



# finalreport

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

**Whole flock OJD vaccination at Merrill**

## **Abstract**

Ovine paratuberculosis or Johne's disease is an insidious mycobacterial infection of sheep capable of causing wasting and death. It has emerged in recent years in Australia as a cause of significant losses in some infected sheep flocks. MLA Project OJD.015 was commenced to enable a detailed longitudinal case study of an attempt to control the disease in a large self-replacing Merino flock at Gunning in NSW with an owner-estimated annual mortality rate up to 25%. Vaccination of the whole flock with exception of a small group in each age cohort was conducted, with documentation of the effects of vaccination and other strategies on mortalities and mycobacterial faecal excretion rates and mortalities in all age groups of animals. The study concluded that the significant decline in mortality risk from 24.2% to 2.85 during the study may only be partially attributed to the increased flock immunity following vaccination. By accompanying vaccination with extensive changes in flock management, including culling of clinical cases, sale of 'at risk' stock and introducing of replacements and reduction in stocking rates, control of the losses can be rapidly achieved in an infected flock with a high prevalence of OJD. This project has enhanced the knowledge and understanding of the performance of this vaccine under Australian conditions in a high OJD prevalence environment and recommends that field evaluation of the performance of the vaccine continue, particularly in low challenge environments.

## **Executive Summary**

Ovine Johne's disease (OJD) is a fatal enteric infection of sheep by *Mycobacterium avium* subspecies *paratuberculosis* 'S' strain (*Mptb.*). OJD in the Australian environment has proven difficult to both diagnose and control. The disease has become increasingly important as despite efforts to control spread by regulation of sheep movements, the disease has continued to spread in Australia. Funds allocated to research the many aspects of this disease have included projects that have investigated the level of losses on affected farms and the efficacy of vaccination in the control of the disease.

Project OJD.015 is a case study commenced in a single large self-replacing flock and former fine wool Merino stud farm at Gunning in NSW. The owner was concerned that rapid increases in losses of about 25% per annum were due to OJD. A study was commenced to measure the level of losses and determine the contribution of OJD to the loss. Further, as the owner was seeking to vaccinate the whole flock with Gudair™ OJD vaccine in an attempt to control the losses, an opportunity was presented to examine the efficacy of this strategy. Whole flock vaccination commenced in May 2000 and was completed in September 2000. To assist the research, a cohort of unvaccinated control sheep from each age group was left, to enable comparisons of mortality and *Mptb.* shedding data between vaccinates and controls over the course of the study.

Over the 4-year study period, adult sheep mortality declined significantly in the Merrill flock. By completion of the study in 2004, mortalities were uncommon, due to a decline in the flock mortality risk from 24.2% to 2.8%, mostly attributed to a decline in the OJD attributable mortality risk from 19.0% to 1.4%. However following commencement of vaccination, significant OJD mortalities did continue, particularly in adult vaccinates. The prevalence of faecal shedders declined from a high to a moderate prevalence following vaccinating of 2-year-old sheep, although shedding at rates considered sufficient for transmission of infection did continue, particularly in wethers. However, a low prevalence of faecal shedding in sheep vaccinated at 3 or 8 months of age, suggests that vaccination is similarly efficacious in these age groups. This finding suggests that vaccination may be beneficial in sheep when delayed to weaner age or older, even when exposed to a heavily contaminated environment since lambing. A decline in the prevalence of *Mptb.* shedders in the unvaccinated control sheep during the project was also observed. However it is considered more likely that a series of management changes, including the culling of clinical cases, sale of 'at risk' stock and introducing of replacements and reduction in stocking rates leading to a decrease in 'sheep-years at risk', would have had more significant impact on pasture contamination with *Mptb.* than the whole flock vaccination strategy.

In conclusion, whilst an increase in flock immunity from whole flock vaccination may have contributed to the control of OJD, it was probably a minor component of the successful control program achieved over such a short time frame. 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. As the study was conducted in a single environment with high mycobacterial challenge, which may have provided sustained stimulation of flock immunity, it is recommended that field evaluation of vaccine performance be conducted in low challenge environments.

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# 1 Background and Industry Context

## 1.1 Introduction

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Johne's disease is an important disease of ruminants worldwide which is often difficult to control and usually fatal. It is caused by enteric infection with *Mycobacterium avium* subspecies *paratuberculosis* (*Mptb*). Aside from production losses due to Johne's disease in farmed animals, recent speculation of a potential zoonotic link with Crohn's disease and hence food safety issues has further enhanced interest in this infection. Ovine paratuberculosis or ovine Johne's disease (OJD) in Australia is caused by the "S" strains of *Mycobacterium avium* subsp. *paratuberculosis* (*Mptb*) (Whittington *et al*, 2000). It was first diagnosed in 1980 on a property in the central tablelands region of New South Wales (Seaman *et al*, 1981) and has gradually spread to other areas of NSW and other states via movement of infected stock or local spread between neighbouring flocks (Sergeant 2001).

Ovine Johne's Disease (OJD) in Australia has become increasingly topical in recent years due to changes in government policies related to controlling the spread of the disease at both a state and national level. Nationally, an OJD Control and Evaluation Program (NOJDCEP) commencing in 1998, was completed in 2004. Recently, a new national program for the control of OJD has begun with vaccination as a major focus of disease control, using Gudair™ (CZ Veterinaria, Porrino, Spain) vaccine imported from Spain. Gudair™ vaccine contains a killed suspension of *Mptb* plus a mineral oil adjuvant and thiomersal as a preservative. It is approved for use by subcutaneous injection high on the neck behind the ear in sheep and goats in Australia. The vaccine is now accepted widely in the region where OJD has become endemic and vaccination has become a major tool for the control program for ovine paratuberculosis in Australia. Work conducted in this trial has contributed to this acceptance of the vaccine by industry.

One of the greatest impediments to control of OJD in Australia has been lack of understanding and acceptance that the disease can lead to significant losses on many infected farms. This led to delays in the diagnosis of OJD on infected farms and failure to implement on-farm disease control strategies. With Gudair™ vaccine not available for wider use until registration in April 2002, prior to that time many producers chose not to report adult sheep losses in fear of a diagnosis of OJD that resulted in imposition of regulatory controls on their stock movements at a time when a viable strategy for on-farm control was not identifiable. Significant mortalities in some flocks resulted.

The Merrill flock near Gunning was particularly affected by OJD, reporting an estimated annual loss in 1999-2000 in the order of 25% of adult sheep. In early 2000, the owner approached the Faculty of Veterinary Science Sydney University to assist, with a request to commence whole flock vaccination against OJD. Support was then sought for inclusion of Merrill in vaccine field trial work and presented to the Chief Veterinary Officer (CVO) and Chief Division of Animal Industries (CDAI) of New South Wales Agriculture. Permission was granted to commence whole flock vaccination at Merrill under the NSW Agriculture permit for experimental use of the vaccine in infected flocks, provided the proposed vaccine research to evaluate the whole flock vaccination strategy would be conducted. Whole flock vaccination commenced at Merrill in May 2000.

Accurate estimation of mortality rate in OJD infected flocks in Australia has proven difficult, with estimations reliant on producer observation and inventory records. The mortality rate was of

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particular importance with the decision to limit pre-permit experimental use of Gudair™ vaccine to flocks with annual mortality rates exceeding 5%. This study proposed a structured approach to more accurately determine mortality rate estimations in a flock, which appeared from anecdotal reports, to have the highest mortality rate attributed to OJD on record. Further, the permission to proceed with the whole flock vaccination strategy provided a unique opportunity to investigate the efficacy of the vaccine in adults in a heavily OJD infected commercial flock. Mortality rates, seroprevalence and faecal excretion of *Mptb.* were to be used as indicators of the effect of whole flock vaccination.

### **1.2 Project objectives**

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- (i) To determine the mortality rate due to OJD in the flock before vaccination and to measure the change in mortality rate following vaccination.
- (ii) To determine the effect of whole flock vaccination on;
  - a. The mortality rate of adult sheep due to OJD.
  - b. The incidence of OJD in young sheep both directly, by protecting them from clinical disease and indirectly, by reducing environmental contamination.
- (iii) To investigate the relationship between the mortality rate in the flock and the effect of vaccination on;
  - a. The seroprevalence of OJD in the flock and in each age group of the flock.
  - b. The proportion of the two-year-old sheep that are excreting *Mptb.*
  - c. The proportion of the flock having lesions of OJD at slaughter.

