



researchreport

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Ovine Johne's Disease Research Report Abstracts March 2006

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Abstract

This publication includes abstracts from reports of the research and development undertaken in Australia as part of the National Ovine Johne's Disease Control and Evaluation Program (NOJDP).

This program commenced in 1998 with the aim of:

1. Determining the extent of OJD infection in Australia,
2. Evaluating if eradication of the disease was feasible and cost effective,
3. Developing management and control options to assist affected producers; and
4. Minimising the spread of the infection during the evaluation phase.

Most of the research projects are now complete and have generated invaluable information to assist the sheep industry in the control of this disease.

Major outcomes from the R&D program include:

- evaluation and registration of an OJD vaccine,
- development of improved diagnostic tests for the disease
- development of scientifically-based pasture and animal management strategies to limit the impact of infection in affected flocks.
- assessment of the feasibility of OJD eradication from flocks
- assessment of the economic cost of the disease to individual producers; and
- better understanding of the role of other animal species in the spread of the disease.

The major areas of research within the NOJDP included:

- Controlling OJD within infected flocks
- Economic impact of OJD
- Eradicating OJD from infected farms
- Cross species transfer of OJD
- Detecting OJD
- Basic research to identify new ways to detect and control OJD
- Preserving genetic material from infected flocks

Abstracts in this report are organized in line with these research themes. Copies of the final research reports can be downloaded from the MLA website (www.mla.com.au) or be ordered by contacting MLA's Animal Health and Welfare team on 02 9463 9288.

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1 Controlling OJD within Flocks

OJD.003/TR.055A/TR.055 Survival of Johne's disease in the environment

NSW Agriculture
June 2001

Abstract

This report covers three MLA projects (TR.055, TR.055A and OJD.003) undertaken by NSW Agriculture to determine how long the sheep strain of *Mycobacterium paratuberculosis* survives in the environment to validate destocking recommendations for eradication of OJD. Survival of the organism was prolonged but finite. In the shade it lasted for 13 months, while in the open in ungrazed pasture it lasted for 7 months. It survived for a shorter period in fully exposed pastures where grazing was simulated but for much longer in water than on pasture. These times were probably underestimates. Liming pasture did not reduce survival and moisture did not increase it. Shade was the most significant factor favouring survival. Further research is necessary to determine the mechanisms of survival, which include dormancy. Decay rates for the organism were determined for short term and long term destocking. These can be used to estimate how much time must be allowed to render pastures safe for control and eradication of OJD, respectively. When estimates of soil ingestion rates by grazing sheep are combined with within-flock OJD prevalence estimates and bacterial shedding rates determined by PFC, it is possible to make property by property recommendations for the purpose of control or eradication of OJD.

Key outcomes:

- *Mycobacterium paratuberculosis* can survive more than 12 months in water or shaded environments
- More than 90% of organisms died off within 6 weeks in exposed environments

OJD.002A Exposure factors leading to establishment of OJD infection and clinical disease

University of Sydney
December 2004

Abstract

This study was conducted in order to provide answers for owners of OJD-infected sheep flocks who wish to minimise the economic and biological impact of the disease on their sheep enterprises. The study has demonstrated that careful management of young sheep can reduce the level of OJD in the flock and reduce the death rate. One key finding is that steps taken to limit the degree of exposure of pre-weaned lambs to infection from pastures will lead to reduced rates of severe infection in those sheep in later years. A second key finding is that continuous exposure to OJD bacteria throughout early life results in higher infection rates than exposure which is limited to either the pre-weaning or the post-weaning period alone. A critical factor in management is to provide 'low risk' pastures to young sheep at weaning to give them a break from exposure to infection which occurs in the lambing

paddocks. We also found that weaned lambs and adult ewes remain susceptible to infection and that there is little evidence for an age-related resistance to OJD.

