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

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 ovine Johne's disease. 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.

Executive Summary

Ovine Johne's disease is transmitted to young sheep mainly by the oral route from faeces from adult sheep. This cycle is hard to break. Control of ovine Johne's disease will depend on management practices that reduce the likelihood of infection transmitting between generations of sheep on a farm, and on preventing spread from farm to farm. While vaccination of lambs is an important strategy, it may not be economically justified or desired on all farms. Many producers also wish to manage pasture and will use grazing management to reduce disease transmission, but only if valid recommendations can be made.

The aim of this project was to determine whether pasture contamination rates and the age of sheep when they are first exposed to the infection influence the occurrence of OJD including its incubation period and the timing of diagnosis. The outcomes were targeted to improve understanding of the development of ovine Johne's disease, in particular the implications of exposure of sheep of different ages to different levels of contamination, and will facilitate development of control strategies based on pasture management.

This project complemented another on OJD epidemiology - preweaning versus post weaning exposure (OJD.002A) - utilising some of the same base infrastructure. The aim of OJD.002A was to determine at what time transmission was most likely to take place, and whether management of the pre-weaning and post-weaning pasture can be altered to reduce transmission. It was concluded that lambs could become infected both before and after weaning.

The specific objectives of the present project were to determine the interval between infection with *M.ptb* and excretion of the organism in relation to the size of the infecting dose and the age of sheep, to determine the effect of pasture contamination rate on the incidence of disease, to compare the efficacy of diagnostic tests (faecal culture, gamma interferon, serology) in young sheep and in the early stages of the disease, and to describe the epidemiology of OJD in a mixed age flock following simultaneous exposure of previously unexposed sheep to infection, particularly in relation to the first detectable evidence of transmission, the clustering of infection within age groups and the assessment of risk of infection of each age group.

The principle conclusions from this study were that post weaning lambs (median age 5.5 months at start of trial) were highly susceptible to infection with *M. paratuberculosis* and if exposed to high levels of contamination a proportion will develop severe infection leading to clinical disease and death. Hoggets and adult ewes are less likely than lambs to develop clinical disease after exposure to *M. paratuberculosis.* Sheep in all age classes may become infected with *M. paratuberculosis* and shed the organism in faeces, but this will happen more often in lambs than in older age classes. Nevertheless, even adult ewes may become infected and later act as a source for transmission of the disease. Lateral spread of OJD is a serious threat to control if control is based solely on management of pasture; it is not necessary for infected sheep to be present in a paddock for transmission of infection to occur if infected sheep are present in neighbouring paddocks. Conventional wire strand fences do not prevent spread of infection. For diagnosis on a flock basis, pooled faecal culture is more effective than the agar gel immunodiffusion assay for detection of the infection at relatively early stages in young sheep. It was surprising that pooled faecal culture detected infection in sheep only 6 months after first exposure to contaminated pasture, when they were 11 months of age.

The recommendations for testing to detect infection were dependent on the age of sheep.. The most appropriate strategy for flock testing after introduction of clean lambs (it is suggested that this include sheep up to 1 year of age) to infected pasture is to undertake PFC at 3 monthly intervals commencing 6 months after introduction. For older animals, PFC can be undertaken at 3 month intervals commencing 12 months after introduction, or alternatively, AGID can be undertaken commencing 18 months after introduction. Testing of potentially infected sheep introduced to infected or clean pasture should be based on prior knowledge of their status, but can commence immediately and then follow the guide above.

For control of OJD the following strategies were recommended. Minimise exposure of lambs to heavily contaminated pasture because they are the most susceptible age class. As adult sheep are more resistant to infection and disease impacts of OJD they should be grazed on contaminated pasture rather than younger animals. Cull adult sheep with clinical signs of OJD, or conduct PFC to obtain evidence of patent OJD infection and manage or cull these mobs to reduce the level of contamination across the farm. Manage pastures by spelling, grazing with adult cattle or grazing with adult sheep in order to reduce the contamination level for lambs, weaners and young sheep. Pasture spelling, which leads to 90% reduction in contamination levels for each month of spelling, should be undertaken where possible to reduce contamination levels from a high level to a moderate or low level. This should substantially reduce the proportion of sheep that develop clinical OJD. In vaccinated flocks, be observant and identify individuals that develop signs of weight loss and cull them to reduce the level of contamination. Maintain boundary fencing to reduce the likelihood of lateral transmission from an infected neighbour. As a single fence line does not prevent lateral transmission, consider double fencing with wide laneways or segregation of critical pastures using a tree belt, gully or other natural feature. Maintain high value lambs in paddocks well away from paddocks known to contain infected sheep.

The results of this study will have immediate impact on the management and control of OJD as they support and extend current recommendations for livestock grazing management by providing experimental data. Some of the current recommendations are based on findings from previous experiments on disease transmission in project OJD.002A, and survival of *M. paratuberculosis* on pasture in project TR.055A, but others are based mainly on professional opinion. There has been no prior research on the impact on OJD occurrence of age at first exposure and levels of pasture contamination. However, it is now possible to state with certainty that lambs should not be exposed to high levels of contamination if clinical disease and relatively high rates of faecal shedding and hence further disease transmission are to be avoided. Lambs in this research project had a median age of 5.5 months. It is still uncertain how long lambs remain fully susceptible but a reasonable working hypothesis would be to avoid grazing sheep on heavily contaminated pastures during the first year of life. Also of great benefit to industry is the finding that hoggets and adult sheep were relatively resistant to the clinical effects of OJD. Even though infection occurred, animals exposed for the first time as hoggets or as adults rarely succumbed to the disease. Hoggets (median age in this study 22.5 months) and adult ewes were relatively resistant. This means that hoggets 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. In the future, strategic recommendations on pasture management, and specific programs of rotational grazing utilising the known susceptibilities of each age class will be combined with vaccination to greatly improve overall management and control of OJD.

Contents

1 Background

Ovine Johne's disease is transmitted to young sheep mainly by the oral route from faeces from adult sheep. This cycle is hard to break. Control of ovine Johne's disease will depend on management practices that reduce the likelihood of infection transmitting between generations of sheep. While vaccination of lambs is an important strategy, it may not be economically justified or desired on all farms. Many producers also wish to manage pasture and will use grazing management to reduce disease transmission if valid recommendations can be made.

The aim of this project was to determine whether pasture contamination rates influence the occurrence of Johne's disease, its incubation period and the timing of diagnosis in sheep of different ages. The outcomes will provide improved understanding of the development of ovine Johne's disease, in particular the implications of exposure of sheep of different ages to different levels of contamination, and will facilitate development of control strategies based on pasture management. If infection can be shown to be strongly clustered within age groups, partial flock destocking may become an option for eradication.

This project complements an existing project on OJD epidemiology - preweaning versus post weaning exposure (OJD.002A)[1], utilising some of the same base infrastructure. The aim of this first experiment was to determine at what time transmission was most likely to take place, and whether management of the pre-weaning and post-weaning pasture could be altered to reduce transmission. It was found that transmission to lambs occurred both before and after weaning.

2 Project Objectives

1. To determine the interval between infection with *M.ptb* and excretion of the organism in relation to the size of the infecting dose and the age of sheep.

2. To determine the effect of pasture contamination rate on the incidence of disease.

3. To compare the efficacy of diagnostic tests (faecal culture, gamma interferon, serology) in young sheep and in the early stages of the disease.

4. To describe the epidemiology of OJD in a mixed age flock following simultaneous exposure of previously unexposed sheep to infection, particularly in relation to the first detectable evidence of transmission, the clustering of infection within age groups and the assessment of risk of infection of each age group.

5. To provide the following outcomes to the satisfaction of MLA:
   - A comparison of infection rates under three levels of pasture contamination: low, medium and high

- A comparison of infection rates of lambs, young ewes and older ewes 2.5 years after first exposure
- A determination of the interval between exposure to infection and excretion in relation to the age of sheep and degree of pasture contamination
- A comparison of faecal culture, cell mediated immunity and serology as diagnostic approaches in sheep recently exposed to infection
- A determination of the efficacy of diagnostic tests soon after infection (0-2.5 years), with particular reference to young sheep
- Recommendations regarding flock testing after introduction of clean sheep to infected pasture. Interpretation of the reverse situation will also be made, and
- Recommendations for control of OJD based on knowledge of age-based clustering of infection.

3 Materials and Methods

3.1 Overview

A total of 840 female Merino sheep of three age groups, lambs, hoggets and adults were obtained from an uninfected property and co-grazed as mixed-age groups in 20 hectare paddocks for 14.5 weeks with infected sheep stocked to provide four levels of contamination, control (very low), low, medium and high (Table 3.1). Each of the contamination treatments was replicated once. The animals remained in the experiment for 2.5 years and were then euthanased and sampled to establish the prevalence of infection with *Mycobacterium paratuberculosis*. Pooled faecal culture and agar gel immunodiffusion assays were conducted on all sheep at regular intervals during the trial, while individual faecal culture, ELISA and gamma-interferon assays were applied to subgroups at some time points.

Table 3.1. Outline of experimental design. Each paddock contained mixed age classes.

Group: age	Treatment: contamination level								
	Control		Low		Medium		High		Total
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	
Stocking rate of donor sheep (sheep/Ha)	0.0	0.0	0.1	0.1	0.35	0.35	0.7	0.7	
Total infected sheep	0	0	2	2	7	7	14	14	48
Lambs	35	35	35	35	35	35	35	35	280
Hoggets	35	35	35	35	35	35	35	35	280
Adults	35	35	35	35	35	35	35	35	280
Total clean sheep	105	105	105	105	105	105	105	105	840

This experiment followed on from an earlier trial, OJD.002A. However, the results from OJD.002A were not available until after the start of this trial. Therefore the experimental design for OJD.028 could not be based on data flowing from the previous experiment.

3.2 Experimental Design

3.2.1 Experimental site

The experimental site was at the University of Sydney farm "Arthursleigh", Marulan, NSW. This property is in the eastern part of the southern tablelands district of NSW and is approximately 20 kms east of Goulburn.

