWt. M + A	38.5(5.0)	39.0(5.0)	38.5(4.5)	38.5(5.5)	40.5(5.5)
Cond Score	2.5	2.5	2.5	2.5	2.5
4 th Year BWt. Surv.	68.0(7.0)	67.0(10.5)	69.5(7.5)	69.5(9.0)	65.0(14.5)
Cond Score	3.5	3.5	3.5	3.5	3.0
BWt. Died	45.0(4.0)	39.5(5.5)	47.0(3.5)	48.5	37.0
Cond Score	2.5	1.5	2.5	3.0	1.0
Year 2 + 3 + 4					
% Total died	25.3	23.8			
% M prog died	20.3	22.4	20.3	20.6	32.0
% A prog died	27.9	25.0			

Grazing Management – Workshop Discussion

Following these presentations a workshop of participants was held to answer the following questions:

1. *What has been achieved by the researchers?*

OJD.002A study:

- Identification of the importance of level of pasture contamination and timing of exposure on infection
- Confirms grazing recommendations that were developed from first principals
- Confirmed the importance of lambing management
- Confirmed that Low contaminated pastures should be given to weaners as priority
- Identified that high mortalities due to OJD do occur.

Kangaroo Island study:

- Proportion of losses attributed to OJD is difficult to estimate.
- Needs to be redone as a new trial – (priority?)
- Checking for low bodyweight can be critical (Londit score)
- Vaccine may have all effect needed.

2. *What key benefit from this research can we communicate to producers?*

OJD.002 study:

- Grazing management strategies are appropriate once the level of pasture contamination is reduced or prior to it rising. Still need vaccination first.
- Grazing management is the key to managing disease in low prevalence flocks, where vaccination is not economical. PFC at 18 month age may be used as a predictor to decide whether vaccination needed.
- Grazing management may prolong the effect of vaccination.
- Change to autumn lambing?
- Need to have different approaches for different enterprise types.

Kangaroo Island study:

- Trace element effects are inconclusive – need to extend study.
- Achieving optimum nutrition levels – may decrease OJD risk?

3. What advice would a farm adviser now be able to provide a producer client?

- Management needs to be regional and beyond the individual farm
- Keep messages simple - don't have too much detail.
- Advice needs to consider objectives.
- Analogous to worm control.
- Hand-out with vaccination recommendations.

4. How could a regional authority, eg RLPB, respond to this information?

- As for 3

5. What still needs to be done/researched?

- Further research on age susceptibility - mature sheep anecdotally susceptible
- Consideration of low prevalence flock options:
 - Grazing management only
 - Grazing management & vaccination
 - Vaccination only
- Further research on cost benefit analysis.

6. Are there any other implications or warnings from this work?

- Can have heavy shedding in younger groups.
- Some grazing strategies harmful eg. cell grazing

7. Have the outcomes of this work been captured in a useful form (scientific publication, advisory note etc) and what actions are required to ensure this occurs?

- OJD.002 requires publication in a scientific journal
- Results need to be translated to regions – further input needed for regional use
- Extension work to focus on farm groups
- Producers not getting these messages yet.
 - NSW RLPB: Newsletters
 - VIC: Support groups, internet, field days, point of vaccination, access to subsidy advice, media
- Kangaroo Island study too broad to publish.

ERADICATION

NOJDP RESEARCH PROGRESS REPORT

EVALUATION OF ERADICATION STRATEGIES FOR OVINE JOHNE'S DISEASE

PROJECT NUMBER: OJD.001

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Background and Objectives

Prior to the registration of Gudair™ OJD Vaccine for use in sheep in Australia in 2002, destocking of infected flocks was the only authorised strategy available to sheep producers to manage ovine Johne's disease (OJD) on their properties. This required the implementation of a formal Property Disease Eradication Plan (PDEP) under the supervision of an accredited veterinarian.

In 1999 this project (Trial 1.1) was funded through the National Ovine Johne's Disease Program primarily to evaluate the efficacy of destock-restock strategies to eradicate OJD from a number of previously infected farms in a range of environments in south eastern Australia. This paper provides a progress report on the results of Trial 1.1 to the end of 2003.

Methods and Results

Methods

1. *Site selection.* Sites were selected on the basis of having an approved PDEP and objective evidence of sufficient infection in the year of destocking for a worthwhile test of the eradication process. PDEPs stipulate the minimum 15 month interval between destocking all infected flocks and restocking the property. They also detail planned interim grazing enterprises and any actions required to mitigate against reintroducing the disease from neighbouring properties (eg double boundary fencing, restrictions on sheep grazing adjacent to the perimeter). Figure 1 shows the location (and current test status) of all sites.

2. *Restocking requirements.* Participants were required to restock with at least 700 low risk sheep within six months of completing an approved 15 month PDEP. Sheep could be purchased from Market Assurance Program (MAP) flocks or flocks tested to MAP equivalence (ie ~95% assurance of detecting $\geq 2\%$ prevalence).

3. *Measuring the result of the eradication process.* The restocked sheep and/or lambs born within the six month restocking interval (monitor sheep) are monitored for evidence of OJD for the next three years. The efficacy of the eradication process will ultimately be determined by culturing at least 10 pools of 50 faecal samples (ie ~ $\geq 99\%$ assurance of detecting $\geq 2\%$ prevalence) at 24 and 36 months after restocking. *Mycobacterium avium* subspecies *paratuberculosis* (Mptb) positive cultures are strain typed to distinguish between S and C strains. Sheep contributing to OJD positive faecal pools are slaughtered and tissue samples are submitted for histopathology from those exhibiting gross lesions consistent with OJD.

4. *Investigations of eradication failures.* These involved whole flock prevalence testing via faecal culture, strain typing of positive cultures, confirming the current MAP status of market assured vendors and where practicable, retesting the flocks of non-MAP vendors. The advice of local veterinarians was also sought on the OJD status of neighbours(hoods) of vendors to all failed Trial sites.

Results

Efficacy of eradication protocol

The results of testing at two and three years after restocking are given in Table 2.

Table 2 Results of testing Trial 1.1 sites two and three years after restocking

Result	Test year				Total
	2001	2002	2003	2004	
Evidence of OJD after 2 years	4/6 (1w)	6/19	7/16 (1w, 1p)	-	17/41
Evidence of OJD after 3 years	-	1/2	6/12 (1 p)	?/10	7/14
Eradication failure to Dec. 03					58.5%

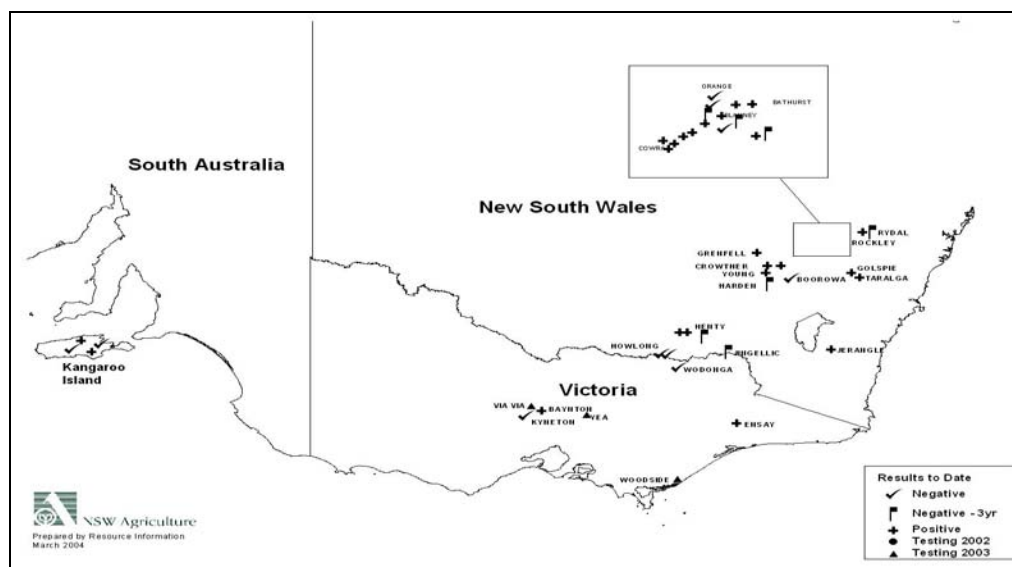
w = withdrawn, p = pending.

Overall 24 of 41 sites tested to date have evidence of OJD. If we consider a negative test at three years after restocking to be definitive, based on these estimates the probability of successful eradication under the protocol imposed by the Trial is low at around 30%.

Geographic distribution of eradication results

The location and current test status of all Trial sites is depicted in Figure 1.

Figure 1 Location and OJD status of participating sites.



There have been eradication failures in all regions represented in the trial. The seven sites that have so far tested clear of OJD at three years after restocking are located in the Central Tablelands (4), the South-West Slopes (1) the Eastern Riverina (1) and the Upper Murray Valley (1) of NSW. There is as yet no obvious association between the probability of failure and geographic location.

Reasons for failure

The reasons for the 24 failures are uncertain but based on the information available to date we have identified four sites that definitely purchased sheep from infected flocks, another four that are considered likely to have purchased from infected flocks, 11 that are inconclusive at this stage and five that are considered to be local eradication failures (ie either incomplete disinfection and/or reinfection from neighbours). Evidence and suspicion of reintroduction in the purchased sheep does not exclude the possibility of local failure as well. Every positive culture from the 18 failed sites strain typed to date has been identified as S strain.

Pre-destock and post-restock prevalence on failed sites

Estimates of apparent prevalence in the year of destocking and in the year of detecting OJD in the restocked sheep are given in Table 3 for the first eight failed sites.

Table 3 Estimates of apparent prevalence prior to destocking and two or three years after restocking

Prevalence	F1	F2	F3	F4	F5	F6	F7	F8
Estimated in year of destock (%) #	20	20	20	32	2	20	20	24
Observed 2 years after restock	6/735	2/600	1/600	3/3250	1/500	2/1450	2/350	4/1050
Estimated 2 years after restock (%)*	2.0	0.8	0.5	0.3	0.5	0.3	1.4	1.0

based on OJD mortality (x 4) in year of destock;* based on PFC sensitivity of 40%.

For each site there has been a substantial reduction in apparent prevalence. This was also observed for the next 14 sites to break down, with an average prevalence in the year of destocking of 16% (range 2 – 40%) and an average prevalence in the year of detection of 0.8% (range 0.5 – 1.75%). The two most recent failures are of interest in that although cultures are not yet complete, both exhibit apparent prevalences in excess of 3%.

Conclusions to Date

1. If we consider a negative test at three years after restocking as definitive, the chances of successful eradication under the conditions imposed by the Trial protocol are poor at around 30%.


2. There is as yet no obvious geographic association with eradication efficacy

3. The process has resulted in substantial reductions in apparent prevalence in the new flocks grazing these sites.

4. With such a high failure rate we must question


- the frequency and distribution of infected flocks that have escaped detection using current MAP assurance thresholds
- the mobility of Mptb within contaminated neighbourhoods
- the longevity of Mptb outside of sheep

5. If systematic eradication by destocking is still on the agenda, these three potential causes of reinfection need to be (re)evaluated experimentally.




Trial 1.1 – NOJDP Research Progress Report

OJD.001 – Evaluation of Eradication Strategies for OJD



Pat Taylor

compiled by
Pat Taylor and
Kevin Thornberry




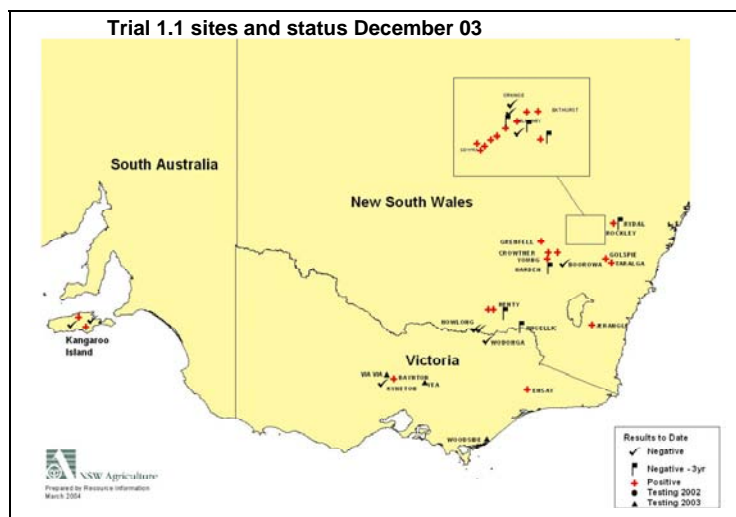
Efficacy					
Results of testing Trial 1.1 sites two and three years after restocking					
Evidence of OJD	Test year				Total
	2001	2002	2003	2004	
After 2 years	4/6 (1w)	6/19	7/16 (1w, 1p)	-	17/41
After 3 years	-	1/2	6/12 (1 p)	?/10	7/14
December 03					58.5%

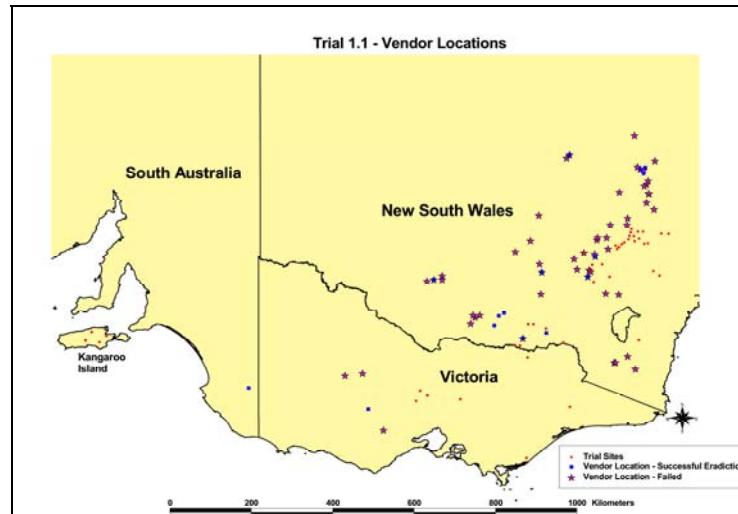
w = withdrawn, p = pending

Reasons for Failure

- 4 definitely purchased from infected flocks
- 4 probably purchased from infected flocks
- 5 local failures
- 11 inconclusive as yet







MEAT & LIVESTOCK AUSTRALIA

Comparison of 5 **local failures** with 7 “**successful**” sites.

