Exposure Factors Leading to Establishment of OJD Infection and Clinical Disease

Epidemiology of OJD-1

Project number OJD.002A
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ABSTRACT

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

ACRONYMS AND ABBREVIATIONS

AFB	Acid-fast bacilli
GLMM	Generalised linear mixed model
H & E	Haematoxylin and eosin stain used in microscopic pathological examination of tissues.
ha	Hectare
M ptb	*Mycobacterium avium* subsp *paratuberculosis.* Equivalent to MAP.
NSW	New South Wales, Australia.
OJD	Ovine Johne's disease. Equivalent to ovine paratuberculosis.
ZN	Ziehl-Neelsen stain used in microscopic pathological examination of tissues

PARTS 1 AND 2 OF THE STUDY

There were two, interlinked components of this study and they are referred to herein as Parts 1 and 2. The main experiment – Part 1 – is described in the body of this report and Part 2 is reported in Appendix 3. Part 2 was a study to examine the susceptibility to infection of adult ewes in comparison to their lambs. The possibility of adding this additional component to the study was proposed after Part 1 had commenced and was initially designed and funded as a variation to the original contract.

EXECUTIVE SUMMARY

1. The aim of this project was to develop grazing management strategies for owners of OJD-affected flocks which would reduce the impact of OJD in their flocks. Over a three year period the benefits of providing low contamination environments for Merino lambs were investigated in an experimental flock in the OJD-endemic area near Goulburn in NSW.

2. We determined that there is a relationship between the level/duration of exposure to *M ptb* on pasture and the level of OJD infection. It is possible to reduce the incidence of severe OJD infections and clinical disease by reducing the exposure of young sheep in the pre-weaning and post-weaning periods. We recommend the following management strategies for infected flocks:

    a. Prepare low-OJD-contamination pastures by removing *M ptb*-shedding sheep for at least three months, preferably including a summer period. Pastures can be left un-grazed, or grazed by adult cattle or by unaffected sheep that are destined for slaughter.

    b. Join ewes over as short a period as possible, so that weaning is not delayed by late-born lambs. Shortly before lambing, and again at marking, remove ewes suspected of having OJD (typically any ewes with unexpectedly low condition score). Move the lambing flock onto the low-contamination pastures shortly before lambing and employ low stocking densities.

    c. Wean the lambs early in order to separate them from the main source of infection (their dams and the pastures contaminated by their dams). Weaning onto pastures which have low levels of *M ptb* contamination will reduce the incidence of OJD. Lambs can be weaned when the youngest are seven weeks old if pastures are highly nutritious.

    d. If low-contamination pastures are scarce it is more important to use them as post-weaning pasture for lambs than for lambing ewes.

    e. Leave weaned lambs in the low-challenge environment for as long as possible. Six months may be ideal, for the paddock could then be prepared for the following year's weaners. During their first nine months, the weaners are expected to have shed very few *M ptb.*

3. Use of a short joining period and weaning when the youngest lambs are seven weeks old is consistent with recommendations for good control of internal parasites as is the preparation of low-OJD-contamination pastures for weaned lambs. Anthelmintic-treated adult cattle or non-lactating adult OJD-free sheep should be used to pre-graze the pasture for three months including a summer period, or six months if summer is not included. There may be an increased risk of parasitism in the weaned lambs if the same pastures are used repeatedly, particularly if the newly weaned lambs graze the pasture for more than six months each year.

4. We experienced difficulty in preventing OJD infection in control groups, highlighting the difficulties faced by producers who attempt to eradicate the disease if there is infection in neighbouring flocks.

5. There were significant production effects due to OJD. Sheep started to lose weight nearly a year before death due to OJD, and by about eight months before death, had lost 4% of their weight (about 1.5 kg). They then lost weight at an accelerating rate and, by the time of death, were 32% lighter (about 12 kg) than sheep free of OJD. On average, sheep in the clinical phase of OJD were 3 – 4 kg lighter than expected and had fleece weights about 0.3 kg lower than expected.

6. Some sheep shed *M ptb* in faeces from as early as 12 months of age. Deaths commenced at 18 months of age and the rate of mortality peaked at around 30 months. Most sheep which shed *M ptb* in faeces died in the subsequent 18 months but a small proportion will survived longer before developing clinical signs and dying. Some sheep can contain the infection for at least 18 months such that there is little, if any, damage to the intestine. It is possible that some infected sheep recover from infection completely.

Details of the experiment are as follows.

7. In Part 1 (the main experiment), sheep were exposed to different levels of *M ptb,* from birth to weaning and/or from weaning onwards. The different levels of exposure were called high (H), medium (M) and low (L). It was estimated that the level of exposure in the H groups was about 10 times higher than in the M groups. The L groups were not deliberately exposed to *M ptb* but accidental contamination at very low levels did occur. The experiment was replicated. The results are summarised in the table below.

			Pre-weaning pasture			Post-weaning pasture					
Nominal description of exposure history	Mean availability of *M ptb*	Infected dam flock	Low	Medium	High	Low	Medium	High	OJD infection rate (%)	OJD severe infection rate (%)	OJD death rate (%)
IHH	494	+			+		+		36.6	12.7	8.3
IHL	476	+			+	+			38.8	10.4	6.0
ILL (m)	311	+			+	+			20.3	11.6	2.9
UHH	309			+				+	28.6	11.4	8.6
UHL	50			+		+			17.4	2.9	1.4
ULH	301		+					+	17.6	5.9	1.5
ULL	0		+			+			18.3	5.6	2.8
Dams of UHL (2)	>50			+		+			>5.6	1.9	0.0

(m) = moved two times to clean pastures during lactation. (2) = part 2 of the experiment.

8. In Part 2, previously unexposed sheep which were the dams of the UHL group and which had been exposed to *M ptb* for the first time from 3-4 years of age, simultaneously with their lambs, were kept for two years before slaughter and post-mortem examination.

9. Two factors contributed to high OJD infection rates in sheep: the level of exposure and the duration of exposure. There was a direct relationship between the level of exposure pre-weaning and the incidence of OJD infection later in life. Some lambs were apparently very susceptible to infection, but it was possible to prevent high rates of infection by reducing the amount of pre-weaning pasture contamination. Continuity of exposure from the pre-weaning to the post-weaning periods resulted in high levels of infection, but this was avoided by weaning onto low contamination pastures. This was not apparent for lambs born to infected ewes then exposed to high challenge pre-weaning and medium challenge post-weaning (IHH vs IHL)

10. Sheep beyond weaning age remained susceptible to OJD infection. Group ULH, which was not deliberately exposed to infection until after weaning, had similar levels of OJD to group UHL, which were co-grazed with infected ewes for four weeks at the start of lambing. Further evidence against a significant age-resistance to infection was provided by the infection rate in dams of UHL sheep in Part 2 of the experiment, although none of these sheep died of OJD.

11. The occurrence of OJD in the control group (ULL) was unexpected and made interpretation of the incidence of OJD in group ULH more difficult. The ULL sheep were inadvertently exposed to extremely low levels of pasture contamination yet had an OJD incidence similar to the groups which were deliberately exposed. This finding highlighted the difficulty of preventing infection of sheep in a clean flock when infected sheep are present nearby and raises the possibility that a small percentage of sheep are particularly susceptible.

12. There were large differences between replicates of some treatment group highlighting the variability associated with the incidence of OJD infection in flocks of Merino sheep.

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BACKGROUND

This project was conceived during early 1999 and commenced in August of the same year. The need for the study arose from two developments in the history of the OJD epidemic in Australia. The first of these was the growing body of evidence that the disease in Merino flocks could have a very substantial direct impact on the productivity and profitability of flocks, in addition to the effects on profitability arising from restrictions on trade which were placed on affected farm businesses. This was in contrast to early assessments of the impact of OJD which found the disease to be relatively minor and similar in impact to OJD in New Zealand flocks[1,2]. The second was the growing realisation and acceptance that eradication of the disease from the country was not possible with the limited resources available to control it[3]. Sheep producers with OJD-affected flocks would have to learn to live with OJD and, in order to continue to run profitable businesses, they would have to institute management strategies to limit the impact of the disease on their flocks.

Reports of the impact of OJD in flocks varied, with some growers claiming that mortalities due to OJD exceeded 10% annually while others, even after ten or more years of endemic infection, were reportedly able to maintain low levels of infection through strategic management of their flocks[4,5].

It was necessary for advisors to make recommendations to try to assist the owners of affected flocks. These recommendations were generally based on field experience and disease management principles, although there was very little evidence to demonstrate the success of the recommended approaches. Central to most recommendations was the belief that lambs would be more susceptible to infection than adults, based on observations made in cattle over many years. Hence, if lambs could be protected from infection during early life then, hopefully, many of them would remain free of clinical disease throughout their adult life even if exposed to infection later.

It is difficult, however, to ensure that lambs have only low levels of exposure to *M ptb*. For most flock owners this strategy would require considerable changes to the way they manage their flocks.

Under the extensive sheep management systems usually practised in Australia, ewes give birth and rear their lambs at pasture. It is not practicable or affordable to separate newborn lambs from ewes and rear them artificially, as is done with dairy cattle. Neither is it affordable to remove ewes and lambs from pasture and feed them a prepared ration. Commercial necessity, therefore, dictates that ewes and lambs must run together at pasture until the lambs can be weaned.

For wool-producing flocks in the medium and high rainfall districts of Australia, it is widely recommended that lambs are weaned when the oldest lamb is about three months of age and moved to a pasture that has been prepared in such a way that contamination with worm parasite eggs and larvae is low. This strategy has advantages for parasite control in lambs and for the recovery of body condition and wool productivity for the ewes. It is a successful strategy and is widely practised. It is generally not feasible to wean lambs at ages less than seven weeks because, at weights below 10 to 12 kg, their nutritional requirements for growth are unlikely to be met by pasture. As lambing typically is spread over a five to eight week period in most flocks, and sheep producers prefer to wean all lambs at one time, when the youngest lamb in the flock is seven weeks of age the oldest will be 12 to 15 weeks.

It is feasible to prepare pastures of low *M ptb* contamination status by ensuring that no infected sheep has grazed the pasture for the previous six to 12 months or more. Sheep-derived strains (S strains) of *M ptb* can survive, in quantities sufficient for cultural detection, for up to 13 months on shaded pasture and seven months in exposed sites[6]. The spelling of pasture from sheep is more likely to result in a low contamination pasture if the six to 12 month period includes some months of hot, dry weather. The pasture could be grazed by cattle (although calves should not be exposed to pastures contaminated by sheep with *M ptb*) or cropped. As an alternative to cattle, it could be grazed by sheep if they are known known to be free of OJD.

Given the difficulties in preparing low *M ptb* contamination pastures in addition to the other constraints on grazing management which exist on most farms, the availability of low contamination pastures on an infected farm is likely to be limited. For example, a sheep producer may be able to prepare sufficient

pasture to graze lambs after weaning, or to allow some or all of the ewes to graze during lambing and lactation, but is unlikely to have sufficient low-contamination pastures for both lambing ewes and weaned lambs.

Assuming that low contamination pastures can be produced but will be in short supply, how should they be used in order to achieve the best result in terms of low OJD-infection rates of young sheep? Should the "safe" pastures be grazed by ewes during lambing so that pre-weaned lambs are protected from high levels of *M ptb* exposure, or should these pastures be reserved for the post-weaning period? There are a number of factors to be considered in attempting to answer this question.

- If the "safe" pastures are grazed by ewes during lambing and lactation, infected ewes in the flock will cause contamination to occur during that time. This level of contamination may be so significant that it outweighs the benefits of having very low levels of contamination at the start of the lambing period.

- If the "safe" pastures are reserved for lambs at weaning at seven to 15 weeks of age, the lambs may already be beyond their most susceptible age and may already have been infected at high levels by grazing contaminated pastures in the pre-weaning period.

- Infection of neonatal lambs may occur directly from ewes, through pre-natal transmission, colostral transmission or through contact with faeces on the teats, udder and perineum of the ewes. This source of infection for lambs may be so important as to outweigh pasture as a major source of infection for lambs. If so, provision of "safe" pastures for lambing may not result in any significant reduction in the incidence of infection in lambs.

- There may be a threshold of contamination above which higher levels of contamination do not result in higher levels of infection in lambs. Pastures prepared in the way described may still exceed that threshold, in which case the reduction in contamination will not result in any useful reduction in disease incidence. It may not be possible, for example, to reduce the infection rates in lambs by reducing the contamination of pastures to levels which are achievable in practice on commercial farms.

- Are the benefits from preparing and using low contamination pastures worthwhile? Do they outweigh the costs of preparing them and the income foregone by compromising other areas of flock management, such as internal parasite control or ewe stocking rates?

Not all animals which are infected with *M ptb* develop clinical signs of disease. There is some evidence that the age of animals when first exposed to *M ptb* influences not only the risk of infection but also the outcome of infection. Animals which become infected at greater ages may be more likely to successfully resist infection, or to overcome infection or to remain sub-clinically infected for long periods of time. Consequently, the age at which lambs are exposed may be related to both the risk of developing clinical OJD and to the age at which clinical OJD develops.

Reports from field observations in NSW suggested that, as OJD became established in flocks over a period of five years or more, clinical cases tended to appear at younger ages[7,8] and that substantial numbers of animals died from OJD before they were two years of age. This was a critical factor determining the cost of OJD in affected flocks, because sheep of this age are, under OJD-free conditions, entering the most productive period of their life. In the mid 1990s, recommendations for limiting the financial impact of the disease included reducing the culling age for older sheep from the flock so that the number of animals perceived to be at high risk of OJD were reduced[9]. Clearly, this action would be ineffective if the peak age for clinical disease was less than three years.

This study (OJD.002A) was designed to address the questions relating to the use of low-contamination pastures. While the experimental design was largely determined by the nature of the questions, the best way to measure the outcome of different types of exposure was not so clear-cut. Ultimately it was decided that the occurrence of infection before or at three years of age was a practical and useful outcome. While the productive lifetime of a Merino sheep extends to five years of age or more, truncation of the experiment at three years of age provided several advantages. These included

- The earlier reporting of results, and lower experimental costs

- A clearer relationship between early exposure and the presence or absence of infection, rather than a relationship complicated by extended periods of exposure resulting from *M ptb* shedding by the experimental animals themselves

- Many naturally infected flocks reported higher incidences of infection in sheep under three years than over three years, so it was likely that a truncated experiment would capture most of the important differences between groups

In addition, by 36 months of age the difference in exposure time between lambs exposed from the time of birth and lambs only exposed after weaning was relatively small – about 100 days, or 9% of their lifetime. It seemed unlikely that the differences in elapsed time since first exposure would account for a significant part of any difference in the infection rate or severity of lesions between experimental groups at 36 months of age.

It was considered important that the study was conducted under relatively natural field conditions and, preferably, in an area where OJD was endemic. These factors were expected to improve the credibility of the results when they were extended to the wider sheep-growing community. Attempts were made to identify a farm on which the study could be conducted. An area of approximately 200 ha was required, and the farm had to be free or nearly free of OJD but prepared to allow the introduction of OJD-infected sheep into the experimental site. Despite public requests in the Goulburn district, the only offer of co-operation was from the University of Sydney, which owned a sheep and cattle property (*Arthursleigh Farm*) near Marulan, NSW. The resident sheep flock on *Arthursleigh* was infected with OJD but there was an area of the farm which had not been grazed by sheep for a period exceeding one year and which could be made available for research.

PROJECT OBJECTIVES

The principal objectives of the experiment were to relate the origin and timing of exposure to infection with *M ptb* in a cohort of young sheep and

1. the prevalence of histopathological evidence of OJD at three years of age,

2. the prevalence and time of onset of sub-clinical disease as measured by liveweight changes and wool production, and

3. the prevalence and time of onset of clinical OJD, in sheep not surviving to three years of age.

A secondary set of objectives was the collection of a 'library' of pasture, faecal, blood and tissue samples from ewes lambing and sheep born on the site, the maintenance of the library and, as funding becomes available, to retrospectively analyse selected samples for examination of any or all of a wide range of epidemiological associations essential to our understanding of OJD in natural field infections.

The aim of the study was to provide management strategies for owners of OJD-affected flocks which would reduce the prevalence of OJD in their flocks and reduce the proportion of the flock which die each year from OJD.

METHODOLOGY

Overview

The experiment required the birth and rearing to three years of age of 490 Merino sheep on a purpose-built experimental site. The sheep were born to ewes which were purchased from two flocks and transported to the site in late pregnancy. After weaning, the ewes were no longer required for Part 1 of the experiment and most ewes were sold. Some ewes (230) were retained and entered Part 2 of the experiment, which is reported in Appendix 3 and not discussed further here. In Part 1, the progeny were retained until three years of age, at which time they were killed.

Experimental design

The principal experimental animals were sheep born on the experimental site and raised to three years of age. They were born to ewes from either an infected flock (I) or an uninfected flock (U) and were raised to weaning on pastures which were either of low contamination (L) or high contamination (H). After weaning, they grazed pastures of low contamination (L) or high contamination (H). Treatments were identified with three letters; the first referring to the ewe flock infection status, the second referring to the pre-weaning exposure contamination level and the third referring to the post-weaning contamination level.

Low contamination pastures were managed in such a way that no deliberate contamination occurred; *ie*, the expected level of contamination was zero. The one exception was the low contamination paddock in which infected ewes lambed – contamination was inevitable in that paddock.

There were seven treatment groups in a two-replicate randomised block design. The seven treatments comprised the factorial combinations of three two-level infection status factors. These factors were (1) ewe infection status (I or U), (2) pre-weaning exposure level (L or H) and (3) post-weaning exposure level (L or H), except for the ILH group, which was omitted. Sheep in group ULL were never deliberately exposed to infection and were therefore considered as 'controls'.

The seven groups were identified as ULL, ULH, UHH, UHL, ILL, IHL, IHH (Table 1a, 1b). At the start of the post-weaning period each treatment group contained 35 or 36 lambs in each replicate (approximately 72 per treatment) and approximately half were ewes and half were wethers. Sheep did not move between replicates and each group remained in the same paddock from weaning until slaughter at three years of age. From weaning the sheep grazed in 5-ha paddocks, at an initial stocking density of 7.0 (if 35 sheep) or 7.2 (if 36 sheep) sheep per ha.

Table 1a Summary of experimental design. Experimental sheep are in one of seven groups according to the OJD status of their dams, their birth pastures and their weaning pastures. The first letter of the description identifies the dam flock status, I (infected) or U (uninfected), the second identifies the pre-weaning exposure, L (low) or H (high), the third identifies post-weaning exposure, also L or H.

Description of exposure history	Infected dam flock	Pre-weaning pasture high contamination	Post-weaning pasture high contamination
IHH	+	+	+
IHL	+	+	
ILL	+		
UHH		+	+
UHL		+	
ULH			+
ULL			

Table 1b Sources of contamination for paddocks used by each group. Grazing was discontinuous in some paddocks within the periods described.

Description of exposure history	Pre-weaning contamination	Post-weaning contamination
IHH	Grazed by Flock I ewes Days 2 to 176	Grazed by Flock I ewes Days 2 to 176
IHL	Grazed by Flock I ewes Days 2 to 176	Grazed by Flock U ewes only
ILL	Ungrazed	Grazed by Flock U ewes only
UHH	Grazed by Flock I ewes Days 2 to 50	Grazed by Flock I ewes Days 2 to 176
UHL	Grazed by Flock I ewes Days 2 to 50	
ULH	Grazed by Flock U ewes only	Grazed by Flock I ewes Days 2 to 176
ULL	Grazed by Flock U ewes only	

Experimental site

Location

The experimental site was at *Arthursleigh Farm*, Marulan, NSW. This property is in the eastern part of the southern Tablelands of NSW and approximately 20 kms east of Goulburn.

Construction

Construction of the facility commenced in June 1999 and was completed by early September of the same year. At the time of the arrival of the ewes on site (early August), the majority of the essential infrastructure was intact. Installation of the reticulated water supply was completed two weeks after the arrival of the ewes on site. The last plots required for grazing in the experiment, paddocks 1, 2 and 3 of each replicate, were finished last and the site was commissioned on 21 September 1999.

Experimental dates

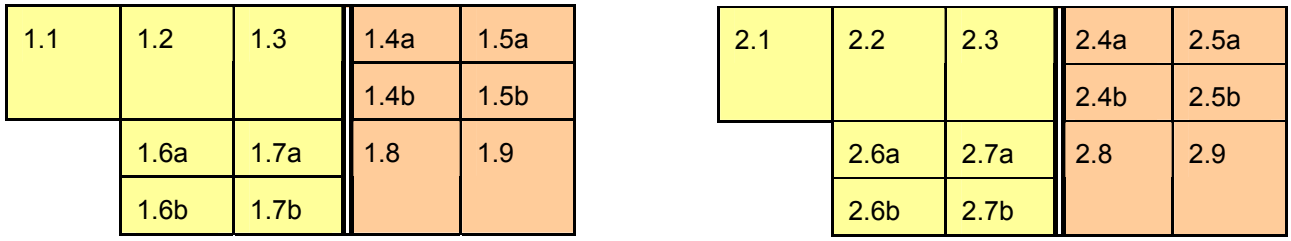
The ewes arrived on the site on 6 August 1999 (Day 1 of the experiment) and the experimental sheep were killed on 10 September 2002 (Day 1132). A detailed timeline for the field experiment is given in Table 7 on Page 20.

Paddock design

The entire site consisted of 200 ha of pasture and was contained within 23 km of secure fencing, of which 19.5 km was erected in 1999 specifically for the project. The site comprised 18 10-ha paddocks, eight of which were subdivided into 5-ha sub-paddocks, and a laneway system. Within the structure of the site, two replicates existed. Paddocks were identified with a numeric system; the first number representing the replicate number (1 or 2) followed by a point and the second number representing the paddock number within each replicate (1 to 9 inclusive). Sub-paddocks were further identified with a letter (a or b). The paddock design and naming convention is illustrated diagrammatically in Figure 1.

In designing the experimental site, erosion gullies were fenced to exclude livestock. The fenced gullies and the site laneways were incorporated in the site design in ways which assisted in the separation of paddocks with different levels of OJD contamination. An accurate plan of the site is provided in Plate 1, Appendix 4. Assignment of paddocks t treatment groups are shown in Table 2.

Figure 1 Diagrammatic representation of the experimental site. The first number in each paddock refers to the Replicate (1 or 2). Within each replicate, Paddocks 1, 2, 3, 8 and 9 were of 10 ha; Paddocks 4, 5, 6 and 7 were divided into parts a and b, each of 5 ha.



Fencing

Fence construction was steel post and ringlock with railway-line strainer posts. In order to reduce the risk of wind-blown faeces and soil causing cross-contamination on paddocks, garden shade-screen material was tied to the ringlock fence along boundaries which separated clean plots from contaminated plots (Plates 2 and 4).

Table 2 Assignment of paddocks to pre-weaning and post-weaning treatment groups. See also plates in Appendix 4.

Paddock number	Area (ha)	Pre-weaning group	Post-weaning group
1	10	IL (for 24 days)	not used
2	10	IL (for 26 days)	not used
3	10	IL (for 76 days)	not used
4a	5	IH	IHH
4b	5	IH	ULH
5a	5	IH	UHH
5b	5	IH	not used
6a	5	UL	IHL
6b	5	UL	ULL
7a	5	UL	ILL
7b	5	UL	UHL
8	10	UH	not used
9	10	UH	not used

Pasture type and paddock topography

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 between near-flat and slopes of approximately 1 in 30 and also varied in aspect (northerly or southerly). Differences between replicates in pasture species, slope and aspect were not systematic (there were paddocks of different types within each replicate) but differences between paddocks within replicates existed.

There were few shade trees on the site and, generally, all or most parts of all paddocks were unshaded. There was a small amount of shade provided by groups of trees in Paddocks 2.4a, 2.5b, 2.6a and 1.7b

Provision of water

With the exception of paddocks 2.8 and 2.9, water was provided by reticulation 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 between paddocks 2.5b and 2.6a. Paddocks 2.8 and 2.9 were supplied by water from a dam in Paddock 2.9 which was also fenced to exclude stock. The sites of the two dams are indicated in Plate 2.

Experimental sheep

Dams of the experimental sheep

Two flocks of pregnant ewes were purchased in July 1999 and moved to the experimental site. The identification and preliminary testing of these two flocks has been described in a Final Report of an MLA-funded project (OJD.002 Phase 1: Epidemiology of pre-weaning versus post-weaning infection, dated 10 May 2000). A brief description of these two flocks follows.

Flock U ewes were from a flock believed to be free of OJD infection. Preliminary serological testing of 480 mixed age ewes had confirmed their negative status. Subsequent testing of ewes from the same flock in 2003 also failed to detect any evidence of OJD infection (Whittington, pers comm).

Flock I ewes were from an OJD-infected flock. Testing of 654 ewes from three age groups (three – five years[a]) revealed a seroprevalence of 5.8% (3.7%, 7.8% and 6.0% in the three, four and five year old respectively). The ewes purchased for the experiment included 168 tested ewes, of which 15.5% were seropositive, and 192 untested ewes, amongst which the expected seroprevalence was 5.4%, based on their age and the estimated seroprevalence in each age group. A further 16 ewes of unknown pregnancy status and unknown seroprevalence but showing clinical signs of OJD were gifted to the experiment by the flock owner. The seroprevalence in the pregnant Flock I ewes (excluding the 16 gifts) was calculated to be 9.9% (Table 3).

Both flocks were of the Hazeldean bloodline of medium wool Merino. In both cases, the owners had been purchasing rams from Hazeldean and breeding their own ewe replacements for over 15 years. The two flocks were considered, therefore, to be closely genetically matched.

All ewes were tested for pregnancy before purchase and, except for the 16 gifted ewes, only pregnant ewes entered the site.

[a] The ages of the ewes are given as their age at lambing in 1999. Strictly speaking, the three year old ewes were approaching 3 years of age in July 1999.)

Table 3 Age distribution and serological status of the ewes from Flocks I and U.

	Flock U	Flock I
Number of ewes purchased	480	360
Seroprevalence of OJD infection	0%	9.9%
Proportion of three year old ewes	27%	25%
Proportion of four year old ewes	25%	38%
Proportion of five year old ewes	25%	36%
Proportion of six year old ewes	22%	0%

Description of the experimental sheep

The experimental sheep were the progeny born to the Flock U and Flock I ewes. They were deemed to have entered the experiment at birth although the final allocation to treatment groups was not performed until weaning, at which time lambs in excess of the number required were removed from the experiment. The sheep included ewes and wethers and were run as mixed sex groups.

Preparation of paddocks prior to lambing

Delivery of Flock I ewes to the site.

Flock I ewes arrived on the site on Day 1. The owner had loaded the ewes onto the truck in such a way that appropriately mixed age groups could be unloaded at three points on the experimental site to minimize the movement of ewes of this flock after delivery. For Replicate 1, 188 ewes were delivered to Paddock 9 (Plate 3). For Replicate 2, 91 ewes were delivered to Paddocks 9 and 97 to Paddock 4b.

Pre-lambing grazing of Flock I ewes

Prior to the start of lambing, paddocks designated to be “contaminated” during the pre-weaning phase of the experiment (Paddocks 4, 5, 8 and 9) were grazed by Flock I ewes. The ewes were grazed in groups of approximately 30 - 180 and were moved periodically during the pre-lambing contamination phase to ensure, as much as possible, equal contamination of all paddocks destined to be contaminated. (The *M-ptb* faecal excretion status of individual ewes was not known at that time because faecal culture results were not available until later.) Ewes were grazed at a known stocking rate for a known time so that a relative level of contamination could be calculated for each paddock. The identification of each animal in the grazing groups was known so that, when faecal culture results became known, the contamination status of each paddock could be estimated retrospectively. Infection rates on the paddocks were initially recorded as "infected sheep days per ha". (The method of retrospectively estimating the availability of *M-ptb* on pasture is described on Page 25.) The movement of Flock I ewes between paddocks and the consequent pattern of contamination, are illustrated in Figure 2.

In order to minimise contamination of laneways, movement of Flock I ewes between paddocks was achieved in one of four ways (Plate 3):

1. by temporarily raising adjoining fences and moving the sheep beneath them (but only when both paddocks were designated high-contamination)

2. moving sheep through adjacent gateways such that the area near the gateway which was subject to contamination was <10m^2. Movements were always conducted quickly. The gateways were

constructed such that they closed on the same fence post, and could be opened to 45º and joined with a short fencing panel, creating a closed pathway around the strainer post at the end of the dividing fence

3. moving sheep directly across laneways, through gates placed opposite each other

4. by trailer, towed behind a vehicle.

Contamination of Paddocks 8 and 9 for treatments UHL and UHH continued until Day 50 even though Flock U ewes had entered the paddocks and started lambing before that time. On Day 50, Flock I ewes were removed from Paddocks 8 and 9 and placed in Paddock 1.

Lambing of Flock I commenced on Day 60. On Day 74 they were moved to Paddock 2 and on Day 100 they made their final move to Paddock 3.

All Flock I ewes stayed within the same replicate from Day 1 until weaning.

Delivery of Flock U ewes to the site.

Flock U ewes also arrived on-site on Day 1. All of these ewes were delivered to paddocks 5a and 5b in Replicate 1.

Dispersion of Flock U ewes to lambing paddocks

Lambing commenced on Day 11 in Flock U. On Day 15, Flock U ewes were allocated to eight groups of 60 ewes at random after stratification on age. These groups were held in Paddocks 6a and b and 7a and b in both replicates, while two groups remained in Paddocks 5a and 5b of Replicate 1 (Figure 3). On Days 25 – 28, four groups of 60 ewes each were placed in Paddocks 8 and 9 in each replicate. Any ewes in these groups which had lambed were replaced with ewes of the same age which had not yet lambed so that all lambs raised in Paddocks 8 and 9 had been born in those paddocks on or after Day 25. Thirty Flock I ewes remained in Paddocks 8 and 9 with Flock U ewes until day 50. Paddocks 6a, 6b, 7a and 7b were stocked with 30 ewes per paddock (6 ewes per ha) from Day 29.

Husbandry procedures

Lambing

Ewes lambed at pasture and were monitored by twice daily inspections. Assistance with delivery of a lamb or lambs was given when required. The number of lambs born each day was not recorded. Median birth dates were calculated from first and last dates of lambing.