Key outcomes:

- Provision of 'low risk' pastures to young sheep at weaning is an important management strategy to reduce infection levels.
- Weaned lambs and adult ewes remain susceptible to infection and there was little evidence for age-related resistance to OJD

OJD.009 Field evaluation of OJD control using Gudair

NSW Department of Primary Industries

May 2005

Abstract

A 5-year field trial was undertaken to determine the efficacy of a killed *M. a. paratuberculosis* (Mptb) vaccine, Gudair™, for control of OJD in Australian merinos. The vaccine stimulated cell-mediated and humoral immune responses. Gudair reduced mortalities due to OJD by 90% and delayed faecal shedding for the first year post-vaccination (pv). Thereafter, the prevalence of shedders among vaccinates was reduced by 90%. The numbers of Mptb excreted by the vaccinated groups were also reduced by at least 90% at most sampling times. However, high levels of excretion by vaccinates occurred on some occasions, and although only 7 of 600 vaccinates died from OJD, all had multibacillary disease. Thus there remains a risk that some vaccinated sheep could transfer the disease. Small reductions in liveweight gain were found in vaccinated lambs in the first year pv, but there was little effect on condition score or wool production. Vaccine injection site lesions were detected in almost 50% of sheep 2 months pv, and these persisted for at least 4 years in 20-25% of vaccinates. Data from this trial enabled the registration of Gudair in Australia in 2002 and underpins the pivotal role of vaccination in the current management of OJD.

Key outcomes:

- Gudair vaccination reduced mortalities due to OJD by 90% and delayed faecal shedding for the first year post-vaccination.
- Gudair vaccination also reduced excretion of *Mycobacterium paratuberculosis* by about 90% in vaccinated sheep
- Some vaccinated sheep will still develop severe clinical disease and may excrete very large numbers of organisms, posing a continuing source of infection for other sheep.

OJD.015 A longitudinal study of OJD and the effects of whole flock vaccination with Gudair

**University of Sydney
March 2005**

Abstract

This study evaluated the level of sheep deaths and the contribution of OJD to these deaths in a large self-replacing Merino flock experiencing high losses (about 25% per annum). The whole flock (with the exception of unvaccinated control sheep from each age group) were vaccinated with Gudair™ between May and September 2000. Over 4-years, adult mortality due to OJD declined significantly. The prevalence of faecal shedders declined from high to moderate following vaccination of 2-year-old sheep, although shedding at rates sufficient for transmission of infection continued, particularly in wethers. A low prevalence of faecal shedding in sheep vaccinated at 3 or 8 months of age, suggested that vaccination is similarly efficacious in these age groups and that vaccination may therefore be beneficial in sheep when delayed to weaner age or older. A decline in the prevalence of shedding was also seen in the control sheep. It is considered likely that a series of management changes on this property (including culling of clinical cases, sale of 'at risk' stock, introduction of replacements and reduction in stocking rates) had more significant impact on pasture contamination with Mptb than whole flock vaccination. The study concludes that by accompanying vaccination with intensive management changes, a significant decline in the risk of OJD mortality can be obtained and rapidly lead to control of OJD in a heavily infected flock.

Key outcomes:

- Whole flock vaccination combined with management changes produced a significant decline in OJD mortality rates in this heavily infected flock.
- Vaccination of lambs at 8 months of age appeared to have a similar efficacy to those vaccinated at 3 months suggesting that vaccination can be delayed to after weaning without jeopardising the level of control achieved
- Vaccination at 2 years of age in the face of severe challenge was less effective than vaccination as lambs.
- Vaccination of adult sheep in a heavily infected flock had no apparent detrimental effect.

OJD.024 Ewe-lamb transmission of ovine Johne's disease

**NSW Agriculture
April 2003**

Abstract

Knowledge of the likelihood of intrauterine or transmammary transmission of *M. a. paratuberculosis* infection in sheep is important in the design of control programs. Little has been published on this aspect of ovine Johne's disease, although studies in cattle have found up to 25% of foetuses from clinically affected cows to be infected. In this study 151 ewes from heavily infected flocks and their late term foetuses were examined using all available antemortem and necropsy tests. Five of six ewes with clinical OJD had infected foetuses. One of 54 subclinically affected ewes and none of 16 apparently

uninfected ewes had infected fetuses. Only two ewes (both clinical cases which also had infected fetuses) had detectable *M. a. paratuberculosis* in their milk or mammary glands. Thus, although intrauterine or transmammary transmission may occur frequently in clinically affected sheep, it is infrequent in subclinically infected ewes or in ewes not detectably infected (even if from a heavily infected flock), suggesting that this mode of transmission is unlikely to significantly affect existing OJD control programs.