NSW Agriculture

Site	Neighbour OJD status	Staged destock	Destock interval (months)	Cultivation during PDEP
NSW 20	2 nd IN property	1	15	Plough
NSW 46	2 nd IN property	1	19	Nil
NSW 61	IN	1	17	Nil
NSW 63	IN	1	15	Nil
NSW 84	IN	1	21	Direct drill
	5/5 IN	0/5	17.4	2/5
NSW17	BJD, Vacc.	2	15	Plough
NSW54	IN	1	15	Plough
NSW83	2 nd IN property	3	15	Nil
NSW109	Cattle	1	16	Nil
NSW110	IN	1	15.5	Nil
NSW62	IN	1	19	Nil
NSW15	2 nd IN property	2	21	Nil
	6/7	3/7	16.6	2/7

MEAT & LIVESTOCK AUSTRALIA

Conclusions to Date

1. If we consider a negative test at three years after restocking as definitive, efficacy ~ 30%
2. There is as yet no obvious geographic association with eradication efficacy
3. The process has resulted in substantial reductions in apparent prevalence in the new flocks grazing these sites.
4. With such a high failure rate we must question
 - the frequency and distribution of infected flocks that have escaped detection using current MAP assurance thresholds
 - the mobility of Mptb within contaminated neighbourhoods
 - the longevity of Mptb outside of sheep
5. If systematic eradication by destocking is still on the agenda, these three potential causes of reinfection need to be (re)evaluated experimentally.

NSW Agriculture

Eradication – Workshop Discussion

Following these presentations a workshop of participants was held to answer the following questions:

1. *What has been achieved by the researchers?*

- Evidence that under commercial conditions, destocking has a low (~30%) probability of eradicating OJD.
- It is very difficult to determine the source of breakdown.
- Destocking can be used to rapidly decrease disease prevalence.
- Trial opened dialogue between infected producers and authorities.
- If trial had not been done, we would still be advising producers that destocking could result in eradication.

2. *What key benefit from this research can we communicate to producers?*

- Destocking has a low probability of success in eradicating this disease
- Partial destocking may be beneficial in reducing disease prevalence on a farm
- Destocking useful if changing enterprise

3. *What advice would a farm adviser now be able to provide a producer client?*

- Think very carefully before embarking on an eradication program by destocking
- Weigh up costs/benefits.
- Ensure producers are aware of the risks if they choose to destock.

4. *How could a regional authority, eg RLPB, respond to this information?*

- Extreme caution should be taken in suggesting destocking for eradication even in very low prevalence areas.
- Don't recommend in other areas.

5. *What still needs to be done/researched?*

- Follow up work on Victorian farms that have destocked for seven (plus) years
- Combine soil survival studies with infectivity for sheep.

- Investigate whether vaccination can achieve eradication?
- With our current tools, further research may be unlikely to yield answers.

6. Are there any other implications or warnings from this work?

- Very difficult to get a robust study design.
- Extremely difficult to design a trial that provides basic information on these farms.
- What determines the success/failure of project, (eg what drives participation and compliance?)
- This disease might be slow and insidious, but:-
 - it is highly infectious
 - we are always years behind the initial infection

7. Have the outcomes of this work been captured in a useful form (scientific publication, advisory note etc) and what actions are required to ensure this occurs?

- Project still underway - results have been presented at ASVS conferences, MLA publications and in "The Land".
- It is believed that producers know of the results, particularly those in endemic areas, but those in very low prevalence areas may be unaware.
- Results should be published in a scientific journal ASAP.
- Local extension as required.
- Stand alone advisory note.
- General media eg "The Land" highlighting that "everyone is at risk".

CROSS SPECIES

South Australia – Cross Species Transfer (KI)

A PREVALENCE SURVEY FOR *M. PTB* IN KANGAROO ISLAND MACROPODS

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Background and Objectives

For several Kangaroo Island sheep flocks infected with OJD, there is no reported history of direct contact with infected sheep via introductions or straying. Heavy pasture contamination with *M.ptb* is probable on endemically infected farms and high densities of several wildlife species co-graze with sheep throughout the year. This raised the suspicion that macropods in particular may play a part in OJD epidemiology on Kangaroo Island. Macropods may possibly passage *M.ptb* and mechanically deliver an infectious dose to sheep, or develop lesions of JD and amplify the organism. Both possibilities pose a risk to control measures in sheep.

A survey to determine the prevalence of *M.ptb* from culture of the ileum and associated lymphatics and the prevalence of lesions consistent with OJD in Kangaroo Island macropods was undertaken in an attempt to shed further light on the role these species may play on the spread of *M.ptb*.

Methods and Results

Site Selection

Site selection was purposive. Eight OJD-infected sheep flocks ranging from endemic to low prevalence were chosen and were thought to reasonably represent the range of OJD prevalences which exist on Kangaroo Island

SAMPLE SIZE

Assumptions

- Sampling was based on shot samples and the direction of the sampling bias is not known. Young animals were assumed to be less likely to show evidence of JD and were deliberately excluded from the study.
- Tissue culture was the screening test. Tissue culture is considered more sensitive than faecal culture. The sensitivity of faecal culture in cattle using BACTEC and PCR is approximately 50%. Since no published data on sensitivity of tissue culture in macropods exists, a conservative sensitivity of 30% was used.
- Since tissue culture is based on physical identification of the pathogen, the test is technically 100% specific, provided extreme care is taken to avoid cross-contamination in the field and laboratory.

Based on the above assumptions, a sample of approximately 500 macropods was required if the minimum prevalence of *M.ptb* of 2% was expected.

FIELD SAMPLING AND DATA COLLECTION

Approximately 60 macropods were euthanased at each study site. Female tammar wallabies weighing 4kg or greater and males weighing 5kg or greater were eligible for sampling since these animals are at least 2 years of age. For kangaroos, only mature animals weighing in excess of 30 kilograms were eligible.

At autopsy, the ileum and associated lymphatics were assessed for the presence of gross lesions. Any lesions present were described and recorded against the ear tag number. Ileocaecal and mesenteric lymph nodes were then dissected and half of these tissue samples were placed in a sterile container for tissue culture. The other half of the lymphatic tissue samples were placed in 10% buffered formalin for histopathology. A 10 cm portion of terminal ileum from each animal was opened, flushed with distilled water to remove gut contents and then placed in a sterile container. A 5 cm portion of adjacent terminal ileum was fixed in the same 10% buffered formalin container used for the lymphatic tissues from that animal. All fresh specimens were chilled and transported to the laboratory within 24 hours of collection.

Between each animal processed, instruments were disinfected with chlorhexidine and alcohol, then rinsed with distilled water. Sterile gloves and scalpel blades were used for each autopsy.

A faecal sample was taken and frozen to -80°C at IDEX-VPS for later testing by faecal culture if required. The faecal samples from all tissue culture and histopathologically positive animals were individually cultured, while the remaining faecal samples from all tissue culture negative animals were cultured in pools of 20.

LABORATORY METHODS

Culture methods for *M.ptb* were conducted using standard procedures for ruminant tissues. Positive cultures were referred to Western Australia for strain typing when necessary.

Standard histopathological techniques were used to assess the presence of visible mycobacteria and other cellular changes consistent with JD infection from all positive cultures or samples with suggestive gross lesions.

Results

A total of 427 wallabies and 55 kangaroos were sampled during the survey. Positive tissue cultures were obtained from eight animals (1.7%) at four sites. Each positive tissue culture result was confirmed as S strain *M.ptb*.

Two of the eight tissue culture positive animals (a female kangaroo and a female wallaby) had lesions which were histopathologically consistent with OJD.

In addition, six animals which were tissue culture negative but had suspicious gross lesions at autopsy, also had histological lesions consistent with OJD and the presence of acid fast bacilli in the terminal ileum and/or associated lymphatic tissue. Attempts to strain type the organisms found in the histological sections were unsuccessful.

The faecal cultures from all tissue culture and/or histopathologically positive macropods were negative for *M.ptb*, as were the pooled faecal cultures for all other animals enrolled in the study.

The results for the study are given in Table 1.

Table 1. Tissue culture and histopathology results for the Kangaroo Island macropod survey.

Site	Species	Tissue Culture	Histology
5	Kangaroo	+ve	Occasional acid fast bacilli in lymph node.
7	Wallaby	+ve	Moderate numbers of acid fast bacilli in lymph node.
1	Kangaroo	+ve	-ve
2	Wallaby	+ve	-ve
2	Wallaby	+ve	-ve
7	Wallaby	+ve	-ve
9	Wallaby	+ve	-ve
9	Wallaby	+ve	-ve
5	Wallaby	-ve	Numerous acid fast bacilli in lymph node.
7	Wallaby	-ve	Severe granulomatous enteritis
7	Wallaby	-ve	Occasional acid fast bacilli in lymph node.
9	Wallaby	-ve	Occasional acid fast bacilli in lymph node.
9	Wallaby	-ve	Moderate numbers of acid fast bacilli in lymph node.
9	Wallaby	-ve	Numerous acid fast bacilli in gut and lymph node.

Conclusions

How commonly the lesions of JD occur in macropods, and under what conditions, are currently unknown. The development of these lesions may be the result of the level and frequency of exposure to *M.ptb* these animals receive when grazing pasture heavily contaminated by infected sheep.


The significance of the survey findings for the Kangaroo Island OJD control program in sheep is still unclear, but the possibility that macropods may be reservoir hosts for *M.ptb* cannot be overlooked. The level of risk to sheep will depend on the prevalence of JD in macropods, the excretion rate of infectious individuals, the size of the infectious dose required for macropods and sheep and the probability of exposure to this infectious dose.

We were unable to culture *M.ptb* from the faeces of any of the tissue culture positive macropods or those with suggestive histological lesions. This could be because the excretion of viable organisms is a rare event in macropods. Alternatively, it could be due to poor faecal culture test sensitivity and/or problems with specimen transport, storage and handling. Macropods with JD lesions could reasonably be expected

to excrete the organism for their lifetimes, and thus be potential sources of infection for sheep within the home range of infected macropods. However, excretion by macropods was not demonstrated in this study.

If transmission of *M.ptb* from infected to susceptible macropods readily occurs and each primary case generates more than one secondary case, then a rise in the prevalence of animals with lesions of JD could be expected in the absence of infected sheep.




On the other hand, macropods may be 'dead-end' hosts of *M.ptb*. If *M.ptb* does not transmit readily between macropods, then only a negligible part of the population will become infected. The delivery of an infectious dose to macropods may only be possible under conditions of special challenge, when they co-graze pasture heavily contaminated by endemically infected sheep. If the excretion rate of *M.ptb* from infected macropods is low, then effective control measures in sheep may result in a decline over time in the prevalence of JD in macropods. The disease in these animals is likely to die out and the risk to control measures in sheep would be negligible.



South Australia – Cross Species Transfer (KI)

Prevalence Survey of *M. ptb* in Kangaroo Island Macropods

Paul Cleland





Macropods may possibly:

- Passage *M. ptb* and mechanically deliver an infectious dose
- Develop lesions of JD and then amplify and excrete *M. ptb* in large amounts

Both possibilities pose a risk to control measures in sheep


Prevalence Survey of *M. ptb* in Kangaroo Island Macropods



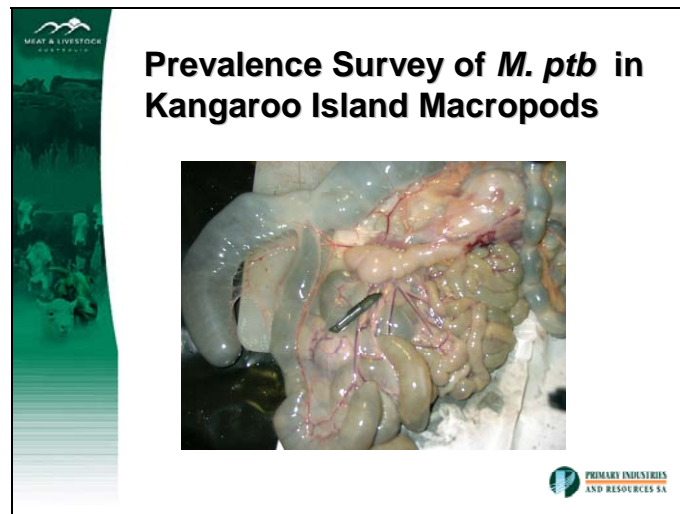
Field Sampling

- Phase 1 – emphasis on detecting infection. Tissue culture used as screening test.
- Phase 2 – emphasis on detection faecal excretion. Gross pathology used as screening test.

Prevalence Survey of *M. ptb* in Kangaroo Island Macropods



Prevalence Survey of <i>M. ptb</i> in Kangaroo Island Macropods (n = 482)			
Site	Species	Tissue Culture	Histology
5	K	+ve	Occasional AFB in l.n.
7	W	+ve	Moderate numbers AFB in l.n.
1	K	+ve	-ve
2	W	+ve	-ve
2	W	+ve	-ve
7	W	+ve	-ve
9	W	+ve	-ve
9	W	+ve	-ve
5	W	-ve	Numerous AFB in l.n.
7*	W	-ve	Severe granulomatous enteritis
7	W	-ve	Occasional AFB in l.n.
9	W	-ve	Occasional AFB in l.n.
9	W	-ve	Moderate numbers AFB in l.n.
9	W	-ve	Numerous AFB in gut and l.n.



Prevalence Survey of <i>M. ptb</i> in Kangaroo Island Macropods				
		Histo.		
n	PFC(n=20)	gross lesions*	Consistent	Suggestive
358W	-ve	34	4	2

*All animals with suggestive gross lesions were tissue and faecal culture negative. One animal with ++++ AFB on histo. yielded *M. genavense* on PCR

Cross Species – Workshop Discussion

Following these presentations a workshop of participants was held to answer the following questions:

1. *What has been achieved by the researchers?*

- Cross species transfer confirmed. Previous research has already identified that the organism has a very wide host range.
- Kangaroos probably play a minimal role in the epidemiology of OJD (S-strain).
- The studies have raised more questions than answers.

2. *What key benefit from this research can we communicate to producers?*

- Macropods are unlikely to pose a high risk of maintaining or spreading S-strain infection in sheep.

3. *What advice would a farm adviser now be able to provide a producer client?*

- Macropods are not a major concern in the control of OJD.
- Goats, cattle, deer, alpaca may still be of concern.
- The role of the C-strain is unclear.

4. *How could a regional authority eg RLPB, respond to this information?*

- As for 3.

5. *What still needs to be done/researched?*

- Studies should be repeated for the C-strain in macropods.
- Additional research needed on the epidemiology of S-strain in cattle, sheep, goats and deer in high risk cross-species environments to allow sound recommendations on risk factors.

6. *Are there any other implications or warnings from this work?*

- Difficult to refute the myth - producers will still believe macropods are important.
- The right risk mix can potentially lead to cross-species infection. Can't say it will never happen!

7. Have the outcomes of this work been captured in a useful form (scientific publication, advisory note etc) and what actions are required to ensure this occurs?

- Not captured in useful format – should be published in a scientific journal. The message should be clearly stated - *Kangaroos pose a minimal risk even in high kangaroo and sheep populations with significant levels of disease (ie on Kangaroo Island).*
- Other cross species work should also be published eg NSW rabbit work.
- Also publish in Tips and Tools and other producer publications.

ECONOMICS

NOJDP RESEARCH PROGRESS REPORT

A STUDY OF THE BIOLOGICAL AND ECONOMIC IMPACT OF OJD IN AFFECTED SHEEP FLOCKS IN NSW

PROJECT NUMBER: OJD.023

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Background and Objectives

Ovine Johne's disease (OJD) 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 on mortality rates attributable to OJD. This 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 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% with some mortality estimates possibly as high as 25%. 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.