Footcare

There was an outbreak of intermediate footrot in Flock I ewes in October 1999. All Flock I ewes were foot-trimmed and foot-bathed in 10% zinc sulphate on Day 77 or 78.

Marking

At marking, lambs were tailed and castrated by knife, ear tagged, vaccinated and had blood samples collected. Marking occurred on 27 October 1999 (Day 83) (Flock U) and 23 November 1999 (Day 110) (Flock I). For marking, ewes and lambs were mustered to a temporary yard constructed immediately outside the gateway of their paddock. Ewes and lambs were separated and the ewes returned to their paddock. Lambs were marked in a cradle and then immediately returned to the paddock.

Identification

All sheep were identified by the application in each ear of a plastic numeric ear tag (same number in each ear). Tags were applied at marking. Lambs born to Flock U ewes were orange in colour, those born to Flock I ewes were yellow.

Weaning

Lambs were weaned between Days 116 and 123 (Flock U) and on Days 175 and 176 (Flock I). Lambs were weighed and allocated to post-weaning paddocks and moved to those paddocks by trailer.

Mulesing

All experimental sheep were mulesed.

Worm control

All ewes were dosed with Ivomec capsules at about the time of lambing between Days 25 and 52. All experimental sheep were dosed with Ivomec capsules soon after weaning between Days 182 and 194 (February 2000). Treatments thereafter were given as shown in Table 4.

Table 4 Anthelmintic treatment of experimental sheep

Day	Date	Product	Comment
188	9 February 2000	Ivermectin capsule	
279	10 May	Ivermectin liquid	
491	8 December	Ivermectin liquid	
557	12 February 2001	Ivermectin liquid	
566	21 February	Closantel	1.4b, 1.7b only
680	15 June	Ivermectin liquid	
790	3 October	Ivermectin liquid	
937	27 February 2002	Ivermectin liquid	

Fly-strike prevention

Fly strike was prevented by the administration of dicyclanil as a spray-on backline treatment (Clik®, Novartis Animal Health) on the dates shown in Table 5.

Table 5 Fly-strike prevention of experimental sheep with dicyclanil

Day	Date	Comment
138	21 December 1999	All ewes and Flock U lambs treated.
175	27 January 2000	Flock I lambs treated
493	30 November 2000	All animals treated.
600	27 March 2001	All animals treated.
820	2 November 2001	All animals treated.

Crutching

All experimental sheep were crutched in May 2000 (Days 292 - 302) and in May 2001 (Days 647 - 652). 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 and the dates of the first day of each shearing event are shown in Table 6. The first and second shearings were performed in a mobile plant which was moved to each paddock so that the sheep did not leave their paddock (Plate 4). Shearing always commenced in the paddocks of lowest contamination and progressed to the paddocks of highest contamination, in order to minimise the risk of moving infectious material on the shearing plant to low infection status pastures. The floor of the mobile plant was swept thoroughly at the conclusion of shearing in each paddock and before the plant was moved from the paddock. Faeces dropped by sheep penned in the plant awaiting shearing was collected on tarpaulins and moved off-site for disposal, in order to prevent the formation of an infection 'hot-spot' at the place in the paddock where the plant had stood.

The third shearing was performed in the *Arthursleigh* woolshed and sheep were sent from there to slaughter on Day 1132.

Table 6 Dates of shearing of experimental sheep.

Day	Date	Comment
403	11- 15 September 2000	First shearing. Wool weights not recorded.
796	9-15 October 2001	Second shearing (WOOL2). Weights recorded.
1117	26-28 August 2002	Third shearing (WOOL3). Weights recorded..

Vaccination

Experimental sheep were vaccinated against clostridial diseases (5 in 1 vaccine) and contagious pustular dermatitis (scabby mouth). Both vaccinations were administered at marking, on Day 83 (Flock U) and Day

110 (Flock I). Vaccination against clostridial diseases (5 in 1 vaccine plus selenium) was repeated between Days 434 and 453 inclusive. Vaccination against caseous lymphadenitis (CLA) was deliberately omitted from the vaccination to avoid any possible cross reaction with mycobacterial antigens in subsequent serological testing.

Supplementary feeding

Lupin grain was used as a supplementary feed source throughout the experiment. Sheep were fed once or twice weekly depending on pasture availability. The grain was trail-fed on the ground, delivered through the fence from a vehicle-drawn trailer which did not enter the experimental paddocks, but remained in the laneways (Plate 4).

Maintenance of paddock infection status

Restriction of sheep to paddocks

The experimental sheep were restricted to their designated paddocks except on a few occasions when deliberate controlled access to the laneway occurred or when accidental breaches of security occurred, as described below. Deliberate access occurred when Flock I (infected) ewes were moved between paddocks through adjacent gateways and through gateways which opposed each other across the laneways. Access was generally for less one minute and involved only a few square metres of the laneway surface on each occasion. The procedures described on Page 12 for movement of Flock I ewes before lambing were followed at all stages of the experiment.

Moving sheep around the site

When sheep were moved greater distances than adjacent paddocks trailers or trucks were used. Sheep were loaded at the paddock gateway (inside their paddock). This occurred principally at weaning and when sheep left the site permanently.

Vehicular and human access to paddocks

Vehicles entered paddocks from time to time for mustering but access was restricted to minimise these occurrences. Whenever feasible, vehicles were parked outside paddocks and materials carried into the paddocks. For repetitive tasks, like supplementary feeding, vehicles remained in the laneways and feed was delivered by hopper into the paddocks through the fence.

Wild animals

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.

Security breaches

Accidental breaches of security occurred on the following occasions:

Day 48 – one Flock I ewe from Paddock 2.4a became caste against the fence dividing 2.4a from 2.6b and rolled under the fence. The ewe was removed the same day and returned to 2.4b and the area around where she had lain in 2.6b was fenced off with portable hurdles which remained in place until the trial was completed. The risk of cross-contamination was considered very small.

Day 70 – three Flock I ewes and four lambs from Paddock 2.4b were found in Paddock 2.6b. They had been in the wrong paddock for at least 14 days and at most 28 days. As Paddock 2.6b was intended to be a zero-contamination paddock, this event represented a possible source of cross-contamination.

Day 76 – (approximately) Flock U sheep in Paddock 2.9 attacked by a pack of six dogs. Several ewes and lambs injured; one ewe and three lambs were euthanased. The dogs were all found and killed the following day. There was no risk of cross-contamination as a result of this event – no sheep were driven from their paddocks.

Day 123 – two lambs from Flock U, raised on low-contamination pastures, were found to have escaped from their post-weaning paddock following weaning a few days previously. They were found in the laneway approaching Paddock 1.3 between 1.2 and 1.5b, caught and returned to their correct paddock. The risk of cross-contamination was considered very small because (a) the laneways were not contaminated except for small areas around a small proportion of gate posts and (b) the lambs were very unlikely to be excreting *M ptb* at that age.

Collection of specimens

Faecal specimens

Faecal samples (1 – 2 g) were collected from the Flock I and Flock U ewes within 50 days of their arrival on-site, again near the time of lamb marking (Days 97 – 103, Flock U; Days 132 – 134, Flock I) and lamb weaning (Days 159 – 173, Flock U; Day 187, Flock I).

Faecal samples were collected from experimental sheep at marking (age of median lamb = 54 – 59 days) and on 17 occasions thereafter. The intervals between sampling events was approximately three months although the frequency of sampling was increased to six-weekly at times when it was expected that infected sheep would be most likely to commence faecal shedding of *M ptb* or to seroconvert. Specimens were collected four times in the first year of the study, six times in the second year, seven times in the third and once at the end of the study. The sampling events were identified as FS1 to FS18 (Table 7).

Blood specimens

Blood samples (5 – 10 ml) were collected from Flock I ewes within 50 days of their arrival on site. Flock U ewes had been blood sampled before delivery. Blood sampling was repeated for ewes of both flocks at the same times as faecal specimens were collected (marking and weaning, as above for faecal specimens).

Blood samples were collected from experimental sheep on the same occasions that faecal specimens were collected. These sampling events were named BS1 to BS18 (Table 7).

Animal handling

All animals were handled using portable yards within their paddocks during sampling and recording procedures. Each animal was identified by its ear tag. Sheep were restrained by the sampler and blood was collected by venipuncture of the jugular vein into plain 10 ml evacuated tubes (BD Vacutainer™, Becton, Dickinson and Company). Faeces were collected by another sampler 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.

Specimen handling

Faeces were placed into 2 ml sterile, screw-top plastic jars. Each jar of faeces and each tube of blood was labelled with the ear-tag number of the sheep, and the date of collection. Blood was allowed to clot at ambient temperatures then placed on ice or in a refrigerated container. Faeces were placed in cool conditions immediately after collection. Serum was separated from blood within 12 hours of collection then stored at –20ºC. Faecal specimens were stored at –76ºC.

Animal ethics

The experiment was conducted with approval of the Animal Care and Ethics Committee of The University of Sydney.

Measurements

Pasture type and availability

Inspection of pasture for estimation of availability and species dominance was carried out, in the first instance, with the assistance of Lori McGarva and Dale Chalker, agronomists with NSW Agriculture, Goulburn. This took place on Days 130 and 138. Each paddock was analysed using two methods.

First, visual estimation of pasture availability was performed with quadrats thrown at random along a predetermined line across each paddock. Second, an electronic pasture probe was used. This device measured only green matter but visual estimation of green and dry matter and bare ground was made at the same time. The results from both methods were combined to estimate the quantity of available pasture. The use of the probe and the advice of the two experts were used to inform the research project manager, who made subsequent assessments of pasture availability by visual methods only.

At this first inspection, in addition to assessing availability, the pasture species in each quadrat were identified and recorded.

Further assessments of pasture availability were carried out 12 times up to and including April 2002. In each 5-ha paddock, five permanent sites were marked out using pegs. The sites were not random because some paddocks contained ungrazed areas (under trees, for example) but were spaced evenly across grazed areas of the paddocks. At each inspection, a visual assessment of total pasture availability (dead plus green) was made, based on the percentage of ground cover and the height of the sward. In addition, each paddock was walked to gain an overall estimation of the changes in pasture growth, pasture species and to assess the grazing habits of each mob. If grazing behaviours were observed to change, the choice of site for inspection was changed.

Liveweight measurements

Liveweights of all sheep present were recorded on 17 occasions between weaning at 93 days of age (Flock U lamb median age) or 98 days age (Flock U lamb median age) and Day 1119. The weaning weight was identified as WEANWT; subsequent weighing events were named WT1 to WT16 (Table 6). Weighing was performed every three months although the frequency was increased when the frequency of blood and faecal sampling was increased.

At all times, except WT16, weighing was performed in the experimental paddock and with no period of restricted feeding before weighing. At WT16 sheep were weighed after shearing and after a period of overnight feed restriction.

Wool weight measurements

Experimental sheep were shorn on Days 404, 798 and 1119 . The weight of greasy wool excluding belly wool was recorded for the second and final shearing only.

Pathological examination

At post-mortem examination of sheep which died or were euthanased during the study or which were killed at the end of the study, two specimens of ileum, ileo-caecal valve, caecum and adjacent lymph nodes were collected, one specimen of each tissue was placed in 10% buffered formol saline and the

other was placed in a sterile container and stored for subsequent bacteriological examination. Fixed tissues were prepared for histopathological examination and adjacent sections were stained with haematoxylin and eosin (H & E) and by the Ziehl-Neelsen (ZN) technique for acid-fast bacilli (AFB).

The system proposed by Perez *et al* (1996)[10] for describing histopathological findings in paratuberculosis in sheep was used. Lesions were classified into one of five categories; 1, 2, 3a, 3b, 3c. Briefly, type 1 lesions consisted of granulomata formed by cells resembling macrophages. Granulomata occurred only in lymphoid tissue (Peyer's patches and lymph nodes) and not in the intestinal mucosa. AFB were not evident. Type 2 lesions were more severe than those of type 1. Granulomata occurred in the basal zone of the lamina propria and in the villi but, if present in those regions, were always related to the presence of granulomata in adjacent Peyer's patches. AFB were only rarely seen and then in very small numbers. In type 3 lesions, granulomata occurred both in Peyer's patches and in mucosal tissue not associated with lymphoid tissue. In type 3a, granulomata were similar to type 2 but larger, more extensive and caused the villi to be larger than normal. AFB were generally visible. Type 3b lesions were characterised by a diffuse granulomatous enteritis formed by epithelioid cells. The structure of the intestinal villi was profoundly disturbed, being thicker and flatter than normal. AFB were always observed in sections and were generally abundant. Type 3c lesions were also of a diffuse granulomatous nature but the predominant inflammatory cells in intestinal mucosa lesions were lymphocytes, infiltrating the entire lamina propria of both villi and basal areas. Langhans giant cells with large numbers of nuclei also occurred. AFB were either absent or present only in small numbers.

Bacteriology

Ewes

Faeces collected from Flock U ewes between Days 25 and 35 were cultured. Samples from 30 ewes were pooled before culture. The groups of 30 ewes included ewes running together in the same lambing group.

Faeces collected from Flock I ewes between Days 41 and 50 were cultured on an individual basis.

Experimental sheep

Faeces collected from experimental sheep at FS5 (Day 394) were cultured in pools of seven or eight animals per pool. All sheep in each pool were derived from the same paddock so five pools were tested for each paddock. The results were reported as BACT12M.

Faeces collected from the experimental sheep at FS8 (Day 565) were cultured individually. The results were reported as BACT18M.

Tissues collected at necropsy from sheep which died during the study or at slaughter were cultured individually.

Bacteriological methods

The double incubation and radiometric culture method described by Whittington *et al* (1998)[11] was used to culture faeces. A small amount (~ 1.5 g) of faeces was broken up in a tube containing 10 ml of sterile normal saline. After standing for 30 minutes, 5 ml from the top of the suspension was added to a tube containing 25 ml of 0.9% hexadecylpyridium chloride (HPC, Sigma Chemicals Pty Ltd) in half-strength brain heart infusion broth (Oxoid Limited) and allowed to stand at 37ºC for 24 hours. After centrifugation (900 g, 30 minutes) the pellet was resuspended in 1 ml of half strength brain heart infusion broth containing vancomycin, nalidixic acid and amphotericin B (Sigma Chemicals Pty Ltd) then incubated at 37ºC for 72 hours. A 0.1 ml inoculum is then withdrawn from near the bottom of the tube and added to the culture medium, which consists of BACTEC 12B medium (Becton, Dickinson and Company) to which has been added 200 µl PANTA PLUS (Becton, Dickinson and Company, 1 ml of egg yolk, 5 µg of mycobactin J (Allied Monitor Inc) and 0.7 ml of water. The vials were incubated at 37ºC for 12 weeks, and the growth index (GI) was determined weekly using a BACTEC 460 machine (Johnston Laboratories). The presence of *M ptb* in vials with a positive growth index was confirmed by PCR for *IS*900.

Pooling of faeces was performed as described by Whittington *et al*, (2000)[12]. Briefly, faeces from all sheep included in the pool were homogenised in an electric blender (Waring MC-3 laboratory blender) before a sample of the homogenate was inoculated into the culture medium as described above for individual samples.

Timeline

The timing of major events in the study are listed in Table 7. Other events are also listed in Table 4(anthelmintic treatment) and Table 5 (flystrike treatment). The timing of movements of Flock I ewes around the site during the pre-lambing contamination phase are shown in Figure 2.

Table 7 Dates of events. Specimens of faeces and blood are identified as FS and BS respectively and the numeric suffix (1 – 18) identifies the order of collection. Similarly, liveweight and wool weight records are identified as WEANWT, WT1 – WT16 and WOOL2 and WOOL3.

Day	*Date*	Activity	Specimens and measurements
1	6 Aug, 1999	Ewes arrived on site	
11	16 Aug	Lambing started, Flock U	
25	30 Aug	Median lamb born, Flock U	
25 - 35	30 Aug – 9 Sep	I'mectin capsule, Flock U	Faeces (1), Flock U ewes
41 - 50	15 – 24 Sep	I'mectin capsule, Flock I	Faeces & blood (1), Flock I ewes
60	4 Oct	Lambing started, Flock I	
67	11 Oct	Lambing finished, Flock U	
74	18 Oct	First move of IL ewes	
78	22 Oct	Median lamb born, Flock I	
83	27 Oct	Marking, Flock U	FS1, BS1, Flock U lambs
95	8 Nov	Lambing finished, Flock I	
98	11 Nov		Faeces & blood (2), Flock U ewes
100	13 Nov	Second move of IL ewes	
118	1 Dec	Weaning, Flock U	WEANWT, Flock U lambs
133	16 Dec	Marking, Flock I	FS1, BS1, Flock I lambs
133	16 Dec		Faeces & blood (2), Flock I ewes
159 - 173	11 – 25 Jan, 2000		Faeces & blood (3), Flock U ewes
176	28 Jan	Weaning, Flock I	WEANWT, Flock I lambs
187	8 Feb		Faeces & blood (3), Flock I ewes
197	18 Feb		FS2, BS2, Flock I lambs
218	10 Mar		FS2, BS2, Flock U lambs
279	10 May		FS3, BS3, WT1
357	27 July		FS4, BS4, WT2

366	6 Aug, 2000	End of 1st year	
394	2 Sep		FS5, BS5
404	13 Sep	First shearing	
436	14 Oct		FS6, BS6, WT3
491	8 Dec		FS7, BS7
524	10 Jan, 2001		WT4
565	20 Feb		FS8, BS8
624	20 Apr		WT5
670	5 Jun		FS9, BS9, WT6
712	17 Jul		FS10, BS10, WT7
732	6 Aug, 2001	End of 2nd year	
756	30 Aug		WT8
768	11 Sep		FS11, BS11, WT9
798	11 Oct	Second shearing	WOOL2
818	31 Oct		FS12, BS12, WT10
888	9 Jan, 2002		FS13, BS13, WT11
948	10 Mar		FS14, BS14, WT12
986	17 Apr		FS15, BS15, WT13
1031	1 Jun		FS16, BS16, WT14
1091	31 Jul		FS17, BS17, WT15
1097	6 Aug, 2002	End of 3rd year	
1119	28 Aug	Final shearing	WOOL3, FS18, BS18, WT16
1132	10 Sept, 2002	Sheep killed	

Definitions

Variables names used in the data-base

The list of variables used for individual sheep records and used throughout this report are described in Table 8.

Table 8 Description of variables used for recording individual sheep data

Variable name	Description	Units and values
WEANWT	Weaning weight	Kg
WT1 to WT16	Liveweight at 16 post-weaning events	Kg
WOOL2, WOOL3	Wool weight at 2nd and 3rd shearing	Kg
BACT18M	Culture result from faeces at 18 months of age	0 = negative, 1 = positive, 9 = no result
BACTPM	Culture result from tissues at post-mortem exam	0 = negative, 1 = positive, 9 = no result
HISTO	Result of histological examination and classification of lesion type at post-mortem exam	0 = negative, 1-5 = positive, 9 = no result
OJDINF	Confirmed case of OJD	Logical true or false
SURVIVED	Survived to the end of the experiment	Logical true or false
NOINFO	No information from post-mortem examination	Logical true or false

Confirmed OJD case

Sheep which had histopathology of the ileum, ileo-caecal valve or caecum characteristic of paratuberculosis, or were tissue culture positive, were considered to be confirmed OJD cases.

Histopathological lesions were classified into one of five categories; 1, 2, 3a, 3b, 3c. If sheep had no lesions characteristic of paratuberculosis they were classified into category 0. The variable HISTO took one of six values, 0 to 5 inclusive, equivalent to classification with the system of 0, 1, 2, 3a, 3b, 3c respectively. For sheep for which no specimens were available, HISTO = 9.

Tissue culture results were classified as BACTPM = 0 (negative result), 1 (positive result), or 9 (no specimens available for culture).

Confirmed OJD cases therefore included animals with HISTO = 1-5 inclusive regardless of BACTPM result, and sheep with HISTO = 0 and HISTO = 9 if BACTPM = 1. For all these sheep, the variable OJDINF was TRUE. For all other sheep, OJDINF was FALSE.

Deaths

Sheep which did not survive until slaughter at the end of the trial were classified as deaths and for these the variable SURVIVED was FALSE. For sheep which were slaughtered at the abattoirs, SURVIVED = TRUE. To this latter group were added sheep which were euthanased before dispatch to the abattoirs because they were judged too thin to be trucked. For analysis purposes they were considered to have survived to the end of the trial.

Confirmed-OJD deaths

Deaths which were subsequently shown to be confirmed OJD cases on the basis of histopathology and/or tissue culture were classified as confirmed-OJD deaths, provided the histopathology was sufficiently severe to cause death and in the absence of clinical or post-mortem findings of other causes of death.

Confirmed-OJD deaths: SURVIVED=FALSE and (BACTPM=1 or HISTO=3, 4 or 5) and NOINFO=FALSE.

Probable-OJD deaths

Sheep for which no post-mortem material was available for examination by histology or culture but which had positive faecal culture at 18 months (BACT18M = 1) and showed clinical signs of OJD before death with no evidence to support alternative diagnoses were classified as probable-OJD deaths. For these sheep, the variable NOINFO was TRUE.

Probable-OJD deaths: SURVIVED=FALSE and BACTPM=9 and HISTO=9 and BACT18M=1 and NOINFO=TRUE.

No-information deaths

Sheep for which no post-mortem material was available for examination and for which faecal culture at 18 months was negative or not performed (BACT18M = 0 or 9) were classified as no-information deaths. For these sheep, the variable NOINFO was TRUE.

No information deaths: SURVIVED=FALSE and BACTPM=9 and HISTO=9 and BACT18M=0 or 9 and NOINFO=TRUE.

Not-OJD deaths

Sheep for which histopathological examination failed to confirm the presence of OJD infection or, in the absence of histopathology, an alternative diagnosis was available, were classified as not-OJD deaths.

Not-OJD deaths: SURVIVED=FALSE and BACTPM=0 or 9 and HISTO=0 or 9 and BACT18M=0,1 or 9 and NOINFO=FALSE.

OJD deaths

For most analyses, the classification ‘OJD deaths’ included confirmed OJD deaths and probable OJD deaths.

Total OJD affected

Confirmed OJD cases and probable OJD deaths were included in the category total OJD affected.

Not affected

Sheep which survived to WT16 (SURVIVED = TRUE) and which had negative findings from histopathology and tissue culture (BACTPM = 0 AND HISTO = 0) were classified as not affected (by OJD).

Survived, no information

Sheep which survived to WT16 (SURVIVED = TRUE) and which had no specimens available for histopathology and tissue culture (BACTPM = 9 AND HISTO = 9) were classified as survived, no information.

Statistical methods

Relationships between treatment effects and OJD infection rates and OJD mortality rates

Two approaches for analysing the data were used, one based on the usual method for factorial designs specifying main effects and interactions, the other using comparisons among subsets of treatments that were chosen after examining the data. Due to the absence of the ILH treatment combination, the usual method of summing across the combinations to get main effect and two-factor totals was inappropriate. Instead, the main effect and two-factor terms were totalled over as many treatment combinations as were present that avoided confounding and preserved balance in the estimates. For these analyses, alternative models were tested which either introduced the main effects for ewe, pre-weaning and post-weaning exposure in that order, i.e. following the sequence of exposures, or ignored that ordering. The method of generalised linear mixed models (GLMM) for binomial data using the logit link function was used in both the factorial and treatment comparison approaches for infection rates and death rates. The method of linear mixed models for normally distributed data was used for the data on time to death. In the mixed models, treatment and sex effects and their interactions were fixed while replicate effects and their interactions with treatment and sex were random. Overdispersion of data in the GLMM models was checked but was not present.

The proportion of deaths during the trial was too low to apply survival analysis methods for time to death. Median age at death was compared between groups using a Mann-Whitney test (SPSS version 11.5 for Windows) as well as GLMM, as described above.

The following comparisons among subsets of treatments were tested as a set of single-degree-of-freedom orthogonal contrasts:

T1 "Control" versus rest: ULL versus the other six treatments

T2 Single high exposure versus multiple high exposure: {ILL,UHL,ULH} versus {IHH, IHL,UHH}

T3 Single high ewe exposure vs single high pre- or post-weaning exposure: ILL versus {UHL,ULH}

T4 Triple high exposure versus double high exposure: IHH versus {IHL,UHH}

Relationships between treatment effects and findings at pathological examination

Treatment effects were assessed using a set of single-degree-of-freedom comparisons among the treatments (contrasts T1-T4 as above plus T5 and T6 as described below). The method of generalised linear mixed models (GLMM) for binomial data using the logit link function was used for BACT18M and the individual HISTO scores (0-5). In some tentative analyses, the method of generalised linear models (GLM) for multinomial data using the generalised logit link function was used to analyse simultaneously the sets of six scores for HISTO. In the GLMM mixed models, the treatment contrast and sex effects and the sex x contrast interactions were fixed while replicate effects and their interactions with treatment (as a

7-level factor) and sex were random. The random effects and interactions were required to be treated as fixed in the GLM models. Overdispersion of data in the GLMM models was checked but was not present.

T5 Single high pre-weaning exposure versus single high post-weaning exposure: UHL versus ULH

T6 Ewe and pre-weaning double exposure versus pre- and post-weaning double exposure: IHL versus UHH

Relationship between OJD pathology and liveweight change and wool production

The method of linear mixed models for normally distributed data was used for the analysis of WT16 (liveweight at 36 months of age) with WT4 as a covariate (WT4 = liveweight at 15 months of age) was considered to be an index of predicted adult bodyweight). The fixed and random terms in the model were the same as those for the above GLMMs.

Multiple linear regression was used to determine the effect of OJD pathology on WOOL3 (greasy wool weight at three years of age) for sheep which survived to the end of the trial. A forward stepwise procedure was used with variables included if the significance of their β value was less than 0.05. WOOL2 (wool weight at two years of age) was included in the wool weight model as an index of innate wool production, assuming that OJD lesions were having no effect on wool weight one year before the shearing event of interest (WOOL3). Multiple linear regression was performed using SPSS version 11.5 for Windows.

Relationship between OJD pathology and risk of death

The existence of associations between OJD pathology, BACT18M and the risk of mortality were examined using the chi-squared test. Where appropriate, the relative risk (RR) was calculated to convey the degree of association -- RR = (a/(a+b))/(c/(c+d)). Approximate confidence limits for the RR were calculated using the method described by Thrushfield[13]. Briefly, the variance of RR (V_{RR}) was approximated as ((b/a)/(a+b)) + ((d/c)/(c+d)). The 95% confidence limits for the estimate of RR were then

$$RR \times \exp(\pm 1.96\sqrt{V_{RR}})$$

Infection rates in sheep used to contaminate paddocks

Ewes from Flock I were used to contaminate paddocks designated as high-infection status for both the pre-weaning and post-weaning periods. Contamination levels were calculated retrospectively when the results of culture of the ewes' faeces collected between Days 41 and 50 became known. While it was recognised that ewes other than those which were faecal culture positive could be excreting *M ptb* in their faeces, although probably at lower levels than those which were detected positive, the proportion of positive ewes was used as an index of the level of *M ptb* excretion by each particular mob of ewes. The daily contamination level was estimated from the number of ewes grazing per ha each day and the proportion of tested ewes from that group which were known to be faecal culture positive. In general, over 90% of ewes in each mob were tested and provided a faecal culture result. Some ewes, however, provided no faecal sample or no faecal culture result.

To predict the level of availability of viable *M ptb* organisms to which the experimental sheep were subsequently exposed, it was assumed that the numbers of viable organisms on pasture declined at a logarithmic rate after deposition. Three different rates were examined:

1. 7.8945% per day which, when applied in daily steps, was equivalent to a 10 fold decline (one logarithm) over 28 days (ie, $(1-.078945)^{28} = 0.1$). Daily survival rate (S) = 0.921055

2. 21.8629% per day, equivalent to a 10^3 fold decline over 28 days. S = 0.781371

3. 38.9459% per day, equivalent to a 10^5 fold decline over 28 days. S = 0.610541

The index of viable *M ptb* availability to which experimental sheep were exposed on Day d was, therefore,

$\sum_0^d (C^+/(C^+ + C^-) * N_d) + S \times (C^+/(C^+ + C^-) * N_{d-1}) + S^2 \times (C^+/(C^+ + C^-) * N_{d-2}) \ldots.$

where C^+ and C^- are the number of culture positive and culture negative ewes in the flock respectively, S is the daily survival rate of *M ptb* organisms and N_d is the number of ewes in the flock per ha on Day d. The index was in units of faecal culture-positive ewes per ha per day, abbreviated to E/D.

The decay rates of one, three or five logarithms per 28 day period was based on the study of Whittington (2001) which found decay rates varying between 0.4 and 2.2 logarithms per month in shaded locations and 1.1 to 6 logarithms per month in unshaded areas. Consequently we considered a rate of one logarithm per 28 days to be a lower limit for the decay rate, three logarithms the most likely rate, and five logarithms to be the upper limit. The period of 28 days in the context of *M ptb* decay rates is referred to throughout this report as 'one month'.

Liveweight change

The weights of all sheep for weights recorded at WT5 and later were converted to an *expected weight* (EW), based on the group mean liveweight and the first adult weight (WT4) recorded for each individual sheep. This approach was used for examining the change in liveweight in sheep affected by OJD and allowed comparisons between sheep in different groups. WT4 was recorded when the sheep were 15 months old and was assumed to be largely free of maternal and birth paddock effects on liveweight. It was expected that, provided ill-health did not intervene, individual sheep would maintain the same ratio of individual liveweight to group liveweight at WT5 – WT16 as they had at WT4

For EW at the nth weight record,

$$EW_n = x_4 \,/\, \bar{x}_4 \times \bar{x}_n$$

where $\bar{x}_n$ is the group mean liveweight at the nth record, and x_4 and $\bar{x}_4$ are the individual and group mean liveweights respectively at the 4th weight record (WT4).

n could take values from 5 to 16 inclusive.

RESULTS

M ptb infection rates in Flock I ewes

Initial infection rates

Faecal samples collected from Flock I ewes between Days 41 – 50 were cultured and the results of this round of testing were used to calculate retrospectively the level of contamination occurring in the paddocks used for the treatments requiring high exposure to *M ptb*. Of the 376 Flock I ewes delivered to the site, faecal culture results were obtained from 345. Of these, 54 (15.7%) were positive. A higher proportion was positive in Replicate 2 (18.2%) than in Replicate 1 (13.1%) (Table 9).