Key outcomes:

- A small proportion of lambs from clinically affected sheep may be infected before birth or through infected milk.
- Pre-natal infection in sheep is unlikely to play an important role in the epidemiology of OJD in most flocks

OJD.028 Epidemiology of ovine Johne's disease 2 – pasture contamination level and age susceptibility

University of Sydney
June 2005

Abstract

To develop pasture management strategies for control of ovine Johne's disease, research was conducted in a flock of 840 sheep to determine whether age of sheep and pasture contamination levels affect OJD. Lambs (median age 5.5 months at the start of the trial) were highly susceptible to infection and should not be exposed to high levels of contamination or a proportion will develop severe infection leading to death. Of great benefit to industry was the finding that hoggets and adult sheep were relatively resistant to the clinical effects of OJD. Even though infection occurred, ewes rarely succumbed to the disease while hoggets were more resistant than lambs. This means that hoggets (median age in this study 22.5 months) and adult sheep can be used to graze contaminated pasture with less risk of clinical impact compared to lambs, and so these classes of sheep can be used to prepare pasture for lambs. Pasture spelling should also be useful. Conventional wire strand fences do not prevent spread of infection so disease control needs to be based on an area-wide approach.

Key outcomes:

- Sheep first exposed to infection as hoggets or as adults are relatively resistant to the effects of disease, compared to lambs (i.e. they will become infected but are less likely to develop clinical disease)
- Adult (uninfected) sheep can be used to graze infected pasture to reduce contamination levels in preparation for grazing lambs or weaners.

OJD.032 A preliminary study on the potential cost at slaughter of OJD vaccination site lesions to the Australian sheep industry

**Central Tablelands Rural Lands Protection Board
October 2004**

Abstract

This report presents the results of a pilot project to predict the risk of discounts being applied in Australia due to OJD vaccination site lesions as vaccine use expands. This includes a review of discounting in New Zealand as well as a preliminary survey of the incidence of lesions and actual discounts applied to slaughtered vaccinates in Australia. Information from New Zealand suggests that the greatest discounts are applied to trimmed high value lamb carcasses that are destined for export in whole carcase form as a result of downgrading to a lower quality grade. In Australia the greatest risk of discounting is likely to be in purebred merino lambs sold into the prime lamb market, but the low proportion exported as carcasses would limit the discount applied. The incidence of lesions observed was 18% for mutton and 65% for lamb carcasses. The value of the trim removed was insignificant, the labour cost of its removal was nil and no carcase was downgraded to a lower value grade. However this study was conducted at a time of low sheep supply in carcasses vaccinated at the recommended site and caution should be exercised in extrapolating these results to oversupplied market situations or in sheep vaccinated at alternate sites.

Key outcomes:

- A high proportion of vaccinated lambs (65%) have detectable lesions at slaughter, but this proportion decreases with age (18% in adults)
- There was no additional cost for trimming and there was no loss of carcase value from trimming or downgrading in this trial.

OJD.038 Identification of risk factors for OJD infection-level in sheep flocks

**University of Sydney
June 2005**

Abstract

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

Key outcomes:

- The level of infection in infected flocks was affected by a range of management and environmental factors, including weaner management and nutrition and soil type.
- Further analysis and investigation is required to clarify some of the specific effects.

2 Economic Impact of OJD

OJD.023 A study of the biological and economic impact of OJD in infected sheep flocks in NSW – 12 farm mortality study

**University of Sydney
September 2005**

Abstract

This study provides industry with factual information about the impact of OJD on farms in southern Australia. This is needed to assess the magnitude of the OJD problem and to direct control and management programs. On 12 farms, the average annual OJD mortality rate based on inventory records from 2002-2004 was 6.8% (range 1.8% to 17.5%), well above the accepted annual mortality rate (from all causes) for Australian sheep flocks. OJD losses accounted on average for 70% (range 17% to 100%) of the estimated financial losses associated with sheep deaths in 2002. In addition this project confirmed that prevalence based on pooled faecal culture (PFC) could be used as an indicator of OJD mortality level and provided support for further investigation of several risk factors in a larger future study (MLA OJD.038). The gross margin model developed provides estimates of on-farm cost of OJD and of vaccination control for wool and sheep-meat enterprises including break-even points for vaccination at various prevalence levels. We recommend that producers, through industry extension activities, be informed of the substantial losses associated with OJD and be encouraged to apply the model to support decision making regarding OJD control.

Key outcomes:

- Average annual mortality rate on 12 farms was 6.8%, ranging from 2% to 17.5%.
- OJD accounted for an average of 70% of losses on these farms.