In addition it was proposed to estimate the change in pasture contamination by OJD from vaccinated adult sheep, by measuring faecal excretion rate of *Mptb.* Following a review of available faecal samples in storage, the 015 Extension project was developed with the additional objectives:

- (iv) To determine the *Mptb.* excretion rates in tagged 1998 drop (2-year-old) sheep collected in 2000, prior to their vaccination in 2000, compared with their excretion rates at ages 3 to 5, in vaccinated and unvaccinated sheep
- (v) To determine the *Mptb.* excretion rates in tagged 1999 drop sheep (3-year-old) vaccinated (at 8 months of age) and unvaccinated sheep, collected in 2002, and compare with the rates in tagged 2000 drop sheep (2-year old) vaccinated (at 3 months of age) and unvaccinated sheep, collected in 2002
- (vi) To examine the relationship between vaccination lesions and presence of OJD lesions by histology

## **2 Methods**

### **2.1 Farm management, inventory, vaccination and 'controls'**

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Paratuberculosis was diagnosed in the Merrill flock in 1996 when annual sheep losses from all causes were estimated to be between 3-5%. At that time the flock grazed 1,420 Ha and comprised about 10,000 adult sheep. In 1997, a cell grazing system was introduced involving management of the flock in mobs of up to 5,000 sheep, in paddocks of about 10 Ha, with a change of paddocks every 2 to 3 days. Weaner sheep were run separately from adult sheep until they were about 15 months of age. Shearing occurs in August-September and lambing in September-October. Ewes were set-stocked during lambing.

In 1999, the year preceding the trial and following a review of his inventory records, the producer estimated the annual mortality rate at Merrill to be about 25%. This was considered to be entirely due to OJD, resulting in a request by the producer to gain access to Gudair™ vaccine. The vaccine was unregistered at the time but field evaluation studies were being conducted in a number of central tablelands flocks, under a permit issued to NSW Agriculture. At the time the study commenced in 2000, the flock enterprise had reduced in size to approximately 1,000 Ha and 9,000 adult sheep. In 2001, significant management changes were implemented to combat the increasingly severe drought conditions. Sheep were sold on several occasions during the year and the remaining sheep were grazed at increased stocking rates to facilitate hand-feeding, provision of water and to enable close monitoring of feed intake and changes in body condition. Supplementary feed included chopped straw, palm kernels, commercial mineral mix and the best available protein supplement.

The study was conducted over a four-year period. This time period was divided into study years with each study year commencing and finishing at shearing in August to facilitate the comparison of post-mortem and inventory data. The study commenced in 2000-2001 as Year 0 and completed in 2003-2004 as Year 3. The property was visited four times in each study year and necropsies were conducted on all sheep dying within a five-day period. The initial sampling period was conducted pre-vaccination. Vaccination of all animals commenced following this sample period and at lamb marking in each subsequent year. All animals in the flock were vaccinated in Year 0, except for a small group of 150 (75 ewes, 75 wethers) ear tagged, numerically identified animals in each age cohort, which remained non-vaccinates for the duration of the trial. The non-vaccinates were always run as part of the main flock, with their vaccinated cohorts and comprise about 7% of each age group born after 1996. For additional comparisons between vaccinates and non-vaccinates over time, commencing in April 2000, 100 vaccinated sheep (50 ewes, 50 wethers) in each age cohort were ear tagged for subsequent identification. Faecal samples were collected from randomly selected sheep in age classes born after 1996 and that were ear-tagged and allocated to vaccinated and non-vaccinated 'control' groups.

Between May and August 2000, the owner vaccinated the entire flock of about 8000 sheep, except for the tagged non-vaccinated 'control' groups described. In the January of subsequent years, only 3-month-old spring born lambs were vaccinated. The first group of these, born in 2000, were faecal sampled for inclusion in subsequent faecal excretion studies. All adults vaccinated throughout the period of the study were identifiable by age from coloured stud tags so it was possible to identify and



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randomly select tagged and non-tagged vaccinated sheep in later years for faecal sampling. Animals from ewe and wether flocks were identified and recorded separately to reflect the property management structure.

Being a field trial in a commercial flock, the trial was managed in partnership with the producer. However the decisions on stocking rate and flock profile throughout the trial were largely made by the producer without close consultation with the research group and in response to a number of factors. These included the increasing severity of the 2002-2003 drought, improved returns for sheep meat, better recognition and culling of groups of sheep at risk of dying of OJD and the desire by the owner to obtain a financial return via salvage slaughter of sheep that would otherwise be of minimal value and were likely to die on the property. These initiatives led to significant changes to the flock profile throughout the trial, initially by replacement of many breeding ewes with purchased wethers and then intensive de-stocking of sheep in Year 2 as a result of the drought. A significant decline in the mortalities was recorded during the trial, suggesting that close attention to flock inventory data was necessary to gain a more accurate estimate of the sheep numbers 'at risk' of developing OJD.

To gain more accurate information on the total number of adults in the flock 'at risk' of developing OJD within a dynamic flock profile, the total number of 'sheep-years at risk' was calculated for each year of the study (see Section 3.2.2 for details). Total sheep numbers in the flock ranged from about 7,500 to almost 10,000 over the period of the study.

### **2.2 Mortality data and mortality risk estimations**

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#### 2.2.1 Mortality data

##### **Animals examined in quarterly necropsy study**

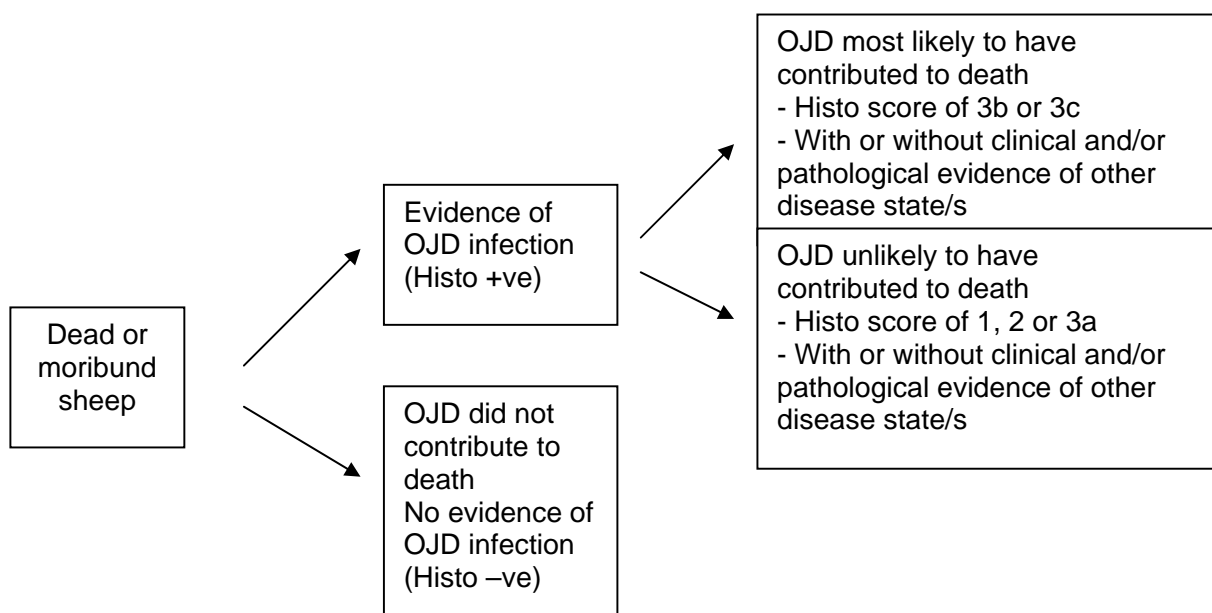
Within each 5-day post-mortem visit, all sheep that died and were collected within this period were submitted for necropsy. A total of 82 sheep were submitted to this procedure over the 4 years of the trial. From these sheep, clinical ante-mortem and gross necropsy information was recorded and samples taken for histopathology to assist with diagnosis and to identify the presence or absence of microscopic lesions indicative of OJD infection. For histological examination, samples were taken of the ileocaecal junction, ileocaecal lymph node and a cross-section of the terminal ileum within 10cm of the ileocaecal junction. These tissues were preserved in 10% buffered formalin, processed by routine methods, stained with both Haematoxylin & Eosin and Ziehl-Neelson stains and examined by light microscopy. Samples of contents were also taken from the abomasum and small intestine as washings for total worm count estimations.

The most likely causes of death were determined from necropsy data and material collected at the time of necropsy. The significance of the presence of OJD to the animal's death was derived following histopathological examination and grading of lesions in tissues by the modified Perez system (Appendix 1). If a slide did not present with any lesions suggestive of OJD it was categorized as NSL, that is, not having a lesion consistent with a diagnosis of OJD. Those slides where a Perez Type 1 lesion was observed, although considered suggestive of OJD, were not included as indicating a diagnosis of OJD as pathology of the intestinal mucosa was not observed in this

category and the subtle lesions present were not likely to have contributed to clinical signs of OJD. Those slides where Type 2 or Type 3a lesions were identified were considered infected with OJD but the lesions were not considered sufficiently severe to have contributed to the death of the animal from OJD. Sheep were then classified according to “the most likely cause of death” by a system we developed that considered all available evidence from the clinical and pathological examinations (Figure 1).

Sheep were classified as OJD having contributed to their death when there was histopathological evidence of severe OJD infection, indicated by the presence of an advanced, diffuse granulomatous enteritis scored as a 3b (multibacillary) or 3c (paucibacillary). Sheep with less severe OJD lesions on histopathology were classified as OJD present but unlikely to have contributed to their death. Sheep with no evidence of OJD infection on histopathology were classified as having died from other causes.