Table 9 Results of faecal culture, Flock I. Samples were collected between Days 41 and 50 (a) Replicate 1 (b) Replicate 2. Within each replicate, ewes were grouped into mixed-age 'grazing groups' of approximately 30 animals (60 in one case) identified as Mobs A to K. The Final paddock is the one in which each group lambed.

(a)

Group ID	Number of ewes (N_0)	Number tested ($C^+ + C^-$)	Number positive (C^+)	Infection rate ($C^+/(C^+ + C^-)$)	Final paddock
A	63	58	6	10.3%	1.1,2,3
B	33	33	6	18.2%	1.4a
C	31	28	3	10.7%	1.4b
D	30	28	3	10.7%	1.5a
E	31	28	5	17.9%	1.5b
TOTAL	**188**	**175**	**23**	13.1%	

(b)

Group ID	Number of ewes (N_0)	Number tested ($C^+ + C^-$)	Number positive (C^+)	Infection rate ($C^+/(C^+ + C^-)$)	Final paddock
F	30	28	2	7.1%	2.1,2,3
G	31	25	5	20.0%	2.1,2,3
H	32	31	5	16.1%	2.4a
I	32	26	6	23.1%	2.4b
J	31	29	6	20.7%	2.5a
K	32	31	7	22.6%	2.5b
TOTAL	**188**	**170**	**31**	18.2%	

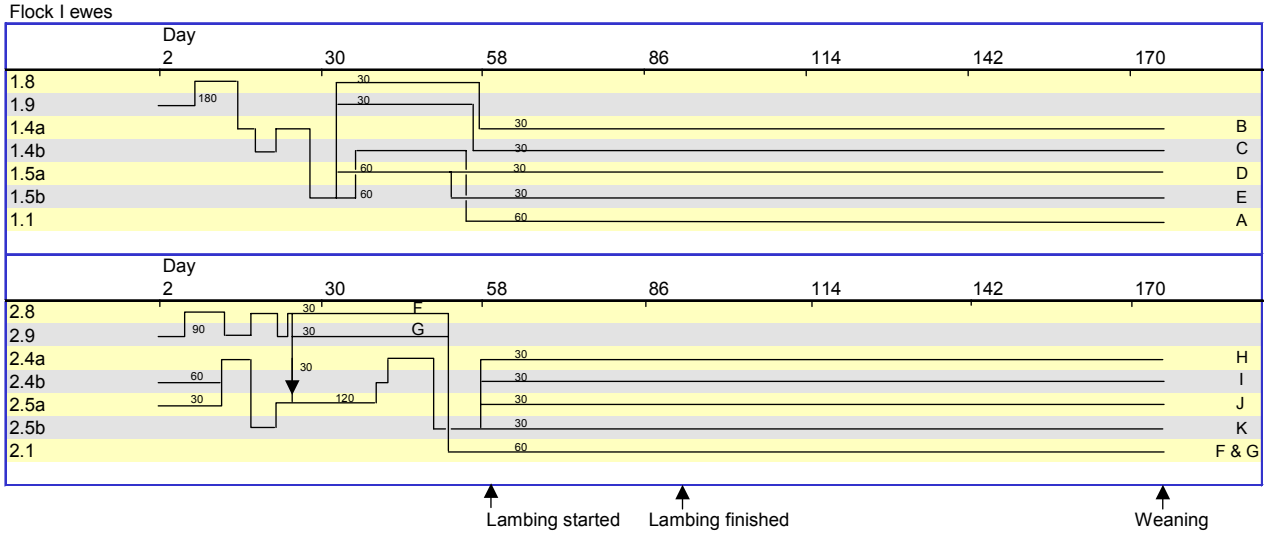
Contamination of pastures by Flock I ewes

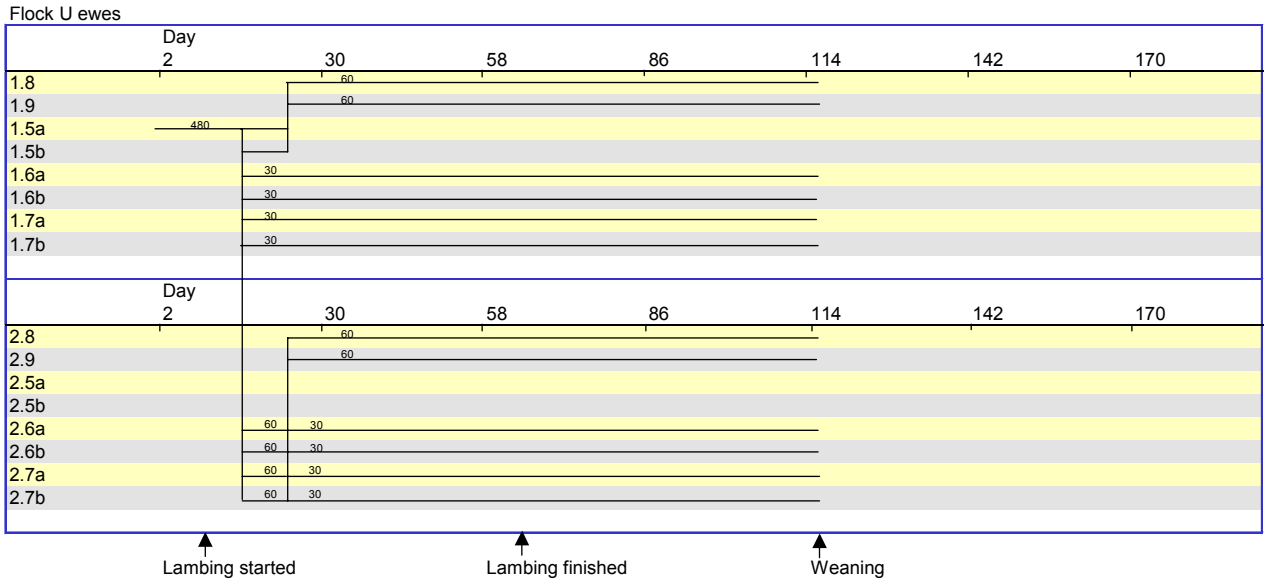
The numbers of faecal culture-positive ewes varied between grazing groups within replicates (Table 9). To some extent, potential variation in contamination levels as a consequence of variation in infection rates between groups was reduced by the pooling of grazing groups and the movement of the pooled grazing groups between paddocks in the days before lambing commenced. The difference in proportion of faecal culture-positive ewes between replicates could not be balanced because ewes did not move between replicates after delivery to the site.

In Replicate 1, the ewes were initially grazed as a group of 180 ewes which was progressively moved across all paddocks for which pre-lambing contamination was required (Figure 2). On Day 33 this group was divided into four parts then, on Day 46 into five parts which retained their integrity until their lambs were weaned on Day 176. In Replicate 2, the ewes were initially grazed in three parts moving between paddocks before final division into five parts on Day 55.

Figure 2 (Top) Diagrammatic representation (time-scale approximate only) of the movements of Flock I ewes, which were used to predict the patterns of contamination with *M ptb* of experimental paddocks. The Flock I ewes were progressively formed into grazing groups, labelled A to K below, which were grazed for set periods in each paddock to achieve desired levels of contamination. The numbers on the figure indicate the approximate size of each grazing group. The ewes left the site after weaning on Day 176.

Figure 3 (Bottom) The movement pattern for Flock U ewes. These ewes were not shedding *M ptb*. Most Flock U ewes left the site after weaning on Day 118.





Predicted levels of M ptb availability on pastures

Three factors – the pattern of grazing of each group of Flock I ewes, the proportion of ewes in each group known to be faecal culture-positive and the expected *M ptb* decay rate – were used to predict the availability of viable *M ptb* on pastures in each paddock at given time points. For the three different estimates of decay rate used, the predicted patterns of *M ptb* availability are illustrated in Appendix 1, Figures 1 to 6. The availability of *M ptb* is given as the number of faecal culture positive ewes/Ha/day (E/D).

Treatment groups IHH and IHL

The patterns of *M ptb* availability in Paddocks 4a, 4b, 5a and 5b were broadly similar to each other and similar between replicates. The availability of *M ptb* was predicted to be relatively stable at (assuming decay rates of three logarithms per month) around 2.9 – 5.5 E/D in Replicate 1 and 4.7 – 6.7 in Replicate 2 (Appendix 1, Figures 3 and 4).

Treatment groups ILL

In Paddocks 1, 2 and 3, lambs were exposed to initially very low levels of *M ptb* availability (when the Flock I ewes first entered the paddock shortly before lambing commenced) then increasing levels of availability over the subsequent 21 – 28 days. With each move to the next paddock, predicted *M ptb* availability on pasture fell briefly to zero then increased. The lamb of median birth date (illustrated in Appendix 1) was exposed to two periods of low *M ptb* availability. The first-born lamb, by contrast, was exposed to three periods of low availability. By the end of the pre-weaning phase, lambs were exposed to levels of availability of a similar scale to that of lambs in Paddocks 4a, 4b, 5a and 5b.

Treatment groups UHH and UHL

In Paddocks 8 and 9, *M ptb* availability was initially moderately high then declined following removal of Flock I ewes. The predicted availability of *M ptb* during the late pre-weaning phase in these paddocks was more strongly influenced by the decay-rate assumptions than in paddocks containing treatment groups IHH, IHL and ILL because of the removal of a continuing source of contamination (Flock I ewes) well before weaning (Appendix 1).

Predicted exposure of experimental sheep to M ptb on pastures

The predicted daily availabilities of *M ptb* for the median-born lamb were summed to produce an area under the curve (AUC) which was an estimate of the total exposure to *M ptb* during the period from birth to weaning. This period was 93 (Flock U) or 98 (Flock I) days. AUCs were estimated for the three different putative rates of decay of *M ptb* – one, three or five logarithms per month (Table 10).

The same approach was taken for the post-weaning exposure of treatment groups IHH, ULH and UHH. The AUC was calculated for the first 120 days of the post-weaning phase (Table 11). After 120 days, the predicted level of *M ptb* availability was very low and was ignored in calculating the AUC (Appendix 1, Figures 1 to 6.). The predicted exposures, based on a decay rate of three logarithms per month, are summarised in Table 12.

The E/D values for ULH and UHH are substantially higher than IHH because these groups actually ran with infected ewes and their lambs during the post-weaning period.

Table 10 Areas under the curve (E/D) for *M ptb* availability during the pre-weaning phase of the study, based on decay rates of one, three or five logarithms every month.

Paddock	Treatment	Replicate 1			Replicate 2		
		1 log	3 logs	5 logs	1 log	3 logs	5 logs
1,2,3	ILL	651	278	162	806	344	200
4a	IHL & IHH	1494	543	305	1299	467	262
4b	IHL & IHH	839	301	169	1826	669	375
5a	IHL & IHH	813	291	163	1597	581	326
5b	IHL & IHH	831	301	169	1840	655	367
8	UHL & UHH	205	50	26	175	42	19
9	UHL & UHH	114	26	14	274	82	44

Table 11 Areas under the curve for *M ptb* availability (E/D) during the first 120 days of the post-weaning phase of the study, based on decay rates of one, three or five logarithms every month.

Paddock	Treatment	Replicate 1			Replicate 2		
		1 log	3 logs	5 logs	1 log	3 logs	5 logs
4a	IHH	177	20	5	152	17	4
4b	ULH	586	187	101	1302	416	226
5a/b	UHH	586	187	101	1131	361	196

Table 12 . Summary data for areas under the curve for *M ptb* availability (E/D) during the pre-weaning and first 120 days of the post-weaning phase of the study, based on decay rates of three logarithms every month. Where multiple pre-weaning paddocks were used, the mean exposure of both (UHL and UHH) or all four (IHL and IHH) are shown.

Treatment	Areas under the curve (predicted level of exposure) in E/D						
	Replicate 1			Replicate 2			Mean of both replicates
	Pre-weaning	Post-weaning	**Total**	Pre-weaning	Post-weaning	**Total**	**Total**
ILL	278	0	**278**	344	0	**344**	**311**
IHL	359	0	**359**	593	0	**593**	**476**
IHH	359	20	**379**	593	17	**610**	**494**
ULL[£]	0	0	**0**	0	0	**0**	**0**
ULH	0	187	**187**	0	416	**416**	**301**
UHL	38	0	**38**	62	0	**62**	**50**
UHH	38	187	**225**	62	361	**393**	**309**

[£] Accidental exposure probably occurred through straying sheep or from adjoining contaminated pasture.

The predicted exposures of the experimental sheep in the pre-weaning period for each treatment group maintained the same ranking whichever decay rate was used to predict exposure (Figure 4). That order was : (IHL and IHH) > ILL > (UHH and UHL).

For the post-weaning period the ranking was also independent of the predicted decay rate, and the order of treatment groups from most exposure to least exposure was: (UHH ≈ ULH) >> IHH ().

Figure 4 Relationship between the treatment groups in terms of predicted exposure to *M ptb* during the pre-weaning period for three different estimates of decay rate for viable *M ptb*, and for each replicate (R1 and R2)

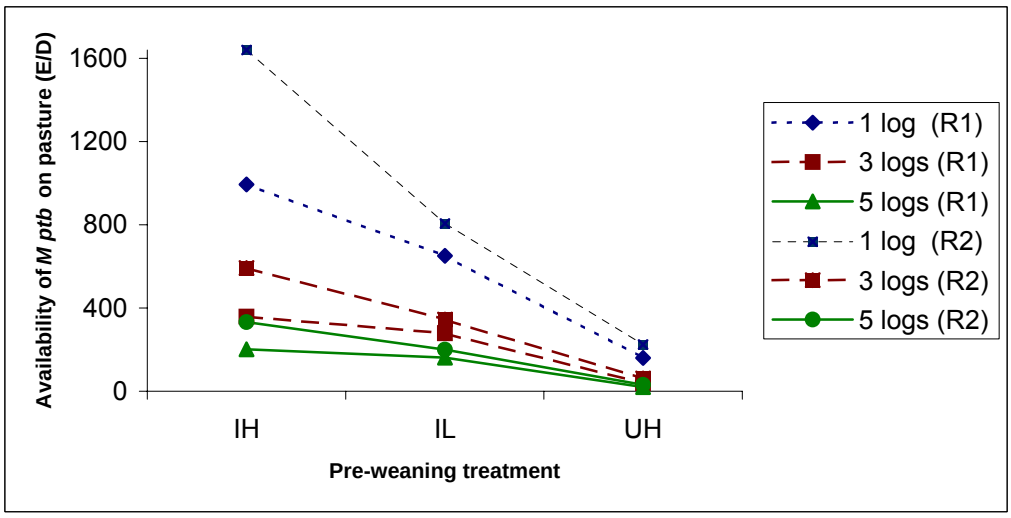
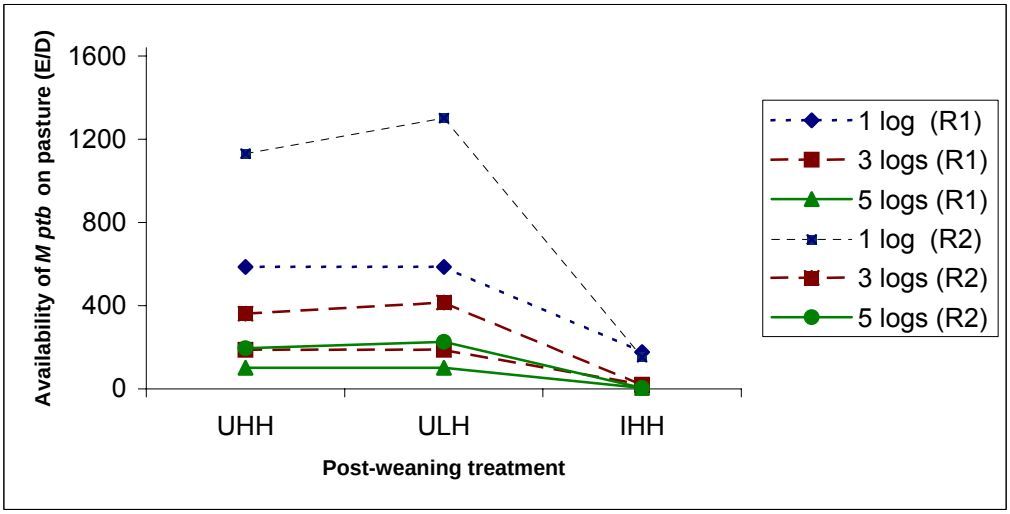


Figure 5 Relationship between the treatment groups in terms of predicted exposure to *M ptb* during the post-weaning period for three different estimates of decay rate for viable *M ptb*, and for each replicate (R1 and R2)



Numbers of lambs present

At marking

No records were made of lambs which died before marking. From Flock U there were 530 lambs present at marking (approximately 110% of the ewes that were delivered) and, from Flock I, there were 257 lambs present at marking (approximately 71% of the ewes delivered) (Table 13). The rate of multiple births was noticeably higher in Flock U than Flock I, which at least in part accounted for the much higher marking percentage in that flock.

The number of lambs present at marking was in excess of the number required for the post-weaning phase of the study. The experimental design required six groups of 35 sheep from Flock I (210 total) and eight groups from Flock U (280 total). Because surplus sheep were available for almost all groups, the initial group size was increased to 36 for all but two groups.

Surplus lambs were either sold at weaning, or made available for a study of cell-mediated immune responses.

Table 13 Numbers of lambs born in each experimental paddock. Flock U on left; Flock I on right.
There were approximately 60 ewes per 10-ha paddocks, or 30 ewes where paddocks were divided into parts a and b, each of 5 ha.

Paddock	Number of lambs tagged	Eartag series	Paddock	Number of lambs tagged	Eartag series
1.6 a and b	70	1159 - 1228	1.3	41	7696 - 7736
1.7 a and b	82	1447 - 1528	1.4a	24	7651 - 7674
1.8	53	1043 - 1095	1.4b	24	7627 - 7650
1.9	64	1096 - 1159	1.5a	21	7675 - 7695
2.6 a and b	69	1378 - 1446	1.5b	26	7601 - 7626
2.7 a and b	77	1301 - 1377	2.3	43	7815 - 7857
2.8	55	1589 - 1600 and 1001 - 1043	2.4a	18	7797 - 7814
2.9	60	1529 - 1588	2.4b	26	7771 - 7796
			2.5a	15	7756 - 7770
			2.5b	19	7737 - 7755
TOTAL	530			257	

At weaning

The allocation of lambs to post-weaning paddocks was done in such a way as to ensure equal or near-equal representation of birth paddocks and sex in each post-weaning paddock, consistent with the factorial and replicate design of the experiment. Where appropriate (treatments IHL, IHH, ULL, ULH, UHL, UHH) similar numbers of each sex from each birth paddock were allocated to each post-weaning treatment. For treatment ILL, this was not possible because lambs were derived from one birth paddock only within each replicate. This led, by chance, to a marked dominance of female lambs in Replicate 1. In all other treatment groups (of 35 or 36 lambs) there were either 17, 18 or 19 lambs of each sex. (Table 14, Table 15).

Table 14 Allocation of lambs to experimental paddocks by birth paddock and sex. Replicate 1.

Birth paddock[a]	Flock[b]	Birth paddock status[c]	Weaning paddock[a]	Weaning paddock status[c]	Female lambs	Male lambs	Total lambs
1.3	I	L	1.7a	L	24	12	36
1.4a	I	H	1.4a	H	6	4	10
			1.6a	L	3	4	7
1.4b	I	H	1.4a	H	5	3	8
			1.6a	L	7	3	10
1.5a	I	H	1.4a	H	2	6	8
			1.6a	L	3	5	8
1.5b	I	H	1.4a	H	4	6	10
			1.6a	L	4	7	11
1.6	U	L	1.4b	H	8	10	18
			1.6b	L	8	9	17
1.7	U	L	1.4b	H	11	7	18
			1.6b	L	11	8	19
1.8	U	M	1.5b	H	10	8	18
			1.7b	L	9	8	17
1.9	U	M	1.5b	H	9	9	18
			1.7b	L	9	9	18
TOTAL					**133**	**118**	**251**

[a]The first number represents the replicate – either Rep 1 or Rep 2. The second number represents the paddock number within each replicate. [b]Flock is either I (infected) or U (uninfected). [c]Contamination status was either low (L), medium (M) or high (H).

Table 15 Allocation of lambs to experimental paddocks by birth paddock and sex. Replicate 2.

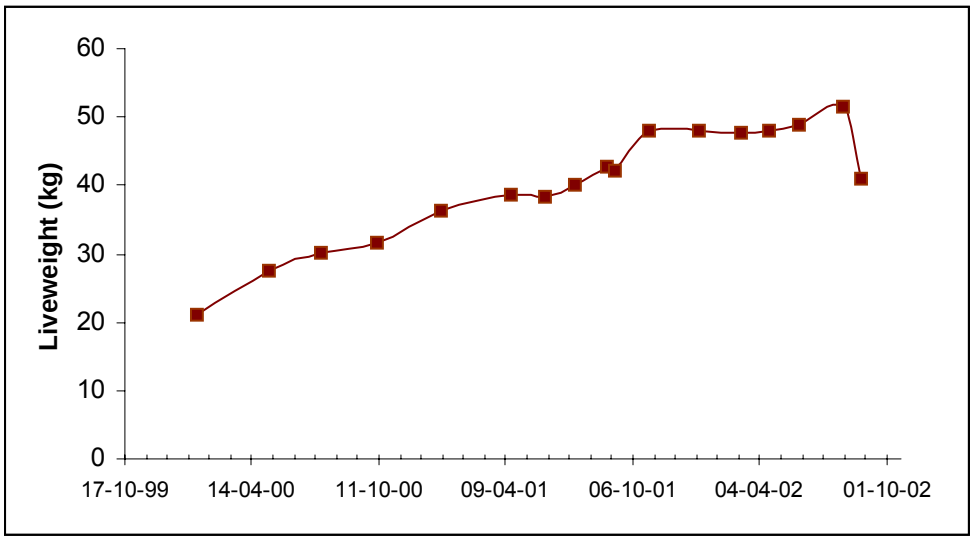
Birth paddock[a]	Flock[b]	Birth paddock status[c]	Weaning paddock[a]	Weaning paddock status[c]	Female lambs	Male lambs	Total lambs
2.3	I	L	2.7a	L	19	17	36
2.4a	I	H	2.4a	H	3	4	7
			2.6a	L	4	3	7
2.4b	I	H	2.4a	H	8	5	13
			2.6a	L	8	4	12
2.5a	I	H	2.4a	H	2	5	7
			2.6a	L	2	6	8
2.5b	I	H	2.4a	H	5	4	9
			2.6a	L	4	5	9
2.6	U	L	2.4b	H	9	9	18
			2.6b	L	10	9	19
2.7	U	L	2.4b	H	9	9	18
			2.6b	L	8	9	17
2.8	U	M	2.5b	H	9	9	18
			2.7b	L	9	9	18
2.9	U	M	2.5b	H	9	8	17
			2.7b	L	9	9	18
TOTAL					**127**	**124**	**251**

[a]The first number represents the replicate – either Rep 1 or Rep 2. The second number represents the paddock number within each replicate. [b]Flock is either I (infected) or U (uninfected). [c]Contamination status was either low (L), medium or high (H).

Liveweights

The mean liveweights of the experimental sheep increased over the course of the experiment from 21.1 kg at weaning to 40.9 kg at WT16 (Figure n). The highest overall mean liveweight recorded was 51.6 kg (WT16).The mean liveweight declined by 10.7 kg between WT15 and WT16. This decline was partly associated with the removal of fleece wool (approximately 5.0 kg) at shearing and partly because WT16 was recorded after a period of overnight fast in the woolshed, in contrast to the normal procedure at previous weighing events where sheep were weighed in each paddock with no fasting period.

Figure 6 Mean liveweight of all experimental sheep on 17 occasions from weaning to 36 months of age. Sheep were weighed in their paddocks (without any period of fast before weighing) at all weighing events except the last, when they were fasted overnight after shearing before weighing.



Wool production

The experimental sheep were shorn first on Days 401 – 405. Individual wool weights at this shearing were measured but are not recorded in the database. It was expected that the variation in lamb birth dates would strongly influence wool weight and make interpretation of the data difficult, because lamb birth date was not recorded. This shearing was considered as an 'evening-up' event, ensuring that all experimental sheep had almost identical inter-shearing periods between the first and second shearings.

The second shearing occurred on Days 793 – 799, approximately 393 days after the first shearing. Wool weights were recorded as WOOL2. Samples from the fleece, in the region of the shoulder of the sheep, were collected and have been stored should further analysis be desired.

The final shearing (WOOL3) occurred on Days 1116 – 1118, approximately 321 days after the second shearing.

Fleece weights were recorded by weighing the fleeces on scales immediately after removal from the sheep but did not include the belly wool (Table 16).

Table 16 Mean greasy fleece weights (free of belly wool) of the experimental sheep at the second and third shearings.

Variable	Number of records	Overall mean weight (kg)
WOOL2	469	4.68
WOOL3	452	5.03

Deaths in experimental sheep before 36 months of age

Overall summary

There were 502 sheep allocated to weaning paddocks for the post-weaning phase of the experiment. Of these, 459 (91.4%) survived to 36 months of age and 43 (8.6%) died in the 32 months between weaning and slaughter at 36 months. A diagnosis of the cause of death was made in 31 of those cases – and 22 (71%) of those were attributed to OJD. Overall, 4.4% of the opening number died of confirmed or probable OJD, 1.8% died of causes other than OJD and 2.4% died without diagnosis (Table 17).

Table 17 Categorisation of deaths between weaning and slaughter at 36 months of age

Category	n	N	% of deaths	% of opening number
(A) Confirmed deaths from OJD	16		37.2%	3.2%
(B) Probable deaths from OJD	6		14.0%	1.2%
Deaths attributed to OJD (A + B)		22	51.2%	4.4%
No information on cause of death		12	27.9%	2.4%
Confirmed death from cause other than OJD		9	20.9%	1.8%
TOTAL DEATHS		43	100.0%	8.6%

Confirmed-OJD deaths

There were 16 confirmed OJD deaths, 15 of which were confirmed by positive histopathology and 1 confirmed by positive tissue culture in the absence of specimens for histopathology. This sheep was also faecal culture positive at 18 months. All 15 cases confirmed by histopathology had lesions of type 3b. Of the 15 cases confirmed by histopathology, 10 had tissues available for culture, all of which were positive (Table 18).

Table 18 Culture and histopathology results from sheep whose death was confirmed as OJD.

Eartag	Group	BACT18M[a]	BACTPM[a]	HISTO[b]	Study day and date of necropsy	Age (days)
1023	UHH	1	9	3b	1008 (9 May 02)	983
1044	UHH	0	9	3b	953 (15 Mar 02)	928
1176	ULH	1	1	3b	960 (22 Mar 02)	935
1334	ULL	1	1	3b	1086 (26 Jun 02)	1051
1532	UHH	1	1	3b	854 (6 Dec 01)	829
1559	UHH	1	1	3b	729 (3 Aug 01)	704
1568	UHH	1	9	3b	1009 (10 May	984
7601	IHH	1	1	3b	950 (12 Mar 02)	872
7653	IHL	1	1	3b	789 (2 Oct 01)	711
7686	IHH	1	1	9	719 (24 Jul 01)	641
7732	ILL	1	1	3b	726 (31 Jul 01)	648
7759	IHL	0	9	3b	918 (8 Feb 02)	840
7784	IHH	0	1	3b	1058 (28 Jun 02)	980
7796	IHL	0	9	3b	988 (19 Apr 02)	910
7797	IHH	1	1	3b	680 (15 Jun 01)	602
7806	IHH	1	1	3b	615 (11 Apr 01)	537

[a]BACT18M – Results of faecal culture at 18 months of age. BACTPM – Results of tissue culture following necropsy. 0 = negative result, 1 = positive, 9 = not tested. [b]HISTO – Histopathology lesion type according to Perez et al (1996) 9 = not tested.

Probable-OJD deaths

Six sheep were classified as probable-OJD deaths. By definition, these were BACT18M-positive. Four were not examined post-mortem; two were examined post-mortem but tissues were lost to follow-up (Table 19). All six showed clinical signs of OJD in the weeks before death.

Table 19 Sheep for which the cause of death was considered to be probably OJD.

Eartag	Group	BACT18M[a]	BACTPM[a]	HISTO[b]	Study day and date of necropsy (if done)	Age (days)
1030	UHH	1	9	9	798 (11 Oct 01). Necropsied	743
1074	UHL	1	9	9	1020 (21 May 02). Necropsied	995
1184	ULL	1	9	9	802 (approx) (Oct 01)	777
7674	IHH	1	9	9	1014 (approx) (May 02)	935
7804	IHL	1	9	9	939 (approx) (Feb-Mar 02)	861
7845	ILL	1	9	9	772 (approx) (Sep 01)	694

[a]BACT18M – Results of faecal culture at 18 months of age. BACTPM – Results of tissue culture following necropsy. 0 = negative result, 1 = positive, 9 = not tested. [b]HISTO – Histopathology lesion type according to Perez et al (1996). 9 = not tested.

No information-deaths

Twelve sheep died without premonitory signs in the experiment and were not examined post-mortem. Four died before 18 months of age, six were BACT18M-negative and two were untested at BACT18M (Table 20). None of the twelve showed pronounced weight loss leading up to their death, or other clinical signs of OJD.

Table 20 Culture results from sheep which died but were not necropsied.

Eartag	BACT18M[a]	BACTPM[a]	HISTO[b]	Estimated study day and date of death
1064	9	9	9	315 (Jun 00)
1137	0	9	9	695 (Jun 01)
1160	9	9	9	468 (Nov 00)
1390	9	9	9	315 (Jun 00)
1477	9	9	9	468 (Nov 00)
7677	9	9	9	792 (Oct 01)
7703	0	9	9	1106 (Aug 02)
7740	0	9	9	1014 (May 02)
7764	0	9	9	939 (Mar 02)
7772	9	9	9	574 (Mar 01)
7802	0	9	9	802 (Oct 01)
7857	0	9	9	802 (Oct 01)

[a]BACT18M – Results of faecal culture at 18 months of age. BACTPM – Results of tissue culture following necropsy. 0 = negative result, 1 = positive, 9 = not tested. [b]HISTO – Histopathology lesion type according to Perez et al (1996). 9 = not tested.