McGregor et al. (2001) developed a protocol to estimate annual OJD mortality rate based on sampling of daily mortalities over four 5-day periods and on stock inventory records of flock numbers over a year. As a consequence of successful application on a single farm since 1999 this protocol was considered suitable for extension and implemented in a study designed to provide accurate estimates of mortality and economic loss associated with OJD in infected flocks. Twelve sheep flocks in southern NSW were studied for 12-months during 2002.

The major objectives of this 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 provide an accurate estimate of the cost of OJD in affected flocks.

Methods and Results

A 12-month observational study was completed on 12 southern NSW OJD-infected farms with flock sizes ranging from 3,500 to 20,000 head. 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. 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.

Biological Impact of OJD

In this study the definitive diagnosis of deaths attributable to OJD was based on histopathology. Sheep were classified as “OJD most likely to have contributed to death” when there was histological evidence of 3b (multibacillary) or 3c (paucibacillary) lesions, indicating advanced granulomatous enteritis (regardless of other findings that may have led to death e.g. drowned in dam, pneumonia). 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 did not contribute to death” when there was no histological evidence consistent with OJD. 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.

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 “malnutrition” category applied to 70 (63%) of the 111 sheep where the most likely cause of death was attributed to causes other than OJD. Between 75-82% of deaths could be attributed to OJD when considering the effect of the dry seasonal conditions experienced during 2002. The “unknown” category includes animals where the cause of death could not be established due to predation or an extended time between death and post mortem.

The distribution of necropsied sheep where OJD contributed to death across age groups and sexes illustrated OJD mortality increased from 1 year of age (10.4%) to peak at 4 years of age (35.6%) and then decrease 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 similar trend among OJD-related necropsies and total necropsies. The majority of OJD-related necropsies occurred in winter (31%) and spring (35%) while fewer occurred in autumn (18%) and summer (16%).

Table 1. Mortality rates for total, OJD and non-OJD losses on the 12 farms in 2002

Area/Farm	Total mortality rate %	OJD mortality rate %	Non-OJD mortality rate%		Proportion of OJD mortalities %
			Malnutrition	Non-malnutrition	
Bungendore/1	7.5	3.8	0.4	3.3	51
Bungendore/2	9.6	6.8	1.9	0.9	71
Bungendore/3	3.1	2.5	0.3	0.3	81
Taralga/1	8.2	8.2	0.0	0.0	100
Taralga/2	8.9	5.4	1.0	2.5	61
Taralga/3	18.2	17.5	0.0	0.7	96
Gunning/1	10.8	8.8	0.5	1.5	81
Gunning/2	7.5	5.8	1.0	0.7	77
Gunning/3	8.0	7.6	0.4	0.0	95
Harden/1	5.5	2.1	2.2	1.2	38
Harden/2	10.9	2.9	6.5	1.5	27
Harden/3	5.4	3.4	1.2	0.8	63
Average	8.6	6.2	1.3	1.1	70

Based on information from the necropsy study and inventory records, the estimated annual mortality rates (total, OJD, and non-OJD) were calculated for the 12 farms in 2002 (Table 1). The average total mortality rate for the 12 farms was 8.6% (range 3.1% to 18.2%) with the average OJD mortality rate being 6.2% (range 2.1% to 17.5%). The average non-OJD mortality rate was categorised as either malnutrition 1.3%

(range 0% to 6.5%) or due to other causes 1.1% (range 0% to 3.3%). The majority of malnutrition mortalities (57%) were located on one farm "Harden/2" while there were no non-OJD mortalities on the farm "Taralga/1". The average proportion of OJD mortalities from the total annual mortalities was 70% (range 27% to 100%).

Economic Impact of OJD

Gross margins were calculated for the 12 farms assuming each farm was free of OJD and then compared with the actual gross margin for each OJD infected farm. The calculated gross margin for an OJD infected flock was found to be on average 6.4% (range 2.2% to 15.4%) less than the gross margin if the flock had not been infected. The average gross margin/DSE for the OJD infected flocks was \$20.58 (range \$10.16 to \$36.36) and when the effect of OJD was removed was \$21.85 (range \$11.77 to \$37.19). The average gross margin/ha for the OJD infected flocks was \$167.75 (range \$66.12 to \$290.86) and when the effect of OJD was removed was \$178.65 (range \$70.60 to \$297.53).

The range of values for gross margin/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 gross margin/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.


Using the necropsy inspection information the average cost of OJD losses on the 12 farms over the 12-month study period was estimated to be \$64,100 (range \$15,569 to \$154,083). The average cost of OJD losses/DSE was \$7.68 (range \$0.84 to \$20.51) while the average cost of OJD losses/ha was \$65.92 (range \$6.75 to \$244.80). The cost of OJD losses accounted on average for 70.1% (range 16.5% to 100%) of the total financial loss related to the sheep necropsied during the 20-day inspection period. 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.

Conclusions to Date



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. On 12 farms, the average OJD mortality rate of 6.2% (range 2.1% to 17.5%) is more than twice the accepted mortality rate (from all causes) for Australian sheep flocks.



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.

Industry groups claiming 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. The data can be used to justify vaccination programs, other control options and the general concept of disease control and prevention. It is also important to note that in this study around 30% of annual mortalities were not attributed to OJD, stressing the importance of maintaining whole flock health management to minimize mortality when OJD is present in a flock, especially in a drought year.




The Biological and Economic Impact of OJD in Affected Sheep Flocks in NSW – OJD.023





Russell Bush



Background

- 12 month observational study
- Detailed description of each farm
 - questionnaires, climatic records and pasture samples
- Mortality estimates
 - inventory and necropsy
 - annual and OJD
- Economic estimates
 - gross margin analysis
 - economic value on mortalities



25/08/2002

Biological impact of OJD					
Area/Farm	Mortality rate %	OJD mortality rate %	Non-OJD mortality rate%		Prop of OJD mortalities %
			Malnutrition	Non-malnutrition	
Bungendore/1	7.5	3.8	0.4	3.3	51
Bungendore/2	9.6	6.8	1.9	0.9	71
Bungendore/3	3.1	2.5	0.3	0.3	
Taralga/1	8.2	8.2	0.0	0.0	100
Taralga/2	8.9	5.4	1.0	2.5	61
Taralga/3	18.2	17.5	0.0	0.7	96
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Harden/1	5.5	5.5	0.0	1.2	77
Harden/2	10.9	2.1	6.5	1.5	27
Harden/3	5.4	5.4	1.2	0.8	61
Average	8.6	6.2	1.3	1.1	70%

Economic impact of OJD \$\$\$\$?


Ave gross margins from 12 flocks with and without OJD

Farm	GM	GM/dse	GM/ha
Without OJD	\$304,317	\$21.85	\$178.65
With OJD	\$289,314	\$20.58	\$167.75

% decrease in GM due to OJD = 6.4%


Estimates of annual economic losses based on the sheep necropsied on 12 farms

	Total losses	OJD losses	OJD %	OJD losses \$/DSE	OJD losses \$/ha
Average	\$95,251	\$64,100	70.1	\$7.68	\$65.92



Outcomes

- OJD contributed to considerable mortality rates (6.2% average) & significant economic loss (\$64,000 average)
- large variability between flocks reflects variation in farm size and management
- data can be used to justify vaccination programs, other control options and the general concept of disease control and prevention
- around 30% of annual mortalities were not attributed to OJD (in a drought year)



NOJDP RESEARCH PROGRESS REPORT

ESTIMATION OF OJD-RELATED MORTALITY ON FLINDERS ISLAND

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Background and Objectives

Flinders Island is the largest of over 50 islands that make up the Furneaux Group, located off the north-east tip of Tasmania. Agriculture is one of the main employment industries on Flinders Island and major products include wool and lamb. There are approximately 85 sheep flocks on Flinders Island, mostly self-replacing Merino enterprises.

Ovine Johne's disease (OJD) was first diagnosed in sheep on Flinders Island in 1996. Some owners of infected flocks subsequently traced in the late 1990s reported that wasting had been a problem for a number of years. Although annual crude mortality rates in OJD infected flocks are reported to vary greatly between farms, sometimes as high as 10-15%, a level of 5% is more common. The apparent flock prevalence of OJD on Flinders Island was approximately 30% at 31 December 2003.

An effective and practical option for control of OJD has not been available for infected flocks on Flinders Island until recent registration of a vaccine for the control of the disease. Owners, however, find it difficult to estimate the cost-effectiveness of the vaccine because there is little information on the economic impact of OJD, in particular the annual mortality rate.

The objective of this study was to estimate the mortality rate attributable to OJD in eight infected flocks on Flinders Island over a 12 month period so that the economic impact could be estimated.

Methods and Results

Eight sheep flocks positively diagnosed with OJD by either histopathology or pooled faecal culture were enrolled in the study. Flocks were self-replacing Merino flocks of greater than 1,000 adult sheep. The protocol developed by McGregor *et al.* (2003) for estimating the annual OJD-related mortality rate was used, which involved sampling daily mortalities in each of the flocks on four five-day 'sampling periods' per annum. A necropsy was performed on any dead or moribund sheep over six months of age during the sampling period according to the methods of McGregor *et al.* (2003). Selected tissues were collected and stored for histopathology, tissue culture and nematode counts.

The most likely cause of death was determined and classified as per McGregor *et al.* (2003). Sheep were classified as having died from 'OJD alone' if there were clinical signs of OJD, histopathological evidence of severe OJD, no evidence of other cause of death and a total worm count of less than 40,000. Sheep were considered to have died 'with OJD' if there was histopathological evidence of OJD accompanied with other diseases. If no evidence of OJD was found, the sheep were determined to have died from 'other causes'.

Each flock was to be sampled in Summer, Autumn, Winter and Spring, commencing in December 2001. The study was terminated after the Summer period primarily because some of the flock owners were

unwilling to continue.

The most likely cause of death was determined for all 26 sheep examined at necropsy during the Summer period from the eight flocks (Table 1).

Table 1. Summary of the likely cause of death for sheep examined from eight farms over 2001/02 Summer period.

No. of sheep examined	No. of sheep and likely cause of death		
	OJD alone	With OJD	Other causes
26	19 (73%)	4 (15%)	3 (12%)

Other conditions observed as occurring in sheep 'with OJD' were parasitism, trace element deficiencies (copper, cobalt and selenium) and broncho-pneumonia. The most likely causes of death diagnosed in sheep with no evidence of OJD were parasitism, trace element deficiencies (copper, cobalt and selenium) and non-granulomatous enteritis.

Conclusions to Date

Inadequate data were obtained to accurately estimate the annual mortality rate attributable to OJD for the Flinders Island flocks in this study.

The results at least suggest that OJD may be implicated, either directly or indirectly, in a large proportion of deaths in infected flocks on Flinders Island, a similar finding to studies undertaken in New South Wales (Bush *et al.* 2003, McGregor *et al.* 2003).

No significant difference was demonstrated between the results of the present study and that of a multi-farm New South Wales study (Bush *et al.* 2003) using a Chi-squared test (Table 2). The 'with OJD' and 'other causes or unknown' categories were combined for this analysis because of the small number of deaths in each of these categories.

Table 2. Necropsy results from the present study (FI) and summer period of a New South Wales (NSW) multi-farm study (Bush *et al.* 2003).

Study	No. of sheep examined	Most likely cause of death (%)		
		OJD alone	With OJD	Other causes or unknown
FI	26	73%	15%	12%
NSW ^a	49	73.5%	8%	18.5%

^a Malnutrition-associated deaths removed, Table 2 of Bush *et al.* (2003).

Unless an intensive, regular sampling program can be undertaken, sufficient and conclusive data will not be available to accurately determine the OJD-related mortality rate for flocks on Flinders Island.

Both adequate resources and support by producers are likely to be paramount for any future OJD-related studies on Flinders Island.

References

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ESTIMATION OF OJD-RELATED MORTALITY ON FLINDERS ISLAND

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WATER and ENVIRONMENT



Background



- First Dx OJD in 1996
- Variable annual mortalities
- 30% apparent flock prevalence
- *Objective: to estimate the mortality rate attributable to OJD*

Results

Summary of the likely cause of death for eight Flinders Island farms, 2001/02 summer

No. sheep examined	Most likely cause of death		
	OJD only	With OJD	Other causes
26	19 (73%)	4 (15%)	3 (12%)

Conclusions

- OJD-related mortality rate
- Comparison to NSW (Bush *et al.* 2003)
- Future studies

Location	No. sheep examined	Most likely cause of death		
		OJD only	With OJD	Other causes
FI	26	73%	15%	12%
NSW	49	73.5%	8%	18.5%

Economics – Workshop Discussion

Following these presentations a workshop of participants was held to answer the following questions:

1. *What has been achieved by the researchers?*

- Losses due to OJD have been quantified.
- ~ 70% of sheep mortalities in infected flocks are due to OJD – but there is large between flock variability.
- Balances earlier reports.
- Demonstrated financial impact in high prevalence flocks.
- Confirmed that owner estimates of OJD mortality are often inaccurate.

2. *What key benefit from this research can we communicate to producers?*

- OJD has the potential for serious economic impact which justifies management measures and makes disease worth keeping out.
- Other aspects of flock management are still critical to reduce non-OJD mortalities

3. *What advice would a farm adviser now be able to provide a producer client?*

- Can put figures on losses.

4. *How could a regional authority, eg RLPB, respond to this information?*

- Encourage regional extension programmes to implement control measures.

5. *What still needs to be done/researched?*

- More content based economic analysis - development of “ready-reckoners” of losses linked to current prices.
- Economic analysis of subclinical losses.
- Investigation to determine if losses increase over time in low prevalence flocks.

6. *Are there any other implications or warnings from this work?*

- Must not ignore the non-monetary impacts of this disease – eg. social, self esteem, pride, duty of care.
- Consequences of not promulgating the findings

7. *Have the outcomes of this work been captured in a useful form (scientific publication, advisory note etc) and what actions are required to ensure this occurs?*

- Australian publication pending
- Tips & tools in preparation

Suggestions:

- Roadshow on OJD to create awareness.
- Producer meetings
- Groups to drive where possible.
- One-on-one group discussion on economics
- Bulletin targeting CVOs, department advisors & other technical people

DIAGNOSIS

GAMMA INTERFERON TO DIAGNOSE OJD

PROJECT NUMBER: OJD.025

David Stewart

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Background and Objectives

The sensitivity and specificity of the interferon- γ (IFN γ) test is being evaluated, in a project funded by Meat and Livestock Australia. Previous reports from Australia and overseas have shown that the IFN- γ assay showed is a promising test for early diagnosis of Johne's disease. Further trials are in progress to validate the accuracy of the test before it can be considered for approval as an official Australian standard diagnostic test for ovine Johne's disease.

Methods

Sensitivity trials

I. The flock consisted of 145 adult pregnant Merino ewes aged approximately 4 years which also were part of another project (OJD.024) run by Richard Whittington, NSW Agriculture Elizabeth Macarthur Agricultural Institute (EMAI). Blood samples were collected from individual sheep prior to slaughter. Sample collection and slaughter were performed by NSW Agriculture. The antigen stimulation of bloods was performed at EMAI and the IFN- γ testing of plasma samples at CLI Geelong. Culture of tissues and histopathology was performed by EMAI.