Construction of the facility commenced in June 1999 for experiment OJD.002A and was completed in early September the same year. It consisted of 200 Ha of pasture contained within 23 km of secure fencing, of which 19.5 km was erected in 1999. Further work to extend and merge existing paddocks was undertaken between November 2001 and February 2002. The new site was completed on 8th February 2002.

The site used for OJD.028 comprised eight 20 hectare paddocks and a laneway system. Within the structure of the site, two replicates existed. Paddocks were identified by contamination (treatment) group and replicate. The paddock design is illustrated diagrammatically in Appendix 2. Erosion gullies were fenced to exclude livestock and these and the laneways were incorporated in ways which assisted in the separation of paddocks with different levels of OJD contamination. Note that paddocks C1 and L1 and paddocks C2A and H2A were separated only by standard fences.

The pasture on the experimental site was unimproved, consisting of native grasses and volunteer introduced species and weeds, including serrated tussock (*Nassella trichotoma*). Paddocks varied in slope from near-flat to approximately 1 in 30 and also varied in aspect (northerly or southerly). Differences between replicates in pasture species, slope and aspect were not systematic as there were areas of different type within each replicate but there were differences between paddocks within replicates.

There were few shade trees on the site and, generally, all or most parts of all paddocks did not have shade. There was a small amount of significant shade provided by groups of trees in control replicate 1.

A reticulated water system was installed to service the original OJD.002A trial paddocks. Water was originally reticulated to a concrete trough in each paddock from a tank above the site, which was in turn supplied by petrol-powered pump from a dam within the site. The dam was fenced-off from stock and lay adjacent to control replicate 1. Reticulation was extended in December 2001 and January 2002 to supply all OJD.028 trial paddocks.

In October 2002, measurements of available dam water were taken and the quality of the water assessed. Due to a lack of significant rainfall plus deteriorating water quality it was necessary to commence transporting water by vehicle from the Wollondilly River on 25th November 2002. Predicting a continuing problem with water supply, new reticulation was installed between 2nd December 2002 and 17th February 2003 to enable water to be pumped from the Wollondilly River. All water for stock was supplied from the Wollondilly River from 17th February until the end of the project.

3.2.2 Experimental sheep

Donor sheep. Infected sheep were sourced from a high prevalence property in the Southern Tablelands of NSW. On 24th December 2001 a total of 781 sheep from this property were bled and serum was tested in the AGID test for antibodies against *M. paratuberculosis*. There was a seroprevalence of 19.8%. The sheep were shorn and treated for lice off shears. Seventy-eight seropositive sheep were selected for inclusion in the experiment as donors, purchased, transported to Arthursleigh on 24th December 2001, held in isolation paddocks within the site and managed using routine farm management pending Animal Ethics Committee (AEC) approval. On 1st April

2002 the donor sheep were allocated randomly to paddocks at rates of 14, 7, 2 and 0 sheep per replicate which represented high, medium, low and very low (control) levels of pasture contamination, respectively. Faecal samples were collected as sheep entered the paddocks to confirm the initial shedding status of each donor sheep by culture. Due to the lengthy incubation period required for isolation of *M. paratuberculosis,* the faecal culture results for donor sheep were not available to the researchers during the period of contamination, and no adjustments to stocking donors were possible.

There was a high mortality rate (42%) among donor sheep between their arrival at Arthursleigh and their allocation to treatment groups with uninfected sheep. There was also a serious mortality problem at the home property (R Churchill, personal communication), but it is possible that the stress of transport and change of location or management increased the rate of progression of the disease. It was therefore necessary to source additional infected animals for replacement of donor sheep expected to die during the period of co-grazing with uninfected sheep. Potential replacement donors were identified during a farm visit for project OJD.015 to another southern tablelands property that had Merino sheep with a high prevalence of deaths due to OJD. These sheep were vaccinated so serology could not be used to confirm infection status. Therefore selection of animals was based on clinical appearance, condition score and existing results from project OJD.015 on the prevalence of faecal shedders in age groups within the flock. Replacement donors arrived at Arthursleigh on 7th May 2002 and faecal samples were collected as sheep were unloaded from the truck. Faeces were submitted for a ZN smear and also stored for culture. Sheep were held in isolation paddocks and treated with Zap for lice control while awaiting ZN smear results. Shedders were identified and added to experimental paddocks as the original donor sheep died. Prescribed stocking rates with infected sheep were maintained in this way over the 14.5 week contamination period. All donor sheep were managed in the same way as the uninfected sheep following their introduction to the experimental paddocks on 1st April 2002. On 11th July 2002 donor sheep were removed from the paddocks. They were held in an isolation paddock within the site and faeces and blood were collected. Donors were slaughtered on 1st August 2002 at Wollondilly abattoir where samples of ileocaecal junction and terminal ileum were collected to provide additional information on their infection status.

Uninfected sheep. Considerable effort was required to locate suitable sheep in the necessary age classes from a single farm with a history of testing for freedom from OJD. In early December 2001 a suitable property in the Armidale district of New South Wales was identified. All sheep in this flock were tested to MN1 status with stud rams tested to MN2. The property had a history of good management including participation in a footrot breeding and eradication scheme and participation in the NEMESIS program for endoparasite control. Joining on the property is from late April with a six-week lambing period expected to occur between 24th September and 4th November each year. Based on this information and to enable age estimates, the median lambing date was estimated to be 15th October. Sheep were selected from lamb, hogget and ewe mobs. The lambs were aged 8-12 weeks, hoggets 2 years and adult ewes 3-6 years. On 24th January 2002, blood samples were collected from 934 non-pregnant females in each of the required age classes. Teeth, skin, foot and age examinations were undertaken and ear tag numbers were recorded. ELISA tests were undertaken by CSL. There were two reactors, which was within the aggregate number of reactors predicted for an uninfected flock based on the known specificity of this test. The two ELISA positive samples were negative in the AGID. 840 of these tested sheep were selected, purchased and transported to Arthursleigh on 11th February 2002. They were held at Arthursleigh on clean pasture that had been grazed only by cattle, in isolation from the experimental site and the OJD-infected

commercial Arthursleigh flock. The sheep were drafted on the basis of age, crutched and drenched in three individual mobs at the Mount Pleasant wool shed adjacent to the Arthursleigh farm on 13th and 14th February. Blood was collected from selected sheep for gamma-interferon diagnostic tests.

Age group mobs were stratified on the basis of body weight and randomly allocated to treatment groups. On 15th February the uninfected sheep were transported to designated paddocks on the experimental site. Sheep were stocked at 105 per treatment group, comprising 35 weaners, 35 hoggets and 35 adults. There were 2 replicates of each of the 4 treatment groups. Animals were set-stocked into the paddocks to which they had been allocated, except for paddocks H2A/H2B and C2A/C2B which were pairs of 10Ha paddocks separated by a laneway and a gully, respectively. The donor sheep in H2A were swapped for the donor sheep in H2B mid-way through the 14.5 week contamination period. Thereafter, sheep in H2A were swapped with sheep in H2B and sheep in C2A were swapped with sheep in C2B annually at each shearing.

Sheep health and numbers were confirmed at the time of allocation of the specified number of infected donor sheep to the experimental paddocks on 1st April 2002. All sheep were again individually examined for any health issues, ear tags were checked and faecal samples were collected on 4th April 2002.

3.2.3 Contamination rate measurement

The prior grazing history of each paddock is provided below in Table 3.2. In summary, there had been no grazing of sheep with OJD on the experimental paddocks for at least 6 months, including 1 summer. In the case of control treatments, there had been no grazing with potentially infected sheep for at least 16 months. Based on data from MLA project TR.055A (Survival of Johne's disease in the environment), the levels of contamination on pasture would be expected to decline by about 90% within 1 to 2 months. Therefore it was assumed that pastures had very low levels of contamination at the start of the trial.

Table 3.2. History of grazing of paddocks prior to trial OJD.028

Paddock	Treatment	History of grazing	Decontamination interval
1.10new	C1	Cattle only for 4 yrs	> 4 yrs
1.10/2.1	C2	OJD infected ewes for 4 weeks in Oct 2000, then low numbers of weaned lambs in early 2001	16 months
1.9new	L1	OJD infected ewes in Aug-Sep 2000, then uninfected ewes until Jan 2001	17 months
2.8/2.9	L2	OJD infected ewes in Aug-Sep 2000, then uninfected ewes, then Arthursleigh sheep (potentially OJD infected) until Aug 2001	8 months
1.8new	M1	OJD infected ewes in Aug-Sep 2000 then uninfected ewes, then infected ewes in autumn 2001, with a few remaining until Jun 2001	10 months
2.10	M2	Arthursleigh sheep (potentially OJD infected) until Aug 2001	8 months
1.2/1.3	H1	OJD.002 infected ewes until October 2001	6 months
2.2/2.3	H2	OJD.002 infected ewes until October 2001	6 months

It was assumed that contamination commenced on the day donor sheep were introduced to the experimental paddocks in proportion to the number of sheep with patent infection and the number of days these sheep were present. The decay rate of *M. paratuberculosis* was not taken into account in the calculation of contamination rates.

Contamination rates for each treatment paddock were expressed as:

> No. patent donor sheep days = sum across donors of the number of days each donor with patent infection was present between 01.04.02 and 11.07.02.
>
> Patent infection was defined as being present for the duration of the contamination period for that animal when it met one of the following criteria: individual faecal culture positive; Perez histopathology score of

3a or 3b; AGID positive and died. Animals that were AGID positive but individual faecal culture negative were defined as having non-patent infection.

3.2.4 Impact of M. paratuberculosis infection

The impact of infection was measured over time in a variety of ways:

- pooled faecal culture (PFC) with sheep in pools of 5
- individual faecal culture (IFC) on sheep present in a PFC positive pool
- agar gel immunodiffusion (AGID) assay
- mortality
- demonstration of infection at post mortem examination after 2.5 years, using culture of intestinal tissues and mesenteric lymph node
- demonstration of infection and severity of infection at post mortem examination after 2.5 years using histopathology. Lesions of score 3a and 3b (Perez system) were defined as severe because groups of sheep with these lesions experienced loss of body condition in an earlier experiment (OJD.002A).