Not OJD-deaths

Nine sheep died and were either necropsied with OJD-negative results, or were diagnosed clinically and by gross necropsy to have died of causes other than OJD (Table 21). Three had negative histopathology with no tissue culture. Two had negative tissue culture but no histopathological examination. (One of these was faecal culture positive at 18 months (February 2001) but tissue culture negative at death in March 2001. Tissues were too autolysed for histopathology.) Three died of haemonchosis in January 2001 and one died of grain overload in September 2002. None of these four had tissues tested post-mortem. The sheep which died from grain overload was faecal culture negative at 18 months.

Table 21 Culture and histopathology results from sheep whose death was determined not to be OJD.

Eartag	BACT18M[a]	BACTPM[a]	HISTO[b]	Study day and date of necropsy & comment
1141	1	0	9	589 (16 Mar 01) Tissues autolysed
1161	0	9	9	1125 (3 Sep 02) Grain poisoning.
1190	9	9	9	524 (Jan 01) Haemonchosis.
1198	9	9	9	524 (Jan 01) Haemonchosis.
1200	9	9	9	524 (Jan 01) Haemonchosis.
7698	0	9	0	918 (8 Feb 02)
7753	9	9	0	557 (12 Feb 01) Enterotoxaemia.
7836	0	9	0	729 (3 Aug 01)
7838	0	0	9	859 (11 Dec 01) Spinal abscess. Tissues autolysed

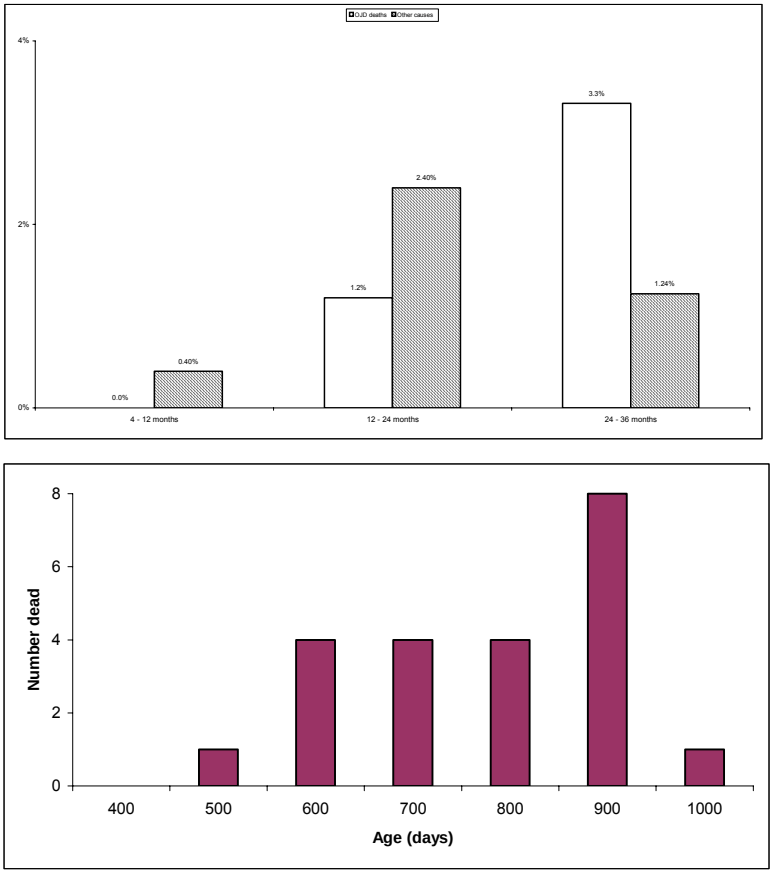
[a]BACT18M – Results of faecal culture at 18 months of age. BACTPM – Results of tissue culture following necropsy. 0 = negative result, 1 = positive, 9 = not tested. [b]HISTO – Histopathology lesion type according to Perez et al (1996). 9 = not tested.

Time of deaths

Overview

Cases classified as OJD deaths (both confirmed and probable) occurred between April 2001 and June 2002, when the sheep were aged between 537 and 1051 days of age[b]. The experiment terminated when the sheep were 1107 (Flock U) or 1054 (Flock I) days of age. More sheep died of causes other than OJD than died of OJD in the first two years of life but, in their third year, most sheep which died were affected by OJD. (Figure 7). Deaths from OJD occurred between 537 and 1051 days of age, with most occurring between 600 and 1000 days of age (Table 22). There was a peak in deaths from OJD between 900 and 1000 days of age (Figure 7 bottom).

Figure 7 Distribution of deaths (all causes) of experimental sheep over time. (Top) The percentages shown relate to the numbers of sheep present at the start of each time period (four months of age (weaning), 12 months of age, 24 months of age). (Bottom) The distribution of ages at death (in days) of sheep dying from OJD, including probable-OJD deaths. There were only 54 days post-1000 days of age for Flock I lambs.



[b] Age is calculated by subtracting the flock median birth date from the study day on which each sheep died. Median birth date was 25 (Flock U) or 78 (Flock I).

Differences in age at death between treatment groups

There were very few OJD deaths in some treatment groups which meant that meaningful comparisons of age at death between all groups were not possible. Valid comparisons could be made between sheep in the two continuous high-exposure groups (UHH and IHH). Sheep which were born from the infected dam flock (IHH) died, on average, 160 days earlier in life than sheep which were born from uninfected ewes (UHH), even if the pasture was contaminated (and cohabited by infected sheep). This observation was not statistically significant when applied to just IHH vs UHH (P=0.11) but, when it was applied to all sheep born to Flock I ewes and Flock U ewes, whatever their pre- and post-weaning exposure, the difference in median age (935 vs 711) was significant (P=0.03) (Table 22a). The differences between groups were less when probable OJD deaths were included (Table 22b) but remained statistically significant (932 > 776, P=0.04)

Further statistical analyses are reported in Appendix 2, Section A.

Table 22 Age at death (with group means and medians) for sheep which died with (a) confirmed-OJD and (b) confirmed-OJD and probable-OJD. Probable-OJD deaths are shown in italics

(a)

	UHH	ULH	ULL	UHL	IHH	IHL	ILL
	704	935	1051		537	711	648
	829				602	840	
	928				641	910	
	983				872		
	984				980		
Mean (each column)	886	935	1051		726	820	648
Mean (groups U and I)	916				749		
Median (each column)	928	935	1051		641	840	648
Median (groups U and I)	935				711		

(b)

	UHH	ULH	ULL	UHL	IHH	IHL	ILL
	704	935	*777*	*995*	537	711	648
	743		1051		602	840	*694*
	829				641	*861*	
	928				872	910	
	983				*935*		
	984				980		
Mean (each column)	862	935	914	995	761	831	671
Mean (groups U and I)	893				769		
Median (each column)	879	935	914	995	757	851	671
Median (groups U and I)	932				776		

Sheep surviving to slaughter at 36 months of age

Overall summary

Four hundred and fifty nine sheep survived to the end of the trial. Twelve were then euthanased because they were too thin to travel to the abattoirs and 447 were slaughtered at the abattoirs.

From the 12 euthanased animals, HISTO results were available from 11 but fresh tissues for culture were only available from one. Thus, these 11 animals had results for HISTO only. The animal for which a BACTPM result was available was the animal with no HISTO result (eartag 7746). This sheep was euthanased following shearing on Day 1119.

At the abattoirs, one animal (abattoir submission number 322, eartag number 7761) provided tissues for culture only. Presumably tissues for histopathology were lost. Five sheep had no result for HISTO or BACTPM. In four cases this was because eartags were lost (eartag numbers 1120, 1464, 7765, 7840); in one case (eartag 1411) viscera were unavailable for sampling.

In total, 441 sheep provided tissues for both HISTO and BACTPM, 11 for HISTO only and two for BACTPM only (Table 23). Both sheep which were tested by BACTPM only were negative in culture, so no survivor was categorised as OJD-affected in the absence of a HISTO result.

In summary, a diagnosis was made in all but five surviving sheep – 454 in total. Of these, the diagnosis for 452 was based on histopathology and/or culture and, in two cases, diagnoses were based on culture alone.

Table 23 Distribution of results from sheep which survived until the end of the trial (36 months of age).

	BACTPM result	No BACTPM result	TOTAL
HISTO result	441	11	452
No HISTO result	2	5	7
TOTAL	**443**	**16**	**459**

Confirmed OJD cases which survived

There were 101 sheep which survived to slaughter at 36 months of age but which were later found to have OJD. This represented 22.2% of the 454 sheep present at 36 months for which a determination of OJD status was made.

Of the 101, 54 had positive histopathology and 47 had positive tissue culture with negative histopathology. Of the cases with positive histopathology, 26 had lesions of type 3a, 3b or 3c. Of these, 24 had a BACTPM result and all were positive. Of the sheep with lesions of types 1 and 2, 75% and 50% respectively were tissue culture positive (Table 24). Of the sheep with no histopathological lesions which had a BACTPM result, 47 of 391 (12.0%) were tissue culture positive.

Table 24 Numbers of experimental sheep classified by histopathological findings (HISTO), bacteriological findings at necropsy (BACTPM) and survival to 36 months. The classes containing sheep which were confirmed cases of OJD are highlighted in italics.

	Survived				**Dead**				**Survived and dead**			
		BACTPM[a]				BACTPM				BACTPM		
HISTO	Total	0	1	9	Total	0	1	9	Total	0	1	9
0	398	344	*47*	7	3	0	0	3	401	344	47	10
1	*16*	4	12	0	0				*16*	4	12	0
2	*12*	5	5	2	0				*12*	5	5	2
3a	*12*	0	11	1	0				*12*	0	11	1
3b	*9*	0	8	1	*15*	0	10	5	*24*	0	18	6
3c	*5*	0	5	0	0				*5*	0	5	0
9	7	2	0	5	25	2	*1*	22	32	4	*1*	27
TOTAL	459	355	88	16	43	2	11	30	502	357	99	46

[a]BACTPM – Bacteriological result from culture of tissues collected at post-mortem examination. 0 = negative for *M paratuberculosis*, 1 = positive, 9 = not tested.

Total OJD cases

Overall summary

There were 101 confirmed cases which survived, 16 confirmed OJD deaths and six probable OJD deaths, making a total of 123 OJD cases out of 485 sheep (454 survivors – see Table 23, 31 deaths – see Table 17) for which a diagnosis was made (Table 25).

Table 25 Contingency tables for estimating the relative risk of death before 36 months in (a) OJD-affected sheep and (b) OJD-affected sheep with type 3b lesions. The relative risk (RR) is shown in the bottom right cell of each table.

(a)

OJD status	Dead	Alive	TOTAL	% dead
OJD affected	22	101	123	(17.9%)
OJD free	9	353	362	(2.5%)
TOTAL	**31**	**454**	**485**	**7.2**

(b)

OJD status	Dead	Alive	TOTAL	% dead
OJD type 3b	15	9	24	(62.5%)
OJD not type 3b	16	445	461	(3.5%)
TOTAL	**31**	**454**	**485**	**18.0**

Of 123 OJD-affected sheep, 22 (17.9%) died before 36 months, compared to 9 of 362 (2.5%) sheep with no lesions of OJD (Table 25a). The difference in proportion was highly significant (X^2=36.4, P<0.001). The relative risk of dying between weaning and 36 months if OJD-affected was estimated to be 7.2 (95% CI; 3.4, 15.2).

All 15 sheep which died and had histopathological confirmation of OJD had type 3b lesions. This represented 60% of the 25 sheep in which type 3b lesions were detected (Table 25b). The presence of type 3b lesions was strongly associated with an increased risk of death when compared to all other sheep for which a diagnosis of OJD status was made. The relative risk of dying if affected with OJD with a lesion of type 3b was 18.0 (10.2, 31.9), compared to all other sheep (X^2=132.9, P<0.001).

In Table 25, sheep for which no information on the cause of death was available were excluded from the analysis. It is possible, however, that some or even all of these 12 sheep were free of OJD and, if so, the estimates of relative risk would be substantially lower than those cited above. For example, if all 12 cases were included (in the shaded cells in the table), the relative risks would have been 3.3 (1.8, 5.6) and 10.6 (6.6, 17.0). These values provide a lower limit for the estimation of the strength of the association between the presence of OJD infection, or a type 3b OJD lesion, and the risk of death. The differences in proportion were still statistically significant (P<0.001).

Forty seven sheep were found to have lesions of types 1, 2, 3a and 3c when examined histologically after slaughter at 36 months, but none of these sheep died before the trial ended (Table 24). There is no suggestion from our data that sheep with lesions of these types are at an increased risk of death compared to unaffected sheep, at least until their lesions develop into a more severe form.

A total of 99 sheep had positive culture from intestinal tissues (BACTPM) at necropsy. This in itself was not a risk factor for death unless associated with a type 3b lesion (Table 24).

Effect of treatment group on lesion type

The distribution of sheep with confirmed OJD (either negative histopathology with positive culture or lesions type 1, 2, 3a, 3b and 3c) is shown in Table 26. There was a tendency for treatment groups in Replicate 1 to have a higher proportion of sheep with low-grade OJD lesions than groups in Replicate 2 – particularly noticeable in groups IHL and IHH.

Severe lesions of OJD (type 3b) occurred at a higher frequency in groups UHH, IHH and IHL, but these also showed a marked difference between replicates with Replicate 2 having more severe lesions than Replicate 1, particularly in UHH. (These values are also represented in Table 30 as percentages of the population at risk, with probable-OJD deaths included as infected sheep with severe lesions.)

The differences between replicates implied that there was either a large amount of randomness to the occurrence of OJD lesions or that there were interactions between replicate and treatment group in some cases. These results made statistical analysis of the results, based on individual treatment groups, difficult (see Appendix 2, Section B.)

Table 26 Frequency of lesion types in surviving and dead sheep, by treatment group

Treatment :	ULL		ULH		UHL		UHH		ILL		IHL		IHH		TOTAL
Replicate :	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
HISTO=0, BACTPM=0	24	33	24	27	26	30	27	22	27	23	16	21	21	23	344
HISTO=0, BACTPM=1	5	1	3	5	4	3	5	3	2	1	4	3	6	2	47
HISTO=1	1	1	0	0	1	1	0	2	0	1	8	0	1	0	16
HISTO=2	1	0	0	0	1	0	0	1	0	1	4	0	2	2	12
HISTO=3a	2	0	1	0	0	0	1	1	1	2	1	1	0	2	12
HISTO=3b	0	1	2	1	0	1	1	4	2	2	2	2	1	5	24
HISTO=3c	0	0	0	0	0	0	0	1	0	1	0	0	2	1	5
TOTAL	33	36	30	33	32	35	34	34	32	31	35	27	33	35	460

Effect of treatment group on risk of OJD

Cumulative incidence of OJD, not including probable-OJD deaths

To calculate the cumulative incidence of OJD, the population at risk included all experimental sheep present at the start of the post-weaning period except those which subsequently died without post-mortem examination or were slaughtered without a diagnostic post-mortem examination ('no-information-deaths' and 'no-information- survived') (Table 27). The time-at risk was the period from weaning to slaughter - 1014 days (Flock U) or 956 days (Flock I). The cumulative incidence, excluding sheep classified as probable-OJD deaths, varied from 37.3% in the IHL group to 15.9% in the UHL group (Figure 8). The incidence in the IHH group (35.2%) was similar to that in the IHL group. Of the 117 confirmed deaths, 60 (51%) were in male sheep.

Details of the statistical analysis are reported in Appendix 2, Section C.

The cumulative incidence of OJD in experimental groups IHH, IHL and UHH (33.2%) was significantly higher than in groups ILL, UHL and ULH (18.1%). (These figures are adjusted for fixed and random effects in the statistical model.)

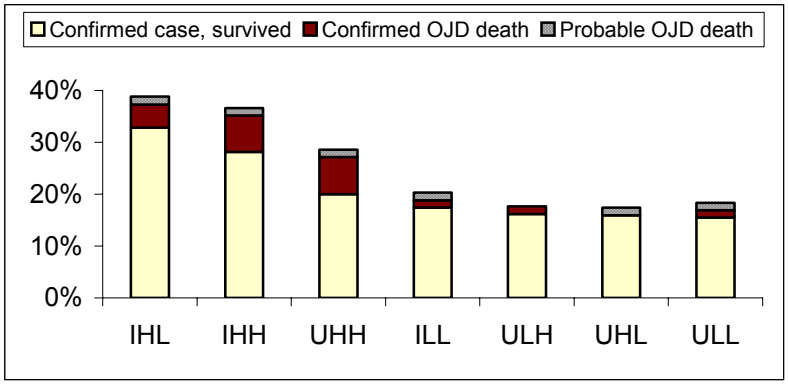
OJD cases including confirmed- and probable-OJD deaths

The analyses for the data that included the probable OJD cases produced the same significance results as those that did not include them, and the interpretation remains the same. The OJD infection rate for sheep exposed to high pre-weaning or continuous exposure (34.2%) was higher than for those exposed to a discontinuous source of infection (18.9%). However, the difference between the OJD incidence for the "control" treatment ULL (17.8%) (corrected in the statistical model and, therefore different from the figure in Table 30) and the average of the six deliberately-exposed groups (26.5%) was not detected as significant (Figure 8).

Table 27 Distribution of OJD cases by treatment group juxtaposed into high (IHL, IHH, UHH) and low infection rate (ILL, ULH, UHL) groups

	IHL	IHH	UHH	ILL	ULH	UHL	ULL	**TOTAL**
A. Confirmed deaths	3	5	5	1	1	0	1	**16**
B. Probable deaths	1	1	1	1	0	1	1	**6**
C. No info deaths	5	0	1	2	3	1	0	**12**
D. Not OJD deaths	1	0	1	3	3	0	1	**9**
E. Confirmed cases, survived	22	20	14	12	11	11	11	**101**
F. No information, survived	0	1	0	1	1	1	1	**5**
G. Opening number	72	72	71	72	72	71	72	**502**
Population at risk (G-C-F)	67	71	70	69	68	69	71	**485**

Figure 8 Cumulative incidence of OJD infection by treatment group. The denominator for incidence is the population at risk shown in Table 27.



Effect of treatment group on risk of death from OJD

Confirmed OJD deaths only

To calculate the OJD mortality rate, the population at risk included all experimental sheep present at the start of the post-weaning period except those which subsequently died with no diagnostic determination (no-information-deaths) (Table 28). The time-at risk was 1014 days (Flock U) or 956 days (Flock I) - the period from weaning to slaughter. The mortality rate, for confirmed deaths only, varied from 7.2% in the IHH group to 0.0% in the UHL group (Figure 9). The mortality rate in the only other group with both pre- and post-weaning exposure (UHH) was similar to that of the IHH group (7.1%). Of the 16 confirmed deaths, 12 (75%) were in male sheep. The OJD death rate for males (3.6%) was higher than that for females (1.1%)[c] (P<0.05).

The statistical analyses performed are described in Appendix 2, Section D.

The OJD death rate was higher for sheep in groups IHL, IHH and UHH (5.5%) than for those in ILL, UHL and ULH (0.8%). The difference between the OJD death rates for the "control" treatment ULL (1.2%) (corrected in the statistical model and, therefore different from the figure in Table 30) and the average of the six deliberately-exposed groups (3.2%) was not detected as significant.

Confirmed and probable-OJD deaths together

The mortality rate (cumulative incidence of mortality), for confirmed and probable-OJD deaths together, varied from 8.6% and 8.3% in the UHH and IHH groups respectively to 1.4% in the UHL and ULH groups (Figure 9 and Table 30). Of the 22 deaths, 14 (63.6%) were in male sheep.

The OJD death rate was higher for sheep in groups IHL, IHH and UHH (7.2%) than for those in ILL, UHL and ULH (1.7%). The difference between the OJD death rates for the "control" treatment ULL (2.5%) and the average of the six high-exposure groups (4.4%) was not detected as significant(Table 29). The difference between the OJD death rates for males (4.7%) and females (2.4%) was not detected as significant (Appendix 2, Section D).

Table 28 Distribution of deaths by treatment group[a] (juxtaposed into high and low infection rate columns)

	IHL	IHH	UHH	ILL	ULH	UHL	ULL	**TOTAL**
A. Confirmed deaths	3	5	5	1	1	0	1	**16**
B. Probable deaths	1	1	1	1	0	1	1	**6**
C. No info deaths	5	0	1	2	3	1	0	**12**
D. Not OJD deaths	1	0	1	3	3	0	1	**9**
E. Opening number	72	72	71	72	72	71	72	**502**
Population at risk (E-C)	67	72	70	70	69	70	72	**490**

[a] First letter of group = Dam flock; I (infected) U (uninfected). Second and third letter = contamination level in the pre-weaning and post-weaning paddocks respectively; L (low) or H (high).

[c] The infection rates quoted in this paragraph are adjusted for fixed and random effects in the model. The fixed effects were sex, T1 and T2 and the random effect was replicate.

Table 29 Number of confirmed- and probable-OJD deaths, by treatment group and replicate

Treatment :	ULL		ULH		UHL		UHH		ILL		IHL		IHH		**TOTAL**
Replicate :	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
Confirmed OJD	0	1	1	0	0	0	1	4	1	0	1	2	2	3	**16**
Probable OJD	1	0	0	0	1	0	0	1	0	1	0	1	1	0	**6**

Figure 9 Cumulative incidence of mortality of sheep (confirmed and probable) by treatment group. The denominator for mortality incidence is the population at risk shown in Table 28.

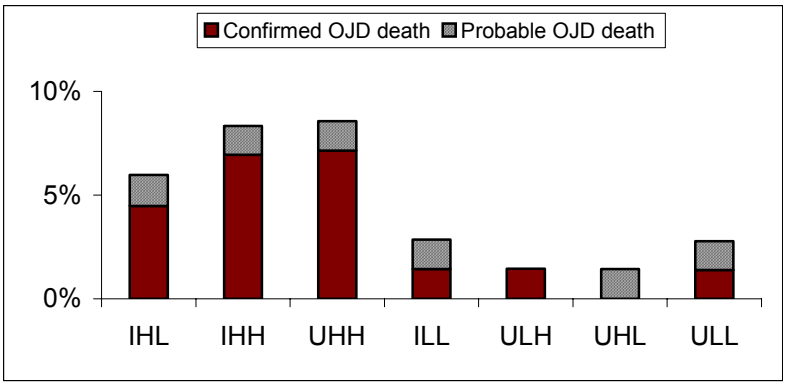


Table 30 Cumulative incidence of OJD infection, severe infection and mortality (confirmed and probable) assuming that probable OJD deaths also had severe lesions (type 3a or 3b). The denominator for infection incidence is the population at risk shown in Table 27. The denominator for mortality incidence is the population at risk shown in Table 28.

	IHL	IHH	UHH	ILL	ULH	UHL	ULL
OJD infection	38.8	36.6	28.6	20.3	17.6	17.4	18.3
Severe infection (3a + 3b)	10.4	12.7	11.4	11.6	5.9	2.9	5.6
Mortality	6.0	8.3	8.6	2.9	1.4	1.4	2.8

Faecal culture results

Results of pooled faecal culture results at 12 months of age (BACT12M)

A total of 70 pools of faeces were cultured. Each pool included faeces from 6, 7 or 8 animals, collected at FS5 (11 - 12 months of age). Nine pools (12.9%) were positive. The most likely number of shedding animals which would result in nine positive pools was 9 or 10 (95% CI; 6, 16).

The positive pools occurred in paddocks in which either the dams were infected (4 cases), the birth paddocks were infected (3 cases) or the post-weaning paddocks were infected (2 cases) (Table 31).

Table 31 Occurrence of culture-positive pools of faeces from experimental sheep at 11 - 12 months of age.

Paddock	6b	4b	7b	5a/b	7a	6a	4a
Treatment	ULL	ULH	UHL	UHH	lLL	lHL	lHH
Rep 1	0	2	1	1	1	2	0
Rep 2	0	0	0	1	1	0	0
TOTAL	**0**	**2**	**1**	**2**	**2**	**2**	**0**

Faecal culture results at 18 months of age (BACT18M)

Overview of BACT18M results

There were 493 sheep alive at 18 months of age. Faecal samples were collected from 481 of these and 27 (5.6%) of these were culture positive for *M paratuberculosis*.

Table 32 Additional findings, outcome and classification of sheep which were BACT18M-positive.

Eartag	Pad-dock	Treat-ment	HISTO	BACT-PM	Outcome	Day of death	OJD free & survived	Conf'd OJD	Prob OJD death	Not OJD death
1023	2.5a	UHH	3b	9	Died 9/5/02	1008		1		
1030	2.5a	UHH	9	9	Died	798			1	
1061	1.5b	UHH	3a	1	Survived			1		
1069	1.7b	UHL	0	1	Survived			1		
1074	1.7b	UHL	9	9	Died 21/5/02	1020			1	
1125	1.7b	UHL	0	0	Survived		1			
1141	1.5b	UHH	9	0	Died 16/3/01	589				1
1176	1.4b	ULH	3b	1	Died 22/3/02	960		1		
1184	1.6b	ULL	9	9	Died 10/9/01	767			1	
1334	2.6b	ULL	3b	1	Died 26/6/02	1056		1		
1532	2.5a	UHH	3b	1	Died 6/12/01	854		1		
1559	2.5a	UHH	3b	1	Died 3/8/01	729		1		
1568	2.5a	UHH	3b	9	Died 10/5/02	1009		1		
7601	1.4a	IHH	3b	1	Died 12/3/02	950		1		
7608	1.4a	IHH	1	1	Survived			1		
7644	1.4a	IHH	0	0	Survived		1			
7646	1.6a	IHL	1	1	Survived			1		
7647	1.6a	IHL	1	1	Survived			1		
7651	1.6a	IHL	3b	9	Survived			1		
7653	1.6a	IHL	3b	1	Died 2/10/01	789		1		
7674	1.4a	IHH	9	9	Died 16/4/01	620			1	
7686	1.4a	IHH	9	1	Died 24/7/01	719		1		
7732	1.7a	ILL	3b	1	Died 31/7/01	726		1		
7797	2.4a	IHH	3b	1	Died 15/6/01	680		1		
7804	2.6a	IHL	9	9	Died 15/1/02	894			1	
7806	2.4a	IHH	3b	1	Died 11/4/01	615		1		
7845	2.7a	ILL	9	9	Died 15/9/01	772			1	
TOTAL							**2**	**18**	**6**	**1**

Relationship between BACT12M and BACT18M results

Of the 27 BACT18M sheep, seven had faeces in positive pools at BACT12M, 16 were in negative pools and four were untested at BACT12M (faeces unavailable at FS5). Of the 23 animals tested on both occasions, seven of 23 (30%) were potentially positive on both occasions (presuming they contributed to the positivity at BACT12M) and 16 of 23 (70%) were negative at BACT12M and positive at BACT18M.

The seven animals in positive pools at BACT12M occurred in five positive pools (two positive pools contained two animals which were later BACT18M-positive). Four of the nine positive pools at BACT12M contained no animals which subsequently were BACT18M positive. In three of these cases, the pools included animals which subsequently died and were unavailable for histopathological examination.

Relationship between BACT18M and treatment group

The BACT18M-positive rate was higher for sheep in groups IHL, IHH and UHH (8.7%) than for those in groups ULH, UHL and ILL (2.7%). However, in this trial the difference between the BACT18M rates for the "control" treatment ULL (2.6%) and the average of the six deliberately-exposed groups (4.9%) was not detected as significant (Appendix 2, Section E.) No effect of sex on BACT18M-positive rate was detected (Table 33).

Table 33 Number of culture-positive faecal samples from experimental sheep at 18 months of age.

Paddock	6b	4b	7b	5a/b	7a	6a	4a
Treatment	ULL	ULH	UHL	UHH	ILL	IHL	IHH
Rep 1	1	1	3	2	1	4	5
Rep 2	1	0	0	5	1	1	2
TOTAL	**2**	**1**	**3**	**7**	**2**	**5**	**7**

Risk of death and BACT18M result

Between the time when faeces were collected from the 18 month-old sheep and the sheep were slaughtered at 36 months of age, 35 sheep died (7.1% of the number alive at 18 months). Thirty three of these had BACT18M results. Of the 27 sheep which were BACT18M-positive, 19 (70%) died before 36 months. Of the 454 sheep which were negative, 14 (3.1%) died (Table 34). The difference was highly significant ($P<0.001$). The risk of dying between 18 months and 36 months was 22.7 times higher for those sheep which were faecal culture positive at 18 months, compared to those which were faecal culture negative[d].

Nineteen BACT18M-positive sheep died before 36 months of age. Twelve of these were confirmed OJD deaths by HISTO and/or BACTPM - six were not examined post-mortem (no HISTO or BACTPM) and one died of causes other than OJD. Consequently, of the 13 BACT18M-positive animals which died and were necropsied, 12 (92%) were confirmed OJD deaths (Table 35).

[d] These numbers vary slightly from those given in the AVA presentation 2003 as a result of a minor re-classification of cases.

Association between risk of death, BACT18M and treatment group

There was a very highly significant effect of BACT18M (P<0.001) on SURVIVAL but the sex and treatment fixed effects were non-significant. The adjusted mean proportion of survivors to the end of the trial for BACT18M negative sheep was 97.9%, compared with 22.7% for BACT18M positive sheep. Non-adjusted figures are given in Table 34.

Table 34 The relationship between faecal culture status for *M paratuberculosis* at 18 months of age and survival to 36 months of age. The relative risk is in the bottom right cell of the table.

Faecal culture status at 18 mo	Dead by 36 months	Alive at 36 months	TOTAL	% dead
Positive	19	8	27	70.4%
Negative	14	440	454	3.1%
TOTAL	**33**	**448**	**481**	**22.7**

Table 35 Distribution of BACT18M results across all deaths.

Classification	BACT18M +	BACT18M -	BACT18M not tested
OJD death	12	4	0
Probable OJD death	6	0	0
Not OJD death	1	4	4
No info death	0	6	6
Total	**19**	**14**	**10**

Risk of death from OJD and BACT18M result

Of the 21 sheep which were BACTPM-positive and later necropsied, 12 (57%) died of OJD before 36 months. Of the 454 which were negative, 4 (0.9%) died of OJD (Table 36a). Sheep which were BACT18M-positive were 65 times (23, 184) as likely to die of OJD before 36 months of age as sheep which were BACT18M negative. If probable-OJD deaths were included, 18 of 27 (67%) BACT18M-positive sheep died of OJD (Table 36b) and the estimate of relative risk was 76 (27, 208).