**Figure 1. Classification of the “most likely cause of death” following post mortem examination**



### **Animals examined in extension of pathology studies**

Additional pathological studies were conducted to further investigate the nature of the lesions in vaccinated and unvaccinated sheep that were at risk of dying from OJD and other causes during the study. This study was of material collected from an additional 198 sheep from the property however due to decomposition, only 184 sheep were considered suitable for histological study. This included three cohorts of sheep that were suffering from ‘wasting’ and had been placed in the ‘hospital mob’. The ‘hospital mob’ consisted of culls from the ‘tail’ of each sheep mob, being mostly sheep at or below a condition score of 2 on a scale of 1 to 5.

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Group 1 comprised 95 moribund sheep that were euthenased and necropsied from the 'hospital mob' at the commencement of the project pre-vaccination in 2000. Group 2 included 11 sheep necropsied from the 'hospital mob' post-vaccination in 2001 and 2002. Group 3 comprised 78 sheep with clinical signs of severe wasting with condition scores of 2 or less. The sheep were sold for salvage slaughter at Wollondilly abattoir with data collection at Wollondilly on 17<sup>th</sup> September, 2001.

These groups provided 95 unvaccinated sheep and 89 vaccinated sheep for comparison. Data recorded included ear-tag colour, age of sheep, size and texture of vaccination injection site lesion if present, and gross signs of thickening of the intestine with or without lymphatic cording, if present. Intestinal lesions were graded on a scale of 0 (nil) to 5 (severe), reflecting lesion severity. Lesion severity of 0 and 1 were considered unlikely to have gross evidence of OJD, with lesion severity of 2 or more, considered likely to have gross evidence of OJD. Samples for histological examination were collected and processed as described. Due to the pace of the slaughter chain at Wollondilly abattoir, numbers of sheep with spread of vaccination site lesions to the prescapular lymph node were not recorded, although samples were taken from several injection site lesions including one with extension of the lesion to the prescapular lymph node. Confirmation of sheep ages at necropsy or slaughter for Groups 2 and 3 was obtained by consideration of the ear tag colours and time of vaccination with Gudair™ vaccine was identified (Table 1).

**Table 1.** Vaccination schedule for sheep inoculated with Gudair™ vaccine at Merrill and examined by histological examination following euthanasia or slaughter.

<b>Sheep vaccinated</b>	<b>Date of vaccination</b>	<b>Months since vaccination at time of collection</b>	<b>Number of sheep in sample population*</b>
2100 lambs (1999 drop)	17/05/2000	16	11
2460 ewes in lamb and all of an adult age	12/08/2000	13	78
3500 wethers all of an adult age	19/08/2000	13	

\*Note: seven sheep had lost identifying ear tags so age unknown and data on gender not collected for all sheep (note only one week separated vaccination dates for sampled adults)

### 2.2.2 Mortality risk estimations

Crude mortality rates were calculated using sheep inventory records prepared from counts of sheep at the start of the year, being at shearing in August. The crude mortality rate was calculated by dividing the number of dead adult sheep identified during the year by the opening number, being the number of sheep present at the start of the year at shearing.

However in Year 2, a marked change in the pattern of sheep sales occurred with significant numbers of sheep sold as a result of the drought. Consequently, we considered that crude mortality rate calculations based on the opening number of sheep seriously under-estimated the risk of mortality for sheep in Year 2. We therefore defined the term 'sheep-years at risk' as a more accurate estimate of the average number of sheep present during each year. By examination of all available inventory records collected throughout the year an estimate of the number of sheep present at the commencement of each month was determined and the average number of sheep present for each

month determined by taking an average of the numbers of sheep present at the start of consecutive months. The 'sheep-years at risk' was then calculated from the average monthly inventory over the 12 months of each study year.

The 'mortality risk' was then calculated by dividing the number of dead adult sheep for the year by the number of sheep-years at risk, and represents the risk of mortality faced by each sheep during the one-year period. In all cases, mortality rates and mortality risk refer to sheep aged 12 months or more at the start of each study year. The OJD or paratuberculosis-attributable mortality risk was calculated by multiplying the mortality risk by the proportion of each year's deaths attributed to paratuberculosis at post-mortem examination.

### **2.3 Faecal excretion prevalence**

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Faecal samples were collected annually from all ear-tagged (individually identified) sheep and from a random selection of non-tagged vaccinated sheep (range  $n = 23$  to  $64$ ) in the ewe and wether flocks and stored at  $-80^{\circ}\text{C}$ . Faecal samples from animals within treatment groups (vaccinated, non-vaccinated; male, female; 3-month-old, 8-month-old, 2-year-old, etc) were randomly allocated to pools ( $n=5$  sheep/pool) for pooled faecal culture, which was undertaken using standard methods. The prevalence of sheep shedding *Mptb.* in faeces was estimated from the total number of pools, number of pools positive and number of samples per pool using the method of Williams and Moffitt (2001). The weeks of incubation required for cultures to develop peak growth index was also recorded, being inversely related to the number of viable organisms inoculated into the culture media, and so to shedding levels in sheep.

Due to the small sample sizes, statistical analyses were not intended to be done but trends were evaluated in data that were presented graphically. Faecal culture was by modified BACTEC 12B radiometric culture and direct confirmation by IS900 PCR. Following a review of available faecal samples in storage, an extension to the project was developed to explore shedding rates in different age cohorts vaccinated at different times. The groups identified for inclusion in the faecal excretion study were animals vaccinated at ages 3 months, 8 months and 2 years, calculated as the median age of each group assuming a joining period of 8-10 weeks.

#### **2.3.1 Excretion experiment 1**

Excretion experiment 1 aimed to determine whether vaccination of sheep older than 3 months of age was effective. This has been an important question raised by producers considering OJD vaccination. A common observation has been that many producers find that the complexity of procedures already conducted at lamb marking means that safe use of OJD vaccine at marking is difficult. OJD vaccinating at weaning or even at later musters is not uncommon. As lambs were vaccinated at 8 months of age at Merrill, this cohort of sheep could assist in providing answers to the efficacy of vaccinating later than the approved recommended age (4-12 weeks).

PFC was conducted on the ear-tagged sheep at 2 years of age, following vaccination as lambs at age 3 months (including both the 2000 and 2001 lamb-drops) or at age 8 months. The ages, times of sample collection and sample sizes were recorded (Table 2). Prevalence of shedding of *Mptb.*, weeks to reach peak growth index, numbers of serological reactors as assessed by ELISA (tested with CSL Parachek) and an estimate of possible mortalities were recorded as the numbers of sheep presenting each year.

**Table 2 Details of animals, vaccination and testing for excretion experiment 1**

<b>Group in Figure 1</b>	<b>2 yr</b>	<b>8 mth</b>	<b>3 mth</b>	<b>3 mth (+1yr)</b>
Year of birth	1998	1999	2000	2001
Year of vaccination	2000	2000	2001	2002
Year of sampling for PFC	2000	2001	2002	2003
Age at vaccination	2 yr	8 mth	3 mth	3 mth
Age at sampling for PFC	2 yr	2 yr	2 yr	2 yr
No. vaccinates sampled (wether flock) <sup>a</sup>	48	46	33	21
No. controls sampled (wether flock) <sup>b</sup>	55	20	53	47
No. vaccinates sampled (ewe flock) <sup>a</sup>	50	30	45	50
No. controls sampled (ewe flock) <sup>b</sup>	60	54	50	35

<sup>a</sup> Number of vaccinates tagged at each time point was 50 in each of ewe and wether groups.

<sup>b</sup> Number of controls (non-vaccinates) tagged at each time point was 75 in each of ewe and wether groups.

### 2.3.2 Excretion experiment 2

This experiment aimed to determine whether shedding rates were lower in sheep vaccinated at age 2 years, compared to non-vaccinated controls. This is a critical question for evaluating the value of whole flock vaccination strategy, particularly as it was apparent that sheep vaccinated as adults continued to die. However producers and their advisors were keen to know whether vaccination of adults may delay or reduce the level of shedding of mycobacteria. This might suggest that adult vaccination could provide a potentially strategic means of hastening de-contamination of the environment with *Mptb*. in medium to high prevalence flocks.

To examine this, the effect of vaccination at 2 years of age, PFC was conducted on faecal samples collected at the time of vaccination of 2-year-old sheep at the commencement of the trial. PFC was also conducted on faecal samples collected at annual intervals until these sheep were age 5 years. Both ear-tagged and randomly sampled animals were included with ages, times of sample collection and sample sizes recorded (Table 3).