OJD.027 Development of computer models to describe the epidemiology of Johne's disease in sheep

**AusVet Animal Health Services Pty Ltd
June 2002**

Abstract

The pathogenesis, epidemiology and options for control of Johne's disease in sheep were reviewed and mathematical models developed to simulate the spread of OJD within infected flocks, and between flocks on a regional basis. The OJD Flock Model simulates spread of OJD within an infected flock for a specified number of years. Effects on flock productivity can be simulated as effects on lambing percentage and on wool production and quality, in addition to the losses due to mortality/premature culling of affected sheep. Although these effects have still not been quantified, the model allows examination of 'what-if' scenarios to estimate the cost of OJD in infected flocks. The model also allows simulation of various control strategies, including management control, vaccination and test & cull options.

The OJD Regional Model simulates spread of OJD between properties within a region. Properties are simulated on a grid of farms, and spread may occur by local spread between adjoining farms, or

through the movement of replacement sheep between farms. The model simulates spread within each infected flock once it becomes infected, as well as simulating the effect of vaccination on prevalence in the flock. Control options that can be used include vaccination, surveillance and quarantine and movement controls on sale/purchase of sheep. The effect of various control strategies can be evaluated by comparing simulations with and without the strategy in place, or with variations of several proposed strategies.

These models provide an opportunity to investigate the effects of assumed realistic values on the rate of spread of infection. In addition, the models allow estimation of the likely costs of disease, and the effectiveness and cost-benefit of proposed control strategies, particularly at the farm level. As more precise estimates of the values of key parameters become available, the models will allow a rapid assessment of the likely impact of these values on our understanding of the disease.

Key outcomes:

- OJD-related mortalities could range from 2-15% per year, depending on environmental factors and time since infection on individual farms
- Mortality rates could take 10-20 years to reach peak levels in most infected flocks
- Vaccination appears to provide the most cost-effective control of OJD in infected flocks
- Widespread vaccination would provide more effective control of OJD than surveillance and quarantine of identified infected flocks.

OJD.027A Modelling of OJD – Extension of project to include economic analysis and preparation of an extension package

AusVet Animal Health Services Pty Ltd
April 2003

Abstract

Modifications were made to the OJD Flock Model (developed in OJD.027) to support economic analysis of control options. A range of scenarios were then developed for both flock and regional models, in consultation with State departmental and industry representatives, and were provided to MLA for incorporation into advisory material for use by affected producers and their advisers.

Key outcomes:

- Vaccination appears to provide the most cost-effective control of OJD in infected flocks
- Vaccination might not be cost-effective in low-prevalence flocks or in the first few years of infection, when losses are relatively low.

3 Eradication of OJD from infected flocks

OJD.001 Evaluation of eradication strategies for ovine Johne's disease

**NSW Department of Primary Industries
February 2005**

Abstract

A field evaluation of the biological efficacy and economic viability of destocking and restocking strategies to eradicate OJD from 44 farms in south eastern Australia was conducted. Farms were destocked of all susceptible species for 15 to 21 months, including two summers, before restocking with at least 700 monitor sheep purchased from flocks complying with OJD Market Assurance Program disease risk assurance standards. Flocks were monitored for evidence of OJD for the next three years. 28 of 41 flocks (68%) monitored to the completion of the study had evidence of OJD infection three years after restocking. Eradication failures appear to be primarily of local origin (ie reinfection from neighbouring flocks and/or incomplete decontamination). Efforts to identify infectious refugia in the summer prior to restocking yielded only one positive culture from 279 samples of topsoil, dam water and dam sediment. There was a 20 fold reduction in the mean apparent prevalence of infection between destocking (18%) and three years after restocking (0.9%) on the reinfected farms. Twenty year simulations of net farm income based on data derived from participating farms revealed that destocking and restocking was less profitable than vaccination as an OJD management option.

Key outcomes:

- Destocking was not a reliable means of eradication of OJD from infected farms under commercial conditions
- Most flocks that failed to eradicate infection were probably re-infected by either reinfection from infected neighbours or by failure of decontamination.

4 Cross Species Transfer of OJD

TR.050 Prevalence of Johne's disease in rabbits and kangaroos

University of Sydney
December 2000

Abstract

Following reports from Scotland that rabbits on JD-infected farms were infected with *M avium* subsp *paratuberculosis* (Mptb) 300 rabbits and 300 kangaroos on 10 NSW farms grazing OJD-affected sheep flocks were examined for evidence of JD between 1996 and 2000. No evidence of JD or Mptb was found in any rabbit. One kangaroo had evidence of low numbers of Mptb in faeces but histopathological examination revealed no evidence of active infection. It was concluded that the bacteria in this animal had been ingested from pasture contaminated by OJD-infected sheep and had survived passage through the gut.