Each of the above measures provided a means to estimate frequency of infection with *M. paratuberulosis* in each age class and treatment. The occurrence of disease, as distinct from infection, was more difficult to ascertain, but mortality rate due to OJD, and presence of severe histological lesions of OJD (Perez score 3a and 3b) were used as measures of disease. The distinction between infection and disease is important because not all cases of infection will proceed to clinical disease.

3.2.5 Biosecurity

The experimental sheep were restricted to their designated paddocks except during transport to and from the Arthursleigh shearing shed and at the conclusion of the experiment. When sheep needed to be moved a contract driver and truck was used. Sheep were loaded at the paddock gateway, inside their paddock. Only one treatment group per load was transported. This occurred for shearing and at the end of the trial. The contractor washed the truck wheels and slats between treatment groups and before returning to the site. Vehicles did not enter paddocks except where emergency access was required to transport an injured or recumbent sheep. Whenever feasible, vehicles were parked outside paddocks and materials were carried into the paddocks. For frequent tasks like supplementary feeding, vehicles remained in the laneways and feed was delivered by hopper into the paddocks through the fence. There was no evidence of rabbits on the site. Wombats were often seen on the site and sometimes caused disturbance to fencing. Occasionally an emu or a wallaby was seen in a laneway.

One accidental breach of biosecurity occurred between allocation of the sheep to their paddocks (checked 04.04.02) and the first sample period (25.06.02). It was discovered that one animal (white tag, control sheep 190) had jumped the fence between Control replicate 2A and the high treatment group replicate 2A (Appendix 2). The sheep was not returned to its original paddock but remained with its new treatment group until the end of the trial.

3.2.6 Husbandry procedures

Animal identification. All sheep were identified by the application in each ear of a plastic numeric ear tag (unique identifier, same number in each ear). Tags were applied and records made of tag numbers when sheep were allocated to treatment groups. A different coloured ear tag was used for each treatment group.

Worm control. All donor and uninfected sheep were drenched with cydectin prior to the start of the trial. A program for endoparasite control throughout the trial included routine monitoring of worm burdens through faecal egg count testing at 6-8 week intervals or as determined by conditions and health of the experimental animals. Faecal egg counts were done on 10 animals per age group across all three age groups in each paddock. Sheep were drenched strategically based on the results of these tests, climate and season. Faecal egg count reduction tests for drench resistance were conducted periodically throughout the trial. Ivomec or cydectin was used routinely on all sheep except where haemonchosis was suspected when closantel was used strategically.

Fly-strike prevention. Fly strike was prevented by the administration of dicyclanil as a spray-on backline treatment (Clik®, Novartis Animal Health) prior to the period of highest fly activity between November and June.

Crutching. All experimental sheep were crutched prior to their entry to the experiment and then each season. Crutching was performed using a mobile shearing plant taken to each paddock.

Shearing. The experimental sheep were shorn three times at approximately 12-month intervals in October 2002 and in September 2003 and 2004. Shearing was performed at the Arthursleigh wool shed for all sheep except control groups which were shorn in their paddocks with a mobile shearing plant. Sheep were transported to the shed within treatment groups and held there on slats overnight. Immediately following shearing they were returned to their paddocks. All sheep were shorn for the final time prior to slaughter. They were transported to the shed and held on slats overnight, shorn and then transported directly to the abattoir following a further night on slats, off shears.

Vaccination. Experimental sheep were vaccinated as lambs against clostridial diseases using 5 in 1 vaccine which was administered at the properties of origin as part of normal husbandry.

Supplementary feeding. Hand feeding commenced on 15th July 2002 due to pasture shortage. As each paddock contained mixed age groups the management of sheep onto hard feed was intensive. Management of feeding rates was governed by pasture availability and body condition of sheep, which was monitored weekly from early November 2002. Combinations of peas, oats, lupins, wheat and hay were mostly used. Selection of feedstuff and rations was based on market price, availability and suitability of feedstuff to provide nutritional requirements. Availability of pasture improved in all paddocks with significant rainfall in mid February 2003. With further significant rainfall and pasture growth in mid - late April 2003, use of hard feed reduced significantly. However, with colder temperatures in early winter, pasture growth slowed and hand feeding resumed. In the later stages of the experiment, pasture availability became critical due to on-going drought conditions. Hay was provided intermittently throughout the experiment for paddocks with extremely low pasture availability.

3.2.7 Collection and handling of specimens

Sample collection times and sheep ages at each time point are shown in Table 3.3.

Table 3.3. Sample collection times, days since first exposure and sheep ages

Sampling period	Sampling date	Days since first exposure	Median age (days)		
			Lambs	Hoggets	Ewes
-1*	11 Feb 2002				
First exposure	01 Apr 2002	0	168	898	1811
1	25 Jun 2002	86	253	983	1896
2	24 Sep 2002	177	344	1074	1987
3	09 Dec 2002	253	420	1150	2063
4	07 Apr 2003	372	539	1269	2182
5	23 Jun 2003	449	616	1346	2259
6	08 Sep 2003	526	693	1423	2336
7	15 Dec 2003	624	791	1521	2434
8	15 Mar 2004	715	882	1612	2525
9	14 Jun 2004	806	973	1703	2616
10	08 Sep 2004	892	1059	1789	2702
Slaughter	13 Sep 2004	895	1064	1794	2707

* samples for CSIRO only

Faecal samples. Sheep were held in a temporary raceway within their paddock and faeces were collected from each individual. Samples of 1 – 2 g were obtained by manual expression from the rectum, using a new latex glove for every sheep. Each animal was released back to the paddock immediately after sampling was completed. The interval between sampling events was approximately three months. The sample container was labeled with individual animal tag number, together with a pre-allocated laboratory accession number and collection date. Where animals were present but no faeces were available for collection, this information was recorded. Faeces were stored on ice bricks in an esky immediately following collection, transported to the laboratory that day, sorted into pools and stored at -80°C as soon as possible following collection.

Blood samples. Blood samples (5 – 10 ml) were collected from all animals for AGID testing. Sheep were restrained by the handler and blood was collected by jugular venipuncture into plain 10 ml evacuated tubes (BD VacutainerTM, Becton Dickinson). Blood was stored on wet ice in the shade and transported that day to the laboratory. Blood samples were left to stand for 3 hours to allow formation of a clot, centrifuged at 2095 x g for 30 minutes and serum was aspirated and stored at -20°C until required for analysis. Heparinised blood samples were collected from selected animals for evaluation of the interferon-gamma assay.

Tissue samples. All sheep that survived to the end of the trial were slaughtered at Wollondilly Abattoir and samples of terminal ileum and mesenteric (caudal jejunal, ileocaecal) lymph node were collected for histopathology and culture for *M. paratuberculosis.* Suitable tissue samples were not available from the majority of animals that died during the trial due to the interval between death and examination.

3.2.8 Laboratory methods

Agar gel immunodiffusion. The AGID was performed as described using the ovine version of the test. [6]

Enzyme-linked immunosorbent assay. Selected serum samples were tested using the CSL Parachek EIA kit by CSIRO. Results will not be presented in this report but instead will be reported by CSIRO in project OJD.025.

Interferon gamma assay. Selected whole blood samples were tested in the CSL Bovigam assay, modified for detection of responses against *M. paratuberculosis.* Results will not be presented in this report but instead will be reported by CSIRO in project OJD.025.

Strategies for faecal culture. In order to test samples from all sheep on a regular basis within a limited budget, pooled faecal culture was used. A pool size of 5 was used throughout the trial. Animals were allocated to pools rather than pools consisting of randomly sampled sheep. Samples of faeces from individual sheep were stored at -80°C, and where a positive result was obtained from a pool, the individuals within that pool were then cultured.

Pooled faecal culture. A standard method was used for PFC.[5] Growth in BACTEC medium was confirmed in all cases by IS*900* PCR and restriction endonuclease analysis.[2] A modification to include ampicillin in BACTEC medium at a concentration of 100 ug/mL was used routinely for all PFC assays conducted on samples from periods 7 to 9 inclusive, and in addition on any samples from periods 1 to 6 that were found to be contaminated. Ampicillin was added to BACTEC vials at the start of week 1 and week 5 of incubation. The rationale for inclusion of ampicillin in the medium was as follows. A high rate of breakthrough growth of irrelevant bacteria in ovine faecal samples from the Arthursleigh farm led to identification of organisms other than *M. paratuberculosis* that survived disinfection in HPC and grew in the presence of vancomycin, amphotericin B and nalidixic acid. On some occasions more than 20% of samples were affected. Ten BACTEC bottles with breakthrough growth of irrelevant microbial flora were subcultured to blood agar, incubated aerobically and examined. Representative colony types were subcultured for purity and submitted to a diagnostic microbiology laboratory for identification. All isolates were considered to be variants of the same colony type. Three isolates were identified using biochemical and other phenotypic tests as *Pseudomonas diminuta*. Antimicrobial sensitivity testing was then undertaken, and the isolates were sensitive to ampicillin, sulphonamide, trimethoprim and neomycin. Ampicillin included in BACTEC medium at a concentration of 100 ug/mL was effective in preventing the growth of this organism and the effect lasted for 4 – 6 weeks. The effect of ampicillin on growth of *M. paratuberculosis* S strain isolates 2624 and 2636 was evaluated using serially diluted suspensions. Growth was delayed for about 1 week at all concentrations of *M. paratuberculosis.*

Individual faecal culture. IFC was undertaken using BACTEC radiometric culture as described. [4] Growth in BACTEC medium was confirmed in all cases by IS900 PCR and restriction endonuclease analysis.[2] Ampicillin was included in media for all IFC conducted on samples from periods 4 to 6 inclusive.