**Table 3 Details of animals, vaccination and testing for experiment 2**

<b>Group in Figure 2</b>	<b>2 yr tagged</b>	<b>3 yr tagged</b>	<b>3 yr random</b>	<b>4 yr tagged</b>	<b>4 yr random</b>	<b>5 yr tagged</b>	<b>5 yr random</b>
Year of birth	1998	1998	1998	1998	1998	1998	1998
Year of vaccination	2000	2000	2000	2000	2000	2000	2000
Year of sampling for PFC	2000	2001	2001	2002	2002	2003	2003
Age at vaccination	2 yr	2 yr	2 yr	2 yr	2 yr	2 yr	2 yr
Age at sampling for PFC	2 yr	3 yr	3 yr	4 yr	4 yr	5 yr	5 yr
No. vaccinates sampled (wether flock) <sup>a</sup>	48	37	30	23	56	19	25
No. controls sampled (wether flock) <sup>b</sup>	55	26	0	18	0	0	0
No. vaccinates sampled (ewe flock) <sup>a</sup>	50	35	0	33	64	9	23
No. controls sampled (ewe flock) <sup>b</sup>	60	37	0	29	0	25	0

<sup>a</sup> Number of vaccinates tagged at each time point was 50 in each of ewe and wether groups.

<sup>b</sup> Number of non-vaccinates tagged at each time point was 75 in each of ewe and wether groups.

## **2.4 Serological prevalence**

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Serum samples were collected following jugular venipuncture from selected unvaccinated sheep only, as a possible indicator of the prevalence of OJD infection in the flock. Despite the known insensitivity of serological tests that examine humoral antibody, as a means of diagnosis of OJD, a profile of ELISA titres in the control sheep through the trial was considered a potentially valuable indicator of numbers of sheep developing clinical OJD, assuming that expression of humoral antibody is most likely an indication of onset of shedding. Samples were not collected from vaccinated sheep as a high rate of seropositive reactions from the vaccination would be expected.

The numbers of animals by age and year of study are detailed in Table 4. The samples were stored at  $-20^{\circ}\text{C}$  until required for analysis and then tested using the CSL Parachek ELISA by CSL, Parkville Victoria.

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**Table 4. Numbers of serum samples tested by Parachek™ ELISA of non-vaccinated sheep by age (both sexes) at sampling and year of study**

<b>Age (yrs)</b>	<b>Year 0</b>	<b>Year 1</b>	<b>Year 2</b>	<b>Year 3</b>
0.6	150	146	142	102
1.6	148	107	123	90
2.6	104	61	83	70
3.6	110	59	43	17
4.6	101		49	16
5.6	98			7

### 3 Results

#### 3.1 Farm management and inventory

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The total number of adults in the flock ranged from about 7,500 to almost 10,000 over the period of the study (Table 5) that commenced in 2000-2001 (Year 0) and was completed in 2003-2004 (Year 3). Significant changes in the flock profile occurred during the study. Replacement of females with purchased wethers and intensive de-stocking of aged sheep of both genders due to OJD and drought, commenced in Year 0 of the study, leaving a low proportion of the flock in the 4-5 year age cohort (Table 5).

**Table 5. Inventory data providing flock profile for the 4 years of the study**

		<b>Year 0</b>	<b>Year 1</b>	<b>Year 2</b>	<b>Year 3</b>
Gender	Age (yrs)	OPEN	OPEN	OPEN	OPEN
Ewes	1-2	3176	2516	2625	2090
	2-3	1588	1258	1313	1045
	3-4	1270	1006	1050	836
	4-5	318	252	262	209
Wethers	1-2	2763	4476	5385	3671
	2-3	1382	2238	2693	1836
	3-4	1105	1790	2154	1468
	4-5	276	448	538	367
Hoggets		2460	2079	1534	1621
Male lambs		1102	977	648	718
Female lambs		1230	1040	767	813
Ram Hoggets		128	62	119	93
Rams		74	70	72	62
<b>TOTAL</b>		8601	9203	9735	7537

Due to increasing severity of the drought, de-stocking intensified in Year 2 and is considered to have contributed to a substantial decline in both sheep numbers and mortalities recorded in Year 3 of the study (Tables 5, 6).



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**Table 6. Summary of inventory figures, sheep-years at risk and mortalities from inventory for all years of the study**

<b>Year of study</b>	<b>Opening sheep numbers</b>	<b>Sheep yrs at risk</b>	<b>Mortalities from inventory</b>	<b>Mortality risk</b>
0	8601	8030	1947	24.2
1	9203	8622	1836	21.3
2	9735	7481	1162	15.5
3	7537	5571	149	2.8

### **3.2 Mortality data and mortality risk estimations**

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#### **3.2.1 Animals examined in quarterly necropsy study**

A total of 82 sheep died and were collected over the 4 years of the trial during the four annual 5-day necropsy periods. Following histological study it was found that the percentage of sheep where OJD contributed to death was 78.7%, increasing to 87.5% of sheep in Year 1, then declining to 78.6 in Year 2 and 50.05 in Year 3. Note however that few animals were available for examination in years 2 and 3 of the study. This data was used to calculate the mortality risk, OJD extrapolated mortality risk and 'other disease' mortality risk (Table 7).

**Table 7. Summary of numbers of animals necropsied in quarterly post mortem studies, plus mortality risk, OJD mortality risk and 'other disease' mortality risk.**

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

Note: inventory records of 'sheep-years at risk' used to derive overall mortality risk, OJD-attributable mortality risk (due to the contribution of OJD) and 'other disease' mortality risk (where it was considered OJD did not contribute to death, plus deaths from other causes) estimations.

#### **Animals examined in extension of pathology studies**

Three cohorts of sheep that were moribund or sold for salvage slaughter at Wollondilly abattoir provided 198 sheep of which 184 were suitable for complete examination. This included 95 unvaccinated sheep in Group 1, with 46% (44) 2.5years of age at necropsy (range 1.5 to 5.5). The 11 Group 2 vaccinated sheep were all over 4 years of age. Of the 78 vaccinated sheep in Group 3,

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46% (36) sheep were 3 years of age (range 2 to 7 years of age). Gross evidence of thickening of the intestine suggesting OJD was present in 79% (75/95) of the Group 1 unvaccinated sheep and in the majority of vaccinated sheep, including 82% (9/11) of Group 2 sheep and 94% (73/78) of Group 3 sheep. However data for histopathological examination of the three groups of sheep categorised according to the modified Perez system (Table 8) indicates that only 75%, 73% and 86% of these 3 groups had histological lesions of OJD respectively. Thus of 184 sheep, 85.3% (157) had gross lesion of OJD, with 79.3% (146) having histological lesions of OJD.

**Table 8. Results of examination of sheep from extended pathological studies**

Sheep Group No.	No. sheep examined	% OJD Diagnosis (Total)	% sheep with histological OJD lesion (Perez category)			% sheep with no diagnosis of OJD	
			3c	3b	3a, 2	1	NSL
1	95	75 (71)	25 (24)	32 (30)	18 (17)	16 (15)	9 (9)
2	11	73 (8)	9 (1)	45 (5)	18 (2)	9 (1)	18 (2)
3	78	86 (67)	56 (44)	21 (16)	9 (7)	14(11)	0

Data presented as percentages with numbers of sheep in brackets

Table 8 includes data on the percentage of sheep that were found not to have a lesion considered capable of causing clinical signs of OJD, which includes those categorized as NSL or having a Perez Type 1 lesion on histological examination, despite presence of intestinal thickening in some at slaughter. Other diagnoses were not made. No effect of age at vaccination on lesion category was observed.

Vaccination injection site lesions were present in 57% of the vaccinated sheep (5/11 of Group 2 and 30/78 of Group 3), mostly located on the side of their neck in the vicinity of the presumed injection site. Extension of the lesion to the prescapular lymph node was noted in several sheep in Group 3 at slaughter. The size of these lesions averaged 2.6cm in diameter and ranged between 0.5 to 5cm. Oil droplets were regularly identified in the vaccine injection lesions on histological examination, accompanying locally extensive severe granulomatous to necrotising cellulitis. Examination of affected prescapular lymph nodes identified lesions of locally extensive severe granulomatous to necrotising lymphadenitis accompanying numerous oil droplets surrounded by macrophages and giant cells.

The pathology data from Group 3 was examined for a possible relationship between the presence and type of OJD lesions and the presence of a vaccination injection lesion. A total of 38.5% (30/78) of these sheep had injection site lesions and of these sheep, 90% had histological lesions confirming a diagnosis of OJD. Lesion category of vaccinates with injection lesions is provided in Table 9.

Comparison of data in Tables 8 and 9 suggest that the lesion type present in Group 3 sheep with vaccination injection site lesions, was similar to that in sheep with or without vaccination injection site lesions.