Table 36 The relationship between faecal culture status for *M paratuberculosis* at 18 months and survival to 36 months of age, (a) excluding probable OJD deaths and (b) including probable OJD deaths.

(a)

Faecal culture status at 18 mo	Confirmed OJD deaths	Survived or died of cause other than OJD	Total	% dead of OJD
Positive	12	9	21	57.1%
Negative	4	450	454	0.9%
Total	**16**	**459**	**475**	**64.9**

(b)

Faecal culture status at 18 mo	Confirmed or probable OJD deaths	Survived or died of cause other than OJD	Total	% dead of OJD
Positive	18	9	27	66.7%
Negative	4	450	454	0.9%
Total	**22**	**459**	**481**	**75.7**

Risk of OJD infection at or before 36 months of age and BACT18M result

Eight sheep which were BACT18M positive survived to 36 months. Two were apparently free of OJD and were clinically normal. Six remained infected and three of these showed clinical signs. One had lesions classified as type 3b and was 23% below its expected liveweight; one had type 3a lesions and had lost 28% of its liveweight; one had type 1 lesions and was also 28% below its expected liveweight. The other three were clinically normal despite tissue-culture positivity in all cases and lesions of type 1 in all cases.

By grouping together sheep which died of OJD and sheep which had OJD when slaughtered at 36 months, it was possible to calculate the relative risk for OJD infection at or before 36 months of age in sheep which were BACT18M positive (Table 37). The RR was 4.0 (3.2, 5.0) if probable-OJD deaths were excluded and 4.1 (3.4, 5.0) if they were included.

Table 37 The relationship between faecal culture status for *M paratuberculosis* at 18 months and the presence of OJD infection at or before 36 months of age. The relative risk is shown in the bottom right cell. (a) Probable-OJD deaths excluded (b) probable-OJD deaths included.

(a)

Faecal culture status at 18 mo	Confirmed OJD	No evidence of OJD	TOTAL	% with OJD
Positive	19	2	21	90.5%
Negative	102	352	454	22.5%
TOTAL	**121**	**354**	**475**	**4.0**

(b)

Faecal culture status at 18 mo	Confirmed or probable OJD	No evidence of OJD	TOTAL	% with OJD
Positive	25	2	27	92.6%
Negative	102	352	454	22.5%
TOTAL	**127**	**354**	**481**	**4.1**

Association between BACT18M and HISTO

There was a significant effect of BACT18M on HISTO for lesion types 0 (P<0.001) and 3b (P<0.001) but not for the other scores (Table 38) (Appendix 2, Section E).

Table 38 Adjusted mean proportions of lesion types which were BACT18M negative or positive.

	0	1	2	3a	3b	3c
BACT18M negative	90.0%	1.7%	2.1%	2.0%	1.9%	0.4%
BACT18M positive	18.3%	6.7%	0.0%	4.0%	65.1%	0.0%

The total sums of percentages do not add to 100% and the partial sums of percentages for lesion scores do not match the category percentages. These are the result of different random effects applying to different scores and between component scores and the corresponding category total.

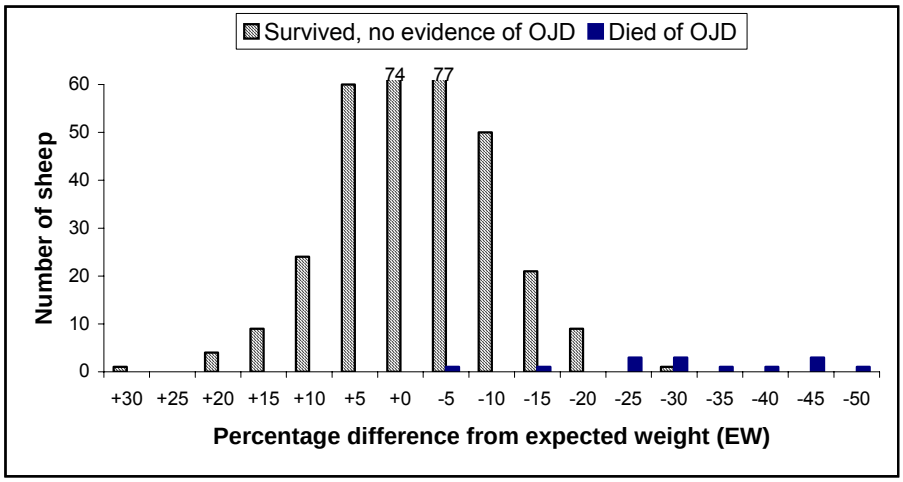
Liveweight change in OJD cases

Use of expected weight (EW) in predicting clinical OJD

Amongst 328 sheep which survived to WT16 with negative histopathology and negative tissue culture, 90% remained within ±13% of their expected weights (EW) and 99% (all but three sheep) remained within ±21%. Of those three sheep only one was more than 20% below its EW (the other two were more than 21% above their EW). Similar results were obtained with all weighing events from WT8 to WT15 inclusive – between 0 and 3 sheep of the 328 uninfected survivors were ever found to be more than 20% below their EW. The specificity was less at WT5, WT6 and WT7 – where 5, 3 and 6 uninfected survivors recorded weights more than 20% below EW.

For the 15 confirmed deaths from OJD, the last recorded liveweight (which could have been any weighing event between WT6 and WT14) was more than 20% below the EW for all but two animals (Figure 10). These two animals were not, however, weighed within 30 days of their death – so their weight loss could have accelerated as death approached. It is clear that a weight loss of 20% below the EW[e] was a good diagnostic predictor for clinical OJD, having a specificity of 99.1% to 100.0% for WT8 to WT16 inclusive and a sensitivity of around 87%.

Figure 10 The distribution of final liveweight expressed as a percentage deviation from the expected weight (EW). Most sheep with no evidence of OJD were within 13% of their EW at WT16. The last recorded liveweights of the 15 confirmed-OJD deaths ranged from –3.4% to –45.1%. A cut-off of –20% was a good predictor of clinical OJD.

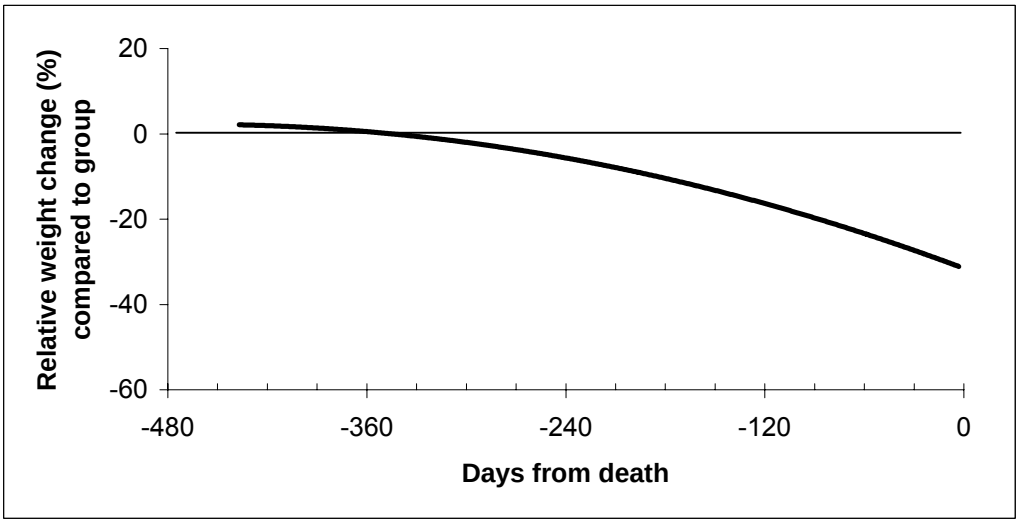


[e] Note that this is a drop below the *expected weight* – which corrects for changes in the liveweight of the group in which the sheep is running. Hence, for a sheep which is of average weight as a young adult, a fall to 20% below the group average would suggest that it has OJD. For a sheep which is 10% above average as a young adult, a fall to 10% below average would suggest the same.

Liveweight change in OJD deaths

Confirmed OJD deaths showed marked weight loss leading up to their death. Of the 15 confirmed deaths[f], none was within 20% of its EW in the 30 days before it died. In a proportion of cases, the weight loss exceed 30% (about 12 kg). The trend in liveweight change for those 15 sheep is illustrated in Figure 11.The average change in liveweight recorded in the last month of their lives for the 11 sheep which were weighed during that period was –31.6%. (No sheep were deliberately weighed when in the terminal stages of OJD – all records were made at normal planned weighing events.)

Figure 11 Change in liveweight of sheep which died of OJD, relative to their expected weight. This is a line of best fit through all data-points available for all sheep, so some sheep are over-represented in the trendline (R^2 = 0.38)



The number of weight records (data points) between WT5 and death varied between individuals from zero to eight. Consequently, the trend-line illustrated in Figure 11 is influenced more by some sheep than others – particularly for weight records more than 300 days before death - and tests of statistical significance are, therefore, inappropriate. Nevertheless, there was strong evidence for a trend of increasing weight loss with time, which became evident at about 240 days in most affected sheep. On average, affected sheep were 4.1% below their EW 240 days before death. For all data points, a linear relationship (with a slope of 0.083) had an R^2 of 0.36 (not shown) while the quadratic relationship illustrated had an R^2 of 0.38, suggesting that the polynomial model provided a slightly better explanation of the observed data than the linear model.

When sheep with 4, 5 or 6 weight records, commencing at a time point close to 240 days before death, were examined separately from those with fewer data, it was acceptable to estimate the expected rate of weight loss by calculating the median slope of the relationship between percentage weight loss and days before death (Table 39). There were 10 sheep for which such data were available - the median slope was 0.134, the range was 0.083 to 0.317 and 80% of the values were between 0.084 and 0.172. This result implies that OJD affected sheep are expected to lose 0.134% of their liveweight daily in the 240 days before death and that they would lose 32.2% of their liveweight over that period. There were five animals with 2, 3 or 4 weight records over a shorter period of time (<<240 days) before death (Table 40). Although the number is limited the observations show the same trend – in fact the higher median slope (0.281) was consistent with the inference from Figure 11 that the rate of weight loss is accelerated closer to death.

[f] The 16th confirmed OJD death occurred soon after WT4 so no corrected relative weight could be calculated.

Table 39 Weight loss in sheep which died with confirmed OJD - for animals with weight records of at least 230 days before death.

Sheep tag number	Number of records contributing to line of best fit	Days before death – earliest record used to calculate line of best fit	Slope of line
1532	5	233	0.172
7759	4	249	0.167
7601	5	239	0.317
1044	5	242	0.111
1176	5	249	0.132
7796	6	233	0.137
1023	5	241	0.147
1568	5	242	0.084
1334	5	240	0.083
7784	5	242	0.115
Median		**242**	**0.134**

Table 40 Weight loss in sheep which died with confirmed OJD - for animals with a limited number of weight records.

Sheep tag number	Number of records contributing to line of best fit	Days before death – earliest record used to calculate line of best fit	Slope of line
1532	2	59	0.281
7601	3	98	0.363
1176	3	105	0.328
1023	3	108	0.261
1334	4	168	0.131
Median		**105**	**0.281**

Liveweight change in surviving OJD cases

Sheep with OJD pathology of type 3a or 3b were predicted to be 3.48 or 3.76 kg respectively lower in liveweight than would have been predicted from their 15 month liveweight, sex and paddock, if they were free of these types of OJD lesions (Table 41). See Appendix 2, Section F for the full results of the statistical analysis..

Table 41 Estimates of differences in weight at WT16 between sheep with HISTO>0 and sheep with HISTO=0 (which had a WT16 of 41.2 ± 1.2 kg).

Lesion type	Difference in weight from unaffected sheep (kg)
1	-0.89 ± 0.96
2	-0.96 ± 1.07
3a	-3.48 ± 1.07
3b	-3.76 ± 1.22
3c	+1.24 ± 1.63

Wool production of surviving OJD cases

The multiple linear regression model for WOOL3 included weaning paddock, WOOL2 (first adult shearing), sex and the presence of type 3a or 3b lesions as significant predictors of final weight. The presence of type 1, 2 or 3c lesions was not significantly associated with final weight. Considered independently, types 3a or 3b did not have a statistically significant effect on WOOL3 but, when combined, they made a significant contribution to the model (P=0.024). SEX was also significant, with wether sheep producing more wool than female sheep, even after adjusting for WOOL2 (P=0.029). The model had an adjusted R^2 of 0.628.

The estimated effect of type 3a or 3b lesions on WOOL3 was –0.291 kg. Sheep with either of these categories of OJD pathology are predicted to produce 0.291 kg less wool than would be predicted from their sex, paddock and wool weight at the previous shearing if they were free of these types of OJD lesions.

Pasture

Pasture species present

The dominant pasture species on the experimental site were Wallaby grasses (*Danthonia* spp), weeping grass (*Microlaena stipoides*), annual grasses (*Vulpia* spp, *Bromus molliformis*), flatweed (*Hypochaeris radicata*), red grass (*Bothriocochloa macra*) and spear grasses (*Stipa* spp).There were also substantial infestations of weeds including serrated tussock (*Nassella trichotoma*) and wire grass (*Aristida ramosa*) (Table 43).

Pasture availability

Pasture availability was recorded on 13 occasions between spring 1999 and autumn 2002. The mean availability in the 14 continuously stocked paddocks ranged from a maximum of 658 kg DM/ha in autumn 2000 to a minimum of 100 kg/ha in late autumn, 2002. Mean pasture availability was typically between 200 and 400 kg DM/ha, but declined to very low levels in late 2001 following the failure of spring rains (Figure 12).

Pasture availability varied between paddocks. Five paddocks consistently had less than 200 kg DM/ha and two paddocks consistently exceeded 500 kg DM/ha (Figure 13).

Figure 12 Mean pasture availability (kg DM/ha) of the 14 continuously stocked paddocks on 13 occasions between spring 1999 and autumn 2002.

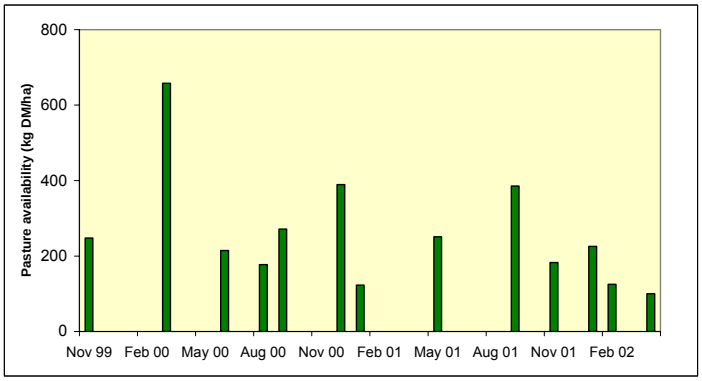
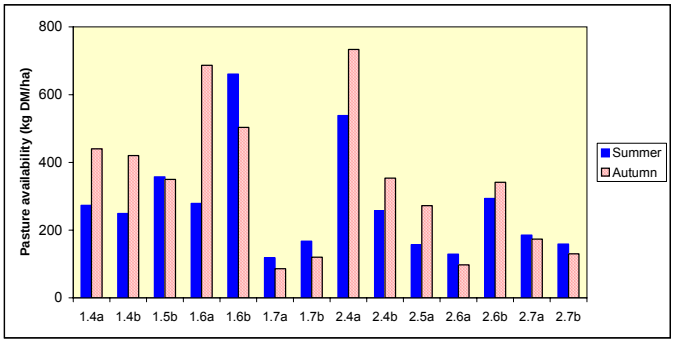


Figure 13 Mean pasture availability (kg DM/ha) of each of the 14 continuously stocked paddocks. Each bar represents the average of three records of pasture availability in three consecutive years.



Supplementary feed

Levels of lupin grain feeding were adjusted in order to minimise the differences in the mean liveweight of each group of sheep. Feeding levels were based on data collected from weighing the sheep and visual assessment of pasture availability. Despite the use of supplementary feed, paddocks with low pasture availability still tended to have sheep with lower liveweights and lower wool weights (Figure 14). There were significant positive relationships between pasture availability and liveweight and pasture availability and woolweight, and strong negative relationships between supplementary feed level and pasture availability, liveweight and woolweight. The strongest relationship existed between supplementary feed level and liveweight (Table 42), indicating that the level of supplementary feed was insufficient to fully compensate for the low levels of pasture availability in some paddocks.

Figure 14 Top; Mean pasture availability (average of three records for summer and autumn and two records for winter from each paddock) and total weight of supplementary lupin grain fed per paddock (/100). Paddocks are sorted in descending order of supplementary grain. Bottom; Group (paddock) mean liveweight (WT16) and mean fleeceweight (WOOL3) of the experimental sheep, sorted in the same order as the top figure. Both liveweight and wool weight are expressed as percentages of the overall mean.

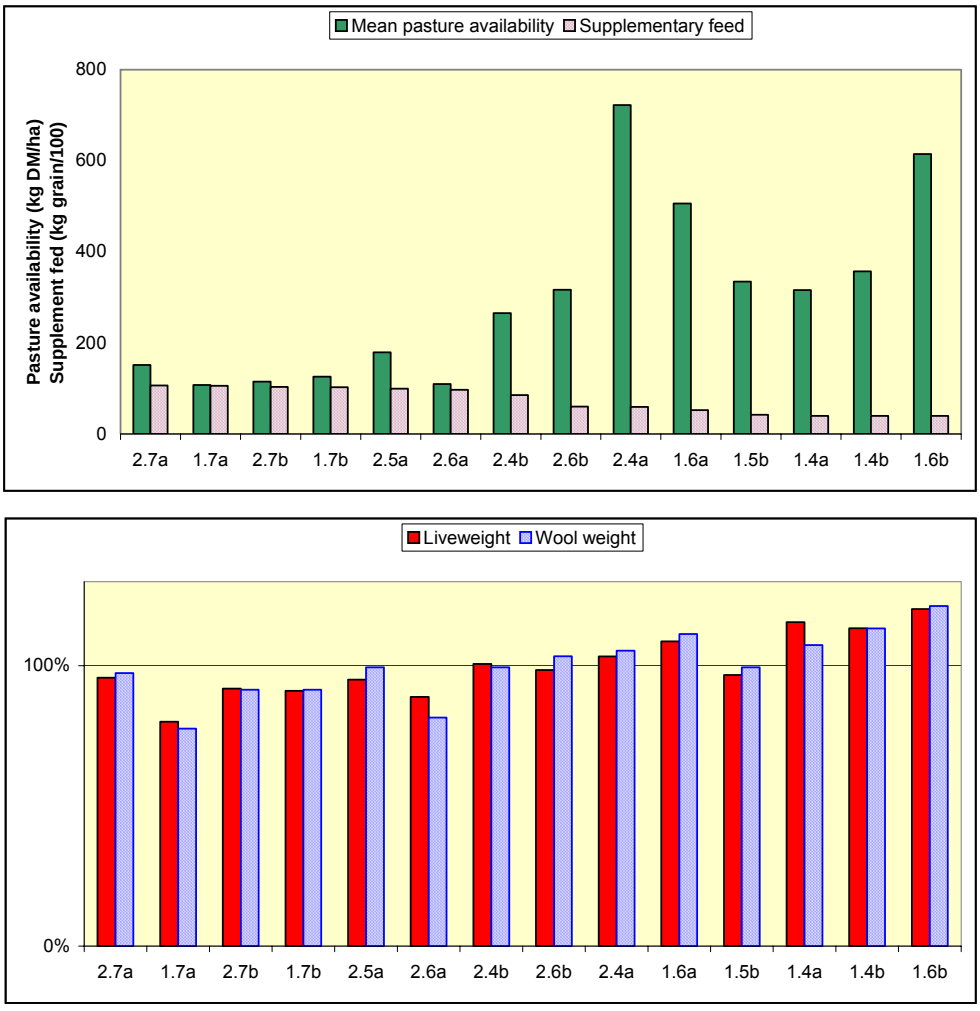


Table 42 Relationships between pasture availability, supplementary feed offered and mean liveweight and wool weight of treatment groups

Variables	Slope	r^2	F	P
Pasture availability and liveweight	Positive	0.52	13.01	<0.005
Pasture availability and wool weight	Positive	0.60	18.04	<0.005
Pasture availability and supplement	Negative	0.57	15.64	<0.005
Supplement and liveweight	Negative	0.69	26.70	<0.005
Supplement and wool weight	Negative	0.64	21.66	<0.005

Table 43 The dominant plant species in each paddock in December 1999.

	Paddock																								
Plant species	**1.1**	**1.2**	**1.3**	**1.4a**	**1.4b**	**1.5a**	**1.5b**	**1.6a**	**1.6b**	**1.7a**	**1.7b**	**1.8**	**1.9**	**2.1**	**2.2**	**2.3**	**2.4a**	**2.4b**	**2.5a**	**2.5b**	**2.6a**	**2.6b**	**2.7a**	**2.7b**	**2.8**
Danthonia spp	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Microlaena stipoides	x	x	x	x	x	x		x	x		x	x			x	x	x		x		x	x	x	x	x
Annual grasses (*Vulpia* spp, *Bromus molliformis*)	x	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x			x				x
Hypochaeris radicata		x		x			x	x	x	x	x	x	x			x		x	x		x	x	x	x	
Bothriochloa macra	x			x	x			x	x	x	x		x				x	x	x	x	x		x	x	
Nassella trichotoma	x	x		x	x	x			x			x	x			x		x	x					x	x
Aristida ramosa			x							x	x							x	x	x	x	x	x	x	
Stipa sp.	x		x			x		x			x		x	x	x		x					x			
Themeda triandra			x							x							x	x							
Panicum effusum								x	x				x												
Rumex acetosella		x						x					x												
Aira sp													x	x											
Cynodon dactylon																				x	x				
Dichelachne sp							x	x																	
Carthamus lanatus																x									
Elymus scaber			x																						
Hordeum spp			x																						
Onopordum acanthium													x												
Thistles (C nutans, O acanthium, C vulgare)								x																	

The plants are sorted in order of the frequency with which they occurred amongst the dominant species in each paddock. We are indebted to Lori McGarva of NSW Agriculture for the survey. An ‘x’ indicates that a plant species was amongst the dominant species in the paddock.

DISCUSSION

Estimates of the availability of *M ptb*

Implications of using estimated *M ptb* decay rates in calculations and analyses

Our initial expectation was that infection would persist for long periods on pasture and, therefore, that pre-contamination of pastures was very important if high levels of bacterial challenge were to be achieved. Pre-contamination was also considered important if some days or weeks were required for *M ptb* bacteria to be released from faecal pellets and to become available to grazing sheep on soil or pasture.

The results of Whittington's experiments, which became available after this trial had commenced, suggested that decay rates of viable *M ptb* were higher than previously suspected. He showed that, in unsheltered areas, decay rates can be at least one logarithm per month and possibly as high as five logarithms. Consequently, in the analysis of the current study, we have used a decay rate of three logarithms per month as the most likely estimate but we have also considered the possiblility that decay rates could be substantially different from that, within a range of one to five logarithms per month.

Low estimates of the decay rate (one logarithm per month) produce relatively high estimates for the duration of *M ptb* availability in the post-weaning period of IHH lambs (which did not co-graze with infected ewes) and high estimates also for the sustained availability of *M ptb* during the late pre-weaning period for the UHL and UHH lambs.

Higher estimates of the decay rate (five logarithms per month) suggest that IHH lambs were exposed to rapidly declining levels of *M ptb* after weaning, and also that pre-weaning exposure in UHL and UHH lambs fell to very low levels well before weaning, following the removal of Flock I ewes. (Appendix 1). In the general sense, high decay rates imply that contemporaneous contamination (co-grazing with patently-infected sheep rather than grazing previously-contaminated pastures) is most important in providing a high-challenge environment for susceptible animals.

In this study, the time of weaning coincided with summer when high decay rates would be expected. Consequently, decay rates of three to five logarithms per month may be more appropriate for estimating availability of viable bacteria than lower rates. Relatively low decay rates may have been expected in the early pre-weaning period (spring) and in the post-weaning period, two to three months after weaning (during autumn and winter).

There were substantial differences in the predicted availability of *M ptb* between some of the treatment groups which were intended to be 'high challenge' groups. In some cases these differences were approximately 10-fold and may have been important in determining the outcome of exposure to infection. For example, the median-born lambs in treatment IHH were predicted to be exposed to 291 – 543 E/D (Replicate 1) or 467 – 669 E/D (Replicate 2) in the 98 day pre-weaning period, then to 20 E/D (Replicate 1) or 17 E/D (Replicate 2) in the first 120 days of the post-weaning period (Table 10 and Table 11). On the other hand, the lambs in UHH were exposed to 26 – 50 E/D (Replicate 1) or 42 – 82 E/D (Replicate 2) in the 93 day pre-weaning period then to 187 E/D (Replicate 1) or 361 E/D (Replicate 2) in the post-weaning period. IHH had a high (pre-weaning) then low (post-weaning) exposure while UHH had a low – high order of exposure. If differences of that magnitude are significant in determing infection rates, it becomes difficult to compare IHH infection rates directly with UHH infection rates.

Consequently, we have, for the purposes of discussion, separated the levels of predicted *M ptb* exposure into three categories (Table 44). These are High, for levels of challenge of 187- 669 E/D, Medium, for levels of 17-82 E/D and Low, for levels <1 E/D.

Table 44 Categorisation of treatment groups by level of exposure to *M ptb*, based on decay rates of three logarithms per month.

		Pre-weaning pasture			Post-weaning pasture		
Nominal description of exposure history	Infected dam flock	Low	Medium	High	Low	Medium	High
IHH	+			+		+	
IHL	+			+	+		
ILL (m)	+			+	+		
UHH			+				+
UHL			+		+		
ULH		+					+
ULL		+			+		

(m) = moved two times to clean pastures during lactation.

Some insights into the factors affecting OJD infection rates are gained by conducting pair-wise comparisons, as follows.

Comparisons of treatment groups

Treatment ULL vs ULH

Summary of results

Lambs in the ULL group were not deliberately exposed to infection and yet one animal in each replicate was BACT18M-positive and died – in one case death was confirmed by histopathology and in the other OJD was the presumptive cause of death. The existence of infection in each group was confirmed by the detection of further cases in surviving sheep – nine further cases in Replicate 1 and two further cases in Replicate 2 (Table 45).

The source of infection for these lambs is unknown. There are, however, some interesting possibilities that can be proposed.

Table 45 ULL vs ULH: comparison of infection rates. *M ptb* exposure is measured in E/D based on *M ptb* decay rates of three logarithms per month.

	ULL (Paddock 6b)		ULH (Paddock 4b)	
	Rep 1	Rep 2	Rep 1	Rep 2
Pre-weaning exposure[g]	0	0	0	0
Post-weaning exposure	0	0	187	416
Infection rates (confirmed)[h]	9/33	3/36	6/30	6/33
Severe lesions (3a & 3b)	0	1	2	1
Confirmed OJD death	0	1	1	0
Probable OJD death	1	0	0	0
BACT18M-positive	1	1	1	0

Possible source of infection in ULL, Replicate 1

In Replicate 1, the lamb which died with probable-OJD (eartag 1184) was born in Paddock 1.6b and weaned into the same paddock. This paddock was adjacent to Paddock 1.8 (220 m fence in common) and Paddock 1.9 (310 m fence in common). Both of these paddocks contained Flock I ewes until Day 50, when the ewes were moved to Paddocks 1.4a and 1.4b. Despite the use of shade cloth on the common fences it is possible that infected faeces moved through the fence and contaminated Paddock 1.6b. It is also worthy of note that the ULH Replicate 1 sheep which died (in Paddock 1.4b) with confirmed OJD was also born in Paddock 1.6b. Lambs for post-weaning ULL and ULH treatments also came from Paddocks 1.6a, 1.7a and 1.7b. None of these died from OJD in either ULL or ULH groups. Paddocks 1.7a and 1.7b were bounded on two sides by ungrazed laneways, one side by each other and another Flock U-grazed paddock, and one side by Paddock 1.3 which was not grazed by Flock I ewes until Day 100. We consider it unlikely that 1.7a or 1.7b were cross-contaminated.

We conclude the most likely explanation for the occurrence of OJD in the not-deliberately-exposed ULL group in Replicate 1 is that sheep 1184 was infected in the pre-weaning period in Paddock 1.6b. This sheep is possibly also the source of infection for other infected sheep in the same group. It is known to have been shedding *M ptb* by 18 months of age. Infection in the nine other positive sheep could have been derived from contamination caused by sheep 1184 or by residual infection remaining from the suspected episode or episodes of cross-contamination of the paddock. These nine sheep included five with no histopathological lesions (but positive tissue culture) two with mild OJD lesions and two with severe lesions. Both of those with severe lesions were born in Paddock 1.7b so we consider that the most likely time of exposure was post-weaning.

Possible source of infection in ULL, Replicate 2

In Replicate 2, the confirmed-OJD death (eartag 1334) was born in Paddock 2.7b. This paddock was considered at very low risk from cross-contamination, with boundaries consisting of an ungrazed double-fenced gully, a Flock U-grazed paddock (2.7a), a double-fenced lane and Paddock 2.3. Only Paddock 2.3 was ever grazed by Flock I ewes, and grazing by them did not commence until Day 100. The Flock U-

[g] The level of deliberate pre-weaning exposure.
[h] Infection rates include animals with positive BACTPM and negative HISTO, and animals with HISTO of 1, 2, 3a, 3b and 3c.

born lambs in 2.7b left that paddock on Day 118. For the post-weaning phase, sheep in treatment ULL in Replicate 2 were paddocked in 2.6b. Although grazed by Flock U sheep until weaning, an episode of cross contamination was recorded for this paddock. On Day 70, (14 October 1999), three Flock I ewes and their four lambs were discovered in this paddock. On checking sampling lists over the previous month it was evident that they had been missing from their correct paddock for 14 – 28 days. The sheep were immediately returned to their correct pasture. It is not known if they were faecal culture positive.

The most likely explanation for the occurrence of OJD in the not-deliberately-exposed sheep in Replicate 2 is that sheep 1334 was infected in the post-weaning period in Paddock 1.6b. It is likely, therefore, that the other two sheep in this group which were found to be infected were also infected in the post-weaning period – either from the cross-contamination episode described or from the shedding of bacteria from sheep 1334. It is significant in considering this possibility that sheep 1334 did not die until Day 1056, which might imply late exposure to infection. This sheep was the only animal in treatment ULL or ULH Replicate 2 which was BACT18M positive.