Culture of intestinal tissues and lymph node. A piece of terminal ileum approximately 2 g and a section of caudal jejunal or ileocaecal lymph node up to 2 g were separately disaggregated in 2 ml of sterile saline in gamma-irradiated disposable bags by beating for 2 minutes in a stomacher (Bagmixer 100 MiniMix ®, Interscience, 30 Ch. Bois Arpents., F78860 St Nom, France). The

homogenates were placed in sterile 5ml tubes and frozen at -80°C until required. A volume of 0.05 mL of intestinal homogenate was pooled with 0.05 mL of lymph node homogenate and inoculated into the same BACTEC medium.[4] Ampicillin was not included in the medium. Growth in BACTEC medium was confirmed in all cases by IS*900* PCR and restriction endonuclease analysis.[2]

Histopathology. Tissues were fixed in 10% buffered formal saline, embedded in paraffin, sectioned at 5µm and stained with haematoxylin and eosin and by a Ziehl Neelson method. [3] To facilitate later studies, fixation in formalin was limited to 48 hours, including time spent in cassettes prior to embedding in paraffin.

4 Results

4.1 Key dates in the trial

Donor sheep were co-grazed with uninfected sheep on decontaminated pasture from 1st April 2002, removed 14.5 weeks later on 11th July, slaughtered on 1st August 2002 and examined for evidence of OJD infection. Uninfected sheep were exposed to *M. paratuberculosis* through co-grazing with donor sheep and were slaughtered approximately 2.5 years later between 13th September and 28th October 2004. Complete temporal details of the trial are provided in Appendix 1.

4.2 Contamination rates of each treatment

There were graded levels of contamination across the treatment groups as shown in Table 4.1. The control paddocks were not intentionally contaminated by grazing donor sheep but shared boundaries with contaminated paddocks (see Materials and methods, also Appendix 2). Only one of the two low contamination paddocks actually received donors with a patent *M. paratuberculosis* infection in the process of random allocation of donors. However, the two medium contamination treatments had very similar contamination rates while the two high contamination paddocks had rates that exceeded those in the other treatments.

Table 4 1. Contamination rates of paddocks in each treatment in trial OJD.028

Treatment	Contamination rate (No. patent donor sheep days)
C1	0
C2	0
L1	0
L2	204
M1	295
M2	306
H1	599
H2	819

4.3 Consistency of responses to M. paratuberculosis between replicates

A number of measures were used to assess the consistency or clustering of cases of *M. paratuberculosis* within replicates, as this was a feature of the results of the previous trial OJD.002A. In general, infection with the organism was distributed among sheep in both replicates (Table 4.2). However, the first replicate in both low and medium treatment groups tended to have fewer cases of infection than the second replicate.

Table 4.2. Distribution of measures of *M. paratuberculosis* infection among replicates of each treatment group. Data are the number of individual sheep with a positive result in the tests shown.

Treatment group	Replicate 1	Replicate 2
Histopathology consistent with OJD		
Control	4	6
Low	0	7
Medium	2	9
High	9	6
Total n = 43		
Individual faecal culture		
Control	5	4
Low	3	6
Medium	1	4
High	18	8
Total n = 49		
Agar gel immunodiffusion		
Control	3	2
Low	5	4
Medium	2	4
High	11	6
Total n = 37		
Culture of intestinal tissues and lymph node		
Control	5	6
Low	1	4
Medium	1	6
High	3	8
Total n = 34		
Mortality due to OJD		
Control	0	0
Low	1	1
Medium	0	0
High	10	4
Total n = 16		

4.4 General features of the results

The pooled faecal culture results suggested a higher prevalence of infection and faecal shedding in lambs than hoggets and ewes, and in high contamination groups compared to other groups, with the greatest numbers of positive pools in the second half of the trial (periods 6-10) (Tables 4.3 and 4.4). There were similar numbers of positive pools in groups with medium, low or control contamination rates, suggesting a major differential effect between so-called "minor" (in this trial = control, low and medium) and so-called "high" contamination.

These results were confirmed by the results of individual faecal culture (Table 4.5 and 4.6). A total of 26 lambs were IFC positive on at least one occasion compared to 19 hoggets and 4 ewes.

Corresponding data for 2 or more occasions positive were 14, 8 and 2. More animals shedding *M. paratuberculosis* were in the high contamination group than in other groups.

There was a slightly different pattern with AGID results (Table 4.7 and 4.8). There was a similar number of AGID reactors among lambs and hoggets, and a lower number among ewes. Ignoring age, the highest number of AGID reactors occurred among sheep in the high contamination group, but the trend did not apply to lambs or ewes. Most of the positive results occurred in the second half of the trial in periods 6-10.

The highest number of mortalities occurred in lambs (35) and ewes (24) compared to hoggets (11), but there were more deaths due to OJD in lambs (10) compared to hoggets (5) and ewes (1) (Tables 4.9 and 4.10). All of the deaths due to OJD occurred in the second half of the trial (periods 6-10) and most occurred in the high contamination group.

Histopathological lesions consistent with OJD were more common in lambs (21) compared to hoggets (15) and ewes (7), with lesions present in animals in each contamination group (Tables 4.11 and 4.12). Severe lesions were also more common in lambs (17) than hoggets (11) or ewes (1). In lambs, most of the severe lesions occurred in animals in the high contamination group, but in hoggets they occurred equally commonly in the control group. Ignoring age, histopathological lesions occurred with similar frequency in sheep in each contamination group, although severe lesions were more frequent in the high contamination group.

M. paratuberculosis was isolated from the intestinal tissues and lymph nodes of similar numbers of lambs (13), hoggets (10) and ewes (11), and ignoring age included animals from each of the contamination groups with similar frequency (Table 4.13).

Overall the results of tissue culture and AGID suggested that infection with *M. paratuberculosis* occurred in sheep of all ages even when there were relatively low levels of contamination. However, disease evidenced by severe histological lesions and mortality was more likely to occur when animals were first exposed as lambs and when animals were exposed to relatively high levels of contamination. Similarly faecal shedding of the organism was more common when animals were first exposed as lambs and was more pronounced following exposure of animals to high levels of contamination. However, faecal shedding, which is an important driver for transmission of the infection, was still initiated in some sheep following exposure even to relatively low levels of contamination, suggesting the existence of highly susceptible individuals within a flock.

4.5 Results relating to specific objectives

4.5.1 Objective 1

To determine the interval between infection with *M.ptb* and excretion of the organism in relation to the size of the infecting dose and the age of sheep.

Due to the occurrence of passive faecal shedding, the situation where sheep ingest *M. paratuberculosis* from pasture and excrete it directly in their faeces without necessarily being infected, a measure of infection was required that provided a high level of confidence that sheep had active infection. This was deemed to be the detection of shedding in two successive sampling periods in the same group of 5 sheep by pooled faecal culture. The chance of this being due to passive faecal shedding was low.

Figure 4.1. The pattern of PFC results by age and treatment group. The shaded bars containing the numeral 1 represent all culture positive pools in each of the 10 sampling periods during the trial. L, lamb, H, hogget, E, ewe.

Treatment	Age	Rep	PFC1	PFC2	PFC3	PFC4	PFC5	PFC6	PFC7	PFC8	PFC9	PFC10
Control	L	1								1	1	1
Control	L	2						1	1	1	1	1
Control	H	1									1	
Control	H	1									1	1
Control	H	2										1
Control	H	2						1	1			
Control	E	1							1			
Low	L	1		1								
Low	L	1						1				
Low	L	2							1			1
Low	L	2							1			
Low	L	2								1		
Low	H	1					1					
Low	E	1				1	1		1	1	1	
Medium	L	1									1	1
Medium	L	2								1		
Medium	L	2									1	
Medium	H	2							1	1	1	1
Medium	H	2			1							
Medium	H	2					1		1	1		
High	L	1						1	1			
High	L	1						1		1	1	
High	L	1				1	1	1	1	1		
High	L	1		1		1	1	1				
High	L	1		1		1		1	1			
High	L	1		1	1	1		1	1			
High	L	2						1	1			
High	L	2		1					1			
High	L	2						1	1	1	1	1
High	L	2				1					1	
High	H	1							1			
High	H	1						1	1	1		
High	H	1	1					1	1	1	1	
High	H	1						1				
High	H	1						1	1			
High	H	2				1		1	1	1		
High	H	2						1				
High	E	1					1	1	1	1		

Faecal shedding occurred for the first time in lambs in September 2002 (PFC2, Figure 4.1), 6 months after first exposure to infected pasture. The median age of lambs at this time was 11 months. Faecal shedding was more frequent in lambs in the high contamination treatment at this time and later. Faecal shedding in hoggets commenced in September 2003 (PFC6, Figure 4.1), 18 months after first exposure, while in ewes it occurred in March 2003 (PFC4, Figure 4.1), 11 months after first exposure.

The design of the experiment did not allow for faecal culture of all individual sheep over time so it is difficult to assess the consistency of shedding in individuals. However, the pattern of shedding suggests that it was intermittent until after the fourth sampling period (Figure 4.2), that is, until after April 2003, when sheep had been exposed to *M. paratuberculosis* for at least 12 months.

Figure 4.2. The pattern of individual faecal culture results over time by age and treatment group for each animal with a positive result. The darker shaded bars (1) represent all culture positive samples, the lighter shaded bars represent samples that were not cultured because an animal had no sample (9) or was culture negative (0). Unshaded areas were samples from an individual that were not cultured because the parent pooled sample was culture negative (4). The code 999 indicates that the animal died after the last sample period but prior to slaughter.