**Table 9. Results of examination of sheep with vaccination injection lesions**

Sheep Group No.	No. sheep with injection lesions	% OJD in sheep with injection lesions (Total)	% sheep with histological OJD lesion (Perez category)			% sheep with no diagnosis of OJD	
			3c	3b	3a, 2	1	NSL
3	30	90 (27)	60 (18)	20 (6)	10 (3)	0	10 (3)

### 3.3 Faecal excretion prevalence

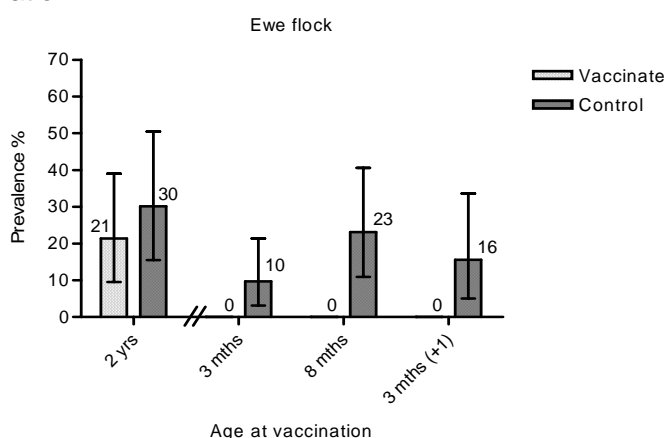
#### 3.3.1 Experiment 1. Efficacy of vaccination at ages 3 months and 8 months

##### Prevalence of faecal shedders

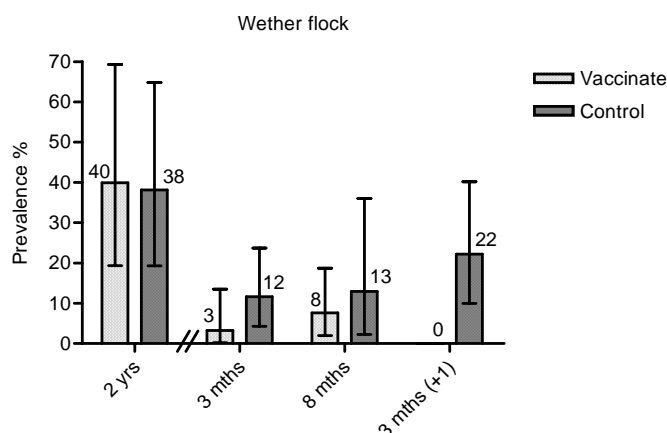
Data for the prevalence of shedding of *Mptb.* in ewes and wethers vaccinated at different ages is shown in Figure 2. The prevalence of shedders in 2-year-old sheep pre-vaccination are included to indicate the prevalence that could be expected in 2-year-old sheep on this farm in the absence of vaccination or other control measures. Those vaccinated at ages 3 months in two successive years and at 8 months, were tested at 2 years of age. The 3 groups, vaccinated at age 3 months in two successive lamb drops and age 8 months, had a lower prevalence of faecal shedders at age 2 years than non-vaccinated control groups (Figure 2). This was particularly evident in the ewe flock where there were no positive pooled cultures in vaccinated groups. Two-year-old sheep in both the ewe and wether flocks had a high prevalence of faecal shedders (20-40%) at the time of vaccination of the 3 and 8-month-old sheep (Figure 2).

**Figure 2. Prevalence of shedding of *Mptb.* in ewes and wethers in groups of sheep vaccinated at ages shown.**

Point prevalences are given by the height of the shaded bars (with the value also shown), with 95% confidence limits indicated by error bars. Note that those vaccinated at 2 years of age were tested at the time of vaccination.



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### Level of shedding and prevalence of ELISA reactors

The weeks of incubation required for cultures to develop peak growth index is inversely related to the number of viable organisms inoculated into the culture media, and so to shedding levels in sheep. The results for weeks to reach peak growth index suggest that sheep vaccinated at 3-8 months of age had lower faecal shedding rates than controls (Table 10).

**Table 10. Level of shedding of *Mptb*, expressed as weeks to peak growth index, for faecal pools in each group for excretion experiment 1.**

Group	Age at vaccination							
	3 mths		8 mths		2 yrs		3 mths (+1 year)	
	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control
Ewes	NA	5-7	NA	4-9	4-12	4-12	NA	5-8
Wethers	7	4-6	7-12	5-6	3-7	4-12	NA	4-8

NA = No growth indices as no positive pools recorded

Note: The level of shedding is inversely related to weeks to peak growth index.

About 6% of 2-year-old sheep unvaccinated sheep were ELISA positive. Too few sheep were tested to draw conclusions about prevalence at 2 years in the controls for sheep vaccinated at 3 and 8 months of age, but there were positive reactors (Table 11).

**Table 11. Number of ELISA reactors in excretion experiment. Data are number positive out of number tested.**

Group	Age at vaccination							
	3 mths		8 mths		2 yrs		3 mths (+1 year)	
	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control
Ewes	NA	1 of 17	NA	2 of 26	NA	3 of 47	NA	2 of 13
Wethers	NA	6 of 17	NA	0 of 7	NA	3 of 50	NA	1 of 20

NA = No animals tested in vaccinate groups

Note: only non-vaccinates were tested.

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The proportion of vaccinated and control sheep presented for sampling in excretion experiment 1 was variable. This was due to problems of incomplete muster at sample collection, culling or sales of tagged sheep, and mortalities due to all causes. However in general a higher proportion of vaccinates than controls was presented for shearing (Table 12). Animals in the 2 years age group were tagged in April 2000 and faecal sampled within one month of vaccination between July and August. As 4% of wether vaccinates were missing at shearing and a higher proportion of ewe and wether control sheep were missing at shearing, it is assumed that up to half of these control sheep may have died. Unfortunately an incomplete muster, due to difficulty of identifying the yellow tags in control sheep by the producer, is likely to have elevated the mortality estimations in control sheep in 2000.

**Table 12. Percentage of 2 yr old sheep not presented for collection of samples for PFC.**

Group	Age at vaccination							
	*2 yrs		3 mths		8 mths		3 mths (+1year)	
	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control
Ewes	0	20	10	33	40	28	0	53
Wethers	4	30	34	33	8	75	58	41

\*Animals in 2 years age group were tagged in April 2000 and faecal sampled within one month of vaccination between July and August, however a number of controls were missing at shearing following an incomplete muster, due to difficulty of identifying the yellow tags by the producer.

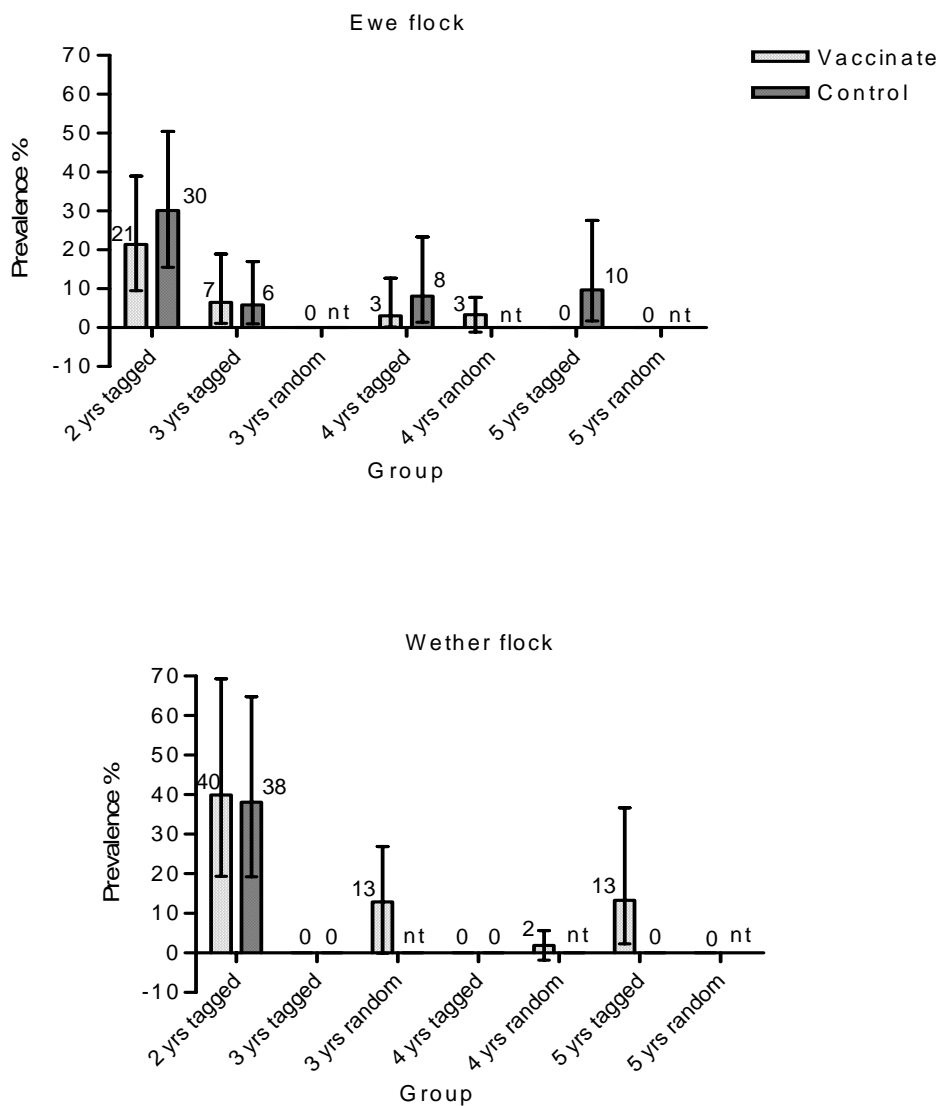
### **3.3.2 Experiment 2. Changes in prevalence of OJD when sheep vaccinated at age 2 years**

#### **Prevalence of faecal shedders**

In excretion experiment 2, vaccine was administered to sheep at 2 years of age, and testing was conducted annually. Between 20 to 40% of 2-year-old sheep were shedding *Mptb*. in 2000 when they were vaccinated (Figures 2, 3).