Assuming that Paddock 2.6b was contaminated by the strayed ewes between Days 40 – 70, other lambs grazing in that paddock at the time would have been exposed to *M ptb* during the pre-weaning phase. Of the lambs born in either 2.6a or 2.6b, five were diagnosed with OJD at slaughter with positive tissue culture. Four of these grazed deliberately contaminated pastures (2.4b - ULH) post-weaning, but had no positive histopathology. The other one remained in Paddock 1.6b and had a type 1 lesion at histopathological examination. Of the lambs born in 2.7a or 2.7b, four were diagnosed with OJD at slaughter or natural death: three grazed in 2.6b after weaning (ULL) and one in 2.4b (ULH). There is no indication from these data that lambs born in any of the four ULL and ULH paddocks were more likely to contract OJD than those born in any other. This lack of association is consistent with the proposition that sheep 1334 became infected after weaning.

Discussion of ULL vs ULH

What is remarkable from the comparison of these two treatment groups (Table 45) is that deliberate exposure of the lambs at and after weaning (treatment ULH) did not result in any substantial increase in infection rates beyond those which probably occurred as a result of very low levels of exposure in the pre-weaning period (Replicate 1) or very low levels in the early post-weaning period (ULL Replicate 2) followed by exposure to organisms shed by the index cases when they commenced shedding at 12 - 18 months of age.

The deliberate exposure of these lambs in the post-weaning period was substantial – the ULH paddocks (1.4b and 2.4b) had been periodically grazed between Days 1 and 50 and then continuously grazed by Flock I ewes from Day 50 to 118. The ULH lambs then co-grazed with Flock I ewes from Day 118 to Day 176. The 'types' of contamination in these paddocks were both long-standing and current so, whether the decay rate applied in calculating *M ptb* availability was accurate or not, it is indisputable that contamination levels were substantial. Particularly in Replicate 2 (where grazing-group I with 23% of ewes known to be shedding *M ptb* had grazed since Day 50 and continued to graze after ULH lambs were weaned and entered the paddock) contamination was high. These results would imply that, with the exception of one or two animals per group (3% - 6%), animals in the post-weaning phase were not very susceptible to infection at the levels of *M ptb* attained on these paddocks. Except for the very susceptible sheep, the few that did become infected either recovered, or developed a persistently infected sub-clinical status with little or no evidence of pathological change at 36 months of age.

Treatment ULL vs UHL

Summary of results

As discussed in the previous section, lambs in ULL were not deliberately exposed to infection and yet two animals were BACT18M-positive and both died before 36 months of age. It was assumed therefore that there was accidental, but very low-level, exposure of some lambs to *M ptb*. By contrast, in group UHL, lambs were deliberately exposed to infection by co-grazing with Flock I ewes for the first 25 days of lambing then remaining in the same contaminated pastures (Paddocks 8 and 9) until weaning. Even if high rates of decay of *M ptb* are assumed, the difference in levels of exposure of the lambs to the bacteria in UHL was many orders of magnitude higher than in ULL.

Discussion of results

Despite this gross difference in exposure, almost identical numbers of lambs became infected with *M ptb* in the two groups (Table 46). The most likely explanation for this result is that there exists a discontinuity in susceptibility within a flock of sheep – a few animals are extremely susceptible while most require much higher levels of exposure or (as discussed below) more continuous exposure to contaminated pastures. The much higher levels of exposure generated in paddocks containing UHL sheep appeared not to exceed the threshold for challenge required to infect the majority of sheep.

Table 46 ULL vs UHL: comparison of infection rates. *M ptb* exposure is measured in E/D based on *M ptb* decay rates of three logarithms per month.

	ULL (Paddock 6b)		UHL (Paddock 7b)	
	Rep 1	Rep 2	Rep 1	Rep 2
Pre-weaning exposure[i]	0	0	38	62
Post-weaning exposure	0	0	0	0
Infection rates (confirmed)	9/33	3/36	6/32	5/35
Severe lesion (3a & 3b)	0	1	0	1
Confirmed OJD death	0	1	0	0
Probable OJD death	1	0	1	0
BACT18M-positive	1	1	3	0

[i] The level of deliberate pre-weaning exposure.

Treatment UHL vs ULH

Summary of results

Lambs in UHL were exposed to infection only in the pre-weaning period while lambs in ULH were exposed only in the post-weaning period (although the infection rate in the ULL group raised the possibility that these lambs also had very low levels of exposure pre-weaning (Replicate 1) or post-weaning (Replicate 2).

Lambs in UHL co-grazed with Flock I ewes (3 ewes per ha) for 25 days (Days 25 – 50) at the start of lambing, then the Flock I ewes were removed. The lambs (the experimental sheep) remained in the same paddock until Day 118. Lambs in ULH co-grazed with Flock I ewes (6 ewes per ha) for 58 days (Days 118 – 176) immediately after weaning, then the Flock I ewes were removed. The experimental sheep then remained in the same paddock until the end of the field study.

The choice of *M ptb* decay rate (one, three or five logarithms per month) has an effect on the relativity between the predicted levels of exposure in the pre-weaning and post-weaning periods. If high decay rates are assumed, the ratio of predicted exposure of ULH to UHL is 5.0:1 (Replicate 1) or 7.2:1 (Replicate 2). At low decay rates it is 3.6:1 (Replicate 1) or 5.8:1 (Replicate 2). The medium rate of decay (three logarithms per month) produces ratios of 4.9:1 and 6.7:1 (Table 47). Particularly with high decay rates, the high level of exposure in the early post-weaning period is substantially more sustained than the high level of exposure in the early pre-weaning period (Appendix 1).

There was a similar number of OJD infections in both groups and a slightly higher number of severe infections in ULH than UHL, but the difference was not statistically or practically significant (Table 47).

Table 47 UHL vs ULH: comparison of infection rates. *M ptb* exposure is measured in E/D based on *M ptb* decay rates of three logarithms per month.

	UHL (Paddock 7b)		ULH (Paddock 4b)	
	Rep 1	Rep 2	Rep 1	Rep 2
Pre-weaning exposure	38	62	0	0
Post-weaning exposure	0	0	187	416
Infection rates (confirmed)	6/32	5/35	6/30	6/33
Severe lesions (3a & 3b)	0	1	2	1
Confirmed OJD death	0	0	1	0
Probable OJD death	1	0	0	0
BACT18M-positive	3	0	1	0

Discussion of results

The differences in level of exposure between the two groups, which are of the order of 4 to 7 fold, make it difficult to validly compare the effects of differences in timing of exposure between them. Nevertheless, it appears that lambs of post-weaning age are no more susceptible to infection than those of pre-weaning age. If the differences in exposure level are ignored, the results suggest that sheep of post-weaning age are not appreciably more resistant to infection either, although infection rates are not significantly different from those of the ULL "controls". In Replicate 1 of ULH at least, infection in the index case could have occurred before weaning as we proposed for group ULL.

Treatment UHL vs UHH

Summary of results

The pre-weaning exposures for UHL and UHH groups were similar for both replicates and identical for both treatments within each replicate, because the lambs were selected at random for both treatment groups from the two birth paddocks (8 and 9) within each replicate (Table 48). The exposure in these paddocks arose from heavy but intermittent contamination in the early days of the experiment (Days 1 – 30) then lower rates of contamination (three Flock I ewes per ha) in the first 25 days of lambing of Flock U ewes. The availability of *M ptb* was predicted to fall at logarithmic rates following the removal of Flock I ewes on Day 50, which accounts for the relatively low AUC quoted for these paddocks – effectively an average level of exposure over a 93 day period which conceals the relatively high levels of *M ptb* availability early in the period.

In the post-weaning period, predicted availability of *M ptb* in Paddocks 1.5b and 2.5a (where UHH grazed post-weaning) was substantial. (It was also very similar in history and predicted level to that in 1.4b and 2.4b where ULH grazed (Table 45). Within replicates, the sheep in UHH had levels of exposure to *M ptb* in the early post-weaning period very similar to those of sheep in ULH (see also Appendix 1).)

Infection rates varied from 14% and 19% in UHL replicates to 21% and 35% in the UHH replicates.

Table 48 UHL vs UHH: comparison of infection rates. *M ptb* exposure is measured in E/D based on *M ptb* decay rates of three logarithms per month.

	UHL (Paddock 7b)		UHH (Paddock 5a/b)	
	Rep 1	Rep 2	Rep 1	Rep 2
Pre-weaning exposure[j]	38	62	38	62
Post-weaning exposure	0	0	187	361
Infection rates (confirmed)	6/32	5/35	7/34	12/34
Severe lesions (3a & 3b)	0	1	2	5
Confirmed OJD death	0	0	1	4
Probable OJD death	1	0	0	1
BACT18M-positive	3	0	2	5

Contrast of infection rate: UHL and UHH

Infection rates in treatment UHL were not significantly higher than in ULL (see previous sections), despite the deliberate and high levels of contamination of the birth paddock in group UHL – particularly in the early part of the lambing period. Death rates and rates of severe infections were very low in UHL. One possible explanation of the failure of early exposure to result in sustained infection in these lambs is derived from the outcome for the three BACT18M-positive animals in Replicate 1. Of these three, one died and was presumptively diagnosed as an OJD case, one survived to slaughter where it was found to be tissue-culture positive but free of pathological evidence of infection and the third animal was negative to both tissue culture and histopathology at examination after slaughter. This finding raises the possibility that a number of animals, possibly in both replicates, became infected but were able to rid themselves of

[j] The figures quoted are the averages for the two birth paddocks within each replicate. See Table 10 for details.

infection or to contain the infection without signs of disease. The nine non-severe cases included seven animals which were tissue culture-positive without pathological change, and two which had type 1 lesions.

By contrast, in treatment UHH seven sheep had severe lesions of OJD and five of these died before the end of the study (Table 48). The contrast of UHH with groups UHL and ULH is intriguing. With similar pre-weaning exposure to UHL and similar post-weaning exposure as ULH (Table 45), the prevalence of infection is higher in UHH than in either UHL or ULH. This is not surprising and implies that more sustained exposure resulted in a higher number of cases. But when only severe infections or deaths are considered, the results indicate that UHH had higher prevalences than predicted from the sum of prevalences from UHL and ULH. The inference we draw from these data is that continuous exposure (pre-weaning plus post-weaning) to high levels of infection has resulted in a higher level of clinical OJD (deaths and severe cases amongst survivors) than would be predicted from simply adding the number of cases resulting from pre-weaning exposure and the number of cases resulting from post-weaning exposure.

This is an even more significant finding against the background of the results from ULL, which suggest that a small proportion of sheep are particularly susceptible to infection. Given that 3% to 6% of lambs are so susceptible that infection is almost inevitable, even at very low levels of pasture contamination, infection in greater numbers of young sheep may be almost completely avoidable by either delaying exposure to weaning age (ULH) or by providing a break from exposure at weaning (UHL). The only group in which significantly higher rates of infection occurred, compared to the highly protected ULL group, was the continuously exposed UHH group.

The different outcome of BACT18M-positive sheep in UHH, compared to UHL is also noteworthy. Seven animals were BACT18M-positive in UHH. Six of these died - four with confirmed-OJD, one with probable-OJD and one died shortly after sampling (19 months of age) with negative tissue culture and no tissues available for histopathology. The seventh BACT18M-positive animal survived to slaughter and was found to have type 3a OJD pathology. The higher rate of BACT18M-positive animals (UHH vs UHL) and the different outcome for those positive animals in UHH groups support the proposition that the continued post-weaning exposure “prevented” the resolution or containment of infection in at least some cases, leading to higher levels of clinical disease.

The relationship between exposure levels and infection rates in UHH is also consistent between replicates – Replicate 2 had higher exposure in the pre-weaning period and particularly the post-weaning period compared to Replicate 1 and, perhaps as a result, had higher rates of BACT18M-positivity, severe infections and deaths.

Treatment ILL vs IHL

Management of ILL

The ILL groups were managed such that the inevitable contamination of the lambing pasture caused by the lambing ewes themselves was kept to a minimum. This was achieved by withholding the ewes from the lambing paddock (which was *M ptb*-free) until a few days before lambing started, then moving the ewes and first-born lambs to another *M ptb*-free pasture 24 days after lambing started, then moving the flock again 26 days later to a third *M ptb*-free pasture, at which time lambing had finished and all lambs were 5 – 50 days old. Despite the similarity in group names, the the levels of exposure in ILL are clearly not comparable with those of ULL and this difference must be taken into account when reviewing the results.

The extensive mass of pasture present in each paddock when the flock first entered following each move may have contributed to a dilution of *M ptb* in these pastures, making the effective level of exposure to the newborn lambs lower than that predicted from faecal contamination alone. In other words, the pasture mass may have had the effect of protecting the lambs from contact with the organisms at soil level. The abundant pasture possibly also encouraged milk production from the ewes, which might have reduced the amount of grazing done by the lambs. There was no evidence for this, however, from the weaning weights of the lambs from these paddock, which were comparable but no better than the weaning weights from other Flock I lambing paddocks.

The estimates for *M ptb* availability in the pre-weaning phase of ILL (Table 49) are based on the AUC calculations described previously (Page 25). They do, however, fail to indicate that the level of *M ptb* availability in the early life of most lambs was low. It was only towards the end of their pre-weaning experience that levels became high and significantly raised the average levels of *M ptb* to which they were exposed (Appendix 1). The differences in AUC between ILL and IHL diminish with higher assumed *M ptb* decay rates (Table 10), because current contamination becomes increasingly more important than long-standing contamination in predicting *M ptb* availability.

Contrast of contamination levels; ILL vs IHL

Sheep in treatment group IHL were exposed to high and sustained levels of *M ptb* contamination. Contamination occurred both before lambing started, with intermittent grazing of large groups of Flock I ewes (Figure 2), and continued throughout lambing and lactation because these ewes were not moved from their lambing pasture until weaning. Thus the lambs raised in treatment IHL were exposed to a relatively high and steady level of exposure while lambs in treatment ILL were exposed to initially low, then increasing levels of exposure.

Table 49 ILL vs IHL: comparison of infection rates. *M ptb* exposure is measured in E/D based on *M ptb* decay rates of three logarithms per month.

	ILL (Paddock 7a)		IHL (Paddock 6a)	
	Rep 1	Rep 2	Rep 1	Rep 2
Pre-weaning exposure	278	344	359	593
Post-weaning exposure	0	0	0	0
Infection rates (confirmed)	5/32	8/31	19/35	6/27
Severe lesions (3a & 3b)	3	4	3	3
Confirmed OJD death	1	0	1	2
Probable OJD death	0	1	0	1
BACT18M-positive	1	1	4	1

Contrast of infection rates; ILL vs IHL

There was effectively no difference in the rate of severe infections between ILL and IHL – in both groups 10% to 11% of sheep had severe infections (11% to 12.5% if the two probable-OJD deaths were included as severe cases) – but there is a trend for cases to be more advanced in IHL than in ILL. Two animals in ILL died during the trial (one with confirmed-OJD and one with probable-OJD) compared with three confirmed cases in IHL. None of the six survivors with severe lesions in ILL showed clinical signs of OJD – their liveweights at WT16 were all >94% of their expected weights. Of the three survivors in IHL, one showed severe weight loss at slaughter (76.6% of expected weight), one showed marked weight loss (84.1%) while the third did not show any weight loss. The occurrence of two animals with lower than expected liveweights suggests that they were more advanced cases and likely to die sooner (had they not been slaughtered) than the survivors with 3a and 3b lesions in ILL.

There was a very high number of mildly affected sheep detected at slaughter in Replicate 1 of IHL. Eight of these had type 1 lesions at histopathology, four had type 2 lesions and four had no lesions but were tissue culture positive. The significance of this is unclear but it may have been a consequence of the existence of four BACT18M-positive sheep in this group causing substantial contamination of the pasture from 18 months of age (or earlier) onwards. All four of these sheep presumably continued to contaminate the pasture; one until it died with confirmed-OJD on Day 789 (2 Oct 2001), the other three until slaughter, when they were found to have lesions of type 3b (one case) or type 1 with positive tissue culture (two cases). The contamination caused by these sheep could have led to the high prevalence of infection in the remainder of the group but, because the remainder were aged 18 months or more when exposed to this 'second wave' of pasture contamination, they may have been able to limit the infection in at least some cases.

Overall, there appears to have been a small improvement in OJD control by attempting to reduce the level of exposure of lambs to infection during the pre-weaning period. Rates of sheep with severe lesions were similar between the two groups and any advantage to ILL by virtue of a lower death rate and lower number of clinical cases at 36 months was small and not of statistical significance. The higher number of sheep with mild lesions may have been a result of high numbers of BACT18M-positive sheep in one replicate, but this seems to have been only a chance event - it only occurred in one replicate and that was the replicate with the lower level of pre-weaning exposure, which would therefore have been expected to have had fewer infected sheep.

Contrast between groups with pre-weaning exposure only; ULL, UHL, ILL and IHL.

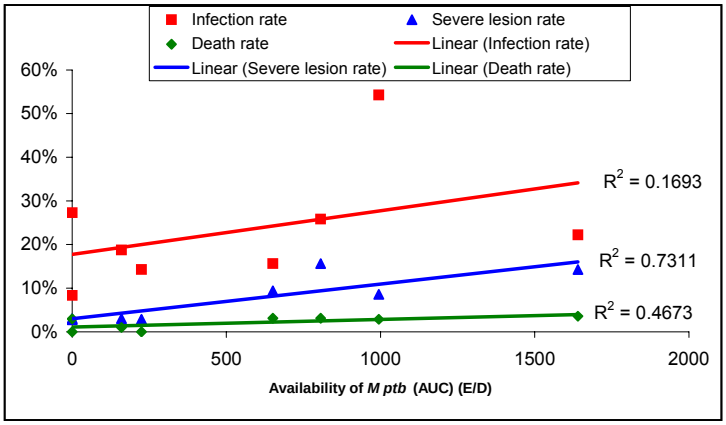
When the treatment groups which had no post-weaning exposure are considered together, and the predicted availability of *M ptb* on those pastures is taken into account, there is a trend of increasing infection rate and increasing death rate with increasing level of pre-weaning exposure observed (Figure 15). This trend is strongest for the rate of severe infections, where the coefficient of determination (R^2) was 0.73 – 0.83, depending on the decay rate used. In fact, the relationship became stronger when higher decay rates were assumed. The relationships were highly significant ($P<0.01$). The weaker relationship between exposure and infection rate (including animals with mild histopathology or no histopathology but infected tissues) was associated with the unexpectedly high number of positive animals in ULL Replicate 1 and IHL Replicate 1. As discussed previously, the animals with mild lesions in these groups may be reflecting exposure to late contamination, caused by shedding from the index cases, and may represent 'noise' in the relationship between exposure and OJD infection. Moreover, if many of these sheep with mild infections were not going to develop clinical disease in the next two years of their lives, the presence of infection in them may not have been serious in terms of effects on flock productivity. (They are of course, important in terms of disease spread.)

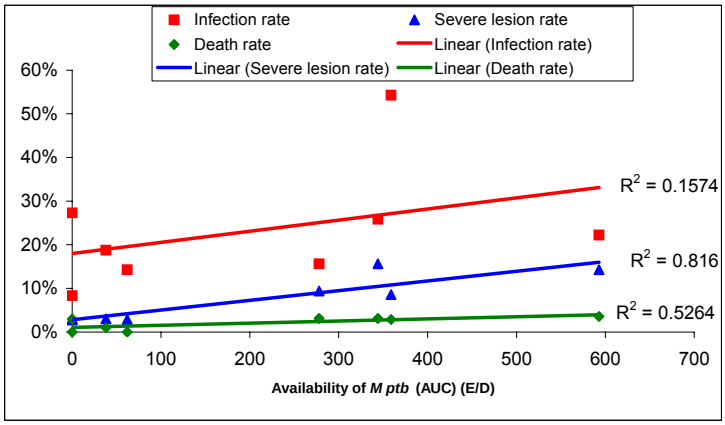
With this in mind, the key relationship, in terms of managing the economic impact of OJD in flocks, is the one between exposure and severe infections and deaths. These relationships seem clear – with, as expected, a positive association with increasing exposure. The slope of the relationship between severe infections and exposure (assuming decay rates of three logarithms per month) was 0.00022. For every additional 100 E/D of exposure in the pre-weaning period, the expected increase in the rate of severe infections at or before three years of age is 2.2%. Note that this relationship is dependent on the method used to calculate *M ptb* availability, in particular, which decay rate is used. Note also that deaths from

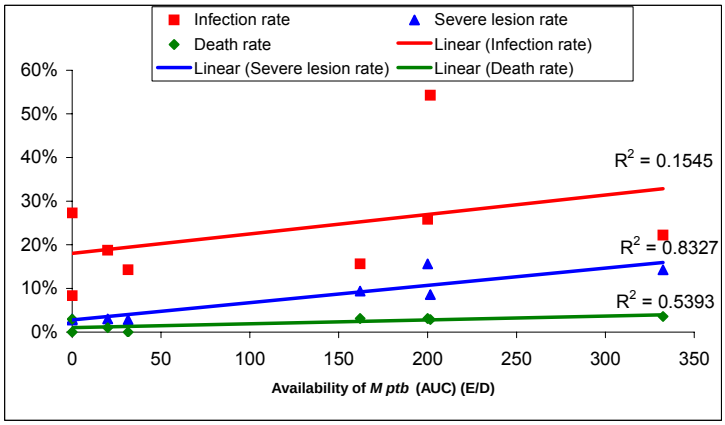
OJD are included in the 'severe infection' category and, in the illustration below and discussion above, probable-OJD deaths are assumed to have had severe infections at death.

In the case of severe infections, the R^2 value is higher when higher rates of decay are assumed. The trend suggests that the rate of severe infections is better 'explained' by a higher decay rate than a lower one. This inference supports the view that contemporaneous contamination presents a higher challenge to susceptible sheep than accumulated contamination.

Figure 15 Plot of predicted *M ptb* availability against the infection rates and death rates in the eight replicates with pre-weaning exposure only (including ULL).Top figure: decay rate of one logarithm per month. Middle: three logarithms per month. Bottom: five logarithms per month.







Contrast of infection rates; {ILL and IHL} vs {ULL, ULH, UHL and UHH}

The numbers of sheep in ILL and IHL with severe infections (six and seven for ILL and IHL respectively) were higher than in the ULL, ULH and UHL groups but similar to that in the UHH group. The explanation we offered for the relatively high number in the UHH group was the continuity of exposure in that group, compared to ULL, ULH and UHL. The relatively high numbers in the ILL and IHL groups may have been a consequence of much higher levels of pre-weaning exposure in these two groups (278 – 593 E/D) compared to ULL, ULH and UHL groups (<1 – 62 E/D), despite the absence of immediate post-weaning exposure.

Treatment IHL vs IHH

Contrast in contamination rates; IHH vs IHL and UHH

Within replicates, equal numbers of lambs from each of the four Flock I high-exposure lambing paddocks were allocated at random to treatment IHL or IHH. Those allocated to IHH remained in one of those lambing paddocks (4a) while those allocated to IHL went to a paddock believed to be free of contamination (Paddock 6a). Unlike group UHH, in IHH there was no continuing source of contamination – all Flock I ewes were removed. Hence, the predicted availability of *M ptb* declined by 99.9% every month after weaning (assuming three logarithms per month for decay) such that the predicted AUC for the first 120 days post-weaning was 20 E/D (Replicate 1) or 17 E/D (Replicate 2) and the availability of *M ptb* after that time was very low (<0.01 E/D). By contrast, in UHH, levels were sustained by the continued presence of Flock I ewes for 58 days and the predicted AUC for the first 120 days post-weaning was ten or twenty times higher than in IHH (Table 50).

At the time when IHH lambs entered their post-weaning paddock, predicted *M ptb* availability was substantial. If the decay rates used in this report are excessive, then the high *M ptb* availability in these paddocks may have been more sustained than predicted. Weaning of Flock I lambs, however, took place in late January. Ambient temperatures are high in January and remain high throughout February. Paddock 1.4a was exposed, with no shade. Paddock 2.4a had a substantial amount of shade in one corner which could have provided shelter for *M ptb* organisms, protecting them from the direct effects of the sun. Overall, however, because it was mid- to late summer, the decay rates used are not considered excessive and the value we consider most likely (three logarithms per month) may even underestimate the true decay rate at that time on those pastures.

Table 50 IHL vs IHH: comparison of infection rates. *M ptb* exposure is measured in E/D based on *M ptb* decay rates of three logarithms per month.

	IHL (Paddock 6a)		IHH (Paddock 4a)	
	Rep 1	Rep 2	Rep 1	Rep 2
Pre-weaning exposure	359	593	359	593
Post-weaning exposure	0	0	20	17
Infection rates (confirmed)	19/35	6/27	12/33	12/35
Severe lesion rates (3a & 3b)	3	3	3	3
Confirmed OJD death	1	2	2	3
Probable OJD death	0	1	1	0
BACT18M-positive	4	1	5	2

Contrast in infection rates; IHL vs IHH

The number of sheep with severe lesions was identical (six, or seven if probable-OJD deaths are assumed to have had severe lesions) in both treatments and the number of deaths (four vs six) was similar. Overall infection rates were consistent across both replicates of IHH and intermediate between the two replicates of IHL. Many of the comments which were applied to IHL in contrast to the ULL, UHL, ULH treatments apply also to the IHH treatment. In that regard, the IHH treatment is almost a replicate of the IHL treatment – perhaps because the amount of, or duration of, post-weaning exposure was so low that it made no significant difference to the outcome.

When ILL, IHL and IHH are viewed as a series of increasing levels of exposure, however, the rates of OJD mortality can be seen to increase steadily – from 3.2% to 6.5% to 8.8% – despite the rate of severe lesions remaining a fairly constant 10% - 12%.

It is unclear what might have happened had the IHH group been exposed to continuing high levels of *M ptb* availability, like the UHH group. Indications from this comparison are that continuing exposure, even at low levels, was associated with a trend towards higher death rates. We can only conclude that failure to provide these lambs with an uncontaminated paddock at weaning has either had no effect on the severity of OJD at flock-level or has made it slightly worse. In this regard, it is not inconsistent with our hypothesis with regard to UHH and UHL that continued exposure to *M ptb* after weaning reduces the opportunity for infected sheep to recover.

Contrast of infection rates and death rates: {ULH, UHL, ILL} vs {IHL, UHH, IHH}

The OJD infection rate – including as cases all sheep which had histological evidence of OJD and/or bacteriological evidence from culture of tissues at post-mortem examination – was found to differ significantly ($P<0.05$) between {ULH, UHL, ILL} and {IHL, UHH, IHH}. This grouping of treatments contrasts those with low or moderate pre-weaning exposure and those with post-weaning exposure only against those with high levels of pre-weaning exposure and those with moderate levels of pre-weaning exposure followed by high post-weaning exposure and those with by high pre- and moderate post-weaning exposure. The cumulative incidences of infection, adjusted in the statistical model, were 18.1% and 33.2% respectively. The unadjusted rates for each group were {19.0%, 16.4%, 20.6%} and {40.3%, 27.9%, 35.3%}.

There was also a significant difference ($P<0.05$) in confirmed-OJD death rate and the BACT18M-positive rate between these two groups. The death rates and the BACT18M-positive rates, both adjusted in the statistical model, were 0.8% and 5.5% respectively for death rates and 2.7% and 8.7% for BACT18M-positive rates.

In summary, apart from the unexpectedly high number of mild OJD lesions in Replicate 1 of IHL, there was a general trend of increasing infection rate with increasing level of pre-weaning exposure and increasing continuity of exposure which was also reflected in the *M ptb* shedding rates at 18 months of age, and the death rates from OJD before three years of age. When the ULL group is considered, there also appears to be evidence that a small proportion of the flock are extremely susceptible to OJD and will contract infection which leads to clinical disease, even if exposure levels are very low. For most of the flock, however, the likelihood of contracting infection increases with the level of exposure, and the likelihood of developing clinical OJD increases if the exposure is sustained after weaning.

Susceptibility to infection

Flock U-derived sheep

Lambs were, in general, resistant to infection with *M ptb* when exposed at low or moderate levels or when exposed over relatively short periods. We found that a small percentage of animals (3% to 6%) became infected when exposure occurred at even very small levels but deliberate exposure to moderately high levels of infection (described as medium level of challenge in this study), or high levels of infection for short periods, did not result in any increase in infection rate. Hence, groups UHL and ULH did not have significantly different incidences of OJD from the control group, ULL. The numbers with infection in each replicate were small (typically < 2), adding to the difficulty of determining any meaningful differences between the low incidence groups. These low rates of infection occurred despite the presence of several infected adult sheep in each paddock for several weeks, before and during the presence of the experimental lambs.

It was only when the levels of challenge with *M ptb* were medium or high during the pre-weaning and post-weaning periods (UHH) that infection rates were significantly raised above the controls.

Age susceptibility

There is some evidence for a decline in susceptibility to infection with *M ptb* with increasing age in cattle. If such an effect were evident in sheep, it could be potentially be exploited by sheep producers to reduce the incidence of OJD in their flocks. In our study, lambs in group ULH were exposed to high levels of infection in the post-weaning period (187- 416 E/D) while lambs in groups UHL were exposed to medium levels (38-62 E/D) only in the pre-weaning period. The lambs exposed at older ages had a higher incidence of severe infections, due to the occurrence of two cases in Replicate 1 of ULH. The small difference (statistically insignificant) in OJD incidence between the two groups could be explained simply by the higher level of exposure in the post-weaning-exposed lambs. If there was an increased resistance in the lambs after weaning, it was readily overcome by the higher level of exposure in the post-weaning environment and does not appear to exist, either at all, or with sufficient strength to be useful in on-farm disease control strategies. This requires further research.

Because the level of challenge between the two groups (ULH and UHL) differed, it is not possible to declare that there was no increased resistance to infection in the weaned lambs. We can observe, however, that we found no evidence for a useful level of resistance in lambs after weaning. Perhaps what is most surprising in this context is the relatively low incidence in the lambs exposed to medium levels before weaning. This might imply that pre-weaned lambs are not highly susceptible and that, as discussed above, persistent challenge over an extended period is at least as important as the level of challenge or the age of the animals in leading to a high incidence of infection.