The prevalence of JD in rabbits and kangaroos on OJD-affected farms in Australia is considered very low (less than 1% of the adult population) or zero. Nevertheless, we recognise that there is a risk that adaptation of the organism to wildlife hosts could occur in future. The positive finding of Mptb in faeces from one kangaroo also implies there is a risk of physical transfer of Mptb from one farm to another by kangaroos. These results, together with overseas findings, suggest that further research should be conducted into the host specificity of strains of Mptb and the species studied should include common domestic farm animals and the rabbit and kangaroo.

Key outcomes:

- Infection of rabbits or macropods is unlikely to be of epidemiological importance under Australian conditions.

TR.054 A survey of potential wildlife reservoirs for *Mycobacterium paratuberculosis*

Department of Natural Resources and Environment, Victoria
June 2000

Abstract

The presence of a common wildlife reservoir of Johne's disease (JD) could render eradication impossible or compromise current control measures. In Scotland, rabbits have been shown to be infected with *M. paratuberculosis* (Mptb). To assess the risk posed to JD control by wildlife this survey examined 310 rabbits, 4 hares and 3 feral goats for the presence of Mptb in 3 different areas where JD was present in livestock. No Mptb was confirmed in any of the animals sampled. The lack of any evidence of JD in the rabbits surveyed suggests that, under the conditions examined, rabbits are unlikely reservoir hosts for Johne's disease. This survey does not rule out that rabbits may occasionally become infected with JD, but if this does occur it is likely to occur in low enough numbers as to not be a significant risk to farmed livestock. Due to the limited range of farms and environments in this survey it is impossible to rule out the presence of a 'focus' or 'hotspot' of JD in rabbits as exists in Scotland.

Key outcomes:

- Infection of rabbits or macropods is unlikely to be of epidemiological importance under Australian conditions.

OJD.005 Cross species transmission of Ovine Johne's Disease – Phase 1

**NSW Agriculture
June 2000**

Abstract

Johne's disease was investigated in fibre goats on several farms. The disease was caused by sheep [S] strains of *Mycobacterium avium* subsp. *paratuberculosis*. The infection appeared to be less severe than the same infection in sheep in that fewer goats than sheep became infected, and fewer goats than sheep developed obvious signs of the infection. However, infected goats shed the organism in their faeces and therefore were able to spread the infection to other goats and sheep. Therefore inclusion of goats in the control program for ovine Johne's disease is justified. A communication program is recommended to advise producers that ovine Johne's disease in goats may not be obvious and that testing should be undertaken to ensure disease is not present. The impact of ovine Johne's disease on the fibre goat industry is projected not to be great due to the small number of herds likely to be infected.

Key outcomes:

- Goats can be infected with OJD, and must be considered in any control or eradication program.
- OJD appears to be less severe (both clinically and pathologically) in goats than in sheep.

OJD.016 Cross species transmission of ovine Johne's disease – Phase 2 cattle

**NSW Agriculture
September 2002**

Abstract

One thousand seven hundred and seventy four cattle from 12 properties were sampled by ELISA and faecal culture to detect Johne's Disease. All properties had a known significant history of Johne's disease in sheep. They were mostly selected from participants in OJD.001 (a study to examine the effectiveness of an eradication strategy) and had cattle known to be susceptible to JD at the time that their properties were infected with OJD. All gave negative results on serology; only one animal from a herd of 349 gave a single positive faecal culture result, with all follow-up investigations being negative, suggesting passive transfer of the organism. Due to the small size of some of the herds tested, and the fact that no confirmed infected animals or herds were detected, it is not possible to give a maximum estimate of the prevalence of OJD in exposed susceptible cattle. However, using information derived

from previous investigations and some additional results in the addendum to this project report, it is known that there are at least 6 cattle herds infected with 'S' strain, in NSW, at the present time.