**Figure 3. Prevalence of shedding of *Mptb.* from ewes and wethers in a group vaccinated at 2 years of age, in randomly selected vaccinated sheep and in ear-tagged non-vaccinated controls at the ages shown.**

Point prevalences are given by the height of the shaded bars (with the value also shown; nt, not tested) while 95% confidence limits are indicated by error bars.



There was a much lower prevalence of shedding of *Mptb.* in these 2-year-old vaccinated sheep in 2001, 2002 and 2003 when the sheep were aged 3, 4 and 5 years respectively (Figure 3). However this applied to both vaccinated and control groups in both ewe and wether flocks and to non-tagged randomly selected vaccinated sheep. In tagged wethers there were no shedders at 3 and 4 years of age, but at 5 years of age more than 10% of vaccinated sheep were shedding. Results from the

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random samples of untagged sheep from the main flock indicated that some vaccinated sheep were in fact shedding *Mptb* at age 3 and 4 years. In wethers, none of the controls appeared to be shedding at 3, 4 or 5 years of age. In ewes, a low prevalence of shedders was observed in vaccinates and controls at age 3 and 4 years, and in controls at age 5 years.

Allowing for the small sample sizes, the results suggest that there were sheep shedding *Mptb* in both ewe and wether flocks throughout the trial. The apparent reduction in the prevalence of faecal shedders between 2 years of age and 3, 4 and 5 years of age in both vaccinated and control sheep is most likely due to the departure due to mortality or culling of clinically affected sheep from the flock after 2 year of age, as reflected in the inventory records (Tables 5,6).

### **Level of shedding and prevalence of ELISA reactors**

The level of shedding was determined from samples from animals individually vaccinated plus vaccinated animals randomly selected from the adult flock at each collection time point, being sheep vaccinated at 2 years and tested when 2, 3, 4 and 5 years of age. The level of shedding is inversely related to weeks to peak growth index. The data for sheep vaccinated when 2 years of age indicate that there is little difference in shedding rates between vaccinates and controls (Table 13).

**Table 13. Level of shedding, expressed as weeks to peak growth index for faecal pools in each group for experiment 2.**

Group	2 yrs		3 yrs		4 yrs		5 yrs	
	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control
Ewes	4-12	4-12	6-7	4-5	5-6	5-6	na	4-7
Wethers	3-7	4-12	11	na	6	na	5	na

na = No growth indices as no positive pools recorded

Serological results indicated that about 6% of 2-year-old sheep were seropositive at the time of vaccination (Table 14) with only one seropositive reactor of the seventeen sheep tested from the 3, 4 and 5-year-old control cohorts.

**Table 14. Number of ELISA reactors in experiment 2. Data are number positive out of number tested.**

Group	2 yrs		3 yrs		4 yrs		5 yrs	
	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control
Ewes	na	3 of 47	na	0 of 6	na	1 of 8	na	0 of 3
Wethers	na	3 of 50	na	nt	na	nt	na	nt

na = note that only non-vaccinates were collected for testing

nt = not available for testing

The proportion of vaccinated and control sheep presented for sampling in excretion experiment 2 was variable. Again this was due to problems of incomplete muster at sample collection, culling or sales of tagged sheep, and mortalities due to all causes. More than 25% of sheep present at 2 years of age were not present at shearing in subsequent years (Table 15). Note that as above (Table 12), the rate of presentation for control sheep aged 2 years was recorded following an incomplete muster, due to difficulty of identifying the yellow tags by the producer. However based on flock mortality figures for that year, up to half of these sheep are considered to have died.

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**Table 15. Percentage of sheep not presented for shearing the year of collection of samples for PFC.**

Group	*2 yrs		3 yrs		4 yrs		5 yrs	
	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control
Ewes	0	13	30	46	30	58	82	64
Wethers	4	24	26	67	50	81	62	100

\*Animals in 2 years age group were tagged in April 2000 and faecal sampled within one month of vaccination between July and August, however a number of control sheep were missing at shearing following an incomplete muster, due to difficulty of identifying the yellow tags by the producer.

### **3.4 Serology**

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Serum available from collections of six differing age groups of unvaccinated control sheep collected over the four years of the study was tested using the CSL Parachek ELISA (Table 16). No serum was available from 4.6 year olds in Year 1 and 5.6 year olds in Year 1 and 2. Despite a reduction in mortalities in years 2 and 3 the seroprevalence in all age groups over this period remained comparable with years 0 and 1 of the study. Note that the 2.6 year old cohort consistently had the highest rate of seroprevalence in all years of the study.

**Table 16. Seroprevalence (Parachek™ ELISA) of non-vaccinated sheep by age at sampling (both sexes) and year of study**

<b>Age (yrs)</b>	<b>Year 0</b>	<b>1</b>	<b>2</b>	<b>3</b>
0.6	2.67	0.68	0.00	4.9
1.6	8.11	8.41	16.26	6.67
2.6	13.46	19.67	22.89	20.00
3.6	9.09	11.86	11.63	17.65
4.6	12.87	NA	16.33	6.25
5.6	10.20	NA	NA	14.29

NA = not available



## 4 Discusson

Due to the severity of OJD losses at the commencement of the study and the increasingly severe drought throughout most of the study period, the owner of this flock instituted a whole flock vaccination strategy, followed by a series of management initiatives. This led to a major change in the flock profile during the study period. Early work confirmed that OJD losses were at a very high prevalence in this flock. However as we became aware of the significant culling of groups of sheep 'at risk' of dying from OJD, it became increasingly apparent that the annual opening and closing numbers obtained from the post-shears inventory were no longer an accurate estimate of the sheep at risk of developing OJD. We decided that consideration of the estimated monthly opening and closing numbers gave a more accurate estimate of the annual 'sheep-years at risk' and enabled observation of an association between the declines in mortalities, with the decline in the 'sheep-years at risk'. It is probable that this association largely reflects the significant changes to the flock profile, particularly in Years 2 and 3.

The quarterly necropsy study with histological confirmation of percentage of mortality due to OJD, was an important method of establishing that the majority of losses were in fact due to OJD and that this data could be related to the annual losses from inventory, rather than other causes of mortality related to seasonal events. The mortality study data documents an increase in the OJD-attributable mortality rate from 78.7% in Year 0 to 87.5% in Year 1, followed by a decline to 78.6% in Year 2 and 50% in Year 3 (although few animals were available for necropsy examination in years 2 and 3 of the study). This and the inventory data were used to calculate that remarkably, the mortality risk declined from 24.2% to 2.8%, with the OJD extrapolated mortality risk declining from 19% to 1.4% during the 4 years of the study. Interestingly there was no significant difference in OJD proportional mortality between years 0 and 2 and insufficient data for Year 3, indicating a reduction in both OJD-related mortality and mortality due to other causes. However, as the quarterly necropsy technique enabled only a small proportion of the total deaths each year to be examined in a relatively short time period, being 5% of the year the estimated values may not be particularly representative of the whole year, particularly later in the study when few animals were available for necropsy.

At the commencement of the trial, almost nothing of the pathology of OJD in vaccinated sheep was known. Comparison of lesions from vaccinated and unvaccinated sheep of varying ages involved examination of three additional cohorts at necropsy or slaughter. Grossly detectable thickening of the intestine suggesting OJD was present in the majority of sheep in all cohorts, however histological examination confirmed that consideration of gross lesions alone regularly over-estimated the prevalence of OJD. Gross intestinal lesions suggesting OJD were found in 85.3% of sheep with 79.3% having histological lesions of OJD. No correlations were found for any of the cohorts between the type of histological lesion and the degree of bowel thickening or the degree of gross signs. Sheep with severe gross pathology were not found to have one particular type of histological lesion while sheep with mild gross pathology were not found to have a different type of histological lesion. This suggests the degree of inflammation and not the type of histological lesion influences the visual appearance of bowel thickening. In addition, no effect of age at vaccination on lesion category was observed.

An unusual finding in this study was the variation in the Perez scores between the vaccinated and unvaccinated groups. The percentage of unvaccinated infected sheep (Group 1) with multibacillary lesions was 32%, with 25% having paucibacillary disease. This compares to vaccinates (Group 3) with 21% having multibacillary lesions and 56% with paucibacillary disease. Other studies have

identified that it is usual to find a greater proportion of animals with multibacillary lesions compared to animals with paucibacillary lesions in an OJD infected population. For example, Clarke and Little (1996) found 69% of a group of unvaccinated sheep to have multibacillary lesions and 31% to have paucibacillary lesions, with Carrigan and Seaman (1990) finding 88% of a group of unvaccinated sheep to have multibacillary lesions and 12% to have paucibacillary lesions.