Flock I-derived sheep

The highest incidences of OJD were recorded in the sheep born in Flock I. These groups (IHH, IHL, ILL) had high levels of pre-weaning exposure and, in addition, some lambs were born and raised by OJD-infected ewes. It is not possible to separate the effects of high pre-weaning exposure from the effects of having an infected dam, but the results are consistent with the hypothesis of a direct and linear relationship between the level of challenge from pasture infectivity and the subsequent incidence of disease. Efforts to reduce the level of exposure of lambs to contamination before weaning (ILL) resulted in the lowest overall infection rate and lowest death rate amongst the three groups, although the rate of severe infections was similar in all three.

Similarly, continuing exposure to medium levels of infection after weaning (IHH) resulted in the highest rate of severe lesions and highest death rate amongst the Flock I-derived groups.

Practical significance for management of OJD-infected flocks

There are several important findings arising from the differences in OJD incidence between treatment groups in this study.

A useful dose-response range is achievable in a commercial farm environment

The range of levels of *M ptb* exposure to which the sheep in this study were subjected produced a range of infection rates between groups of 16% to 40%. There was, therefore, a marked dose-response to the variation in challenge levels. While this was an important outcome for our experiment, it was not clear at the beginning of the study if this would be the case. There was effectively no experience of OJD which related the level of contamination necessary to achieve significant levels of infection - or to produce variable levels of infection. It was possible that all levels of challenge which we produced could have exceeded the threshold for infection for all, or nearly all, sheep. If so, there could have been high levels of infection in all groups, with little difference between them. Alternatively, the levels of infection we achieved may have been so low that few sheep became infected in any groups.

This was, in fact, a point of concern at the start of the study and one reason that considerable effort was directed to high levels of pre-contamination in paddocks intended for high exposure treatments. It was also an important finding because the medium and high levels of challenge that we created are likely to be of similar magnitude to those occurring on commercial farms. The high levels of contamination, for example, were produced by the contemporaneous grazing of adult ewes, of which around 16% were known to be shedding *M ptb*, at stocking rates of six ewes per hectare. This level of contamination would be expected on infected farms with moderately severe outbreaks of OJD. (For comparison, in the flock studied in project OJD.015, which was considered to have a very severe outbreak of OJD, over 40% of the two-year old sheep were shedding *M ptb* and stocking rates were typically greater than ten sheep per hectare.)

The medium levels of *M ptb* exposure in our study were produced by similar levels of grazing with infected sheep as those producing the high levels of contamination, but the grazing preceded the exposure of the experimental (susceptible) sheep by some weeks. The level of challenge was predicted to be an order of magnitude lower in these paddocks, based on an assumed decay rate of viable *M ptb* of three logarithms per month. That this was a valid assumption was demonstrated by the lower incidence of OJD in sheep exposed to this type of pasture infectivity.

Based on these observations, it is our view that planned management of grazing on OJD-infected commercial farms could produce pastures which are sufficiently low in infectivity to result in significant reductions in OJD incidence compared to the incidence which might be expected if no OJD control plans were in place. Our findings are, therefore, highly relevant to the real commercial farm environment.

The challenge from OJD-contaminated pastures falls significantly in a relatively short period

The model of *M ptb* decay that we used provided a plausible explanation for the variation in infection rates between some groups. If this model is correct, or approximately so, we could expect to see heavily contaminated pastures, if they are protected from further *M ptb* contamination for, say, three months during hot weather, become so low in *M ptb* availability as to be relatively 'safe' for further grazing. While these pastures are unlikely to become so low in *M ptb* availability that no sheep will become infected, the low levels of *M ptb* intake from the pasture may lead to a relatively low incidence of severe cases of OJD in subsequent years.

The benefits of providing pastures of low *M ptb* infectivity

The mechanisms by which most sheep exposed to low or medium levels of *M ptb* availability resist the development of severe infections and clinical OJD are unclear. There are several possibilities, including

1. under conditions of very low *M ptb* availability, it may be that most sheep do not come in contact with the bacteria at all, or ingest numbers of bacteria which do not exceed the threshold for an infective dose. This may result from chance alone, where the distribution of *M ptb* is highly localised in a paddock, and only a small proportion of sheep ingest the herbage which is heavily

contaminated with bacteria. Alternatively, the ingestion of the contaminated pasture may relate to specific grazing habits, which may differ between individual sheep in a flock. This has been suggested as a reason for differences in intake of nematode larvae between sheep.

2. under conditions of low or medium levels of *M ptb* availability, it is likely that most sheep will ingest the bacteria but the threshold for an infective dose may differ between sheep due to innate factors, possibly associated with non-specific cell mediated immune responses. Consequently, some, more resistant sheep, are able to prevent an infection from establishing.

3. under conditions of low, moderate or discontinuous exposure to *M ptb*, some sheep which do become infected are able to mount a successful immune response which leads to recovery from infection or the transformation of a life-threatening infection into a long-term sub-clinical infection.

In our study, the high incidence of OJD in group UHH, compared to groups ULH and UHL, lent support to the proposition in point 3 above. In these three groups, pre-weaning exposure was classified as low (near zero), in group ULH, or medium, in groups UHL and UHH. Post-weaning exposure was classified as low (near zero) in group UHL or high, in groups ULH and UHH. It seems unlikely that point 1 above could apply to the medium level of pre-weaning exposure or the high level of post-weaning exposure. We consider that most, if not all, sheep would have ingested *M ptb* bacteria during the period that they were exposed to medium or high levels of *M ptb* availability. The difference in infection rates, severe infection rates and death rates between groups ULH and UHH cannot be adequately explained by the threshold hypothesis (point 2 above), but can be explained by the recovery hypothesis in point 3. Both groups were exposed to near identical levels of *M ptb* in the post-weaning period, so the threshold would have been exceeded in a similar proportion of sheep in both groups. The lower incidence in UHL and ULH implies that more sheep in those groups were able to deal successfully with the infection because their exposure was restricted to the pre-weaning or post-weaning period only.

Some sheep may be extremely susceptible to OJD

There appears to be a small proportion of Merino sheep which is particularly susceptible to infection and, for these animals, the 'threshold' of challenge is easily exceeded and a successful immune response does not eventuate, even if the challenge period is brief. We estimated that 3%–6% of sheep fell into this category. This proposition is consistent with reports from natural transmission of the disease, where low numbers of introduced infected sheep transfer infection into a previously uninfected flock. The disease is often difficult to detect in the recently infected flock for several years because so few animals are infected. These first cases, however, lead to amplification of infection over time and cause higher levels of pasture infection when they enter the faecal-shedding phase of the disease. As a consequence of the higher levels of pasture contamination, a higher proportion of the flock becomes infected.

Management of ewes at lambing

We found that the level of exposure to *M ptb* in the pre-weaning phase is important in determining the incidence of OJD. While we consider that the extreme lengths that we pursued to provide clean pastures for ILL lambs would not be practical, given that infection rates in that group still reached 20%, the principle may still be worth pursuing in other ways. For example, it may be possible to (a) join ewes for a short period only, such as 4–5 weeks, (b) identify one paddock for lambing which is left uncontaminated by infected sheep for several months before lambing starts then, (c) all ewes which are suspected of clinical OJD infection could be removed from the lambing flock, in order to reduce the number of those which are shedding *M ptb*, before (d) placing the lambing flock on the 'safe' pasture as close to the start of lambing as possible. Finally, (e) the lambs are weaned as early as possible and placed on another 'safe' pasture.

During the period that the pasture is not grazed by infected sheep, it could be grazed by adult cattle, by unaffected sheep, or left ungrazed. It should not be grazed by calves because experience has shown that young cattle can become patently infected with *M ptb* derived from sheep. If unaffected sheep are used, they should be considered to be an infected group after grazing the pasture for some weeks and it is to be expected that at least some of the group will commence shedding *M ptb* at around 12 months after first exposure. This may not be of practical consequence if the previously unaffected sheep are destined for slaughter within a few months of grazing the contaminated pasture.

In many OJD-affected Merino flocks, reproduction rates are low and flock owners have difficulty in producing sufficient replacement females to maintain the ewe flock without having to retain unproductive old ewes. In such flocks, advice to reduce the length of joining will present difficulties because reductions in joining time will reduce the lambing percentage. We propose that flock owners could continue to use extended joining times (eg, eight weeks) but use ram harnesses with crayons applied after four weeks. The ewe flock could then be divided into two groups based on crayon marks, with one group lambing first for four weeks then a second, smaller group lambing later. Each group could then be treated as described above.

Management of lambs at weaning

If lambs that are born in an infected flock are separated from the ewe flock at weaning (at as young an age as practicable) and placed on a pasture that had not been contaminated with *M ptb* for several months, we would expect that a proportion of the sheep which had been infected with OJD before weaning would either recover from the disease entirely or enter a sub-clinical state which might persist indefinitely.

Merino lambs can be satisfactorily weaned at 11–12 kg liveweight provided the pasture onto which they are weaned is highly nutritious. A green, legume-dominant pasture is generally satisfactory. Most Merino lambs will have reached this liveweight by six or seven weeks of age, assuming a birthweight of 3 kg, and a daily growth rate of 200 g/day. For graziers who wish to mules lambs at marking time, it is possible to allow lambing to occur over five weeks, mark and mules two weeks later, leave lambs without disturbance for five weeks, then wean. At this time the lambs will range in age from 7–12 weeks.

Preference for low-contamination pastures, if scarce.

The recommendations described above advise that low OJD-contamination pastures should be made available to both lambing ewes and to lambs at weaning. In some flocks this will not be possible, and only one low-contamination pasture may be available to the flock. We would advise that, in this event, the low-contamination pasture should be made available to the weaned lambs. We advise this, not because pre-weaned lambs are less likely to contract OJD, but because the difference in exposure to the bacteria that can be achieved by preparing low contamination pastures is likely to be greater in the case of post-weaned lambs than in lambing ewes. This is due to the fact that, despite efforts to remove clinical cases, some infected ewes are likely to remain and cause contamination of the lambing pasture. The benefits from efforts to prepare a low-contamination pasture, in terms of reduction of exposure, are likely to be greater for the post-weaning paddock. In the absence of any evidence for an increased resistance to infection after weaning, we expect there to be a greater response in reduction of OJD incidence from providing a clean weaning pasture than providing a clean lambing pasture.

Duration of low-challenge grazing after weaning

It is not clear how long these young sheep would need to be protected from further exposure but, in our study, shedding of organisms from the few patently-infected animals in these groups commenced at 12–18 months of age and did not lead to high levels of severe infections in their cohorts by three years of age. Hence it might be practicable and useful to recommend that weaned lambs continue to graze the same or other ‘safe’ pastures for six to nine months after weaning.

Interaction of OJD control and internal parasite control

The above recommendation to have a short joining period and to wean Merino lambs when the youngest in the lamb flock is seven weeks of age is consistent with recommendations for good control of internal parasites. In addition, the preparation of low-OJD-contamination pastures for weaned lambs is consistent with good parasite control if anthelmintic-treated adult cattle or non-lactating (eg, wethers) adult OJD-free sheep are used to pre-graze the pasture for three months including a summer period or six months if summer is not included.

OJD-free wethers are, however, unlikely to be available in most OJD-affected flocks. In the past, adult wethers have been recommended for the preparation of low-risk (for internal parasites) weaning pastures but this practice may not be appropriate in OJD-affected flocks.

In OJD-affected flocks, the cohort with the lowest prevalence of *M ptb*-shedding is the flock of weaned lambs, from 3–12 months of age. While sheep of this cohort would be appropriate, for OJD control, to graze the designated weaning paddock at least up to three months before weaning, they will cause substantial contamination of pastures with worm eggs. Frequent anthelmintic treatment to control their parasite burdens is not advisable, because of the risk of accelerating the development of anthelmintic resistance and because it may suppress the development of parasite immunity in the sheep. In most instances, if these sheep are removed from the weaning pasture after six months, the contamination they have caused will not be serious for the following year's weaners. There remains, however, the problem of which animals should graze the weaning pasture in the following six months.

Clinical and sub-clinical effects of OJD

Clinical OJD

Weight loss in sheep dying of OJD

This study provided the opportunity to investigate the weight change in sheep which subsequently died of OJD. The weight change in 15 sheep which died with OJD confirmed by histopathology was examined closely. Two results emerged. First, sheep which were dying from OJD showed large variation in liveweight over the last eight months of their lives, in some cases alternately gaining and losing weight. Second, despite the fluctuations in liveweight over that period there was a over-riding trend towards increasing weight loss, from about 4% of liveweight at 240 days before death to, on average, approximately 32% at death. The rate of loss appeared to accelerate from a point 120 to 150 days pre-death, but there was marked variation between individuals. The sheep in this experiment weighed, generally, 40 to 50 kg, so a loss of 4% of liveweight was about 2 kg, and a loss of 32% was about 14 kg.

This prolonged course (eight months) is greater than is generally reported for the clinical course of OJD. The early weight loss, perhaps up to 5 kg or 10% of liveweight, may not be evident clinically, particularly if not associated with faecal soiling of the perineum. In most sheep dying of OJD, losses of 10% of liveweight were evident by 180 days pre-death and in all sheep by 45 days pre-death. Four animals lost 40% or more of their liveweight; in two cases they survived for four months after that weight was recorded. We found, therefore, that the clinical course of OJD is typically four to six months.

Histological lesions in OJD mortalities

All sheep which died of OJD and were examined post-mortem had type 3b lesions of the intestinal tissues. The association between type 3b lesions and death was statistically highly significant. We would conclude that sheep which die naturally from OJD are very likely to have type 3b lesions.

Sub-clinical OJD

Histological lesions and weight loss

Not all sheep with type 3b lesions died of OJD – nine of the 24 with that form of histopathology survived to the end of the trial. On average, these nine sheep were significantly lighter than their flockmates. The statistical model predicted that sheep thus affected would be 3.76 kg lighter than unaffected sheep. This finding is consistent with the observations of weight loss in sheep dying from OJD and would suggest that these nine survivors were likely to die and that many of them were in the final 240 days of their life. The nine survivors with type 3b lesions had final weights (WT16) which ranged from 5.2% above to 23.4% below their expected weights. Only two were above their expected weight and four were >10% below.

Twelve sheep had type 3a lesions at post-mortem examination after slaughter. Lesions of this type were also statistically significantly associated with weight loss, but not with death. The statistical model predicted that sheep with 3a lesions would be 3.48 kg lighter than unaffected sheep. The 12 sheep (all were survivors) with type 3a lesions had final weights (WT16) which ranged from 17.9% above to 27.9% below their expected weights. Only three were above their expected weight and four were >10% below.

These observations suggest that sheep which are losing weight as a consequence of OJD infection are likely to have type 3a or type 3b lesions and that type 3a lesions will become 3b lesions at some time before the sheep dies. Lesions of these two types seem incompatible with continuing normal health and productivity although some animals, possibly in the early stages of developing these severe types of pathological change, were apparently of expected liveweight. It is possible that OJD infections develop to type 3a or 3b severity before the disease produces significant weight loss.

Sheep with lesions of type 1 (n=16), type 2 (n=12) or 3c (n=5) had liveweights at slaughter which were not significantly different from unaffected sheep. Similarly, sheep with no histopathological evidence of OJD but with positive tissue culture also had liveweights not significantly different from unaffected sheep. There was no evidence from our study, therefore, that lesions of these types affected the health or productivity of the sheep or predisposed them to death. Nor is it clear whether or not these lesions were progressive.

Histological lesions and reduced wool production

The results for the association between lesion type in sheep surviving to WOOL3 and wool production (greasy fleece weight) at WOOL3 were similar to those for liveweight. Those animals which had either type 3a or 3b lesions at slaughter were predicted to produce 0.291 kg less wool than their unaffected flock mates. An effect on production of this magnitude (about 6% of annual production) was presumably the result of reduced rates of wool growth for some weeks or months before the final shearing. This is consistent with the finding with regard to types 3a and 3b and liveweight change, which suggests that some animals are in a pre-clinical phase of disease while others are in an advanced clinical phase.

Overview of the effects of OJD on productivity of sheep flocks

This study is the first to provide data on the effects of OJD on the productivity of Merino sheep in Australia. The information of the incidence of mortality, the prevalence of lesions of varying severity and the effects of those types of lesion on productivity, can be examined in an economic model to estimate the financial cost of OJD at different rates of flock infection. Furthermore, because we also have information relating particular management strategies to the likely infection rates in the flock, it will be possible to examine the likely cost:benefit of the management strategies.

SUCCESS IN ACHIEVING OBJECTIVES

The principal objectives of the experiment were to relate the origin and timing of exposure to infection with *M ptb* in a cohort of young sheep and

1. the prevalence of histopathological evidence of OJD at three years of age

We found that there was a complex relationship between the exposure history to OJD contamination of lambs and the prevalence of OJD at three years of age. In general, the higher the level of exposure, the higher the prevalence of infection, but some additional factors influenced the outcome. Even when contamination of the environment was very low, a small proportion of the flock became infected and caused secondary contamination of the pastures, leading to infection in about 18% of the flock by three years of age. There were relatively few deaths when contamination levels were low. When contamination of the environment was high, up to 39% of the flock became infected at or before three years of age and up to 9% of the flock died of OJD before that age. There was no evidence that any useful level of age-resistance to infection had developed in lambs by the age of weaning. The continuity of exposure to OJD contamination was relatively more important in determining the incidence of OJD than the age of the sheep when exposed.

2. prevalence and time of onset of sub-clinical disease as measured by liveweight changes and wool production, and the prevalence and time of onset of clinical OJD, in sheep not surviving to three years of age.

Sub-clinical disease, measured by liveweight changes, was only evident in animals that subsequently died from OJD or which had histo-pathological lesions of Perez type 3a and 3b when killed at three years of age. In sheep which died from OJD, sub-clinical effects were evident for up to one year before death. There was no detectable evidence of sub-clinical effects on liveweight or wool production of infections associated with lesions of types 1, 2 or 3c, or in sheep with no histological lesions but *M ptb*-infected tissues. Deaths and, therefore, sub-clinical disease tended to occur earlier in sheep which were exposed to high levels of infection in the pre-weaning environment and were raised in an infected dam flock. The median age of death from OJD of sheep in the infected flock was 776 days, compared to 932 days in the uninfected flock.

3. A secondary set of objectives was the collection of a 'library' of pasture, faecal, blood and tissue samples from ewes lambing and sheep born on the site, the maintenance of the library and, as funding becomes available, to retrospectively analyse selected samples for examination of any or all of a wide range of epidemiological associations essential to our understanding of OJD in natural field infections.

Samples were collected every three months and have been stored, except for those which have been analysed. At some times during the study the frequency of collection of specimens was increased to every six weeks approximately. These specimens are available for further analysis. The value of samples currently stored at –80°C will need to be assessed as there are on-going costs in their maintenance. Serum samples will be tested in 2004 as part of a variation to this project to study sub-clinical biochemical effects of OJD. Pasture samples have not been stored; methods to detect/enumerate *M ptb* on standing pasture were not successful and there are no results for pasture culture included in this report.

4. The aim of the study was to provide management strategies for owners of OJD-affected flocks which would reduce the prevalence of OJD in their flocks and reduce the proportion of the flock which die each year from OJD.

The study has demonstrated that there are management strategies which owners of OJD-infected flocks can implement which will reduce the incidence of OJD. Key findings are (1) the direct relationship between pasture contamination levels and the incidence of disease, over a range which is relevant to the levels which occur or can be achieved on most farms, (2) the importance of providing low-contamination grazing to weaned lambs even if they have been exposed to OJD before weaning, and (3) the opportunities to reduce the infectivity of pastures to low levels in a few months with beneficial effects on the subsequent incidence of OJD.

IMPACT ON MEAT AND LIVESTOCK INDUSTRY

Since OJD was first diagnosed in Australia, there have been many reported observations of the way in which OJD has affected flocks – in some cases severely and in other cases less severely – and of the way that some graziers have managed to reduce the impact of the disease in their flocks. These anecdotes have provided the only clues so far to the factors which are responsible for variations in the impact of the disease between flocks, and to the steps that owners of affected flocks should take.

Now that this study has been completed, these observations can be re-evaluated in the light of our findings and the recommendations can be refined. Broadly, the common sense approach advised by most field workers has been vindicated, but some additional insights are now available. These insights relate specifically to the extreme difficulty in preventing low levels of infection in a flock, the continuing susceptibility of lambs beyond weaning age, the benefits of providing low-contamination grazing to weaned lambs and the relatively short time needed to render a contaminated pasture substantially lower in infectivity by removing infected animals. Also important to efforts to reduce the disease incidence through management is the finding that the incidence of disease in a flock varies with the level of contamination, particularly that caused by contemporaneous grazing, at levels which are relevant to commercial sheep farming.

Vaccination against OJD was not evaluated in this study but other studies have examined its use. It is likely that the combined used of strategic vaccination and grazing management, as described here, will provide additive benefits in OJD control. These combined strategies remain to be tested.

This study has not directly addressed issues relevant to eradication of OJD from flocks but the difficulty that we experienced in preventing infection in the control flocks highlights the difficulties faced by producers who attempt to eradicate the disease if there is infection in other nearby flocks.

CONCLUSIONS AND RECOMMENDATIONS

The understanding of OJD epidemiology in young sheep which has been derived from this study will allow the development of guidelines for OJD control in infected flocks. These guidelines should focus on strategies to protect pre-weaned lambs from exposure to *M ptb* as much as possible, recognising the inherent difficulties of achieving this in the face of infection in the ewe flock and to then protecting weaned lambs from exposure. The importance of providing a ‘safe’ pasture to weaned lambs is a critical discovery, for this is a relatively straightforward practice on most farms. This knowledge should be considered by graziers and their advisors and developed into specific farm management plans.

The outcome for sheep owners of adopting an OJD-control program is expected to be a reduction in the incidence of severe OJD infection in their flocks. It is possible that this strategy, when used in conjunction with vaccination, will improve the level of control achieved by vaccination, although that hypothesis was not tested in our study.

The knowledge gained from this study is potentially useful for all breeders of replacement sheep in infected flocks, including self-replacing flocks, Merino flocks breeding crossbred ewes as meat lamb dams, and ram-breeding flocks. The improved understanding of the relative susceptibility of non-exposed weaned lambs is useful also for graziers buying OJD-free lambs and introducing them onto OJD-infected farms.

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Technical assistance

Mr Craig Kristo was responsible for all the *M ptb* culture work carried out at the JL Shute building until 2002 and for the treatment of the ewes with footrot. Mrs Anna Waldron and Dr Om Dhungyel undertook culture work later in the trial, including the tissue cultures. Final year veterinary science students provided skilled assistance at Goulburn abattoir at the end of the trial.

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Shearer

Mr Neil Taylor was the extremely careful and patient shearer who sheared all the experimental sheep from late 2000 until late 2002.

Those who constructed the experimental site

The site was planned by Dr Kym Abbott and Mr Steven Burgun, in conjunction with surveyors Flood and Poidevin of Goulburn. The site was constructed by Arthursleigh Farm staff (Messrs Brian Farrell, Dick Bryant, Rodney Baxter) under the supervision of Mr Burgun. A gravity fed watering system with concrete troughs in each paddock was installed by Dr Abbott, Messrs Kristo and South to a design by Mr Neil Southorn of Orange Agricultural College. Fenced out gullies were planted with 500 tube-stock native shrubs and trees by students of the Faculty of Agriculture on 8 April 2000. The areas between the exclusion fencing and gully lips were sown to *Phalaris tuberosa* in May 2000 and, on 27 September, approximately 24 kms of tree-lines were direct seeded by Mr Brian Cumberland of Greening Australia.

The agronomists

Estimation of pasture availability and identification of the major pasture species was carried out by Ms Lori McGarva and Mr Dale Chalker (NSW Agriculture) in December 1999.

Suppliers of Animal Health Products

Novartis Animal Health Australia Pty Ltd supplied Clik™ which protected the experimental sheep from fly-strike throughout the trial. Merial Australia Pty Ltd supplied Ivomec™ which was used strategically for control of internal parasites throughout the study period.

The owners of the experimental site

The University of Sydney, owners of Arthursleigh Farm, through its Farm Advisory Committee approved the use of a portion of Arthursleigh for the experiment. We particularly acknowledge the support of Mr Burgun and Mr Grant Fulton in our efforts to obtain permission to use the site.

The owners of Flocks 1 and 2

Mr and Mrs George Gundry of Collector and the Bruis family of Cooma were extremely co-operative vendors of Flock I and Flock U ewes. They provided patient and thorough assistance in the preliminary testing, selection and dispatch of the ewes from their flocks.

Organisers and participants in the field day

A field day for producers and advisers was held on 4 May 2000. The meeting was held in the Arthursleigh woolshed and was hosted jointly by The University of Sydney, NSW Agriculture and the Goulburn Rural Lands Protection Board. Attendance exceeded 165. The field day program focussed on control of the disease within infected flocks and sought to provide information to producers with affected flocks and to those producers who wished to prevent infection in their flocks. A booklet was produced for all attendees and for distribution by the Board after the meeting. Speakers included Drs Richard Whittington, Kym Abbott, Geoff Eppleston, Ms Helen McGregor, Messrs Bill Johnson, Terry Hayes and Cliff Kelly. Sponsorship was provided by Novartis Animal Health Australia Pty Ltd and Merial Australia Pty Ltd.

The statistician

Mr Paul Nicholls performed the most demanding parts of the statistical analyses done on the data collected during the experiment.

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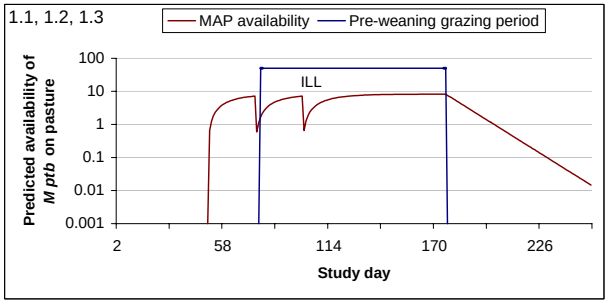
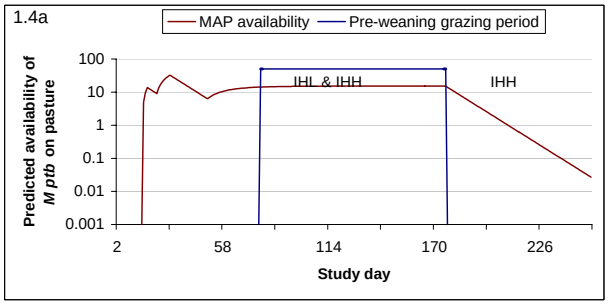
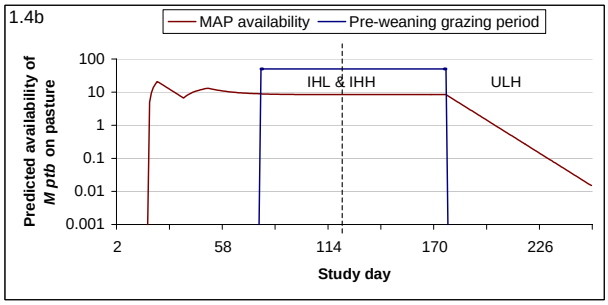
Appendix 1

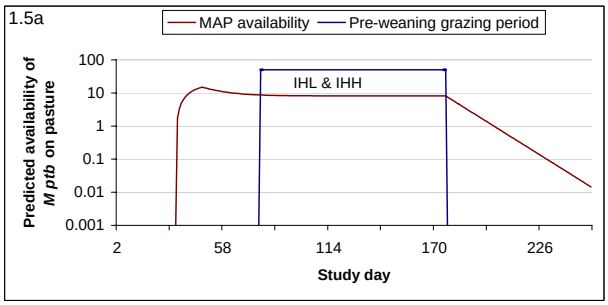
Figures of *M ptb* contamination

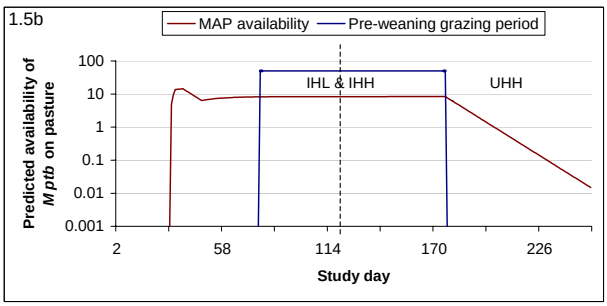
Figure 1 Predicted availability of *M ptb* on pastures grazed by experimental sheep - Replicate 1. Units of M ptb availability are E/D and reflect decay rates of viable *M ptb* of one logarithm every 28 days. The period during which the lamb with the median birth date grazed in the pre-weaning period is indicated by the blue rectangle. The treatment groups derived from each pre-weaning grazing are indicated in red within the blue rectangles. Paddocks 4a, 4b and 5b were used for post-weaning treatments – the treatment groups are indicated on the relevant figures outside the blue rectangle. The dotted lines (Paddocks 4b and 5b) indicate the time points when lambs of groups ULH and UHH were placed on these pastures.

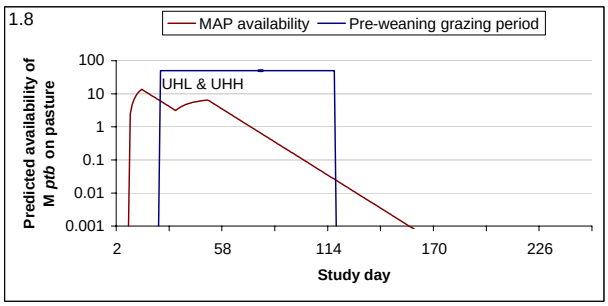












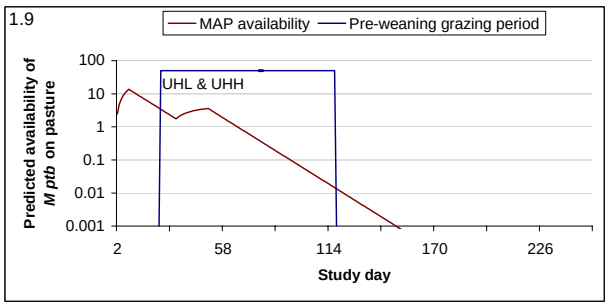


Figure 2 Predicted availability of *M ptb* on pastures grazed by experimental sheep - Replicate 2. Units of *M ptb* availability are E/D and reflect decay rates of viable *M ptb* of one logarithm every 28 days. The period during which the lamb with the median birth date grazed in the pre-weaning period is indicated by the blue rectangle. The treatment groups derived from each pre-weaning grazing are indicated in red within the blue rectangles. Paddocks 4a, 4b and 5a were used for post-weaning treatments – the treatment groups are indicated on the relevant figures outside the blue rectangle. The dotted lines (Paddocks 4b and 5a) indicate the time points when lambs of groups ULH and UHH were placed on these pastures.