TAG	Tx	Age	Rep	IFC1	IFC2	IFC3	IFC4	IFC5	IFC6	IFC7	IFC8	IFC9	IFC10
16	C	L	1	4	4	4	4	4	4	4	0	1	0
18	C	L	1	4	4	4	4	4	4	4	1	9	1
46	C	L	2	4	4	4	4	4	9	1	1	1	1
71	C	H	1	4	4	4	4	4	4	4	4	1	4
82	C	H	1	4	4	4	4	4	4	4	4	1	9
107	C	H	2	4	4	4	4	4	4	4	4	4	1
122	C	H	2	4	4	4	4	4	0	1	0	4	4
123	C	H	2	4	4	4	4	4	0	1	1	4	4
168	C	E	1	4	4	4	4	4	4	1	4	4	4
292	L	L	2	4	9	4	4	4	4	1	1	9	1
298	L	L	2	4	4	4	4	4	4	1	0	4	0
300	L	L	2	4	4	4	4	4	4	1	0	4	999
302	L	L	2	4	4	4	4	4	4	0	4	4	1
303	L	L	2	4	4	4	4	4	4	0	4	4	1
308	L	L	2	4	4	4	4	4	4	4	1	4	999
348	L	H	1	4	4	4	4	1	4	4	4	4	4
393	L	E	1	4	4	4	1	9	0	0	9	9	4
395	L	E	1	4	4	4	1	1	1	9	1	1	4
523	M	L	1	4	4	4	1	4	4	4	4	1	1
560	M	L	2	4	4	4	4	4	4	4	4	1	4
615	M	H	2	4	4	4	4	4	4	1	1	1	1
631	M	H	2	4	4	4	4	0	1	9	1	4	4
635	M	H	2	4	4	4	4	0	0	1	0	4	4
753	H	L	1	4	4	4	1	4	9	1	9	4	4
754	H	L	1	4	4	4	0	4	0	1	0	4	4
758	H	L	1	4	4	4	4	4	0	1	1	0	4
759	H	L	1	4	4	4	4	4	1	9	9	1	4
767	H	L	1	0	4	4	0	1	1	1	1	4	4
769	H	L	1	0	4	4	1	0	9	0	0	4	4
770	H	L	1	0	4	4	0	1	9	1	9	4	4
772	H	L	1	1	4	4	4	4	4	4	4	4	4
777	H	L	1	4	1	4	0	1	1	9	999	4	4
783	H	L	1	0	0	1	0	4	9	1	9	4	4
784	H	L	1	4	0	0	1	4	9	1	0	4	4
786	H	L	2	4	4	4	4	4	1	1	4	4	4
807	H	L	2	4	4	4	4	4	9	0	0	0	1
810	H	L	2	4	4	4	4	4	1	1	1	1	999
811	H	L	2	4	4	4	2	4	4	4	4	1	4
823	H	H	1	4	4	4	4	4	4	1	4	4	4
826	H	H	1	4	4	4	4	4	9	1	1	4	4
830	H	H	1	4	4	4	4	4	9	1	0	4	4
832	H	H	1	0	4	4	4	4	2	1	1	1	4
835	H	H	1	1	4	4	4	4	9	0	0	0	4
855	H	H	1	4	4	4	4	4	1	1	999	4	4
857	H	H	2	4	4	4	0	4	1	1	0	4	4
858	H	H	2	4	4	4	1	4	1	1	1	4	4
860	H	H	2	4	4	4	0	4	9	1	0	4	4
881	H	H	2	4	4	4	4	4	1	4	4	4	4
895	H	E	1	4	4	4	4	1	1	1	9	4	4

4.5.2 Objective 2

To determine the effect of pasture contamination rate on the incidence of disease.

Disease was defined as the occurrence of mortality due to OJD or the presence of severe histological lesions of OJD (Perez score 3a and 3b). Mortality due to OJD in animals exposed for the first time as lambs or hoggets occurred almost exclusively in the high contamination group. Only one ewe died, and this animal was from a low contamination group. Of the 16 animals that died of OJD, 14 were from a high contamination group (Table 4.10).

4.5.3 Objective 3

To compare the efficacy of diagnostic tests (faecal culture, gamma interferon, serology) in young sheep and in the early stages of the disease.

PFC results were positive in lambs in the high contamination group at the second sampling in September 2002 when the median age was 11 months, approximately 6 months after initial exposure. Serology was not performed until the fourth sampling period, when the lambs were 18 months old, and two were positive at this time. As five pools of faeces from the lamb group were culture positive at the fourth sampling (Figure 4.1), PFC is likely to be more sensitive than the AGID at this early time point. Note that a pool size of 5 was used in this trial, compared to the usual pool size of 50 in routine diagnostic testing. A pool size of 5 may have higher sensitivity than a pool size of 50.

ELISA and interferon-gamma results will be reported by CSIRO in project OJD.025. AGID results in this trial did not appear to be highly related to faecal culture results in this trial. Further evaluation of AGID in relation to ELISA will be undertaken to determine the specificity of both tests, but this was beyond the scope of the present project and cannot be undertaken until the CSIRO project is complete.

4.5.4 Objective 4

To describe the epidemiology of OJD in a mixed age flock following simultaneous exposure of previously unexposed sheep to infection, particularly in relation to the first detectable evidence of transmission, the clustering of infection within age groups and the assessment of risk of infection of each age group.

The results suggest that transmission of *M. paratuberculosis* occurred very quickly after first exposure. Patent infection was established within 6 months of first exposure in lambs,. Although shedding was probably intermittent at this stage, there was the potential for transmission from these animals to occur at that time.

Clustering of *M. paratuberculosis* infection has been observed on some farms. In this trial infection was observed in all age groups but patent infection leading to transmission was more frequent in animals exposed for the first time as lambs and hoggets than in animals first exposed as adults. Fourteen lambs were individual faecal culture positive on at least two occasions compared to 8 hoggets and only 2 ewes. The pattern of results for AGID was similar in that ewes had a low rate of

seroconversion implying a low rate of multibacillary infection. The mortality pattern in this trial and presence of severe histological lesions at slaughter 2.5 years after first exposure also suggested a degree of clustering. However, patent infection and disease occurred in all age groups. Therefore clustering is not absolute.

It is important to note that relatively few donor sheep led to contamination and transmission of *M. paratuberculosis* in this trial. It is also important to observe that infection was spread between paddocks across conventional fenced boundaries. Therefore while clustering may be an issue, its effect may be lessened by lateral spread.

The risk of infection and therefore transmission of OJD was greater when lambs were exposed to contamination compared to hoggets and ewes. The risk of infection was greater where there were high levels of contamination.

4.5.5 Objective 5

To provide the following outcomes to the satisfaction of MLA:

1. A comparison of infection rates under three levels of pasture contamination: low, medium and high
2. A comparison of infection rates of lambs, young ewes and older ewes 2.5 years after first exposure
3. A determination of the interval between exposure to infection and excretion in relation to the age of sheep and degree of pasture contamination
4. A comparison of faecal culture, cell mediated immunity and serology as diagnostic approaches in sheep recently exposed to infection
5. A determination of the efficacy of diagnostic tests soon after infection (0-2.5 years), with particular reference to young sheep

Points 1 to 5 have been addressed above.

6. Recommendations regarding flock testing after introduction of clean sheep to infected pasture. Interpretation of the reverse situation will also be made

Recommendations for flock testing after introduction of clean sheep to infected pasture will depend on the age of the sheep. For animals introduced as lambs, which may include sheep up to 1 year of age, PFC should be undertaken at 3 monthly intervals commencing 6 months after introduction. For older animals, PFC can be undertaken at 3 month intervals commencing 12 months after introduction, or alternatively, AGID can be undertaken commencing 18 months after introduction. These recommendations are made based on data in Tables 4.1 and 4.5.

Testing of potentially infected sheep introduced to infected or clean pasture should be based on prior knowledge of their status, but can commence immediately and then follow the guide above.

7. Recommendations for control of OJD based on knowledge of age-based clustering of infection.

It is possible to recommend several approaches to control OJD based on the data from this study:

- Minimise exposure of lambs and hoggets to heavily contaminated pasture

- As adult sheep are more resistant to infection and disease impacts of OJD, if clean pastures are scarce, they should be grazed on contaminated pasture rather than younger animals
- Cull adult sheep with clinical signs of OJD, or conduct PFC to obtain evidence of patent OJD infection and manage or cull these mobs to reduce the level of contamination across the farm
- Manage pastures by spelling, grazing with adult cattle or grazing with adult sheep in order to reduce the contamination level for lambs, weaners and young sheep
- In vaccinated flocks, be observant and identify individuals that develop signs of weight loss and cull them to reduce the level of contamination
- Maintain boundary fencing to reduce the likelihood of lateral spread from an infected neighbour. As a single fence line does not prevent lateral spread, consider double fencing with wide laneways or segregation of critical pastures using a tree belt, gully or other natural feature. Maintain high value lambs in paddocks well away from paddocks known to contain infected sheep.

Table 4.3. Pooled faecal culture results in each period by age and treatment group. There were 5 sheep in each pool. The age of the animals in each group at each time is shown (months). Data are the number of pools.

Collection date/ Treatment	Lambs		Hoggets		Ewes	
	No. tested	Positive	No. tested	Positive	No. tested	Positive
Age at first exposure (mths)	5.5		22.5		58.5	
25-Jun-02						
Age of sheep (mths)	***8***		***25***		***61***	
Control	14	0	14	0	14	0
Low	14	0	14	0	14	0
Medium	14	0	14	0	14	0
High	14	0	13	1	14	0
Total	**56**	**0**	**55**	**1**	**56**	**0**
24-Sept-02						
Age of sheep (mths)	***11***		***28***		***64***	
Control	14	0	14	0	14	0
Low	13	1	13	0	14	0
Medium	13	0	14	0	14	0
High	14	3	14	0	14	0
Total	**54**	**4**	**55**	**0**	**56**	**0**
09-Dec-02						
Age of sheep (mths)	***14***		***31***		***67***	
Control	14	0	14	0	14	0
Low	14	0	14	0	14	0