II. The blood samples were provided by Professor Richard Whittington, University of Sydney and were obtained prior to slaughter and autopsy of 232 Merino sheep from a terminated experiment (project OJD.002). The blood samples were air freighted to Geelong prior to antigen stimulation. Culture of tissues and histopathology were performed by the University of Sydney.

Specificity trial

The flocks were located in regions of low prevalence of OJD. Five of six flocks were enrolled in SheepMAP at the level of MN1 or 2. There was no history of OJD in neighbouring flocks. In each flock, 120 sheep were sampled consisting of 40 lambs, 40 yearlings and 40 adult 3-year-old ewes. Three of the flocks were located in NSW and one each in WA, Vic and SA. Blood samples were collected from individual sheep on each of the properties in 3 NSW Rural Lands Protection Boards (Riverina, Dubbo and Armidale) and one property in WA, SA and Vic. For the Riverina, Dubbo and Armidale flocks, the blood stimulation and culture phase was performed at the CLI Geelong, RVL Orange and the CLI, Armidale laboratory, respectively and the IFN- γ testing at CLI Geelong. For the WA flock, the IFN- γ test was performed by the WA Department of Agriculture. Pooled faecal cultures were performed on 6 pools of 20 sheep by NSW Agriculture EMAI (Riverina, Dubbo and Armidale flocks), RVL Orange, WA Department of Agriculture, IDEXX Laboratories, Adelaide and the Department of Primary Industries Victoria, Attwood.

Longitudinal trial

The IFN- γ test is being evaluated in a 3-year controlled longitudinal experiment on naturally infected sheep in collaboration with the University of Sydney. The trial is part of another study (OJD.028) run by the University of Sydney examining the effects of age of sheep and exposure to 4 different levels of pasture contamination. The infection status of the sheep is being monitored during the trial by faecal

culture, serology and the IFN- γ test. At the completion of the trial, the sheep will be slaughtered and autopsies performed by the University of Sydney to obtain tissue samples for culture and histopathology.

IFN- γ test

The Johnin PPD, provided by CSL Limited, was from 2 different sources (A and B). Stimulation of blood samples and the IFN- γ ELISA (BOVIGAM™, CSL Limited) was performed according to the manufacturers instructions. The interpretation criteria for a positive result included: the mean OD values for avian PPD stimulation minus the nil antigen mean optical densities (A-N), Johnin PPD minus nil antigen OD (J-N) and the OD values for J-N minus A-N (J-A) with a difference of either 0.05 or 0.1 being required (J-N \geq 0.05; J-N \geq 0.10; A-N \geq 0.05; A-N \geq 0.10; J-A \geq 0.05; J-A \geq 0.10).

Results

Sensitivity trial I (see also MLA R&D Update July 2002)

IFN- γ test cut-point OD \geq 0.05. Estimated sensitivity for A-N, J-N and J-A was 54%, 67%, and 52%, respectively when the comparative reference standard was combined culture and histopathology. For J-N, Sensitivity was 65% for all culture positives and 81% for all histopathology positives. For both A-N and J-A, the comparative sensitivity estimates for culture and histopathology were 54% and 64%, respectively,

For J-N, there were 33% apparent false positives among the IFN- γ test positive / reference standard test negative sheep. By comparison, for the A-N and J-A cut-points, there were 18% and 13% apparent false positives.

IFN- γ test cut-point OD \geq 0.1. Estimated sensitivity for A-N, J-N and J-A was 54%, 46% and 32%, respectively with combined culture and histopathology as the standard, culture only 37%, 50% and 35%, respectively and histopathology only 64%, 58% and 42%, respectively.

For J-N, there were 13% apparent false positives among the IFN- γ test positive / reference standard test negative sheep. By comparison, for the A-N and J-A cut-points, there were 18% and 4% apparent false positives.

The proportion of culture and histopathology positive sheep in this trial (145 sheep) was 31.7% and 24.8%, respectively. The period between completion of collection of blood and commencement of antigen stimulation was ~2 hours (road transport from Goulbourn to Camden). Johnin A was used for stimulating the blood cultures.

Sensitivity trial II

The sensitivity of the IFN- γ test in this trial was very low. For both A-N and J-N \geq 0.05, sensitivity was 8% when the reference standard was culture and histopathology combined. For culture alone, A-N and J-N sensitivity were both 8.3% and for histopathology alone, A-N and J-N and sensitivity were both 25%. The proportion of culture positive sheep in this trial (232 sheep) was 20.7% compared to histopathology (3.4%). The period between completion of collection of blood and commencement of antigen stimulation was ~ 6.5 h (road and air transport from Marulan to Geelong). As Johnin A was unavailable, Johnin B was used for this trial.

Specificity trial

IFN- γ test cut-point OD \geq 0.05. With Johnin A and using the cut-points J-N or J-A for the Riverina, flock the specificity of the IFN- γ test was 99.2% and 100%. Specificity was slightly lower for this flock with Johnin B (J-N 98.3%, J-A 98.3%). For the WA flock, with Johnin A, the corresponding specificities were 93.3% and 100%, respectively but with Johnin B, specificity was higher for the J-N cut-point (97.5%). Only Johnin B was available for the Dubbo, SA, Vic and Armidale trials. Specificity for J-N and J-A was

98.3% and 99.2% (Dubbo), 100% and 100% (SA), 99.2% and 100% (Vic) and 85% and 100% (Armidale).

Three of 6 flocks (WA, Vic and Armidale) had differing A-N responses compared to those for Johnin A and B. In the WA flock, A-N Specificity (95%) was higher than Johnin A (93.3%) but lower than for Johnin B (97.5%). In the Vic flock, specificity of Johnin B-N (99.2%) was higher than A-N (93.4%) and in the Armidale flock specificity for A-N (84.2%) and Johnin B-N (85%) was similar.

The specificity of the IFN- γ test over the 6 flocks for A-N, Johnin B-N and J-A was 95.3%, 96.1% and 99.3%, respectively.

IFN- γ test cut-point OD ≥ 0.1 . Five of the 6 flocks had a specificity of 97.5-100% irrespective of the PPD antigen stimulant or interpretation of the cut-point (A-N, Johnin B-N, J-A). The Armidale flock had a slightly lower and similar specificity for both A-N and Johnin B-N (96.7%).

The specificity of the IFN- γ test over the 6 flocks for A-N, Johnin B-N and J-A was 99.2%, 99.2% and 99.9%, respectively.

Comparison of age groups cut-point OD ≥ 0.05 . On the Armidale property, there was a relatively high number of reactors in the group of 3 year old ewes (11/40 and 9/40) and 18 month old ewes (8/40 and 9/40) for the A-N and Johnin B-N, respectively but no reactors in the lambs. For the remaining 5 flocks, there were fewer reactors for the mature ewes (0-4 and 0-2) and yearlings (0-3 and 1-2) for these 2 cut-points and only one reactor (Vic) for A-N in the lambs. The age specificity for mature ewes was 92.1% (A-N), 94.2% (J-N) and 99.6% (J-A). For yearlings, specificity was 94.2%, 95% and 99.2% and lambs 99.6%, 100% and 100%, respectively.


Comparison of age groups cut-point OD ≥ 0.1 . There was 1-3 reactors for both A-N and J-N cut-point in the mature ewes and yearlings and no reactors in the lambs. The age specificity for mature ewes was 98.3% (A-N), 98.8% (J-N) and 100% (J-A). For yearlings, specificity was 99.2%, 98.8% and 99.6% and lambs 100%, 100% and 100%, respectively.

Longitudinal trial

This trial is still in progress and will terminate in the latter part of this year. The preliminary results indicate that a cell-mediated immune response has been induced in 3 of the 4 groups of sheep which was evident after 14 months of exposure to infection and that the proportion of IFN- γ responders in a particular group may be indicative of the level of exposure to infection.



Conclusions to Date

- The specificity of the IFN- γ test has been estimated in 6 uninfected flocks in different regions in 4 states. The false positive rate was higher in some flocks and varied according to the age class of sheep indicating greater probable exposure to cross-reacting environmental mycobacteria.
- Different interpretation criteria can be used to alter the specificity and sensitivity of the IFN- γ test for which there is a reciprocal relationship. If an OD difference of 0.1 is exploited for A-N and J-N specificity for the Armidale flock increased for both from ~ 85% to ~ 97% and the overall flock specificity increasing from ~ 96% to 99%. Similar estimates for sensitivity and similar apparent false positive rates were obtained in sensitivity trial I when the J-N ≥ 0.1 (46% and 13%), A-N ≥ 0.1 (54% and 18%) parameters were compared with J-A ≥ 0.05 (52% and 13%). For J-A ≥ 0.1 , although the false rate was only 4%, the sensitivity estimate was also reduced to 32%. J-N ≥ 0.05 had the highest sensitivity (67%) in comparison to A-N ≥ 0.05 (54%) and J-A ≥ 0.05 (52%) but the J-N apparent false positive rate (33%) was greater than A-N (18%) or J-A (13%).
- Factors responsible for the low sensitivity in trial II require further investigation. Decline in viability of blood cells over the longer time required for transport to the laboratory and that the potency of Johnin B may be lower than A may have contributed to at least part of this difference.



Gamma Interferon to Diagnose OJD

OJD.025

David Stewart



The Gamma Interferon Test for Diagnosis of Ovine Johne's Disease

- Specificity trials on 6 uninfected sheep properties in the SE of Australia and WA.
- Sensitivity trial I in an infected commercial sheep flock in NSW (NSW Agriculture)
- Sensitivity trial II in an infected experimental flock in NSW (University of Sydney)
- A longitudinal (3 year) trial on an infected experimental flock (University of Sydney)





Liveness Industries

Estimated % specificity of the IFN test in 6 Merino flocks (0.05 cut-point)							
Criteria	Riverina	WA	Dubbo	SA	Vic	Armidale	Overall
A-N	100	95	99.2	100	93.4	84.2	95.3
¹ J-N	99.2	93.3	NT	NT	NT	NT	
¹ J-A	100	100	NT	NT	NT	NT	
² J-N	98.3	97.5	98.3	100	99.2	85.0	96.1
² J-A	98.3	100	99.2	100	100	100	99.3

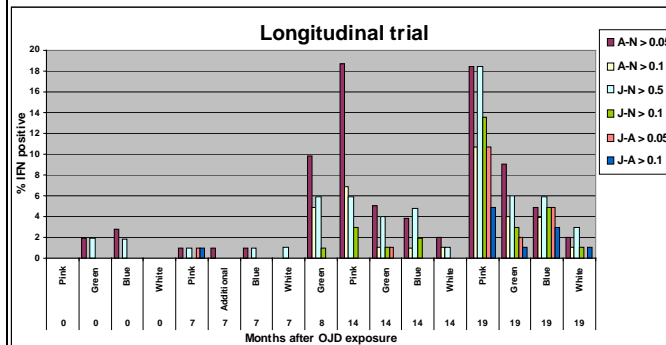
Estimated % specificity of the IFN test in 6 Merino flocks (0.1 cut-point)							
Criteria	Riverina	WA	Dubbo	SA	Vic	Armidale	Overall
A-N	100	97.5	100	100	100	96.7	99.2
² J-N	99.2	98.3	99.2	100	100	96.7	99.2
² J-A	99.2	100	99.2	100	100	100.0	99.9

Age % specificity (0.05 cut-point)				Age % specificity (0.1 cut-point)			
	Ewes	Yearlings	Lambs		Ewes	Yearlings	Lambs
A-N	92.1	94.2	99.6	A-N	98.3	99.2	100
² J-N	94.2	95.0	100	² J-N	98.8	98.8	100
² J-A	99.6	99.2	100	² J-A	100	99.6	100

Estimated % sensitivity of the IFN test in a Merino flock in comparison to the reference standard of 54 culture positive / histopathology positive sheep (Sensitivity trial I)

Criteria 0.05 cut-point			Criteria 0.1 cut-point		
	Number IFN +ve	% sensitivity		Number IFN +ve	% sensitivity
A-N	29	54	A-N	29	54
¹ J-N	36	67	¹ J-N	25	46
¹ J-A	28	52	¹ J-A	17	32

¹CSL A Johnin PPD



Livestock Industries



Conclusion

- Similar specificity for Avian and Johnin PPDs but more false +ves with OD 0.05 than 0.1 cut-point
- Sensitivity varied from > 50% (trial I) to 8% (trial II)
- Potential factors contributing to test performance:
 - Variation in potency of Johnin PPD (A vs B)
 - Proximity of the property to the laboratory



Livestock Industries

ABATTOIR SURVEILLANCE

DETERMINATION OF INDIVIDUAL ANIMAL LEVEL SENSITIVITY OF ABATTOIR SURVEILLANCE FOR OJD

PROJECT NUMBER: OJD.029

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Background and Objectives

The purpose of this project was to determine the sensitivity of OJD inspectors in detecting ovine Johne's disease when examining viscera in real - life conditions in a meatworks.

Abattoir surveillance of sheep intestines for OJD has occurred in 21 meatworks in all states except the NT. It is now a requirement under the National Standard Definitions and Rules for sheep for maintenance of Control or Protected Zone status that 20% of flocks are subjected to abattoir surveillance. Since late 1999, over 18 million sheep have undergone this method of examination. Less than 4% of the lines examined were detected as positive. Obviously there are many sheep that are examined by abattoir surveillance that are found to have no detectable lesions of OJD. If the sensitivity of this technique were known at an individual animal level then it would be possible to use the negative data that has been generated. This would be a very efficient and economic means of providing data for a flock assurance scheme.

Previous work examining the sensitivity of abattoir surveillance includes MLA Project OJD-007 conducted by NSW Agriculture which examined line - level sensitivity of inspection and part of a larger project performed at VIAS – Attwood. The NSW work looked at 35 lines of sheep from infected farms. Inspectors detected gross lesions suggestive of OJD in 97% of the lines. The VIAS – Attwood work compared gross examination of viscera by untrained staff (not under abattoir conditions) with histopathology. The sensitivity from this work was 30.6% (95% CI 22.1 – 40.3). As some of the staff assessing viscera in this trial had never seen a set of sheep viscera, this result should be interpreted with caution.

Methods and Results

Potentially eligible sheep farms were sought from districts in NSW with known high prevalence of OJD in their flocks. A bounty of \$1000 was provided as an incentive for farmers to become involved in the project. Farmers had to subject their sheep to serology, agar gel immunodiffusion test (AGID) paid for by the project, and agree to consign their sheep to Southern Meats in Goulburn over a specified 2-day period if they were deemed suitable for the trial.

Initial eligibility criteria for farms to enter the trial included: a presumed high prevalence of OJD from previous abattoir surveillance data or on-farm flock profiling, a minimum line size greater than 150 and sheep aged 2 years or older. For statistical reasons (to maintain narrow confidence intervals around the point estimate of sensitivity) it was important to gain an overall true prevalence of OJD for sheep in the

trial of over 10%. It would have been possible to set a lower prevalence but, as histopathology was the main cost of the trial and was performed on all viscera, the cost of such a trial would have been prohibitive. Sheep to be consigned to the works were blood tested by private practitioners and subjected to an AGID for *M. paratuberculosis*. The minimum seroprevalence for the trial was 3% in the line of sheep which, allowing for a sensitivity of around 30% for this test, would indicate a prevalence of about 10%.