Key outcomes:

- Cattle can become infected with OJD but infection appears to be a relatively uncommon event.
- Grazing of young calves on pastures contaminated with OJD should be avoided

5 Detecting OJD

TR.060 Development of a rapid cost effective test for ovine Johne's disease based on testing of pooled faeces

NSW Agriculture
June 2000

Abstract

This project was undertaken to develop and evaluate a rapid, cost-effective, flock test for *Mycobacterium paratuberculosis* in pooled faecal samples, based on hybridisation-capture polymerase chain reaction (HC-PCR). However, a simpler direct technique (DPCR) was found to be more sensitive than HC-PCR. About 67% of culture positive pooled faecal samples were positive when tested using DPCR. In a blind trial, 83% of 12 farms identified by culture of pooled faecal samples were detected using DPCR. The cost of DPCR is no greater than that of other flock detection strategies. The test is suitable for use in the National Ovine Johne's Disease Control and Evaluation Program. A constraint exists in that Veterinary Committee does not recognise the results of DNA-based tests for *M. paratuberculosis* as being definitive. The costs of follow-up testing to confirm infection are high. Recommendations are made to improve the test and reduce its cost.

Key outcomes:

- Direct PCR on pooled faeces provides a less sensitive but more rapid alternative to pooled faecal culture for flock-testing for OJD.
- Further research and refinement of the test is likely to result in improved sensitivity of direct PCR on pooled faeces

TR.073 Pilot study: Tracer weaner trial for ovine Johne's disease

NSW Agriculture
June 2000

Abstract

Eradication of ovine Johne's disease requires destocking sheep and spelling land until *Mycobacterium avium* subsp. *paratuberculosis* (Mptb) has died out. There is no simple way to assess the residual level of contamination or the risk this poses to livestock. Consequently small groups of Merino weaners were orally dosed with Mptb. Within 7-14 weeks the organism could be cultured from various locations in the gastrointestinal tract and associated lymph nodes. In addition, the results of a skin test were positive in 66% of weaners in which the organism had established an infection and were negative in uninfected sheep. The results of tests for gamma interferon were positive in some infected weaners but also in some of the controls. An ELISA test was not useful at this early stage of infection. Overall, the results suggest that weaner sheep could be used as sentinels in an infected environment, but this requires validation in a controlled field trial. Furthermore, the results suggest that the infectious dose of Mptb may be higher than previously thought.

Key outcomes:

- The OJD organism could be cultured from tissues of weaner sheep within 7-14 weeks of oral exposure.

OJD.007 Evaluation and comparison of two methods of abattoir surveillance for detection of ovine Johne's disease

NSW Agriculture
June 2001

Abstract

This project was designed to determine whether properties infected with OJD could be identified by routine monitoring of cull sheep at abattoirs for lesions of OJD or presence of *Mycobacterium avium subsp. paratuberculosis*. Abattoir inspectors detected gross lesions suggestive of OJD in 34 (97%) of 35 trial lines. The only trial line in which lesions were not detected by inspectors was a line of cross-bred ewes introduced as adults to a property on which OJD had only been reported at low prevalence. Pooled intestinal culture (PIC) was also conducted on 10 randomly selected sheep from each line. PIC detected as OJD positive only 18 (53%) of the 34 lines detected by visual examination. The results of this trial indicate that visual and tactile monitoring of the viscera of cull sheep at slaughter for lesions suggestive of OJD is a highly sensitive and reliable strategy for the detection of infected flocks in which deaths from OJD are occurring. Whether abattoir screening will be sufficiently sensitive for routine detection of flocks that have been recently infected with OJD or flocks with persistent low prevalence of infection is less certain.

Key outcomes:

- Visual and manual examination of sheep intestines at slaughter (abattoir surveillance) is a highly effective method for detection OJD infection in infected flocks.

OJD.014 Field evaluation of the tracer weaner model

NSW Agriculture
November 2002

Abstract

Experiments were conducted on two separate farms in the endemic area for OJD to determine whether tracer animals could be used to detect infective levels of S strain *M. a. paratuberculosis* (Mptb) on pasture. Culture from tissues was shown to be the most sensitive method for the detection of early infection in sheep after natural exposure to S strain Mptb. The organism was detected in at least one naïve introduced sheep from every potentially exposed group, 6 to 12 months post-exposure. Antemortem diagnostic tests (skin testing, IFN- γ and faecal culture) were shown to have low sensitivity at this early stage of naturally acquired disease. The prevalence of infection early after exposure was similar in sheep first exposed as neonates, as weaners or as adults. Lambs born from an infected flock became tissue culture-positive sooner than naïve lambs suckling uninfected ewes introduced to the same infected environment. These findings suggested that groups of naïve sheep used as tracer animals and tested by tissue culture at slaughter after 6 months exposure, might be useful to assess pasture infectivity in disease control programs.