Collection date/ Treatment	Lambs		Hoggets		Ewes	
	No. tested	Positive	No. tested	Positive	No. tested	Positive
Medium	14	0	14	1	14	0
High	14	1	14	0	14	0
Total	**56**	**1**	**56**	**1**	**56**	**0**
07-Apr-03						
Age of sheep (mths)	***18***		***35***		***71***	
Control	14	0	14	0	14	0
Low	14	0	14	0	14	1
Medium	14	0	14	0	14	0
High	14	4	14	1	14	0
Total	**56**	**4**	**56**	**1**	**56**	**1**
23-Jun-03						
Age of sheep (mths)	***20***		***37***		***73***	
Control	14	0	14	0	14	0
Low	14	0	14	1	14	1
Medium	14	0	14	1	14	0
High	14	2	14	0	14	1
Total	**56**	**2**	**56**	**2**	**56**	**2**
08-Sep-03						
Age of sheep (mths)	***23***		***40***		***76***	
Control	14	1	14	1	14	0
Low	14	1	14	0	14	0
Medium	14	0	14	0	14	0
High	14	6	14	6	14	1
Total	**56**	**8**	**56**	**7**	**56**	**1**
15-Dec-03						
Age of sheep (mths)	***26***		***43***		***79***	
Control	14	1	14	1	14	1
Low	14	2	14	0	14	1
Medium	14	0	14	2	14	0
High	14	5	14	5	14	1
Total	**56**	**8**	**56**	**8**	**56**	**3**
15-Mar-04						
Age of sheep (mths)	***29***		***46***		***82***	
Control	14	2	14	0	14	0
Low	14	1	14	0	14	1
Medium	14	0	14	2	14	0

Collection date/ Treatment	Lambs		Hoggets		Ewes	
	No. tested	Positive	No. tested	Positive	No. tested	Positive
High	14	3	14	3	14	1
Total	**56**	**6**	**56**	**5**	**56**	**2**
14-Jun-04						
Age of sheep (mths)	***32***		***49***		***85***	
Control	14	2	14	2	14	0
Low	14	0	14	0	14	1
Medium	14	2	14	1	14	0
High	14	3	14	1	14	0
Total	**56**	**7**	**56**	**4**	**56**	**1**
08-Sep-04						
Age of sheep (mths)	***35***		***52***		***88***	
Control	14	2	14	2	14	0
Low	14	1	14	0	14	0
Medium	14	1	14	1	14	0
High	14	1	14	0	14	0
Total	**56**	**5**	**56**	**3**	**56**	**0**

Table 4.4. Aggregated pooled faecal culture results by age and treatment group. Data are the total number of positive pools in each age and group over the trial, derived from Table 4.3.

Group	Lambs	Hoggets	Ewes	Total
Control	8	6	1	15
Low	6	1	5	12
Medium	3	8	0	11
High	28	17	4	49
Total	**45**	**32**	**10**	**87**
Total period 1-5	11	5	3	19
Total period 6-10	34	27	7	68

Table 4.5. Individual faecal culture results for animals in each age class and treatment group. Individual faecal cultures were undertaken on those animals present in a culture positive pool. Data are the number of culture positive animals.

Collection date/ Treatment	Lambs	Hoggets	Ewes	**Total**
Age at first exposure (mths)	5.5	22.5	58.5	
25 Jun 2002 (1)				
Age of sheep (mths)	***8***	***25***	***61***	
Control				
Low				
Medium				
High	1	1		2
Total	***1***	***1***	***0***	***2***
24 Sep 2002 (2)				
Age of sheep (mths)	***11***	***28***	***64***	
Control				
Low				
Medium				
High	1			1
Total	**1**	**0**	**0**	**1**
09 Dec 2002 (3)				
Age of sheep (mths)	***14***	***31***	***67***	
Control				
Low				
Medium				
High	1			1
Total	**1**	**0**	**0**	**1**
07 April 2003 (4)				
Age of sheep (mths)	***18***	***35***	***71***	
Control				
Low			2	2
Medium	1			1
High	3	1		4
Total	**4**	**1**	**2**	**7**
23 Jun 2003 (5)				
Age of sheep (mths)	***20***	***37***	***73***	
Control				
Low		1	1	2
Medium				
High	3		1	4
Total	**3**	**1**	**2**	**6**
08 Sep 2003 (6)				

Collection date/ Treatment	Lambs	Hoggets	Ewes	**Total**
Age of sheep (mths)	***23***	***40***	***76***	
Control				
Low			1	1
Medium		1		1
High	5	4	1	10
Total	**5**	**5**	**2**	**12**
15 Dec 2003 (7)				
Age of sheep (mths)	***26***	***43***	***79***	
Control	1	2	1	4
Low	3			3
Medium		2		2
High	9	8	1	18
Total	**13**	**12**	**2**	**27**
15 Mar 2004 (8)				
Age of sheep (mths)	***29***	***46***	***82***	
Control	2	1		3
Low	2		1	3
Medium		2		2
High	3	3		6
Total	**7**	**6**	**1**	**14**
14 Jun 2004 (9)				
Age of sheep (mths)	***32***	***49***	***85***	
Control	2	2		4
Low			1	1
Medium	2	1		3
High	3	1		4
Total	**7**	**4**	**1**	**12**
08 Sep 2004 (10)				
Age of sheep (mths)	***35***	***52***	***88***	
Control	2	1		3
Low	3			3
Medium	1	1		2
High	1			1
Total	**7**	**2**	**0**	**9**

Table 4.6. Aggregated individual faecal culture results by age and treatment group. Individuals were tested when the pooled culture in which they were present was culture positive.

Group	Lambs		Hoggets		Ewes		Total	
	No. positive in at least 1 period	No. positive in at least 2 periods	No. positive in at least 1 period	No. positive in at least 2 periods	No. positive in at least 1 period	No. positive in at least 2 periods	No. positive in at least 1 period	No. positive in at least 2 periods

Group	Lambs		Hoggets		Ewes		Total	
	No. positive in at least 1 period	No. positive in at least 2 periods	No. positive in at least 1 period	No. positive in at least 2 periods	No. positive in at least 1 period	No. positive in at least 2 periods	No. positive in at least 1 period	No. positive in at least 2 periods
Control	3	2	5	1	1	0	9	3
Low	6	1	1	0	2	1	9	2
Medium	2	1	3	2	0	0	5	3
High	15	10	10	5	1	1	26	16
Total	**26**	**14**	**19**	**8**	**4**	**2**	**49**	**24**

Table 4.7. AGID results for animals in each age class and treatment group. The age of the animals in each group at each sampling is shown (months).

Collection date/ Treatment	Lambs		Hoggets		Ewes	
	No. tested	Positive	No. tested	Positive	No. tested	Positive
Age at first exposure (mths)	5.5		22.5		58.5	
07-Apr-03						
Age of sheep (mths)	***18***		***35***		***71***	
Control	66	0	64	0	65	0
Low	66	0	68	0	67	0
Medium	67	1	65	0	65	0
High	68	1	69	0	68	0
Total	**267**	**2**	**266**	**0**	**265**	**0**
23-Jun-03						
Age of sheep (mths)	***20***		***37***		***73***	
Control	68	0	68	0	64	0
Low	68	1	68	0	69	0
Medium	66	1	67	0	62	0
High	68	0	69	0	70	0
Total	**270**	**2**	**272**	**0**	**265**	**0**
08-Sept-03						
Age of sheep (mths)	***23***		***40***		***76***	
Control	67	0	67	0	62	0
Low	66	1	70	1	70	1
Medium	64	1	67	0	64	1
High	69	2	69	4	68	0
Total	**266**	**4**	**273**	**5**	**264**	**2**
15-Dec-03						
Age of sheep (mths)	***26***		***43***		***79***	
Control	65	0	66	0	63	0
Low	65	5	69	2	68	2
Medium	65	1	67	0	62	1
High	64	4	67	4	66	0
Total	**259**	**10**	**269**	**6**	**259**	**3**
15-Mar-04						
Age of sheep (mths)	***29***		***46***		***82***	
Control	64	1	68	0	64	2
Low	66	2	67	1	68	1
Medium	65	0	68	1	60	0

Collection date/ Treatment	Lambs		Hoggets		Ewes	
	No. tested	Positive	No. tested	Positive	No. tested	Positive
High	60	2	64	7	63	0
Total	**255**	**5**	**267**	**9**	**255**	**3**
14-Jun-04						
Age of sheep (mths)	***32***		***49***		***85***	
Control	63	1	61	2	64	0
Low	58	1	69	0	63	1
Medium	62	1	66	2	63	1
High	59	1	63	3	64	1
Total	**242**	**4**	**259**	**7**	**254**	**3**
08-Sep-04	35		52		88	
Age of sheep (mths)						
Control	60	0	66	1	63	0
Low	58	2	67	0	64	1
Medium	63	0	65	0	62	0
High	54	0	63	1	63	1
Total	**235**	**2**	**261**	**2**	**252**	**2**

Table 4.8. Aggregated AGID results by age and treatment group.

Group	Lambs			Hoggets			Ewes			Total		
	No. tested	No. positive in at least 1 period	No. positive in at least 2 periods	No. tested	No. positive in at least 1 period	No. positive in at least 2 periods	No. tested	No. positive in at least 1 period	No. positive in at least 2 periods	No. tested	No. positive in at least 1 period	No. positive in at least 2 periods
Control	69	1	1	68	2	1	67	2	0	204	5	2
Low	65	5	4	68	2	2	68	2	2	201	9	8
Medium	69	1	1	67	3	0	68	2	1	204	6	2
High	63	7	3	61	9	7	70	1	1	194	17	11
Total	**266**	**14**	**9**	**264**	**16**	**10**	**273**	**7**	**4**	**803**	**37**	**23**

Table 4.9. Deaths in each period. The number of deaths occurring in each group since the previous sample collection date is shown. Three animals present at the last sample collection in period 10 were not identifiable at slaughter (405, 646, 675) due to a lost eartag, or gut samples removed by a meat inspector; they are not shown here.