There were 3 inspectors: X, Y and Z who varied in their experience with working in abattoirs inspecting sheep viscera for OJD. Inspector X had previously worked for 14 years as a meat inspector, had been working full - time as an OJD inspector for NSW Agriculture for 2.5 years, and had undergone the formal NSW training program for inspection. Inspector Y was located in Western Australia, had undergone formal training for OJD inspection and worked for 3 years as an inspector but only 20 – 30 full days per year. Inspector Z was based in Victoria, had been a meat inspector for 15 years, had not undergone formal training and had been working for 6 months as an inspector on a full - time basis.

Ideally, to test the hypothesis that different exposure to OJD infected sheep affects the ability of inspectors to detect the disease (ie the sensitivity of the test), replicate inspectors from each different prevalence area would be enlisted. Logistically this was not possible due to space constraints on the line at the works. The inspectors were aware that they were involved in the trial but did not know ahead of time what proportion of the sheep were positive nor which individual sheep were. The inspectors were instructed to simply “call the lesions as they saw them”, as they would in their own work place.

All sheep intestines (runners) were tagged sequentially with numbers from 0001 to 1200. The task of the inspector was to read the tag number and give a diagnosis for each set of viscera as it moved along the line. Their results were recorded on small cassette recorders using an amplifying microphone attached to their lapel. The inspectors were separated from each other by between one and four workers on the line, and so were unable to see or hear the other inspectors making their assessment. Their observations were therefore independent. The line speed in the works was 10 runners per minute and in nearly all cases the inspectors were able to examine every set of guts as they presented on the line. At the end of the consigned “trial” sheep, the inspectors (without foreknowledge) examined 200 sheep that were not part of the trial but were from a farm where OJD was not known to occur – the “dummy run”. This farm was located in the Protected Zone for OJD, but was not a MAP flock and had no extra testing for OJD conducted on this property.

Following inspection on the line, all runners fell down into the “runners room” where they were individually bagged (with tag attached) and then processed by Department of Primary Industry animal health staff into 10% formalin.

The samples were dispatched to Gribbles Veterinary Pathology Group in Melbourne and processed generally as 6 tissue pieces on 3 slides with both haematoxylin and eosin (H&E) stains and Ziehl - Neelsen (ZN). As per the Australian and New Zealand Standard for Diagnostic Tests (ANZSDT's) if there was no suggestion of OJD pathology on the haematoxylin and eosin (H&E) slide and the tissue appeared well preserved, then there was no need to examine the ZN slide. However if autolysis was present or there were any signs of pathology consistent with OJD then the instruction was to examine the ZN processed slides for 10 minutes per tissue to establish the whether acid fast organisms were present. A positive diagnosis was made where one or more ZN – staining organisms were viewed. A negative diagnosis was made when there was no pathology consistent with OJD on H&E and an inconclusive diagnosis was made where there was pathology consistent with OJD on H&E but no organisms could be viewed on ZN slides.

Pathologists at VIAS Attwood re-read approximately 10% of cases read by Gribbles. This was used as a quality control exercise and, it was hoped, to resolve some of the inconclusive pathology results.

The sensitivity for each inspector was calculated with 95% binomial exact confidence intervals. Inconclusive results and sheep for which all 3 inspectors did not make a diagnosis were excluded from analysis.

In total, there were 1196 sheep viscera that passed along the chain at the abattoir during the 2 days of the trial. Thirty-nine viscera were excluded because either identification tags were lost during processing

or a diagnosis was not made by one or more inspector. Twenty-six sets of viscera were excluded because the final histopathology result was inconclusive.

Histopathological examination showed that the line prevalence varied from 6.6% to 25.0%: Line 1a – 8.3%, Line 1b – 25.0%, Line 2 – 15.3%, Line 3 – 6.6%, and Line 4 – 8.9%.

The sensitivity of inspection (including 95% binomial confidence intervals) was:

Inspector X: **87.3%** (82.5 – 91.2)

Inspector Y: **74.1%** (66.5 – 80.7)

Inspector Z: **52.5%** (44.1 – 60.2)

The specificity overall was:

Inspector X: **98.3%** (97.2 – 98.9)

Inspector Y: **97.0%** (95.8 – 98.0)

Inspector Z: **99.9%** (99.4 – 100.0)

There were a total of 158 histopathology positive (H+) sheep. Seventy – seven of these (49%) were detected by all three inspectors while 16 (10%) were not detected by any of the three inspectors. Of the remainder, 23 (15%) were detected by just one inspector while 42 (27%) were detected by two of the three inspectors. Pairwise, the inspectors agreed on the diagnosis for about 94% of sheep, ranging from 90–97% across the five lines of sheep.

The number of positive inspector diagnoses was examined for sheep where the pathologist registered a maximum count against the number of acid fast organisms in one or more tissues examined (these "severe" cases also registered a maximal count for "epithelioid macrophages"). There were 55 "severe" cases that fitted this criterion. The remaining 103 cases were classified as "mild".

Only 4 of the severe lesions were given a negative diagnosis by all three inspectors. Inspector X diagnosed all the remaining 51 severe lesions as positive, with 40 of these given as positive by all three inspectors. There were 6 severe lesions diagnosed as positive by X and Y, and 4 by X and Z.

Negative control line

The trial included 200 animals from a flock with no known history of OJD (Table 5). Only one animal (and by only one inspector) was classified as positive from this negative control line.

Conclusions to Date

Along with other diagnostic tests and testing regimes for OJD such as pooled faecal culture, serology (AGID or ELISA) and individual faecal culture, negative abattoir surveillance data can be built into an overall confidence-of-freedom program.

Testing two animals with a 50% sensitivity test has approximately the same chance of detecting disease as testing one animal with a 100% sensitive test. If a fraction of a point (equal to the sensitivity of the test) is assigned for each animal inspected/tested, the total number of points gained approximates the equivalent number of 100% sensitive tests.

Such a points-based system could be used to combine the confidence from several different types of tests. For example, to achieve a target expressed as a number of "100%-sensitive" tests that should be done in a year, the results from lines of sheep subjected to abattoir surveillance could be "topped up" with a part-round of pooled faecal culture tests or sufficient serological check tests. Alternatively, the confidence obtained by a specified regime of MAP testing could be increased by including the points resulting from the amount of abattoir monitoring. The measure of assurance of freedom from disease



would be obtained by totalling the number of points allocated for each "round" of testing, provided that the number of sheep tested and the test sensitivity were known.

The MAP protocols are based on detecting disease when the within-flock prevalence is 2%. The sensitivities of the tests used have been lowered to reflect the lower likelihood of a detectable response in a lower prevalence flock.

If the relative sensitivity of abattoir inspection relative to histopathology is taken to be 50% for a low prevalence flock, the overall sensitivity is $37.5\% = 75\% \text{ (estimated sensitivity of histopathology)} \times 50\%$. Hence, 525 animals need to be examined to give the same level of confidence as 7 PFC pools.

The use of OJD abattoir surveillance for the purposes of negative flock assurance depends on use of a sheep identification system and properly trained and accredited inspectors. Logistically there may be some administrative details to attend to before the system could be used. However once these issues were addressed, producers could benefit from the assurance information gained from abattoir surveillance. How negative data fits in with other programs such as MAP would depend on national agreement.

It is apparent that abattoir surveillance is potentially a highly sensitive methodology. In low prevalence lines it is also highly specific (although specificity is less important as positive cases are resolved with histopathology).







Abattoir Surveillance

OJD.029 Determination of Individual Animal Level Sensitivity of Abattoir Surveillance for OJD

Tracey Bradley



DEPARTMENT OF PRIMARY INDUSTRIES



Determining the Sensitivity of Abattoir Surveillance for OJD




Brief methodology:

- Approximately 1200 viscera from infected farms with seroprevalence (AGID) of about 3%
- Numbered viscera examined by 3 OJD inspectors under normal conditions in works (X from NSW, Y from WA and Z from Vic)
- Diagnoses recorded into tape recorder - entirely independent
- Histopathology follow - up (gold standard) of all viscera
- "Dummy Run" of 200 viscera from uninfected property



Results

- Overall sensitivity estimates: (95% confidence intervals)
Inspector X - 87.3% (82.5 - 91.2)
Inspector Y - 74.1% (66.5 - 80.7)
Inspector Z - 52.5 (44.1 - 60.2)
- Overall Specificity estimates:
Inspector X - 98.3% (97.2 - 98.9)
Inspector Y = 97.0% (95.8 - 98.0)
Inspector Z = 99.9% (99.4 - 100.0)
- "Dummy" run - of 200 sheep - only one inspector detected one positive



Conclusions

- Sensitivity of inspection varies considerably with inspectors (Z had not undergone formal training with lower Se and higher Sp)
- Most sensitive inspector (X) least affected by variation in line prevalence
- Inspector X also least affected by lesion severity
- Sensitivity of technique lower at lower prevalence (limitation of all work looking at the sensitivity of diagnostic tests)
- Training and accreditation of inspectors important
- Average sensitivity of about 70% for this trial
- Ab surveillance data could be used with other tests such as serology and PFC for overall confidence of freedom program
- Comparison with MAP: 525 sheep by ab surveillance (using 50% Se X 75% for histo) equivalent to 7 PFC pools or 875 AGID

INFECTED FLOCK PROFILE-A CASE STUDY

LONGITUDINAL STUDY OF THE SPREAD OF OVINE JOHNE'S DISEASE IN A SHEEP FLOCK IN SOUTHEASTERN NEW SOUTH WALES

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Background and Objectives

The study farm located in southeastern NSW comprises 1739 hectares of improved pasture. The main enterprise is sheep breeding, but around 700 beef cattle are also grazed and 250-300 hectares are cropped annually. The sheep flock consists of a commercial Bond¹ breed flock of approximately 3000 breeding ewes and a Bond stud of about 300 ewes. The flock was established when the current owner bought the property in the late 1960s. Sheep are segregated based on age and run in separate mobs, up to six years old, after which they are culled for slaughter. The commercial and stud ewes are run separately and mobs are grazed in a rotational pattern, fitting in with pasture availability and the cropping program. The entire flock shares one shearing shed and a main set of yards. Lambing is in April/May; wether lambs are sold to slaughter as prime lambs from December onwards.

Other than a once off purchase of some Merino sheep in 1993 and a once off purchase of five Dorset rams in 1997 no sheep are routinely introduced. In 1993 the owner decided to commence breeding his own Merino wethers to graze on a separate property. He purchased 410 mixed age fine wool Merino ewes from farm X, located in what is now recognized as the high prevalence OJD area in NSW. Ten fine wool rams were also purchased from another property. These Merino ewes and rams were run on the main property, separate from the Bond commercial and stud flocks, however the yards and shearing shed were shared. In January 1996 the owner sold 287 of the Merino ewes to another farm (farm F) and the remainder to slaughter. The progeny from these Merino ewes and the merino rams that remained on the study farm were sold to slaughter in December 1996.

In 1996 OJD was confirmed on farm X, where the above described merino sheep were bred. In accordance with OJD policy in NSW at that time, trace forward investigations commenced, both on farm F and the study farm, because they were both identified as having purchased and run sheep from a known infected flock. Initially testing was conducted on farm F, since it still had some of the merino sheep bred on farm X that were alive and OJD was confirmed in these merino sheep. Testing then commenced on the study farm in 1997 based on the knowledge that known infected merino sheep had grazed on that farm. Testing was required on three occasions over a four-year period to confirm infection and in March 2000 OJD was confirmed on the study farm in homebred 5-year old Bond ewes. Test results to that stage indicated that infection was clustered and confined to one age group only; the age group that was <12 months old when exposed to assumed *Mycobacterium paratuberculosis* (Mptb) contamination shed by the introduced merino sheep.

There is very little objective information regarding the spread of Mptb within an infected flock. It is assumed that most sheep are exposed via pasture, and that all animals in a flock have an equal chance

¹ Australian sheep breed which evolved in early 1900 as a dual purpose breed using merino ewes and Lincoln rams

of becoming exposed. Surveillance programs to detect infection entail sampling based on this assumption and ignore the possibility that infection may be confined to subgroups or mobs within a flock.² In addition, recommendations for control of OJD are based on whole flock destocking or quarantine and other trading restrictions to reduce further transmission. These make no allowance for the possibility that infection is clustered within a flock. The availability of pooled faecal culture (PFC)⁴ has enabled relatively inexpensive testing of whole flocks, to better determine the source and distribution of infection.

The aim of this study was to apply whole flock testing over time to determine the prevalence, distribution and spread of infection in a recently infected flock, with a view to plan intervention strategies for disease control. As a result of this study the procedure is now being applied in Australia using PFC and is referred to as an infected flock profile (IFP).

Methods and Results

On farm investigation and monitoring for ovine Johne's disease was performed between 1997 and 2002 in the sheep flock on the study farm. Tests used were serology (AGID), pooled faecal culture (PFC) and histopathology. From 1997 to 2000 partial flock testing was done and from June 2000 to October 2002 annual whole flock testing, using PFC was performed to determine the distribution and prevalence of infection (infected flock profile). The prevalence of OJD based on sero-reactors was estimated as the proportion of gel test reactors assuming 100% test specificity with 95% exact binomial confidence limits calculated using a computer (Statmate, Graphpad Software, San Diego, California, USA). The prevalence of sheep shedding Mptb was estimated from the number of PFC positive pools, with 95% confidence limits.⁸ Results are presented in table 1 and figure 1.

Faecal shedding of Mptb commenced in homebred sheep around six to seven years after introduction of putative infected merino sheep in 1993. These merino sheep are the assumed source of infection on the study farm and were present from 1993 until 1996, however environmental contamination contributed to these sheep was likely to be present from 1995 to 1997 on the study farm.

For at least seven years there was clustering of infection and shedding within one or two age groups only. Sheep in these age groups appeared to have been exposed to mycobacterial contamination at an early age (<12 months) and commenced shedding at five years of age or older. Groups that were exposed to contamination, as adults did not shed detectable levels of Mptb during the study period.

Conclusions to Date

Observations and test results support the following scenario: 1) Infection was introduced onto the study farm with Merino sheep purchased in 1993 and this source of contamination was removed in 1996; 2) Shedding in infected merino sheep occurred prior to their detection, and infective concentrations of Mptb originating from the Merino sheep were present in the environment on the study farm from 1995 to 1997. This is based on a 12-month survival period on pasture after removal of the infected sheep³ 3) Only sheep less than 12 months old when exposed to Mptb developed patent infections, that is progressed to shed Mptb, during the study period; 4) Shedding commenced when sheep were five years or older, a significant longer time period than the commonly reported 12 months incubation period.