Initial interpretation of this observation was related to the hypothesis that the paucibacillary lesion may represent a more successful immunological response of the sheep to infection. Thus such sheep, whilst still dying of OJD, could have a much lower rate of excretion of *Mptb*. prior to death. However the time of data collection, time after and extent of vaccination in the flock, severity of infection on the property, and sampling errors could all be involved in the obvious difference in the pathological character of the groups. The observation deserves some discussion.

Firstly, it is possible, although unlikely, that the observation of a predominance of paucibacillary OJD in vaccinates could simply be a sampling error and that the ratio of the lesions would be similar to that in other flocks if more animals were examined. Secondly, it is more likely that these results reflect a bias in the distribution of lesions in the overall population of sheep due to temporal differences and/or effects of the drought, where the clinical condition of the sheep was as much due to malnutrition and other factors as it was to OJD (whereas previous studies were primarily of clinical cases of OJD without the confounding effect of drought conditions). Further, it is also possible that the finding of more sheep with paucibacillary lesions in Group 3 may represent a more rapid demise of OJD infected sheep with multibacillary lesions. In addition, as these cohorts were collected at different times, the time of data collection could have affected the type of histological lesion. Group 1 sheep were collected at the start of the investigation and Group 3 collected almost 18 months later, although in such a high prevalence environment, it may be assumed that levels of exposure are comparable in both Groups 1 and 3, despite the 18-month difference in collection times. An even further possibility is that Group 3 sheep are demonstrating a post-exposure vaccination response, with the vaccine possibly acting like a booster in the high *Mptb*. challenge environment from intensive grazing of Merrill. Perhaps this observation was a reflection of a property with a high infection status where the challenge potentially boosts the immune response, directing the developing lesions in a paucibacillary direction? However, in sheep where the disease is already too far advanced and the antigenic load is too great, a sufficient immune response is not created and the lesion develops in a multibacillary direction. If this observation is an immunological effect of vaccination in a high challenge environment, we might expect to see this finding on other high infection properties.

The finding of a higher rate of paucibacillary lesions in vaccinated sheep prompted a separate project examining pathological findings on 56 adult-vaccinated sheep from a different property with a high infection status. However in this case the dominant histological lesion was multibacillary (unpublished observations). In conclusion, as this is a field observational study with numerous management factors involved in animal selection, plus the numbers of sheep examined were a limited proportion of the flock, we are reluctant to speculate further on the many potential contributing factors to this observation.

Vaccination injection site lesions were present in 43% of the sheep, mostly located on the side of their neck in the vicinity of the presumed injection site. Extension of the lesion to the prescapular lymph node was noted and oil droplets were regularly identified in the vaccine injection lesions on histological examination, accompanying locally extensive severe granulomatous to necrotising

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cellulitis. Examination of affected prescapular lymph nodes identified lesions of locally extensive severe granulomatous to necrotising lymphadenitis. These findings concur with other studies conducted on injection lesions from vaccinates (Windsor *et al*, 2005). The type of OJD pathology present in sheep with vaccination injection site lesions, was compared to that in sheep without vaccination injection site lesions. However no trends were noted, with no correlation between the presence of a vaccination injection site lesion and the presence of gross pathology or histological lesion type of OJD. This indicates that the presence of a vaccination injection site lesion is unlikely to influence the degree of bowel thickening or the formation of a particular type of histological lesion. Further, the presence of a palpable vaccination injection site lesion does not appear to be a useful indicator that adult sheep vaccinated with Gudair® have sufficient protection against the effects of OJD to alter the clinical outcome.

The results of faecal excretion experiments 1 and 2 indicate that the prevalence of faecal shedders in this flock declined from a high to a moderate prevalence following vaccinating of 2-year-old sheep. However shedding did continue, particularly in wethers. In contrast, data on the prevalence of faecal shedding in sheep vaccinated at ages 3 months and 8 months, appears to suggest that vaccination is similarly efficacious in these age groups, particularly in the ewes. This finding suggests that vaccination can be beneficial in reducing excretion rates in sheep when delayed to as late as 8 months of age, even when there is likelihood that they have been exposed to a heavily contaminated environment since lambing. Interestingly, all cohorts of control sheep that were age 3 months and 8 months when non-vaccinated, when faecal sampled at age 2 years in 2002, had a lower prevalence of *Mptb.* shedders than 2-year-old sheep in 2000. This would appear to indicate a decline in the prevalence of shedders in the unvaccinated sheep during the project. Although the whole flock vaccination strategy may have contributed to a reduction in the level of environmental contamination with *Mptb.*, it is considered more likely that the management changes were the predominant influence. These included the culling of clinical cases, changing the flock profile by sale of 'at risk' stock and introducing of replacements, plus significant reduction in stocking rates, leading to a decrease in 'sheep-years at risk'. All would have had a major impact on levels of pasture contamination with *Mptb.* The management changes commenced in early 2000 and their practice intensified over the trial period so that by the completion of the trial, mortalities were uncommon.

The serological and faecal culture results, particularly the Year 3 seroprevalence of 20% in 2.6-year-olds and the faecal culture results showing persistence of shedding in non-vaccinated animals throughout the study, suggest that despite declining mortality rates, a continuing high level of infection was present in this flock throughout the study period. However, it would appear that even though the prevalence of OJD infection persisted through the study, by instituting dramatic changes in management practices combined with increasing effect of vaccination of the younger flock, the owner achieved a substantial reduction in mortality risk, particularly in the final two years of the study.

The results of this study do provide illumination of the complexity of the question of whether whole flock vaccination with Gudair™ vaccine is a useful strategy in high prevalence flocks of adult sheep in Australia. The data were derived from a 'real world' study where the socio-economic reality of survival of a family business overwhelmed the scientific objectives of the project. Clearly, the management changes instituted by the owner are very likely to have greatly influenced the mortality rate. With the serological and faecal results suggesting that a high level of OJD infection persisted in the flock despite the rapid decline in mortality rate, it is very likely that the change in flock profile was a more significant impact on mortality rate than the effect of whole flock vaccination. However it is

also probable that vaccination may reduce *Mptb.* excretion rates in the survivors and thus contribute to a decrease in contamination levels, consequently reducing the risk of exposure of the infection to newborn lambs or other sheep. In conclusion, whilst a significant increase in flock immunity as a result of whole flock vaccination could be a factor that supported the control of OJD in the flock, it was probably a minor component of the successful control program achieved over such a short time frame.

## 5 Success in achieving objectives

Determining the success or otherwise of the project requires consideration of the context in which the trial was initiated and the reality of the often differing expectations of the rural and scientific communities. The trial was initiated largely in response to demands from the rural community to assist a critical OJD mortality problem at a time of strident calls from the rural community for an alternative to regulation of OJD. Despite complete lack of information on the potential performance of the vaccine in Australian sheep in the Australian environment, there was immense pressure on regulatory policy-makers to permit the use of whole flock vaccination at Merrill, irrespective of concerns that there was little scientific justification for this strategy. The objectives of OJD.015 are listed below with comments on the achievement of each:

- (i) To determine the mortality rate due to OJD in the flock before vaccination and to measure the change in mortality rate following vaccination.

This was achieved with annual flock and OJD-attributable mortality rates determined successfully from a combination of quarterly necropsy studies and examination of flock inventory data. Data indicate a drop in flock mortality rate from 24% to less than 3% and a drop in OJD-attributable mortality rate from 19% to 1.4% over the four years of the study.

- (ii) To determine the effect of whole flock vaccination on;
  - (a) The mortality rate of adult sheep due to OJD.
  - (b) The incidence of OJD in young sheep both directly, by protecting them from clinical disease and indirectly, by reducing environmental contamination.

These objectives were not able to be determined in isolation from the significant management changes that were introduced by the owner in response to both his critical mortality situation and the increasingly severe drought throughout much of the study period. However the project did clearly demonstrate that a combination of management changes leading to a marked reduction in 'sheep-years at risk' with vaccination was able to markedly reduce mortality rates of all ages of sheep on the property and reduce environmental contamination with *Mptb*.

- (iii) To investigate the relationship between the mortality rate in the flock and the effect of vaccination on;
  - (a) The seroprevalence of OJD in the flock and in each age group of the flock.
  - (b) The proportion of the two-year-old sheep that are excreting *Mptb*.
  - (c) The proportion of the flock having lesions of OJD at slaughter.

These objectives were met. The seroprevalence of the majority of age groups of control sheep was determined over time, suggesting that it remained consistently high in all age groups throughout the trial, with the 2.6year old cohort consistently having the highest rate of seroprevalence in all years of the study. The excretion rate of 2-year-old sheep over time was determined and a sheep vaccinated

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at age 3 months in two successive lamb drops and age 8 months, had a much lower prevalence of faecal shedders at age 2 years than both non-vaccinated control groups and sheep vaccinated at 2 years of age. The proportion of a sample of the flock having lesions of OJD at slaughter at the abattoir or on-farm was high, being 85.3% with thickening of the bowel suggesting gross lesions of OJD, with 79.3% confirmed with histological lesions of OJD.

The 015 Extension project was developed to estimate the change in pasture contamination by OJD from vaccinated adult sheep, by measuring faecal excretion rate of *Mptb.* from stored faecal samples. The additional objectives were:

(iv) To determine the *Mptb.* excretion rates in tagged 1998-drop (2-year old) sheep collected in 2000, prior to their vaccination in 2000, compared with their excretion rates at ages 3 to 5, in vaccinated and unvaccinated sheep.