Collection date/ Treatment	Lambs	Hoggets	Ewes
25-Jun-02			
Control	0	0	0
Low	0	0	0
Medium	0	0	0
High	0	0	0
Total	**0**	**0**	**0**
24-Sept-02			
Control	0	0	0
Low	1	0	0
Medium	1	0	0
High	0	0	0
Total	**2**	**0**	**0**
09-Dec-02			
Control	0	1	0
Low	1	0	0
Medium	1	0	2
High	1	0	1
Total	**3**	**1**	**3**
07-Apr-03			
Control	0	0	3
Low	0	0	0
Medium	0	1	2
High	0	0	0
Total	**0**	**1**	**5**
23-Jun-03			
Control	0	0	0
Low	0	0	0
Medium	2	0	1
High	0	0	0
Total	**2**	**0**	**1**
08-Sep-03			
Control	1	0	1

Collection date/ Treatment	Lambs	Hoggets	Ewes
Low	0	0	0
Medium	0	0	1
High	0	0	0
Total	**1**	**0**	**2**
15-Dec-03			
Control	0	0	0
Low	0	1	0
Medium	0	0	1
High	0	0	0
Total	**0**	**1**	**1**
15-Mar-04			
Control	3	0	1
Low	2	0	2
Medium	0	0	0
High	4	3	3
Total	**9**	**3**	**6**
14-Jun-04			
Control	3	0	0
Low	6	0	1
Medium	1	1	0
High	3	3	1
Total	**13**	**4**	**2**
08-Sep-04			
Control	0	0	1
Low	1	0	1
Medium	0	0	0
High	4	1	1
Total	**5**	**1**	**3**

Table 4.10. Total mortality during the trial for animals in each age class and treatment group. Death due to OJD was determined by post mortem examination and histopathology (lesions ≥3a), or ascribed to animals that died after at least two positive ante-mortem tests (AGID and/or individual faecal culture).

Treatment	Lambs		Hoggets		Ewes		Total	
	No. dead	Deaths due to OJD	No. dead	Deaths due to OJD	No. dead	Deaths due to OJD	No. dead	Deaths due to OJD
Control	7		1		6		14	0
Low	11	1	1		5	1	17	2
Medium	5		2		7		14	0
High	12	9	7	5	6		25	14
Total	**35**	**10**	**11**	**5**	**24**	**1**	**70**	**16**
Total period 1-5	8	-	2	-	11	-	21	0
Total period 6-10	27	10	9	5	13	1	49	16

Table 4.11. Histopathological lesions in animals in each age class and treatment group. Lesions of OJD were grades 1, 2, 3a, 3b, or 3c according to Perez, with one animal having a lymph node lesion only.

	Lambs			Hoggets			Ewes			Total	
Treatment	Examined	OJD lesions	Not examined	Examined	OJD lesions	Not examined	Examined	OJD lesions	Not examined	Examined	OJD lesions
Control	66	3	4	69	6	1	66	1	3	201	10
Low	60	3	10	69	1	1	65	3	5	194	7
Medium	65	6	5	68	4	2	61	1	9	194	11
High	63	9	7	64	4	6	66	2	5	193	15
Total	**254**	**21**	**26**	**270**	**15**	**10**	**258**	**7**	**22**	**782**	**43**

Table 4.12. Severe histopathological lesions in animals in each age class and treatment group. Severe lesions of OJD were grades 3a and 3b according to Perez.

Treatment	Lambs		Hoggets		Ewes		Total	
	OJD lesions	Severe lesions	OJD lesions	Severe lesions	OJD lesions	Severe lesions	OJD lesions	Severe lesions
Control	3	2	6	5	1	0	10	7
Low	3	3	1	1	3	1	7	5
Medium	6	3	4	2	1	0	11	5
High	9	9	4	3	2	0	15	12
Total	**21**	**17**	**15**	**11**	**7**	**1**	**43**	**29**

Table 4.13. Tissue culture results in animals in each age class and treatment group.

Treatment	Lambs			Hoggets			Ewes			Total	
	Examined	M. patb isolated	Not examined	Examined	M. patb isolated	Not examined	Examined	M. patb isolated	Not examined	Examined	M. patb isolated
Control	63	3	7	69	6	1	63	2	6	195	11
Low	59	1	11	69	2	1	65	2	5	193	5
Medium	65	4	5	68	2	2	61	1	9	194	7
High	58	5	12	63	0	7	65	6	0	186	11
Total	**245**	**13**	**35**	**269**	**10**	**11**	**254**	**11**	**20**	**768**	**34**

5 Success in Achieving Objectives

Each of the objectives for this project was met.

The interval between infection with *M.ptb* and excretion of the organism in relation to the size of the infecting dose and the age of sheep was determined. In lambs exposed to high levels of contamination, this interval was 6 months, while in ewes and hoggets it was 11 to 18 months. Shedding was delayed generally in animals exposed to lower levels of contamination.

The effect of pasture contamination rate on the incidence of disease was determined. Disease occurred almost exclusively in animals exposed to high levels of contamination. Sub-clinical infection occurred in animals exposed to lower levels of contamination.

The efficacy of diagnostic tests (faecal culture, gamma interferon, serology) in young sheep and in the early stages of the disease were compared. Gamma interferon and serology comparisons were facilitated by supply of materials to CISRO for project OJD.025. Pooled faecal culture was more effective in detecting early infection than serology, and revealed infection in lambs at 11 months of age, 6 months after their first exposure to infection.

The epidemiology of OJD in a mixed age flock following simultaneous exposure of previously unexposed sheep to infection was described, particularly in relation to the first detectable evidence of transmission, the clustering of infection within age groups and the assessment of risk of infection of each age group. Transmission of infection occurred soon after exposure, enabling shedding within 6 months of first exposure. Clustering of infection in lambs exposed to high levels of contamination occurred, but was not complete, with infection detected in all age classes and in sheep exposed to contamination levels ranging from very low (control) to high. Lambs exposed to high levels of contamination presented the greatest risk of infection.

The following outcomes were provided: a comparison of infection rates under three levels of pasture contamination: low, medium and high; a comparison of infection rates of lambs, young ewes and older ewes 2.5 years after first exposure; a determination of the interval between exposure to infection and excretion in relation to the age of sheep and degree of pasture contamination; a comparison of faecal culture, cell mediated immunity and serology as diagnostic approaches in sheep recently exposed to infection; a determination of the efficacy of diagnostic tests soon after infection (0-2.5 years), with particular reference to young sheep; recommendations regarding flock testing after introduction of clean sheep to infected pasture and interpretation of the reverse situation; and recommendations for control of OJD based on knowledge of age-based clustering of infection.

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

The results of this study will have immediate impact on the management and control of OJD as they support and extend the current recommendations for livestock grazing management by providing experimental data. Some of the current recommendations are based on findings from previous experiments on disease transmission in project OJD.002A, and survival of *M. paratuberculosis* on pasture in project TR.055A, but others are based mainly on professional opinion. There has been no prior research on the impact on OJD occurrence of age at first exposure and levels of pasture contamination. However, it is now possible to state with certainty that lambs should not be exposed to high levels of contamination if clinical disease and relatively high rates of faecal shedding and hence further disease transmission are to be avoided. Lambs in this research project had a median age of 5.5 months. It is still uncertain how long lambs remain fully susceptible but a reasonable working hypothesis would be to avoid grazing sheep on heavily contaminated pastures during the first year of life. Also of great benefit to industry is the finding that hoggets and adult sheep were relatively resistant to the clinical effects of OJD. Even though infection occurred, animals exposed for the first time as hoggets or as adults rarely succumbed to the disease. Thus hoggets (median age in this study 22.5 months) and adult ewes were relatively resistant. This means that hoggets 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.

In the future, strategic recommendations on pasture management, and specific programs of rotational grazing utilising the known susceptibilities of each age class will be combined with vaccination to greatly improve overall management and control of OJD. Cost-benefit analysis of the relative merits of vaccination and grazing strategies will inform management decisions on individual farms. When used together with risk-based approaches to trading, this will lead to improved disease control outcomes on a local, regional and national basis.

7 Conclusions and Recommendations

The principle conclusions from this study are that:

1. Lambs are highly susceptible to infection with *M. paratuberculosis* and if exposed to high levels of contamination a proportion will develop severe infection leading to clinical disease and death.

2. Hoggets and adult ewes are less likely than lambs to develop clinical disease after exposure to *M. paratuberculosis.*

3. Sheep in all age classes may become infected with *M. paratuberculosis* and shed the organism in faeces, but this will happen more often in lambs than in older age classes. Nevertheless, even adult ewes may become infected and later act as a source for transmission of the disease.

4. Lateral spread of OJD is a serious threat to control based solely on management of pasture; it is not necessary for infected sheep to be present in a paddock for transmission of infection to occur if infected sheep are present in neighbouring paddocks. Conventional wire strand fences do not prevent spread of infection.

5. Pooled faecal culture is more effective than the agar gel immunodiffusion assay for detection of the infection at relatively early stages in young sheep. It was surprising that pooled faecal culture detected infection in sheep at 11 months of age only 6 months after first exposure to contaminated pasture. Note that a pool size of 5 was used in this trial, compared to the usual pool size of 50 in routine diagnostic testing. A pool size of 5 may have higher sensitivity than a pool size of 50.

The recommendations from this study are:

1. Testing to detect infection
    - The most appropriate strategy for flock testing after introduction of clean sheep to infected pasture will depend on the age of the sheep. For animals introduced as lambs (it is suggested that this include sheep up to 1 year of age), PFC should be undertaken at 3 monthly intervals commencing 6 months after introduction. For older animals, PFC can be undertaken at 3 month intervals commencing 12 months after introduction, or alternatively, AGID can be undertaken commencing 18 months after introduction.
    - Testing of potentially infected sheep introduced to infected or clean pasture should be based on prior knowledge of their status, but can commence immediately and then follow the guide above.

2. For control of OJD the following strategies are recommended

- Minimise exposure of lambs to heavily contaminated pasture
- As adult sheep are more resistant to infection and disease impacts of OJD they should be grazed on contaminated pasture rather than younger animals
- Cull adult sheep with clinical signs of OJD, or conduct PFC to obtain evidence of patent OJD infection and manage or cull these mobs to reduce the level of contamination across the farm
- Manage pastures by spelling, grazing with adult cattle or grazing with adult sheep in order to reduce the contamination level for lambs, weaners and young sheep
- Pasture spelling, which leads to 90% reduction in contamination levels for each month of spelling, should be undertaken where possible to reduce contamination levels from a high level to a moderate or low level. This should substantially reduce the proportion of sheep that develop OJD.
- In vaccinated flocks, be observant and identify individuals that develop signs of weight loss and cull them to reduce the level of contamination
- Maintain boundary fencing to reduce the likelihood of lateral spread from an infected neighbour. As a single fence line does not prevent lateral spread, consider double fencing with wide laneways or segregation of critical pastures using a tree belt, gully or other natural feature. Maintain high value lambs in paddocks well away from paddocks known to contain infected sheep.