Clustering of patent infection in age groups of sheep that were exposed as lambs was a feature of the epidemiology of Mptb transmission on this farm, providing indirect evidence of finite duration of survival of Mptb on pasture and the influence of age on the susceptibility of sheep to develop patent Mptb infection. Transmission of Mptb on this farm occurred very slowly and this was related to the long incubation period (exposure to shedding interval) of five years. Better understanding of disease spread within flocks over time through flock profiling using PFC will also help in devising surveillance strategies (including testing protocols for market assurance testing) that account for clustering of infection as well as very slow transmission of infection through a flock.

Opportunities for disease control and risks to effective surveillance are apparent retrospectively. On this farm, it may have been possible to stop transmission of OJD through timely implementation of disease management such as partial flock culling, selective grazing management and vaccination. If these disease control strategies had been implemented earlier than was possible on this farm, disease

transmission may have been stopped.

In retrospect, action that could have been taken in 1996/97 that may have led to elimination of the infection from the study farm would include:

- Implementation of an IFP immediately upon identification of the infected introduced sheep
- Culling of these sheep and their progeny
- Culling of home bred sheep born during the preceding and following 12 months, corresponding to the period of contamination, and prior to reaching 12 months of age
- Implementation of a vaccination program of all remaining sheep and annually of lambs at marking time
- An IFP about 5 years after commencement of the program to monitor flock infection status

Selective culling of age groups, grazing management and vaccination was used on the study farm and the owner plans to vaccinate ewe lambs annually at marking or weaning. Based on recent research results with Gudair® vaccine under Australian conditions, vaccination on this farm is likely reduce the levels of Mptb contamination as well as delaying the onset of shedding.¹³ It may be that in very low challenge situations such as this, vaccination eventually prevents shedding of detectable levels of Mptb. Further testing would be needed to confirm this.

This study demonstrates how difficult effective OJD surveillance may be unless exact history is known and the correct age groups or mobs are tested or unless testing occurs over a long period of time. Clustering of infection reduces the confidence in a negative test outcome if sampling is random. This has significant implications for market assurance program testing² and surveillance testing with a view to assess the OJD risk of a flock or mob of sheep intended for sale.

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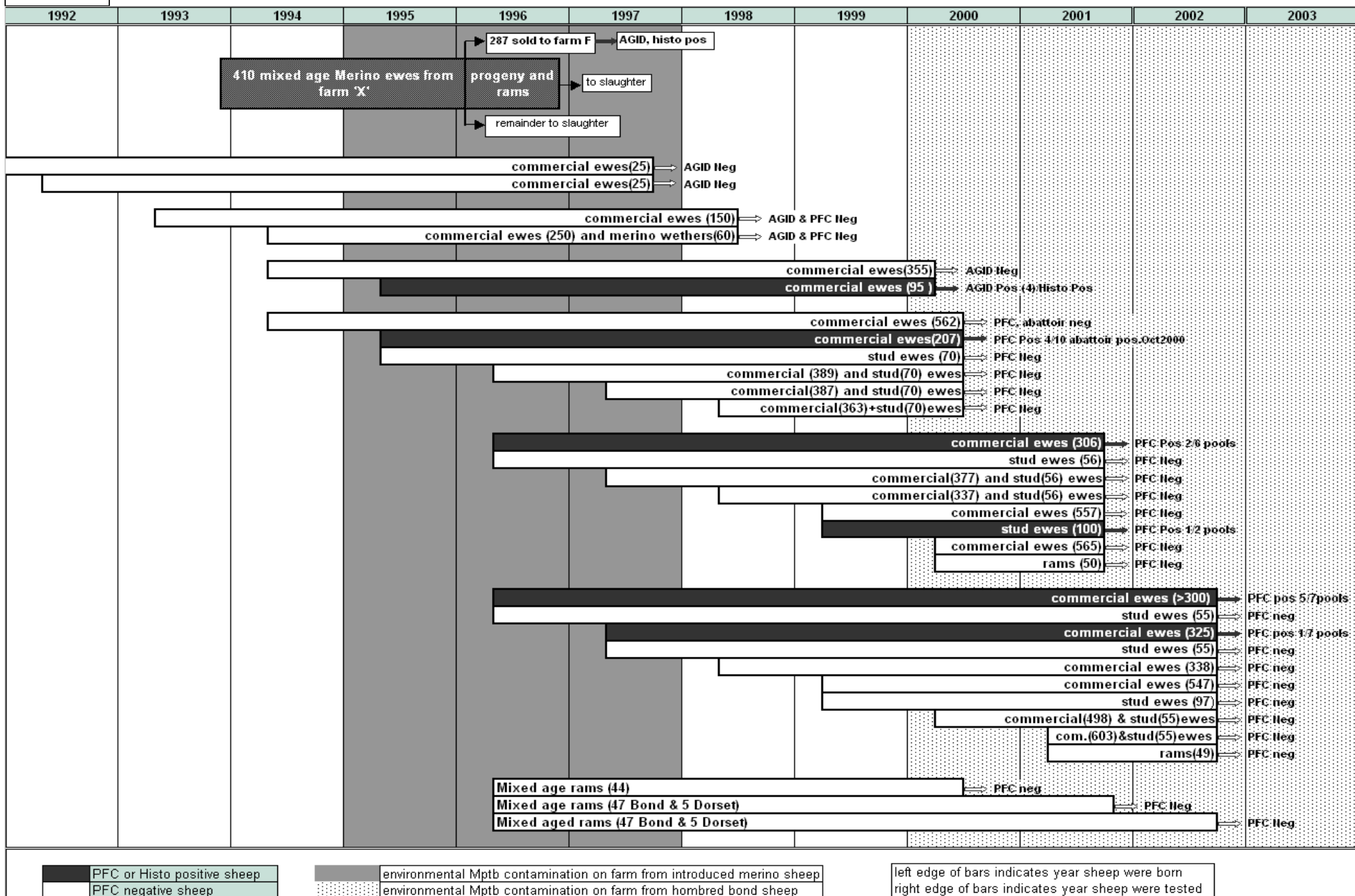
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Table 1. The prevalence of OJD infection based on AGID and PFC test results, for groups of samples where either test was positive.

Test date	Year birth	of	Sample size	Breed	No. sheep/pool	Test	Results	Prevalence of sero-positive or faecal culture positive sheep % (95% C.L.).
22/10/1997	1991/90		239	Merino	na ¹	AGID	9 positive	3.8 (1.7 – 7.0)
10/3/2000	1995		95	Bond	na	AGID	4 positive	4.2 (1.7 - 11.9)
16/6/2000	1995		202 (10 pools)	Bond	20-22	PFC	4 of 10 pools positive	2.5 (0.1 - 5.0)
29/9/2001	1999		100 (2 pools)	Bond	50	PFC	1 of 2 pools positive	1.4 (0 - 4.1)
3/10/2001	1996		306 (6 pools)	Bond	50-56	PFC	2 of 6 pools positive	0.8 (0 - 1.9)
8/10/2002	1996		350 (7 pools)	Bond	50	PFC	5 of 7 pools positive	2.5 (0.2 – 4.8)
8/10/2002	1997		325 (7 pools)	Bond	26(1 pool), 49- 50	PFC	1 of 7 pools positive	0.3 (0 - 0.9)

¹ not applicable

Figure 1







Infected Flock Profiling

Longitudinal Study of OJD spread in a sheep flock (Infected flock profile)

1997-2002

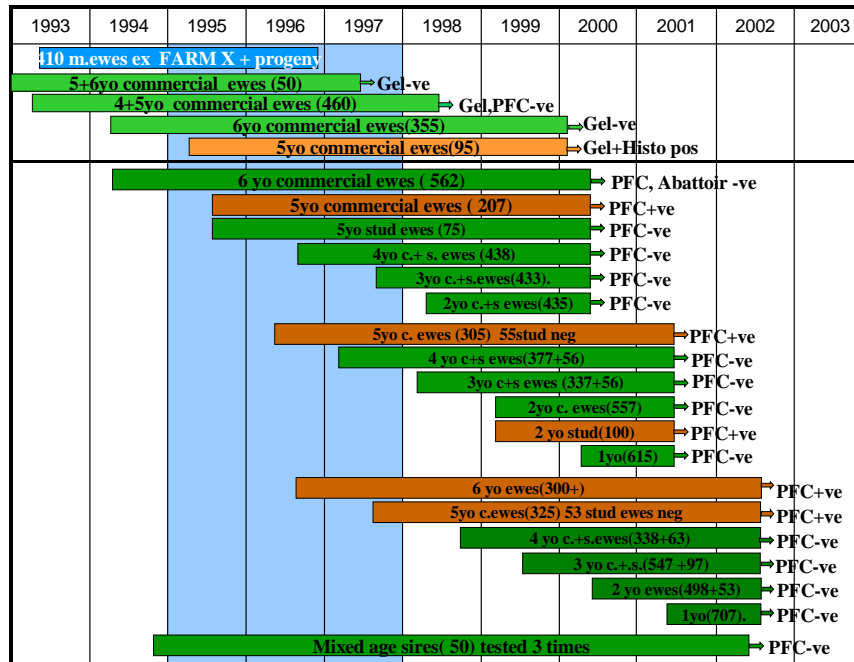



Luzia Rast



Study Farm - Background


- Mixed farming enterprise
 - Self replacing Bond commercial and stud flock (3000 ewes)
- Investigation commenced in 1997 due to trace forward
- OJD infection confirmed in apparent isolated age group of homebred sheep in 2000.
- Annual whole flock testing by PFC('00,'01,'02) to establish OJD prevalence and transmission
- Based on results-disease management strategies implemented (partial flock culling, selective grazing, vaccination)





CONCLUSIONS

- Clustering of infection over long period (implications for surveillance and MAP testing)
- Slow transmission of infection through flock
- Age (breed?) resistance
- Late onset of shedding(5 yo)
- Disease control implemented early may interrupt disease transmission
- Need for IFP



NOJDP RESEARCH – “BIOPSY TRIAL”

INDIVIDUAL ANIMAL TESTS FOR OVINE JOHNE'S DISEASE

PROJECT NUMBER: OJD.020

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Background and Objectives

Most of the tests used in the OJD program are flock tests. Due to the insidious nature of the disease, in particular the very long incubation period and the delay in faecal shedding and seroconversion, antemortem individual animal testing has usually been considered to be impractical and unreliable. Individual lethal tests such as histopathology and tissue culture, have mostly been used as follow-up tests as part of flock evaluation. There is a need for an accurate test or testing strategy for individual animals, particularly where it is desired to show with confidence that a given individual is not infected. This is important for studs detected with OJD and in trace-forward investigations. It may be possible to provide, as part of a risk assessment, a classification for individual sheep from within an infected flock. Experiments in the UK where sheep or cattle apparently recovered from infection, and on-farm observations in Australia indicate that Johne's disease affects only a proportion of animals within an infected flock. Some animals, although exposed to the infection, either do not become infected, or eliminate the infection, and pose no risk to other livestock.

In order to better exploit existing tests and develop new strategies for their use in individual animals, greater understanding of the progression of the infection in individuals was required, as well as better knowledge about the behaviour of tests at different stages of the disease. In this longitudinal study, the results of tests (including intestinal biopsy) at each time point were used to define the progression of disease in individual sheep. Test results were then correlated with the final infection status determined by necropsy examination at 3 years of age. This was the first study of this kind in sheep. The field evaluation of the tracer weaner concept, which investigated the detection of infection soon after exposure, had been undertaken on the same heavily infected property. Together, the two trials provided a picture covering the entire disease process from infection to clinical phase.

The specific objectives of the current study were

- Evaluate testing strategies for individual sheep at different stages of the disease
- Provide information about the best tests to use in different age classes of sheep
- Evaluate tests for early diagnosis of OJD
- Assess the predictive value of early test results for later infection status
- Observe progression of infection and possible recovery in individual sheep using biopsy of gut tissues
- Provide preliminary data on the value of gut biopsies for early diagnosis

Methods and Results

The trial used 77 unvaccinated fine-wool Merino sheep on a heavily OJD affected property near Goulburn, NSW. These sheep were born in September 2000, and necropsied at three years of age in September 2003. Early results of the tracer weaner studies indicated that about 20% of sheep had intestinal infection detectable by culture of intestinal tissues 6 months after first exposure, which enabled manageable numbers of sheep to be used in the current trial. Trial sheep were grazed with 150 3-year-old ewes, about 10% of which were AGID positive, thus ensuring an ongoing high level of exposure to *M. a. paratuberculosis*. Each lamb was examined at each time point using a range of tests including clinical assessment, AGID, ELISA (by CSL), IFN- γ (EIA performed at CSL), skin testing, individual faecal culture, d-PCR on faeces, and biopsy of terminal ileum and mesenteric lymph node (both for culture of tissues and histopathology). Details of the testing regimen, some preliminary results, and the biopsy surgery technique are already published in the Australian Sheep Veterinary Society Proceedings, Cairns Conference, Volume 13, 2003: McConnel et al (pp 63-65), and Whittington et al (pp 66-68).

Most sheep were biopsied twice and some three times. The sheep tolerated surgery well, and five different veterinarians successfully performed the procedure. A summary of the results is given in the following table. Results from the 36-month examination are interim, in that culture was not complete for some samples at time of writing.

Test results at each sampling time.

(Percentage of sheep positive for each test)

	Age at sampling (months)					
	9	12	18	24	30	36
Clinical samples						
Faeces culture	10	1	9	14	13	18
Faeces D-pcr	4	4	4	13	9	18
IFN-g	8	1	13	12	46	17
Skin test	ns	ns	ns	31	12	3
AGID	0	0	3	5	10	6
ELISA	ns	ns	8	14	10	11
Biopsy samples						
Tissue culture	ns	20	40 ^a	23 ^b	ns	33 ^c
Histopathology	ns	5	40 ^a	25	ns	35 ^c
Combined	ns	20	45 ^a	29	ns	39 ^c
Necropsy exam						
Combined						59
OJD clinical signs						
Cumulative OJD deaths	0	0	0	4	11	15

^a Results are from only 20 sheep, selected for signs of infection at the previous sampling

^b Results from only 64 sheep, contamination of batch of Bactecs

^c These are results for mock biopsies collected at necropsy examination

ns Not sampled

Individual sheep were classified with regard to final OJD status at 3 years of age as follows:

Died OJD = had died or euthanased with severe clinical OJD, confirmed by lab testing (11 sheep)

Severe OJD = not died OJD, diffuse histological lesions, type 3b or 3c (6 sheep)

Mild OJD = confirmed by culture and/or histology, lesions not diffuse (30 sheep)

Uninfected/n = culture, dpcr and histo negative at all times, no immunological reactions (11 sheep)

Uninfected/r = culture, dpcr and histo negative at all times, immunological reaction (5 sheep)

Recovered = uninfected at final sample, previously infected (11 sheep)

Three sheep had died in paddock and are not included above. Two of them had confirmed OJD infection at their last sample.