Key outcomes:

- The OJD organism could be cultured from tissues of weaner sheep within 6-12 months following exposure to contaminated pastures.
- Other tests at this time had a low sensitivity for detection of infection.

OJD.020 Individual animal tests for ovine Johne's disease

University of Sydney
March 2004

Abstract

Routine diagnostic tests for ovine paratuberculosis have poor sensitivity in the early stages of the disease, and transmission often occurs before detection. Currently there are no tests to accurately confirm early infection in individual sheep. Such tests are required to provide trading opportunities for producers who may have valuable stock at low risk of infection. Surgical biopsy is one means of disease detection using relatively sensitive laboratory procedures, but was unproven. 77 sheep grazing on a heavily infected farm were examined at 12, 18 and 24 months of age by histopathology and culture of biopsied ileum and mesenteric lymph nodes. Results from biopsy were compared to those from routine tests (ELISA, AGID, IFN- γ , skin testing, faecal culture and direct PCR) applied at six-monthly intervals, and to necropsy findings at three years of age. A total of 170 biopsies were performed without serious complications, and the samples collected were adequate for culture and histological assessment of paratuberculosis. Overall, 36% of sheep were shown to be uninfected at 3 years of age. Of these, 16 were uninfected at all sampling times and 11 sheep had recovered (ie. they had been infected at an earlier sampling). The remaining 64% of sheep were classified at necropsy as infected. These included 36 sheep with subclinical OJD (6 of these had severe diffuse lesions, and 30 had milder lesions), and 11 sheep which had died of OJD. Biopsy was consistently the most sensitive non-lethal 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 of individual valuable sheep from infected stud flocks. Because some sheep do recover from early infection with *M. a. paratuberculosis*, early testing at 12 months of age, even with a sensitive test such as culture of biopsied tissue, would not be predictive of later infection status. By 18 to 30 months old, positive test results for CMI, humoral immunity or faecal excretion, were often highly associated with later death from OJD, although not often with infection status at 3 years. The lack of statistical association with final infection status reflects the low sensitivity of these tests at this early stage of the disease.

Key outcomes:

- Tissue culture from surgically biopsied tissues was the most sensitive (non-terminal) method for early detection of OJD in naturally infected sheep but still only detected two thirds of infected sheep.
- A significant proportion of naturally infected sheep recovered from infection and were not detectably infected at 36 months of age, despite having been confirmed as infected on previous testing occasions.

OJD.022 Evaluation of PFC and AGID as flock-screening tests for OJD

**AusVet Animal Health Services Pty Ltd
November 2001**

Abstract

A Monte Carlo simulation model was developed to estimate the sensitivity of pooled faecal culture (PFC) and the agar-gel immuno-diffusion test (AGID) as flock-screening tests for OJD under a range of scenarios. The flock-sensitivity of a test is the level of confidence of detecting a specified prevalence of infection. The mean flock-sensitivities for a Check Test (sample size = 100) were 67% and 42% for PFC and AGID respectively, and for a Sample Test (sample size = 350 for PFC, 500 for AGID) were 98% and 93% respectively. When large flocks were sampled, sample sizes of 300, 350 and 450 provided a flock-sensitivity for PFC of about 95%, 98% and 99% respectively, to detect infection if present at a prevalence of 2% in the sampled population. Sample sizes for the AGID to provide equivalent sensitivity to PFC were generally 2 – 3 times the PFC sample size, depending on the assumed animal-level sensitivities of the tests. When whole-flock testing was simulated, AGID flock-sensitivity was generally poor for smaller flock-sizes and low prevalence or animal-level sensitivities, whereas PFC flock-sensitivity remained high. The flock-sensitivity for PFC was $\geq 98\%$ for all combinations of flock size, prevalence, percentage of paucibacillary lesions and animal-level sensitivities tested compared to $\geq 68\%$ for the AGID.