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10 Appendices

10.1 Appendix 1
Calendar of activities on farm

Dates refer to week commencing; most activities were multi-day.

24.12.01 Infected sheep. 781 sheep were bled on a farm at Crookwell (Owner - Prell) and serum was tested in AGID. There was a seroprevalence of 19.8%. The sheep were shorn and treated for lice off shears. 78 seropositive sheep were purchased, transported to Arthursleigh on 24th December 2001 and held in paddocks.

21.01.02 Uninfected sheep. Considerable effort was required to locate suitable sheep in the necessary age classes from a single farm. 934 prospective clean sheep in the required age classes were bled on a property at Armidale NSW (JM Stephens, Waranee) on 21st January 2002. ELISA tests were undertaken by CSL. There were two reactors, which was within the aggregate number of reactors predicted for an uninfected flock based on the known specificity of this test. The two ELISA positive samples were negative in the AGID. 840 sheep were purchased and transported to Arthursleigh on 11th February 2002.

11.02.02 Drafted uninfected sheep based on age, drenched and collected blood for IFN-G for CSIRO.

01.04.02 Combination of infected and uninfected sheep. The infected sheep were allocated randomly to paddocks at rates of 14, 7, 2 and 0 infected sheep per replicate representing high, medium, low and control levels of pasture contamination, respectively. Faecal samples were collected to confirm the shedding status of each infected sheep by culture. Clean sheep were stocked at 105 per replicate, comprising 35 weaners, 35 hoggets and 35 adults. Each replicate paddock was 20 Ha.

04.04.02 Faecal samples were collected from all sheep (i.e. recipients and donors), ear tags were checked and general condition and health was confirmed individually.

26.04.02 Due to high mortality in the donor group it was necessary to source additional donors. During a project visit for OJD.015 Merrill, potential replacement donors were identified for OJD.028 and ear tagged. These were vaccinated animals in the Merrill adult flock so that serology could not be used to confirm infection status. Identification was based on clinical appearance/condition score and knowledge of the frequency of faecal shedders in age groups within the Merrill flock. Transport to Arthursleigh was arranged.

07.05.02	Replacement donors arrived at Arthursleigh and faeces were sampled off the truck. Faeces were submitted for a ZN smear and also stored for culture. Sheep were held in an identified "infected" paddock from the OJD.002A trial and were treated with Zap for lice control while awaiting ZN smear results. Shedders were identified and added to paddocks in OJD.028 as the original donors died and were confirmed as missing.
13.05.02	Faeces were collected for a worm egg count and all sheep in OJD.028 were drenched. This routine worm egg count is done on 10 samples per age group across all three age groups in each paddock i.e. 30 per paddock.
25.06.02	Faeces were collected from all animals for pooled faceal culture and worm egg count.
11.07.02	Donors were removed from all paddocks. They were held in an OJD.002A "infected paddock" and faeces and blood was collected. Donors were slaughtered within two weeks at an abattoir where relevant samples were collected to confirm infection status.
15.07.02	Hand feeding commenced due to pasture shortage. As each paddock contained mixed age groups the management of sheep onto hard feed was intensive..
12.09.02	Faeces were collected for a routine worm egg count as above and all sheep were drenched.
24.09.02	Faeces were collected for a post-drenching worm egg count and pooled faecal culture. Blood was collected from all sheep. Blood samples for the gamma interferon assay were submitted to CSIRO Geelong. The post drench worm egg count was conducted based on knowledge of the possibility of resistance/efficacy problems in the area these sheep have come from. Drench efficacy tests will be carried out periodically to assess the efficacy and choice of drench type used, based on 10-15 samples only per paddock taken on a random basis.
Hand feeding Jul 02 – Sep 02	The feeds used since the start of hand feeding were primarily lupins, but sheep were later managed onto fuel peas for cost reasons. Feeding rates averaged 1.2-1.7kg/head/week depending on pasture availability. Rates were increased to 1.5-2.5 kg/head/week over the last 2 weeks of November. Pasture availability measurements were taken every 14-21 days to ensure our feeding regimes reflected the availability of pasture feed.
07.10.02	Shearing of all sheep. Infected mobs were transported to and held on slats in the Arthursleigh woolshed within their allocated exposure groups. Control sheep were shorn in their paddocks with a mobile machine.
07.10.02	Water measurements for volume and quality commenced.

25.11.02	Start to transport water by tanker as required from Wollondilly River.
02.12.02	Faecal egg count all sheep. All counts were low (<450 EPG). Apply fly strike prevention (CLICK®) to all sheep in trial.
02.12.02	The existing site dam capacity was increased from approximately 1.2Ml to 2.0Ml
09.12.02	Collected individual faecal samples from all sheep for pooled culture.
13.01.03	Faecal egg count all sheep. All counts were low (<400 EPG).
17.02.03	Reticulation from the Wollondilly River was completed. Water was pumped from the river to a second 10,000-litre tank newly installed. Water will continue to be pumped from this source until dam water returns to an acceptable level to ensure good water quality for all stock.
07.04.03	Faecal egg count all sheep. All counts were low (<250 EPG). Collect blood from all sheep. Gel test sera stored at Camden. G IFN bloods transported to CSIRO Geelong on day of bleed. This bleed was 2 weeks later than scheduled due to wet weather, shearing at Arthursleigh and need to coordinate with CSIRO for the GIFN assay.
14.04.03	Crutch all sheep in Low, Medium or High challenge paddocks.
28.04.03	Crutch all Control sheep.
Hand feeding July 02 – April 03	Pasture availability improved in all paddocks with significant rainfall in mid February. Pasture growth/composition and DM availability was monitored weekly from early November 02 and the hard feed regime was managed accordingly. The sheep were fed peas and/or oats depending on market price and availability at the following rates: peas 0.7 - 1.5kg/head/week; oats 0.2 – 1.0kg/head/week. With further significant rainfall and pasture growth in mid - late April, use of hard feed reduced significantly.
26.05.03	Haemonchosis was suspected in 3 treatment groups due to clinical signs of anaemia and sub-mandibular anaemia. A worm egg count (WEC) was conducted on all sheep. Sheep in clinically affected groups were drenched immediately. Affected treatment groups had moderately high egg counts 450-600 epg. Sheep in all other paddocks had <450 epg. Sheep in all remaining paddocks were treated with Cydectin drench.
23.06.03	Individual faecal samples were collected from all sheep for PFC and blood was collected for AGID tests. Sera were stored for testing at Camden.
07.07.03	WEC all paddocks. Low counts were obtained from sheep in all paddocks (<400 epg)

08.09.03	Blood was collected from all sheep. Sera were stored at Camden while G-IFN bloods were transported to CSIRO Geelong on the day of collection. Individual faecal samples were collected from all sheep for PFC.
18.09.03	WEC all paddocks. Moderately high counts were obtained from all paddocks (400-650 epg). It was arranged to drench all sheep off shears.
29.09.03	All sheep were shorn. Infected mobs were transported and held on slats in the Arthursleigh woolshed within their allocated exposure groups. (Control sheep will be shorn week of 20.10.03 in their paddocks). All sheep were drenched with Cydectin off shears prior to returning to them their paddocks.
Hand feeding Apr 03 – Sep 03	This continued governed by pasture availability and body condition of sheep. Peas were fed at rates within the range 1-2 kg/head/week throughout this period. Hay was provided from mid-August for paddocks with extremely low pasture availability. Rates were increased for all sheep off shears by 0.2-1.2 kg/head/week. Water continued to be pumped from the Wollondilly River to meet animal requirements.
21.10.03	Control sheep shorn in paddocks.
09.11.03	All sheep treated with CLIK™ for fly strike prevention. Worm egg count samples collected and tested, 10 per mob. Low counts in all paddocks.
15.12.03	Individual faecal samples were collected from all sheep for PFC and blood was collected for AGID tests
12.01.04	Faecal samples collected from all mobs for worm egg count. Low-moderate counts in all mobs (0-450 epg), and with consideration of conditions and available nutrition a decision was made to drench all mobs.
22.01.04	All sheep drenched with Cydectin.
15.03.04	Individual faecal samples were collected from all sheep for PFC and blood was collected from all animals. Sera were stored at Camden for AGID tests, while heparinized bloods for gamma interferon tests were transported to CSIRO Geelong on the day of the collection.
12.04.04	Infected mobs of sheep were transported to shed for crutching. Sheep were held on slats in the Arthursleigh woolshed overnight within their allocated exposure groups. Control sheep were crutched in their paddocks from 14.04.04.
14.06.04	Individual faecal samples were collected from all sheep for PFC and blood was collected for AGID tests. Faecal samples were collected from all mobs for worm egg counts. Low counts were found in all mobs.

06.09.04	Infected mobs transported to the Arthursleigh woolshed for shearing. Sheep were held overnight prior to shearing on slats in their allocated treatment groups. Individual faecal samples were collected from all sheep for PFC and blood was collected from all animals. Sera were stored at Camden for AGID tests, while heparinized bloods for gamma interferon tests were transported to CSIRO personnel who were working at EMAI on the day of the collection.
18.10.04	Control sheep transported from paddocks to Arthursleigh woolshed in groups, shorn, and transported to Wollondilly abattoir the next day for slaughter 3 days after removal from paddocks.
Hand feeding Oct 03 - Oct 04	Pasture was virtually non-existent due to on-going drought conditions. Peas, lupins wheat and oats were fed through this period both as single feeds and in various mixes at 1.0-3.5 kg per head per week. Hay was provided intermittently for paddocks with extremely low pasture availability. Rates were gradually increased through this period to a maximum of 3.2 to 3.5 kg per head per week fed in 3 feeds every second day. Water continued to be pumped from the Wollondilly River to meet animal requirements.

10.2 Appendix 2
Paddock Map