Analysis and Conclusions to Date

Full analysis of results will be possible once results are finalised over the next month. Some preliminary analyses and interim conclusions are given below:

Faecal culture. At 9 months, eight (10%) sheep were positive, an unexpectedly high result, given that only a single animal was positive by IFC at 12 months. Passive excretion (associated with short winter pasture??) may partially explain these results. There was no association with final OJD status. Four of these sheep had no evidence of infection at necropsy, although 2 of the 4 had infection in tissue detected by biopsy at 12 months. Faecal culture status at 18, 24 and 30 months, however, was highly predictive of a fatal outcome. At 18 months, 7 sheep were IFC positive. One later died in paddock and was not included in analysis, and the remaining 6 all died of OJD. At 24 months, 11 were IFC positive, 2 later died in paddock and the other 9 all died of OJD. At 30 months, 9 were IFC positive; 6 died of OJD, and the other 3 were infected at necropsy.

Tests for humoral immunity. As expected, early results in these tests were not helpful, with all sheep negative at 12 months. Of 2 sheep AGID positive at 18 months, one died of OJD and the other recovered. The 4 sheep AGID positive at 24 months all died of OJD. Positive ELISA results at 18, 24 and 30 months were highly associated with death from OJD, but not significantly associated with final infection status (3 of 6 positives at 18 months later died of OJD, as did 5 of 11 at 24 months, and 4 of 7 at 30 months).

Tests for cell mediated immunity. Unexpectedly, 6 sheep were IFN- γ positive at 9 months, but there were no significant associations of these results with final OJD status. Three of these sheep were also positive at this time by faecal culture, and all were confirmed to be infected on at least one occasion. One sheep was positive at 12 months, and it later died of OJD. Positive IFN- γ results at 18 and 24 months were highly associated with later death from OJD, but not significantly associated with final infection status (6 of 9 positives at 18 months later died of OJD, as did 5 of 9 at 24 months). Many sheep (46%) were IFN- γ positive at 30 months, but there was no significant correlation with final disease status. Skin test results at any time were not significantly associated with either final infection status or with death from OJD.

Results from biopsy examinations. Culture of tissues obtained by biopsy at 12 months detected many more infected sheep than any other antemortem test. However, there were no significant associations between these results and final infection status at 3 years of age. Histopathology at this age was not helpful. At 18 months of age, histopathology and culture from biopsies detected the same numbers of infected sheep (and mostly the same sheep), but because only a sub-sample of the sheep were tested, analysis of predictive value of biopsy at this age was impossible. At 24 months, biopsy detected about twice as many infected sheep as other tests, and biopsy results were highly predictive of both final infection status and fatal outcome. All 13 biopsy culture positive sheep were still classified as infected at 3 years of age, and 10 of these had died of OJD. Mock biopsy at 3 years detected 59% of infected sheep, more than twice the detection rate of any other antemortem test.


Taken together the above results and analyses support the following conclusions:

Biopsy is a practical technique, about the same level of expense and difficulty as ovariohysterectomy in a large dog. At all time periods, it was the most sensitive technique for identification of infected sheep, although even at 36 months it detected only 2/3 of infected sheep. It may be useful as an additional tool in the management for individual valuable sheep from infected stud flocks.

Many sheep (15% in this study) recover from early infection with *M. a. paratuberculosis*. Thus, any early testing (eg at about 12 months of age), even with a highly sensitive test such as culture of biopsied tissue, may not be predictive of later disease status.

By 18 to 24 months old, positive test results for either cell mediated or humoral immunity, or for faecal excretion, are highly associated with later death from OJD, although not with infection status at 3 years. Skin testing was not helpful. Biopsy results at 24 months detect twice as many infected sheep as other tests, and are very highly associated both with later infection status and death from OJD.


Apparently anomalous results may occur for some tests at some sampling times eg high numbers of IFN- γ positive and IFC positive sheep at 9 months, and IFN- γ at 30 months. The reasons for these results are not apparent.




Biopsy Trial

OJD.020 – Individual Animal Tests for OJD

Leslie Reddacliff



MEAT & LIVESTOCK
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Individual animal tests for OJD

Background

- Most tests in current use are flock tests
- Only reliable individual test currently is necropsy
- Individual tests needed in particular situations – studs, trace-forwards


Current study

Longitudinal study using biopsy to observe progression of infection or recovery in individual sheep

- Provide preliminary data on the value of gut biopsies for early diagnosis
- Evaluate predictive value of early test results for later infection status
- Evaluate testing strategies for individual sheep at different stages of the disease


Methods

- 77 unvaccinated sheep on a heavily infected property
- Followed from 9 months to 3 years of age
- Biopsy at 12, 18 (sub-sample), 24 and 36 months (necropsy, mock biopsy)
- Testing every 6 months
 - Faecal examination (IFC and D-PCR)
 - Humoral immunity (ELISA and AGID)
 - Cell-mediated immunity (IFN-G and skin testing)
- Severely clinically affected animals killed and necropsied
- Results at each time were compared with final disease status at 3 years
 - Follow-on from tracer studies of very early infection
 - IFN-G and ELISA courtesy of CSL



Surgery site – biopsy scars on ileum

Test results at each sampling time						
(Percentage of surviving sheep positive for each test)						
	Age at sampling (months)					
	9	12	18	24	30	36
Clinical samples						
Faeces culture	10	1	9	14	13	18
Faeces D-pcr	4	4	4	13	9	18
IFN-g	8	1	13	12	46	17
Skin test	ns	ns	ns	31	12	3
AGID	0	0	3	5	10	6
ELISA	ns	ns	8	14	10	11
Biopsy samples						
Tissue culture	ns	20	40	23	ns	33
Histopathology	ns	5	40	25	ns	35
Combined	ns	20	40	29	ns	39
Necropsy exam						
Combined						59
OJD clinical signs						
Cum OJD deaths	0	0	0	4	11	15



Conclusions

- Biopsy is a practical technique
- Many sheep (15% in this study) recover
- Positive tests at 18 to 24 months are highly associated with death from OJD
- Biopsy detects many more infected sheep
- Even biopsy detects only 2/3 of infected sheep at 3 years

MOLECULAR BIOLOGY OF OJD

PROGRESS IN OUR UNDERSTANDING OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* USING MOLECULAR BIOLOGY PARTICULARLY FOCUSING ON STRAIN CHARACTERISATION

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Background and Objectives

After researching Johne's disease (JD) for more than 100 hundred years our understanding of how *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the etiological agent, functions and causes disease is limited. However, what understanding we do have was greatly increased with the development of new research techniques particularly in the fields of molecular biology and genomics.

The identification of IS900, a DNA sequence unique to MAP, greatly improved our ability to detect and confirm the presence of MAP in a range of biological samples as well as improving our epidemiological understanding of the disease. IS900 polymerase chain reaction (PCR) in combination with radiometric culture has rapidly become one of the most sensitive and specific diagnostic tests for JD. IS900 based restriction fragment length polymorphism (RFLP) analysis, a DNA fingerprinting technique, has been used extensively in epidemiological studies and identified two broad groups of MAP, those that primarily infect sheep (S strains) and those that primarily infect cattle (C strains) plus many sub-types within each group. Another DNA sequence present in both *Mycobacterium avium* subsp. *avium* (MAA) and MAP, IS1311, has been successfully used to rapidly differentiate between the two species and between the strains of MAP. Unfortunately, little is understood about the differences between the S and C strains of MAP that explain respective host specificity. A number of other genetic elements have been identified in MAP but have yielded little in explaining how this organism causes disease and/or functions in the environment, *in vivo*, or *in vitro*. However, recent advances in the fields of molecular biology, genomics and proteomics are providing opportunities to overcome these limitations allowing researchers to explore new and revealing aspects of microbial pathogens in ways that were previously unachievable. One of the key areas of JD research has been the sequencing of the entire MAP genome. Recently completed and now publicly available, the genome sequence provides a valuable blue print reference for all JD researchers working in the fields of molecular biology, genomics and proteomics.

The aims and objectives of my PhD program and this review are to highlight the methods and results of my investigation in to the microbiological differences between the sheep and cattle strains of MAP that may explain host adaptation and pathogenicity. These results will be discussed with reference to other research groups undertaking similar studies in the fields of molecular biology, genomics and proteomics.

Methods and Results

Representational difference analysis (RDA). RDA is a molecular biology technique used to identify differences between two closely related DNA samples. In this study RDA was used to identify differences between the DNA from the S and C strains of MAP. Three differences were identified of which two were confirmed.

The first of these revealed a single base difference in the sheep and cattle strains of MAP. The second difference was used to identify a region of DNA that appears to be deleted from the S strain but present in the C strain. Continued analysis of this region is in the closing stages. To date the results indicate the DNA region of about 24, 000 base pairs has been deleted from the S strain. This region appears to contain 16 genes including 3 with functions similar to other microbial genes while the remaining 13 have no known function. The completion of this work has been greatly assisted by the availability of the completed genome sequence.

Gene sequencing analysis. At the time this study commenced 27 genes (some confirmed and some putative) had been deposited in Genbank for MAP. PCR was undertaken in an attempt to amplify each of these genes from both the S and C strains of MAP. The PCR products were sequenced and compared in an attempt to identify if any differences existed between the same gene from the different strains. From these 27 genes, 18 have been successfully amplified resulting in 6880 base pairs of DNA being sequenced from the sheep strain and 7020 base pairs from the cattle strain. Nine differences across 5 genes have been identified. It would appear that the majority of these genes are highly conserved in both strains of MAP, a situation similar to studies undertaken on *Mycobacterium tuberculosis*.

Proteomic analysis. Two-dimensional electrophoresis (2DE) has gained popularity as a technique to identify differences between two closely related biological samples, at the protein level. 2DE is being used in this study to identify unique proteins or differential expression of proteins from the sheep and cattle strains of MAP grown under the same *in vitro* culture conditions.

After having problems in identifying a procedure for extracting proteins from MAP, a technique was eventually found. One-dimensional SDS-PAGE and 2DE have been successfully undertaken to identify proteins that are either unique to one strain or differentially expressed in one strain compared to the other. This work is still in progress and at the point of protein identification.

Microarray analysis. Like 2DE, microarrays are increasingly being used to identify differences between biological samples. Once again, microarray technology was used in this study to identify differences between the S and C strains of MAP. The S and C strains were compared indirectly by comparing each strain independently with the K10 MAP strain, the strain used to generate the MAP genome sequence. These experiments have identified 94 genes including those genes identified by RDA, confirming the presence of a large genomic deletion in the S strain of MAP compared to the C strain. These differences are currently being further evaluated and confirmed.

Conclusions to Date

The molecular biology section of the 7th International Colloquium on Paratuberculosis introduced what might be considered the next generation of molecular biology based research on MAP. This colloquium saw the introduction of the MAP genome, microarray technology and proteomics. Since then several studies using these techniques have been published offering new and exciting fields of inquiry into MAP and how it causes disease.

Studies have been undertaken to compare the MAP and MAA genomes in an attempt to identify MAP specific DNA regions⁽¹⁾. The DNA sequences identified in these studies have been used to identify proteins that are currently being evaluated as potential antigens for new and improved diagnostic immunoassays⁽²⁾. RDA and other new and improved molecular biology techniques have been used to identify what appears to be a 38-kilobase pathogenicity island unique to MAP and characterise the differences between S and C strains of MAP^{(3) (4) (5)}. Dohmann et al. (2003) have successfully identified the first extensive DNA regions present in the S strain but absent from the C strain. The results from these studies will help extend our understanding of the extent to which these strains have diverged from

each other and MAA plus offer new targets for DNA based diagnostic assays. Furthermore, new theories on the evolution of these species and sub-species will evolve. New strategies, combining a range of molecular biology techniques, have been used to further our epidemiological understanding of the disease by comparing MAP isolates from a range of hosts and geographical locations ⁽⁶⁾.


The methods and results presented in this review of the PhD currently being undertaken, demonstrate significant outcomes that will directly contribute to and complement the findings from the other studies described above. Further characterisation of the deletion identified by RDA in this study will hopefully identify key differences between the S and C strains of MAP that may explain the different host specificities of these strains and enhance our understanding of how this organism causes disease. Moreover the results from this study will hopefully contribute to our ability to control Johne's disease amongst the many farm animal industries it affects.

Acknowledgments

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Molecular Biology of Ovine Johne's Disease

Molecular Biology Research of
Mycobacterium
Avium Subsp. *Paratuberculosis*


Ian Marsh

 NSW Agriculture



 The University of Sydney

- Research initiatives undertaken for PhD
- Recent advances in JD research using molecular biology






Johne's Disease

Over 100 years of research on Johne's Disease
Major advances in the 10-15 years
Limited understanding of *Mycobacterium avium* subsp. *paratuberculosis* (*M. ptb*) and the disease it causes

NEED to learn more about
basic biology of *M. ptb*
how it causes disease
host specificity
environmental and *in vivo* survival
dormancy

Vaccine development
Improved diagnostics



PhD Objectives

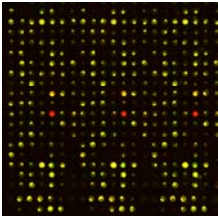
Find differences between the sheep and cattle strains of *M. ptb* that explain host specificity and survival in the environment


Representational difference analysis (RDA)
3 DNA regions identified
Large deletion in the S strain including up to 16 genes
Single base difference

Gene sequencing analysis
approx 7000 bp sequenced
9 differences over 5 genes

Proteomics
Differences observed
requires identification of spots

Microarray
94 genes identified
confirmation under way





Other Molecular Biology Studies

Genome sequence (Bannantine and Kapur)
***M.ptb* and *M. avium* genome comparisons (Bannantine *et al.*, 2002)**
New antigens (Bannantine *et al.*, 2004)
Comparisons between S and C strains (Stevenson *et al.* 2002)
Unique DNA regions in the S strain (Dohmann *et al.*, 2003)
Advanced epidemiological studies (Motiwala *et al.*, 2003)

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Meat and Livestock Australia
University of Sydney - Prof. Richard Whittington & OJD group
NSW Agriculture - Dr Leslie Reddacliff & Microbiology staff
CSIRO - Dr Mark Tizard
USDA - Dr John Bannantine

Diagnosis – Workshop Discussion

Following these presentations a workshop of participants was held to answer the following questions:

Diagnosis – Infected Flock Profiling

1. *What has been achieved by the researchers?*

- Confirmation that it is very difficult to confirm a negative diagnosis
- Identification that shedding may be delayed in crossbreds

2. *What key benefit from this research can we communicate to producers?*

- None at this stage

3. *What advice would a farm adviser now be able to provide a producer client?*

- None at this stage

4. *How could a regional authority, eg RLPB, respond to this information?*

- None at this stage

5. *What still needs to be done/researched?*

- More profiles need to be conducted in other geographical areas (particularly in crossbred flocks)

6. *Are there any other implications or warnings from this work?*

- It is very difficult to confirm a negative diagnosis
- Shedding may be delayed in crossbreds

7. *Have the outcomes of this work been captured in a useful form (scientific publication, advisory note etc) and what actions are required to ensure this occurs?*

- Scientific paper currently in preparation

Diagnosis – Molecular Biology of OJD

1. *What has been achieved by the researchers?*

- Identification of distinct differences between C and S strains of M. ptb.

2. *What key benefit from this research can we communicate to producers?*

- Provides support for the advice to “clean” pastures for sheep with cattle.