This objective was met. A much higher rate of excretion *Mptb.* was found in 1998-drop sheep collected in 2000, compared to that in both 2-year-old vaccinated and control sheep when 3, 4 and 5 years of age. However rather than suggesting the vaccine reduced the prevalence of faecal shedders, the results are considered most likely due to the departure due to mortality or culling of clinically affected sheep from the flock after 2 year of age, as reflected in the inventory records.

(v) To determine the *Mptb.* excretion rates in tagged 1999-drop sheep (3-year old) vaccinated (at 8 months of age) and unvaccinated sheep, collected in 2002, and compare with the rates in tagged 2000-drop sheep (2-year old) vaccinated (at 3 months of age) and unvaccinated sheep, collected in 2002.

This objective was met. Excretion rates of 2-year old sheep vaccinated at age 3 months in two successive lamb drops and age 8 months, had a lower prevalence of faecal shedders at age 2 years than non-vaccinated control groups, particularly in the ewe flock, where there were no positive pooled cultures in vaccinated groups.

(vi) To examine the relationship between vaccination lesions and presence of OJD lesions by histology.

This objective was met however no evidence of a relationship between vaccination injection lesions and presence of OJD lesions was observed.

## **6 Impact on meat and livestock industry**

Prior to the introduction of Gudair™ vaccination, OJD in Australia was widely considered as one of the major animal health problems facing the livestock industries. OJD had effectively divided the sheep industry, with lobbying for regulation by the OJD-free areas and more intense lobbying for de-regulation in infected areas by producers financially compromised by trading restrictions. Enormous political sensitivity and emotion had been generated in the NSW sheep industry with intense criticism of the regulatory authorities and personnel involved in managing the disease.

OJD control by regulation was seen as a punitive strategy compromising the livelihood of affected producers and eradication by de-stocking was widely disputed as a viable disease control alternative. Many affected producers were promulgating OJD vaccination as their most palatable option of OJD control. Whole flock vaccination was considered an option for the most desperate. The owners of Merrill were particularly affected by this, having lost their stud business and possibly their reputation for quality sheep management. At the time this project was conceived, they considered OJD vaccination as their only possible strategy for rescuing their business and whole family future from what they perceived was a very dire and seemingly hopeless situation. Although other disease control strategies were discussed and doubts cast as to the efficacy of vaccinating adults in such a high prevalence environment, the owners of Merrill were desperate to pursue this strategy. Arguments were prepared and accepted by the CVO and DCAI to support the strategy provided research data could be generated.

As it transpired, institution of vaccination appeared to partially alleviate the enormous stress suffered by the owners of Merrill and other disease control strategies could then be instituted. Thus the immediate impact on the livestock industry of commencing this trial was the positive influences generated by providing a rescue strategy for a property in deep trouble with OJD losses.

The situation at Merrill generated keen interest from local and regional producers and the Merino industry in particular. Many of these producers were also suffering economic losses from the mortalities, plus the economic and psychological pressure from regulatory controls and the stigma attached to a positive diagnosis of OJD. For this reason, ongoing results from the project, which were keenly sought by industry and producers throughout the study period, were presented annually at conferences and meetings. The positive results of the this project and the related vaccine trial project (OJD.009) and the eventual registration of the vaccine in April 2002, generated a palpable release of 'OJD pressure' in the rural sector. The positive results emerging from both studies confirmed that sheep producers now had a tool that would allow OJD to become another disease and management issue and the continuous barrage of letters in *The Land* against the OJD program largely faded.

Annual updates of the project have been presented at conferences in Australia between 2001 and 2005, at the International Sheep Veterinary Congress in Greece in 2005 and at the International Colloquia on Paratuberculosis in Spain in 2002 and Denmark in 2005 (Appendix 2). In addition, informal advice on progress of the project has been provided on a continuing basis to industry personnel including animal health policy makers and at numerous producer meetings in eastern Australia. Use of Gudair™ appears already to be a routine practice in many infected flocks and at the time of writing over 7million doses had been sold.

## **7 Conclusions**

In a flock with a very high prevalence of mortalities, investigations confirmed that the majority of losses were attributable to OJD, supporting a strategy to study whether whole flock vaccination with Gudair™ would alleviate the losses. Due to this being a longitudinal field trial on a real world property, flock management decisions were made independently of the aims and objectives of the trial and we were unable to separate the effect of changing the flock profile from the effects of vaccination.

This trial examined sheep in a number of successive generations in only one very high prevalence flock. A small but significant number of unvaccinated control sheep were present in all cohorts and this was considered necessary to facilitate measurement of the effects of vaccination between vaccinates and controls. However the usual practice on most properties is to vaccinate all lambs over successive years, so the problem of several hundred unvaccinated sheep being a source of potential contamination with *Mptb*. is ultimately avoided. The trial design was further complicated at Merrill by owner management decisions in response to the overwhelming mortality rate and drought, leading to extensive culling and replacement of ill and 'at risk' sheep. Therefore research to monitor the use of vaccination in successive generations where unvaccinated control sheep are not retained and 'at risk' sheep not readily disposed of, is important to demonstrate what Gudair™ is likely to be doing in real world situations, particularly on farms of differing OJD prevalence.

In a sense this project was a "worst-case scenario" from the perspective of OJD, being by far the highest level of OJD-attributable mortality recorded during the current epidemic of OJD in Australia. As it can be expected that vaccination will be more effective when combined with the type of management procedures that occurred at Merrill, then when used alone, the trial has proven to be a very valuable demonstration of what can be achieved when extreme management and vaccination strategies are applied. The results of other studies and field experience over many years will be necessary to gain a clearer picture of what can be achieved by vaccination alone.

High levels of excretion by vaccinates with multibacillary OJD prior to death indicates there is considerable risk that some vaccinated sheep could transfer the disease. This has implications for translocation of vaccinated sheep to very low prevalence areas, and implies that sustained vaccine use will be necessary to avoid recrudescence in heavily infected flocks.

Specific recommendations from this trial are:

1. Continue on-going research into the efficacy of vaccination over several generations of sheep on farms of varying OJD prevalence.
2. Review the likely effectiveness of on-going research into the efficacy of vaccination and duration of immunity in low challenge environments
3. Consider the value of extension of the limitations of vaccination in control programs for OJD.
4. Consider the value of extension of the role of flock management in addition to vaccination in the control of OJD



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## **9 Appendices**

### **9.1 Appendix 1: OJD histological lesion classification system**

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This classification system was modified from Perez et al. 1996.

#### **Type 1 Lesions**

##### **Location**

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

#### **Type 2 Lesions**

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

#### **Type 3 Lesions**

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

##### **SubType 3a**

- Type
  - Lesions sporadic, multi-focal
  - AFB are seen in granulomata in the mucosa
- Location
  - PPs and associated mucosa:
    - lesions are similar to Type 2
    - granulomata in the lamina propria are larger, extend from PPs, involve more villi, cause enlargement of villi
  - Areas of mucosa not associated with PP's:
    - granulomata are small and well delineated in lamina propria of villi and/or the basal area

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- Submucosa and serosa:
  - foci of inflammatory cells (mostly lymphocytes and macrophages) are seen around lymphatic and blood vessels
- MLNs: granulomatous lesions

### **SubType 3b (Multibacillary)**

- Type
  - Diffuse granulomatous enteritis creates a mosaic formed by macrophages, epithelioid cells, a few giant cells (2-3 nuclei), small numbers of lymphocytes and other leukocytes
  - AFB in abundance, numbers in mucosa > than in lymphoid tissue.
- Location
  - PPs: granulomata in the interfollicular areas, follicles and domes, with infiltrates giving a mosaic-like appearance, among lymphoid aggregates
  - Mucosa:
    - villi thickened, apices flat and wide, fused, fewer crypts due to infiltration
    - in some sheep the mucosa is less thickened, epithelioid cells are seen in the villi (mostly the apex) and diffuse enteritis is due to confluence of numerous small granulomata
  - Submucosa:
    - lymphocyte and plasma cell infiltrates, initially perivascular, but extending to the muscular layer, with lymphatics dilated and thrombi composed of macrophages seen within
  - Serosa:
    - Lymph-angitis/angiectasis, perivascular lymphocyte/plasma cell aggregates
  - MLN's:
    - multi-focal or diffuse granulomatous lymphadenitis
    - subcapsular sinuses usually contain macrophages
    - serosal lesions similar to those in the gut serosa

### **SubType 3c (Paucibacillary)**

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

**NSL lesions** – No significant lesion

## **9.2 Appendix 2. Publications arising from this project**

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Throughout this study, the results of the trials have been keenly sought by both producers and industry. The project collaborators have regularly attended producer meetings and discussed the interim results of the project. Updates of this and related projects have also been presented regularly, usually as conference proceedings. Final publication of the results in peer-reviewed literature is planned.

Publications arising directly or indirectly from the project are listed in reverse chronological order.

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