Although the AGID appears to perform reasonably well in higher prevalence flocks, its flock-sensitivity in low prevalence or recently infected flocks is likely to be very low, unless sample sizes 2 – 3 times those used for PFC are used. This is particularly important in Australia at present, as the majority of flocks being investigated outside the Residual Zone (endemic area) are likely to be relatively recently infected and still have only a low prevalence of infection. In these circumstances, PFC should be the preferred test, and larger sample sizes or whole-flock testing should be considered to maximise flock-sensitivity. In higher prevalence flocks, such as many of those in the Residual Zone, the AGID will provide a satisfactory flock-sensitivity and a more-rapid result than PFC, particularly if biased sampling is used to maximise animal-level sensitivity and provided sample sizes are adequate.

Key outcomes:

- Pooled faecal culture is substantially more sensitive as a flock-screening test than serology using comparable sample sizes.
- A sample size of about 875 is required for serology to provide a similar flock-sensitivity to PFC with a sample size of 350.

OJD.025 Validation of the gamma-interferon test for diagnosis of ovine Johne's disease

**CSIRO Livestock Industries
September 2005**

Abstract

In theory, the interferon- γ (IFN) test has potential for improved control of ovine Johne's disease (OJD) by early detection before contamination of pasture and transmission of infection. To validate the IFN test, a project has been completed for determining specificity and sensitivity. Because of non-specific IFN responses, raised cut-points were required to achieve high specificity ($\geq 98\%$). This resulted in reduction of sensitivity to below 50%, limiting its application for early detection or certification from disease freedom. The assay may have application in a test and cull program as a surrogate test for faecal shedding and the removal of sheep with severe disease but this approach to control of OJD will require further confirmation. A major limitation for adoption is the widespread use of vaccination precluding the use of immunological tests for diagnosis. Other limitations, apart from relatively low sensitivity, include cost of the test as well as a narrow window of ~6 hours between the commencement of blood sample collection and laboratory initiation of the assay so that test result validity is not compromised. Thus, in its current format, it is unlikely, that the IFN assay, apart from possibly in stud flocks, will have wide application in the sheep industry.

Key outcomes:

- The gamma interferon assay is more sensitive than AGID or ELISA for the detection of OJD but is less specific.
- Lack of specificity, cross-reaction with vaccination and technical issues requiring rapid processing of samples limit the usefulness of this test under commercial conditions.

OJD.029 Determination of individual animal-level sensitivity of abattoir surveillance for ovine Johne's disease

**Department of Primary Industries Victoria
September 2002**

Abstract

This project aimed to estimate the sensitivity of abattoir surveillance for ovine Johne's disease (OJD) at an individual animal level. Currently abattoir surveillance for OJD occurs in 21 meatworks around Australia and since late 1999 over 16 million sheep have been examined by this method. Abattoir inspection is an extremely efficient and economical method of regional surveillance for OJD but could also be used for negative assurance purposes if the sensitivity were known. The trial involved three inspectors working in different OJD prevalence areas and compared their diagnoses with the gold standard of histopathology for approximately 1200 sheep from known infected properties. The sensitivity level for the best inspector was 87.3%. A 'dummy run' of presumed negative sheep yielded a very low false positive rate. This trial has established that for the lines of sheep examined, an average sensitivity of 70% is applicable. However for the purposes of a flock negative assurance system in a low prevalence flock, a sensitivity level of 50% would be more appropriate. This potential use of this methodology for flock assurance purposes relies on an accurate identification system for sheep.

Key outcomes:

- On average, three inspectors were able to detect 70% of infected sheep from heavily infected flocks using visual and manual examination at slaughter.
- The ability of individual inspectors to detect OJD-infected sheep was highly variable, depending on experience.
- The sensitivity of this technique in lower-prevalence flocks is still unclear.

6 Preserving Genetic Material

OJD.012 Assessing the risks of transmitting OJD in the semen of rams by artificial insemination – A pilot study

Central Tablelands Rural Lands Protection Board
November 2000

Abstract

The impact on stud breeders of the current national program to limit the spread of OJD within the Australian sheep industry could be reduced if they could safely use semen from within their studs. Trading semen would be a means of maintaining some cash flow, and artificial insemination could be used to re-establish the studs' genotype within any destocking program.

This research has shown for the first time that semen collected from rams clinically infected with OJD can contain *M Ptb*, the bacteria that causes OJD, and that it is likely that bacteria could be transferred to the reproductive tract of ewes at mating or AI. These results indicate that quality assurance guidelines for the safe use of semen from infected flocks need to be developed further.

Key outcomes:

- Semen from infected rams with clinical signs of OJD can be infected with the OJD organism.
- Clinically affected rams could pose a risk of transmitting OJD through natural service or AI programmes