3. *What advice would a farm adviser now be able to provide a producer client?*

- As for 2

4. *How could a regional authority, eg RLPB, respond to this information?*

- As for 2

5. *What still needs to be done/researched?*

- Important to continue to test that strain differences hold up.

6. *Are there any other implications or warnings from this work?*

- As for 5

7. *Have the outcomes of this work been captured in a useful form (scientific publication, advisory note etc) and what actions are required to ensure this occurs?*

- Yes

Diagnosis – Biopsy Trials

1. *What has been achieved by the researchers?*

- Recovery from infection can occur.
- Biopsy only detects 2/3 of infected animals.
- Harder to diagnose infected individual animals than infected flocks

2. *What key benefit from this research can we communicate to producers?*

- N/A

3. *What advice would a farm adviser now be able to provide a producer client?*

- N/A

4. *How could a regional authority, eg RLPB, respond to this information?*

- N/A

5. *What still needs to be done/researched?*

- More analysis of results.

6. *Are there any other implications or warnings from this work?*

- At present, biopsy is a better research tool than for on-farm use.

7. *Have the outcomes of this work been captured in a useful form (scientific publication, advisory note etc) and what actions are required to ensure this occurs?*

- Yes

Diagnosis – Abattoir Surveillance

1. *What has been achieved by the researchers?*

- Good estimate of abattoir sensitivity
- Benchmark for abattoir surveillance
- Learning and training lessons - guidelines for training & accreditation.
- Comparative cost
- Utility in general scheme of diagnostics
- Abattoir surveillance is an appropriate test for surveillance.

2. *What key benefit from this research can we communicate to producers?*

- Reliability of tools in disease surveillance & recognition.
- Demonstration of negatives is as important as positives
- Abattoir surveillance provides an even playing field for producers
- Producers should request feedback on samples that go to abattoir

3. *What advice would a farm adviser now be able to provide a producer client?*

- As for 2

4. *How could a regional authority, eg RLPB, respond to this information?*

- As for 2
- Promote area assessment
- Promote producers to send sheep to an abattoir that tests.
- Take advantage of negative surveillance results.
- Monitoring vaccination results – abattoir surveillance provides mechanism to capture results.

5. *What still needs to be done/researched?*

- Is animal ID system working? - Better traceability.
- Something other than eyeball, eg transparency of terminal ileum

- Maintain quality control - ongoing training & QA
- Sensitivity in lower prevalence flocks

6. *Are there any other implications or warnings from this work?*

- Producers avoid abattoirs that conduct surveillance.
- QA of inspectors.
- Harder to get inspectors
- Chains may get faster due to increased mechanisation.

7. *Have the outcomes of this work been captured in a useful form (scientific publication, advisory note etc) and what actions are required to ensure this occurs?*

Suggestions:

- Standard operating procedure, QC
- Formal accreditation of inspectors
- Publication/advisory meeting to producers
 - media
 - tips & tools

Diagnosis – Gamma Interferon Test

1. *What has been achieved by the researchers?*

- Comprehensive evaluation of the γ -IFN test
- Results reaffirm opinion that test has limited application

2. *What key benefit from this research can we communicate to producers?*

- Producers can be satisfied that research into diagnostic tests is underway and this test has been examined carefully.

3. *What advice would a farm adviser now be able to provide a producer client?*

- N/A

4. *How could a regional authority, eg RLPB, respond to this information?*

- N/A

5. *What still needs to be done/researched?*

- New antigens??

6. *Are there any other implications or warnings from this work?*

- Variability in Johnin – consider again in the future?

7. *Have the outcomes of this work been captured in a useful form (scientific publication, advisory note etc) and what actions are required to ensure this occurs?*

- Scientific publication when completed

WESTERN AUSTRALIA

OVINE JOHNE'S DISEASE PROGRAM IN WESTERN AUSTRALIA

UPDATE ON THE OJD PROGRAM IN WESTERN AUSTRALIA

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Background

Western Australia (WA) was declared a Free Zone for ovine Johne's disease (OJD) in October 1999. However, the State now exceeds the limit of cases in the criteria for a free zone and the status is being reviewed by the Animal Health Committee which makes recommendations on zone status to the Primary Industries Standing Committee.

Surveillance for OJD in WA includes the targeted surveillance of flocks/herds that have previously imported sheep and goats from the eastern states (higher risk animals). On these properties, a representative group of older animals are tested using a pooled faecal culture.

Surveillance in WA also includes the inspection of randomly selected, single source lines of sheep at abattoirs. All sheep in such a line are examined for signs of the characteristic changes to the gastrointestinal tract caused by the disease. Suspicious tracts are then sampled for microscopic examination and culture.

Incidents

Until July 2003, OJD had been detected in three animals including one goat and one sheep imported from the eastern states and one goat that was the offspring of an imported animal. Each animal was detected on a separate occasion through the import surveillance program. None of the infected animals showed any clinical signs of disease or visible lesions on autopsy. Infection was found by microscopic and/or culture testing of tissues and faecal samples. In the first two cases the properties were destocked and neighbour and trace herds/flocks quarantined for up to three years until suspicion was resolved by testing. The third property is undergoing further testing as a suspect herd.

As at 4 March 2004, seven flocks (totalling over 82,000 sheep) have been detected with OJD in the current 2003/04 incident. On five of the properties, OJD has been diagnosed only in sheep purchased within the last 14 months from the first two properties quarantined. One hundred and forty four properties are under investigation (47 neighbours, 10 trace-backs, 36 trace-forwards and 51 properties investigated and assessed as requiring no further action).

The evidence indicates that OJD has been present in WA for seven years or more and that none of the infected flocks found to date is the source of infection. Evidence from the current outbreak and the experience in other parts of Australia indicate that there is likely to be a number of other infected flocks in WA and hundreds of traces.

Infected properties have been quarantined. However, given the likelihood of further infected properties and tracings, the decision was taken to gather further information rather than quarantine all neighbour and trace properties and initiate eradication on infected properties. In the interim, owners of these properties have agreed to dispose of sheep only to export or directly to abattoirs.

Future Program

Benefit cost analysis using assumptions about the spread of disease in WA has found that with present technology, it is not technically, logistically, economically, or socially feasible to eradicate OJD from the state.


A broad based industry and Department OJD Advisory Committee is currently developing strategies for an OJD Program in WA, within the agreed framework of the new National OJD Program due to commence on 1 July 2004. Consistent with the National OJD Program, WA will maintain a vision to control OJD and eliminate it by socially acceptable means, if that becomes technically, logistically and economically feasible.

Issues being considered by the Advisory Committee include:

- owner access to self protection measures such as vaccination;
- the level of regulatory restrictions or controls, if any, to apply to known infected and at risk flocks and other control measures;
- the development of conditions for the movement of sheep and goats to WA that recognise the State's current status and harmonise with movement conditions intended by the National OJD Program;
- the source of funding to carry out the various elements of an OJD program in WA including surveillance, investigations and testing, communications and extension, printing and distribution of animal health statement forms, establishment of the Market Assurance Program and administration and delivery of the program in WA.

The Department has initiated moves for the training and accreditation of private veterinarians to enable flock owners to enter the voluntary national Market Assurance Program. The Department is also developing processes for the ordering and use of vaccine.

Industry and the Department will arrange workshops and seminars to provide information to owners and other stakeholders about OJD and the agreed OJD program in WA, once finalised.




GOVERNMENT OF
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Agriculture

OJD in WA

Fiona Sunderman


- Free Zone since October 1999
- Targeted and abattoir surveillance
- 3 previous incidents involving single imported or related animals
- Destocked infected properties
- Tested neighbours and traces



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Current Incident

- 7 infected properties
 - 2 neighbours
 - 5 trace forwards
- Source has not been determined
- In WA for at least 7 years
- Infected properties quarantined
- Net cost of attempted eradication in WA estimated to be about \$70 million
- No funding mechanism



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Incident Statistics - Tracing

(24-2-04)

• No Infected Properties	7
• No sheep on Infected Properties	82,000+
• No Suspect Properties for testing	144
• No Neighbours for testing	47
• No Trace Backs for testing	10
• No Trace Forwards for testing	56
• No of traces investigated NFA	51



OJD Advisory Committee

- Industry and Government
- Eradication?
- Vaccination?
- Quarantine?
- Movement conditions
- Funding

OJD Forum Attendees List

The Attendees are as listed below

Animal Health Australia	Lorna Citer Ralph Hood
Armidale Rural Lands Protection Board	Geoff Green John Macfarlane
Australian Agriculture Fisheries & Forestry (AFFA)	Graeme Garner Jane Bennett
Australian Wool Innovation	Scott Williams
Ausvet Animal Health Services	David Kennedy Evan Seargent
Central Tablelands Rural Lands Board	Jeff Eppleston
CSIRO Livestock Industries	David Stewart Mark Tizard
CSL	Jan Tennent
Dairy Australia	Robin Condron
Department of Agriculture WA	Debby Cousins Fiona Sunderman
Department of Primary Industries Water and Environment (TAS)	Cameron Bell
Department of Primary Industries (VIC)	Alison Lee Chris Schroen Maryanne Martin Tracey Bradley Tristan Jubb
District Veterinarians	Alexandra Stephews Elizabeth Braddon Greg Simpson Helen Walker Jim McDonald John Macfarlane Luzia Rast Robert Templeton Tony Morton
Goulbourn RLPB	Jason Moroney
Meat and Livestock Australia	Eileen Leung Gilly Simos Ian Jenson Karen Fox Michael Goldberg Miriam Daniel Peter Rolfe Skye Voveris Terry Longhurst

NSW Agriculture

Barbara Moloney
Bruce Christie
Col Langford
Ian Links
Ian Marsh
Julie Bolam
Laurie Denholm
Leslie Reddacliff
Marilyn Evers
Martin McLoon
Pat Taylor
Paul Forbes
Scott Seaman

Primary Industry & Resources South Australia

Paul Cleland

Private Veterinarians

David Rendell
Debby Lehman

Producers

Denise Cunningham
Felicity Henderson
Frank Tobin
Ken Baldry
Richard Molesworth

Ruth Davis Consulting

Ruth Davis

University of Melbourne

John Larsen

University of Sydney

David Emery
Deborah Taylor
Helen McGregor
Jenny Ann Toribo
Katrina Bosward
Kumidika de Silva
Lyrisa Di Fiore
Om Dhungyel
Peter Windsor
Richard Whittington
Russell Bush

Appendix 1

SUMMARY OF THE RESULTS & OUTCOMES OF OJD RESEARCH PROJECTS 2004

PROJECT	MAJOR OUTCOMES
DETECTING OJD	
Pooled Faecal Culture (PFC) as a flock screening test	➔ <i>PFC now the main test for screening flocks for OJD infection. The introduction of this test has saved the national program alone approximately \$4 M in costs of disease surveillance.</i>
Comparison of PFC and AGID blood tests to detect OJD infected flocks	PFC proved more sensitive than serology at detecting OJD infection when similar numbers of sheep were sampled ➔ <i>Increased numbers of sheep must now be sampled when AGID is used as a flock screening test. The integrity of Market Assurance Programs has been improved.</i>
Evaluation of the effectiveness of "tracer" lambs to detect on-farm infection	Tracer lambs require exposure to infected pastures for at least 6 months before infection can be detected. ➔ <i>Tracer lambs are a useful for testing the success of destocking and as a research tool, particularly vaccine evaluation.</i>
Evaluation of Abattoir Surveillance to Detect OJD	Abattoir monitoring of cull sheep for visible signs of OJD proved to be a reliable method for detecting infected flocks in regions with a high and moderate prevalence of the disease. ➔ <i>Abattoir monitoring now routine in all states</i> ➔ <i>Negative results are now an accepted part of the assessing OJD status of flocks in the ABC risk based trading scheme now implemented across Australia.</i>
CROSS SPECIES TRANSFER OF OJD	
Transmission of OJD via goats, cattle and wildlife.	Cattle can occasionally be infected with sheep strain of JD. Goats can spread the disease to sheep. Marsupials can be infected in unique and unusual situations. Management options now include: ➔ <i>Avoid grazing young cattle on pastures grazed by infected sheep</i> ➔ <i>Include goats in all OJD control or eradication programs</i> ➔ <i>Wildlife are not considered to pose a threat to OJD control on most farms</i>
CONTROLLING OJD WITHIN FLOCKS	
Survival of OJD organisms in the environment	Most OJD bacteria die within 6 weeks in the environment. A small number may survive for over a year in shaded areas ➔ <i>Recommendations can now be made on grazing management and eradication, particularly spelling areas for 6 weeks in most climates will reduce pasture contamination with bacteria by 90%.</i>
Vaccine evaluation	Vaccination at weaning reduces subsequent deaths from OJD and delays the onset of bacterial shedding in dung ➔ <i>A vaccine is now registered for the control of OJD and is being used as a management tool to reduce disease transmission. It will reduce mortalities by 90% and shedding of bacteria in faeces by (worst case). 300,000 doses of vaccine have been sold.</i>
When does infection commence?	Infection status of ewes and lambing paddocks has been found to be critical in the transmission of OJD. ➔ <i>Prepare low risk lambing and weaning paddocks to reduce</i>

	<i>disease transmission within a flock.</i>
Direct transmission of OJD infection from ewes to lambs.	<p>Ewes showing signs of OJD can infect their lambs before birth or via milk. Direct transmission is rare in ewes that do not have obvious signs of disease.</p> <p>→ <i>Cull ewes promptly at the first sign of OJD infection</i></p> <p>→ <i>Removing lambs from ewes at birth will not eliminate lamb infection entirely, but will reduce the risk (high value animals)</i></p>
ECONOMIC IMPACT OF OJD	
Economic effects of the disease on-farm	<p>→ Deaths associated with OJD have been confirmed as very significant on some farms and 90% of total deaths in adult sheep.</p> <p>→ Mortalities in adult sheep range from 3-16% pa and the gross margin per hectare is reduced by the same amount 20% where infection is present for > 5 years.</p>
Assessing the spread of infection within and between flocks	<p>Computer simulation model developed to predict the spread of OJD and compare cost-effectiveness of various control strategies.</p> <p>→ <i>An established OJD infection costs producers on average \$17,000 per year (2000 ewe self replacing merino flock)</i></p> <p>→ <i>Lamb vaccination is an economically effective control option</i></p> <p>→ <i>Management strategies which reduce contact of susceptible sheep with infected faeces can lower flock mortality rates</i></p>
PRESERVING GENETIC MATERIAL	
Risk of OJD Transmission in Ram Semen	<p>Ram semen found to be infected with OJD organisms.</p> <p>→ <i>A policy for the use of reproductive material from OJD infected or suspected flocks developed by NSW Agriculture</i></p>
ERADICATING OJD FROM INFECTED FARMS	
Evaluation of destocking programs	<p>Some farms, which were restocked after a period of decontamination, have developed new infections.</p> <p>→ <i>Eradication is not possible or cost effective unless great care is taken to source uninfected sheep.</i></p> <p>→ <i>Eradication is difficult where surrounding farms are infected</i></p> <p>→ <i>Destocking for 15 months and over 2 summers is usually enough time for infection to die off on pasture.</i></p>