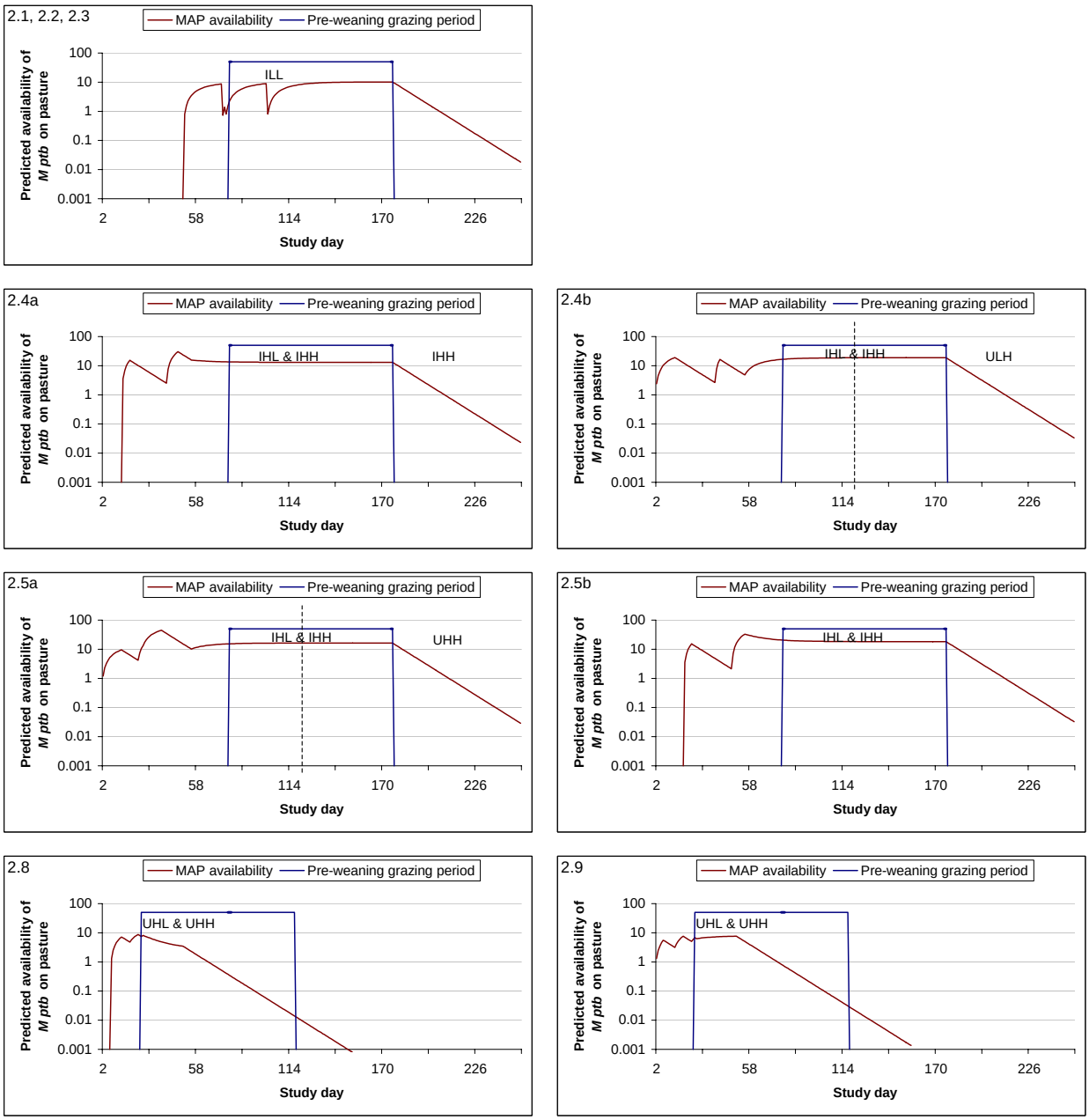
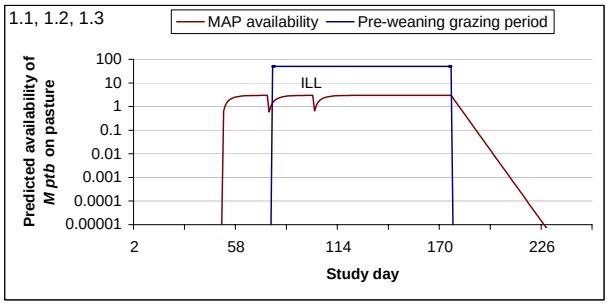
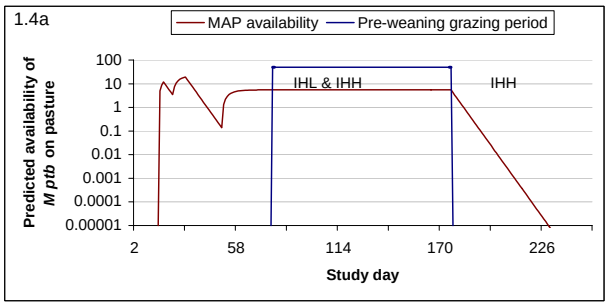
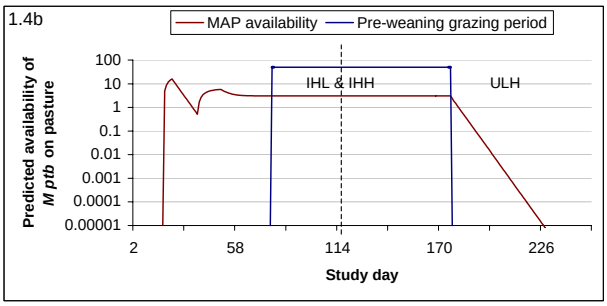
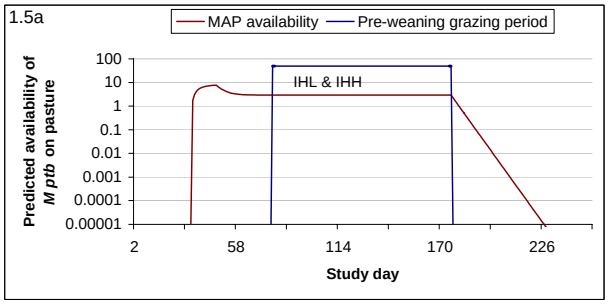


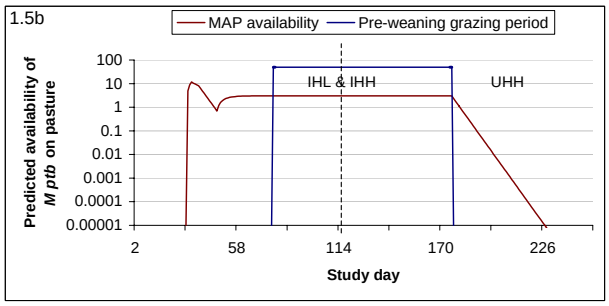
Figure 3 Predicted availability of *M ptb* on pastures grazed by experimental sheep - Replicate 1. Units of *M ptb* availability are E/D and reflect decay rates of viable *M ptb* of <u>three</u> logarithms every 28 days. For other details, see caption on Figure 1.

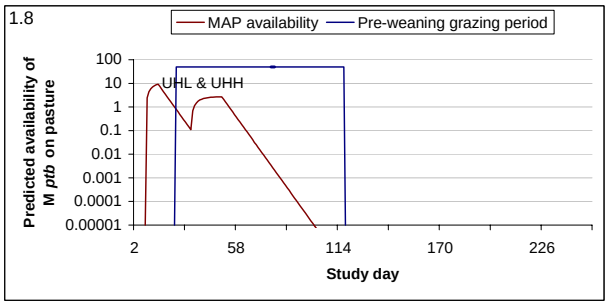












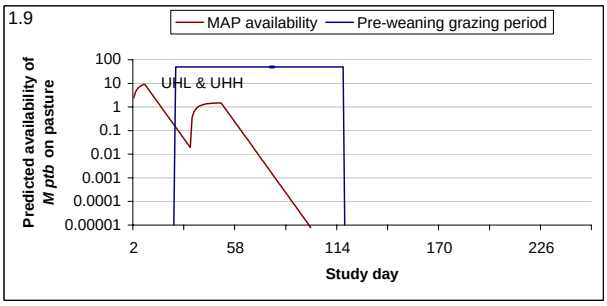


Figure 4 Predicted availability of *M ptb* on pastures grazed by experimental sheep - Replicate 2. Units of *M ptb* availability are E/D and reflect decay rates of viable *M ptb* of <u>three</u> logarithms every 28 days. For other details, see caption on Figure 2.

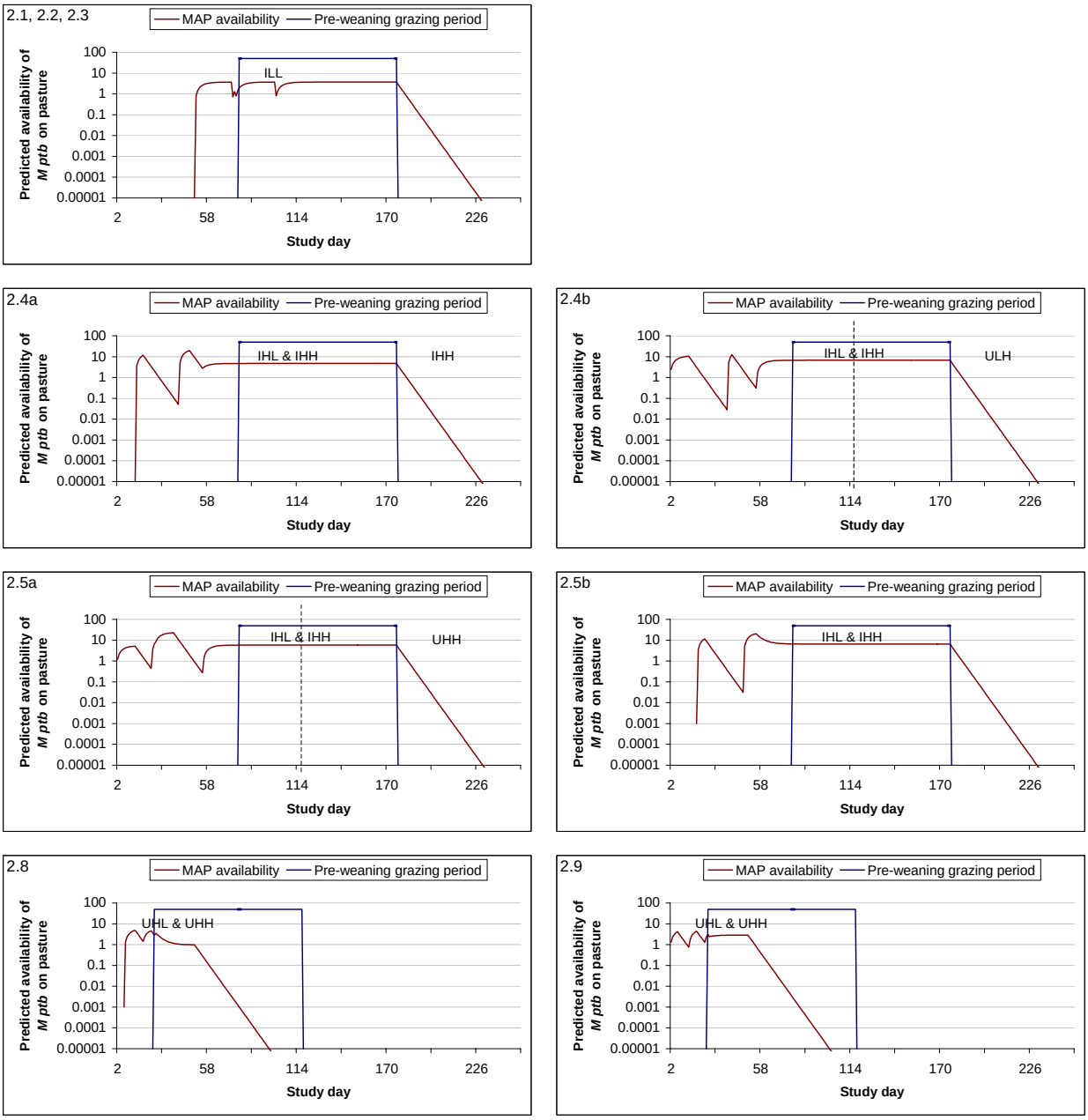
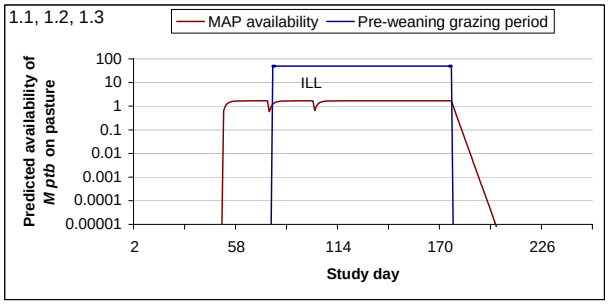
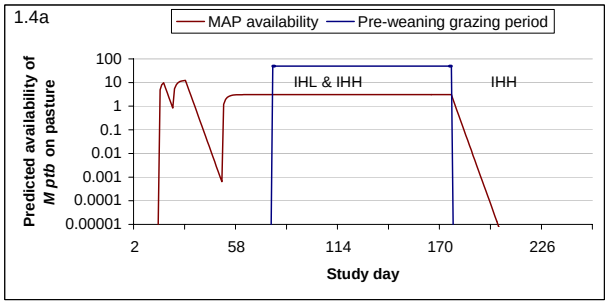
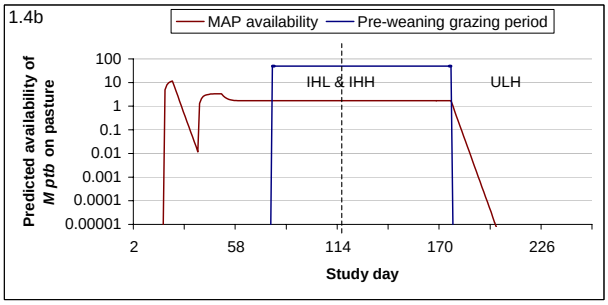
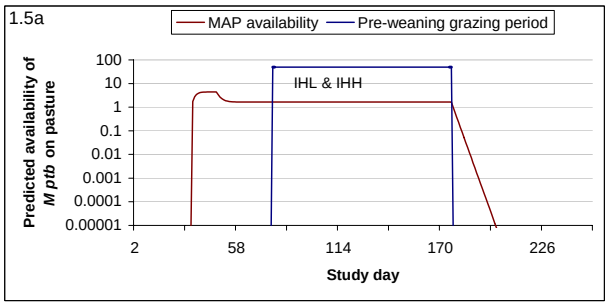


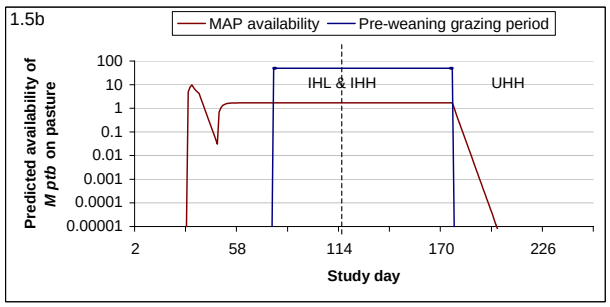
Figure 5 Predicted availability of *M ptb* on pastures grazed by experimental sheep - Replicate 1. Units of *M ptb* availability are E/D and reflect decay rates of viable *M ptb* of <u>five</u> logarithms every 28 days. For other details, see caption on Figure 1.

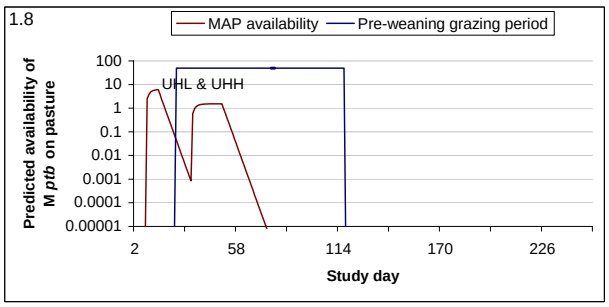












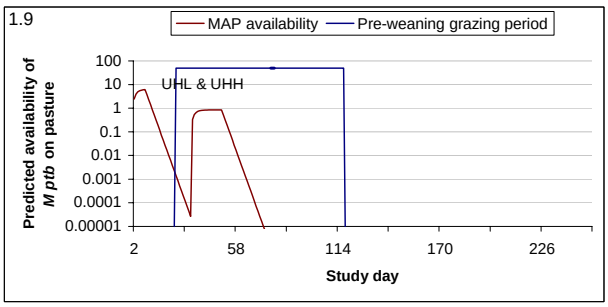
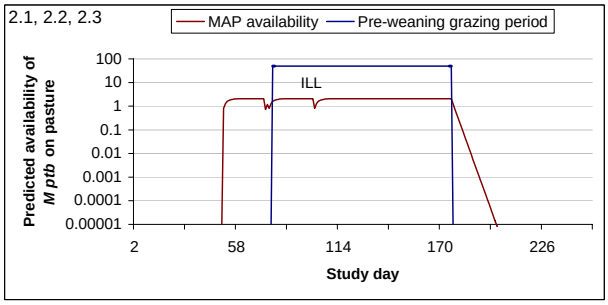
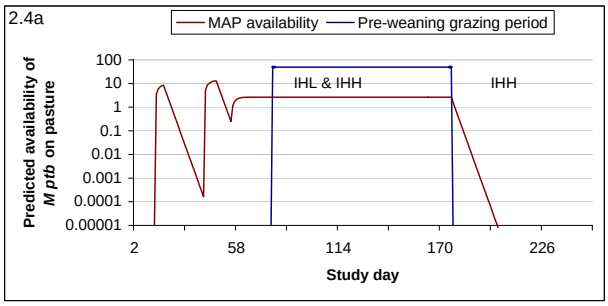
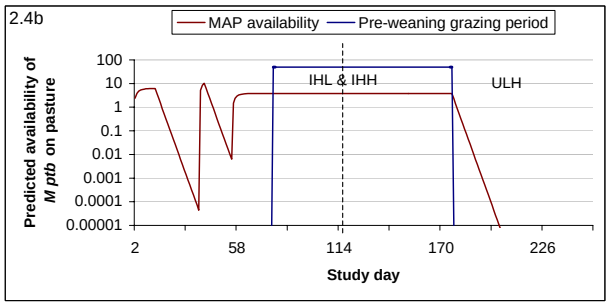
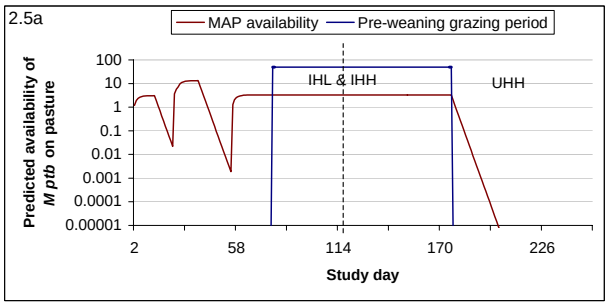


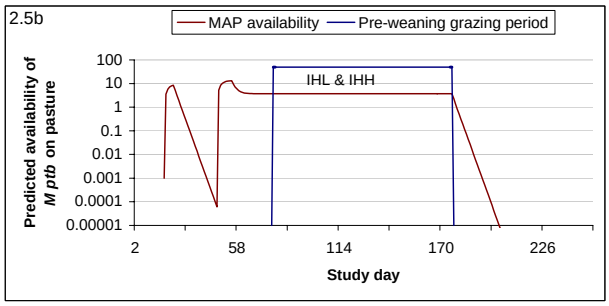
Figure 6 Predicted availability of *M ptb* on pastures grazed by experimental sheep - Replicate 2. Units of *M ptb* availability are E/D and reflect decay rates of viable *M ptb* of <u>five</u> logarithms every 28 days. For other details, see caption on Figure 2.

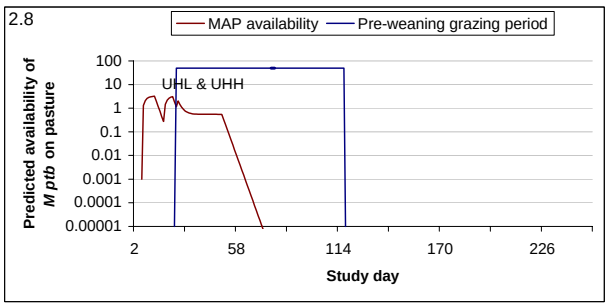


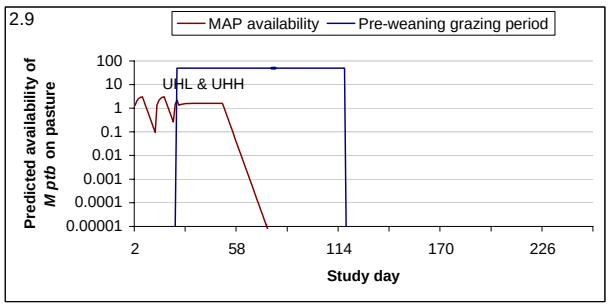












Appendix 2

Statistical analyses

Section A.

Differences in age at death between treatment groups

There were very few OJD deaths in some treatment groups which meant that meaningful comparisons of age at death between all groups were not possible. Valid comparisons could be made between sheep in the two continuous high-exposure groups (UHH and IHH). Sheep which were born from the infected dam flock (IHH) died, on average, 160 days earlier in life than sheep which were born from uninfected ewes (UHH), even if the pasture was contaminated (and cohabited by infected sheep). This observation was not statistically significant when applied to just IHH vs UHH (P=0.11) but, when it was applied to all sheep born to Flock I ewes and Flock U ewes, whatever their pre- and post-weaning exposure, the difference in median age (935 vs 711) was significant (P=0.03). The differences between groups were less when probable OJD deaths were included but remained statistically significant (932 > 776, P=0.04)

When analysed using generalised linear mixed models and the four contrasts T1 to T4[a], no effects of treatment or sex were detected as significant (all four contrasts and sex P>0.20) and there were no random effects. When probable-OJD deaths were included, the T3 contrast was detected as significant (P<0.05) but the other contrasts and the sex effect were non-significant (P>0.20) and there were no random effects. The T3 contrast only involved four values, 648 and 694 days for ILL compared with 995 days for UHL and 935 days for ULH. There were insufficient data to accept that the statistically significance result for T3 indicated a real difference in average time to death between the ILL treatment and the UHL and ULH treatments.

Section B.

Effect of treatment group on lesion type

In the GLMM binomial analyses, there were no significant treatment or sex fixed effects for any of the individual lesion scores or categories but there were random effects. The replicate x treatment interaction effects were very highly significant (P<0.001) for lesion types 0, 1 and 2, but were non-significant (P>0.20) for lesion types 3a, 3b and 3c. There were also moderate replicate x sex effects for lesion type 3a and small replicate x sex effects for lesion type 0.

In the GLM multinomial fixed effects analyses for the sets of six lesion scores there were some highly significant replicate x treatment contrasts (mainly replicate x T2) and in the presence of these interactions the treatment contrasts themselves were either weakly significant (0.05<P<0.10) or non-significant. These tentative analyses supported the results of the GLMM binomial mixed model analyses that showed some strong replicate x treatment random effects but no significant fixed effects of treatments or sex. There were marked differences between replicates in the frequencies of lesion types for treatments ILL, IHH, IHL and UHH, which are the basis for the replicate x treatment interactions.

[a] T1 = ULL vs all other treatments. T2 = single high-exposure vs multiple high-exposure: {ILL,UHL,ULH} vs {IHH, IHL,UHH}, T3 = single high-exposure from dam vs single high pre- or post-weaning exposure: ILL vs {UHL,ULH}, T4 = triple high exposure vs double high exposure: IHH versus {IHL,UHH}

Section C.

Effect of treatment group on risk of OJD

For statistical examination of the differences between groups, factorial design analyses were used initially but models with different sets of main effect and interaction terms, or the same terms introduced in different orders, did not necessarily produce similar levels of statistical significance for a given term. The presence of random replicate x treatment interaction effects further compounded the difficulty. It was concluded that these analyses did not provide the basis for an adequate interpretation of the data.

When the effects structure of the treatments was ignored (*ie* simply regarding treatment as a factor with seven different levels), a model with treatment and sex fixed effects and replicate, replicate x treatment and replicate x sex random effects indicated that the treatment factor (on six degrees of freedom) was non-significant (P>0.20) but that replicate x treatment interaction effects were significant (P<0.05). However, when the replicate x treatment effects were dropped from this model, the treatment factor was then significant (P<0.05). This suggested that there could be significant differences between particular subsets of the treatments if those subsets did not have appreciably different effects in the two replicates.

An analysis which included the single-degree-of freedom treatment contrasts T1-T4[b], sex and their four interactions as fixed effects and replicate and replicate x treatment (as the seven-level factor) as random effects showed the contrast T2 was significant (P<0.05) but that T1, T3 and T4 were non-significant (P>0.20), as was the effect of sex (P>0.20) and all the sex interactions (P>0.10 for sex x T2, P>0.20 for the rest). As before, the replicate x treatment interaction effects were significant (P<0.05).

It can be concluded that the OJD infection rate was higher for sheep exposed to multiple sources of high-exposure (33.2%) than for those exposed to a single high source (18.1%). However, in this trial the difference between the OJD infection rates for the "control" treatment ULL (16.9%) and the average of the six high exposure groups (25.6%) was not detected as significant[c].

Section D.

Effect of treatment group on risk of death from OJD

Confirmed OJD deaths only

Analyses based on the factorial design structure were not attempted, but those based on either the unstructured treatment factor with six degrees of freedom or on treatment contrasts T1 to T4 were performed. In the former analysis, there was a significant effect of sex (P<0.05) but the treatment factor was non-significant (P>0.20). The treatment x sex interaction was non-significant (P>0.20) and, although there were some small random replicate effects, there were no replicate x sex or replicate x treatment random effects. In the latter analysis, as well as the effect of sex, the contrast T2 (single high-exposure vs multiple high-exposure) was significant (P<0.05), but the contrasts T1, T3 and T4 were non-significant (P>0.20). As the single-degree-of-freedom T2 contrast accounted for almost all of the variation among the seven treatments, it is appropriate to accept that the effect associated with that contrast was statistically significant and that the OJD death rate was higher for sheep exposed to multiple sources of high exposure (5.5%) (IHL, IHH, UHH) than for those exposed to a single high source (0.8%) (ILL, UHL, ULH). The difference between the OJD death rates for the "control" treatment ULL (1.2%) and the average of

[b] T1 = ULL vs all other treatments. T2 = single high-exposure vs multiple high-exposure: {ILL,UHL,ULH} vs {IHH, IHL,UHH}, T3 = single high-exposure from dam vs single high pre- or post-weaning exposure: ILL vs {UHL,ULH}, T4 = triple high exposure vs double high exposure: IHH versus {IHL,UHH}

[c] The infection rates quoted in this paragraph are adjusted for fixed and random effects in the model. The fixed effects included were T1 ("control" vs "rest") and T2 (multiple vs single exposure) and the random effects were replicate and replicate x treatment group. (Note that T1 was present in the model even though it was not significant, in order to estimate means for "control" and "rest".

the six deliberately-exposed groups (3.2%) was not detected as significant. The OJD death rate for males (3.6%) was higher than that for females (1.1%)[d].

Confirmed and probable-OJD deaths together

Results including probable-OJD deaths were analysed in the same way as the results including only confirmed deaths. In the analysis with the unstructured treatment factor, neither the sex effect nor the treatment factor were significant (P>0.20) and, although there were no replicate x treatment random effects, replicate x sex random effects were present. The sex effect remained non-significant (P>0.10) when the replicate x sex effects were dropped from the model. In the analysis with the contrasts and the replicate x sex effects included, contrast T2 was significant (P<0.05), but T1, T3 and T4 were non-significant (P>0.20). For the same reason given for the analysis based on confirmed deaths only, it is appropriate to accept that the effect associated with the T2 contrast was statistically significant. The OJD death rate was higher for sheep exposed to multiple sources of high exposure (7.2%) than for those exposed to a single high source (1.7%). However, in this trial the difference between the OJD death rates for the "control" treatment ULL (2.5%) and the average of the six deliberately-exposed groups (4.4%) was not detected as significant. The difference between the OJD death rates for males (4.7%) and females (2.4%) was not detected as significant.

Section E.

Faecal culture results at 18 months of age (BACT18M)

Relationship between BACT18M and treatment group

Treatment contrast T2 was significant (P<0.05) but the other contrasts, including T1, were non-significant (P>0.20) and the effect of sex was non-significant (P>0.20). There were some moderate replicate and replicate x sex random effects, but no replicate x treatment effects. The BACT18M-positive rate was higher for sheep exposed to multiple sources of high exposure (8.7%) than for those exposed to a single high source (2.7%). However, in this trial the difference between the BACT18M rates for the "control" treatment ULL (2.6%) and the average of the six high exposure groups (4.9%) was not detected as significant. No effect of sex on BACT18M-positive rate was detected.

Association between risk of death, BACT18M and treatment group

GLMM binomial analyses were performed on SURVIVAL with a model comprising the fixed effects of sex, treatment contrasts T1 and T2 and BACT18M (as a two-level factor) and the random effects of replicate, replicate x sex and replicate x treatment (as a seven-level factor). There was a very highly significant effect of BACT18M (P<0.001) on SURVIVAL (i.e. a strongly significant association between faecal culture BACT18M result and SURVIVAL) but the sex and treatment fixed effects were non-significant (P>0.20). The replicate x treatment interaction effects were very highly significant (P<0.001) and there were some small replicate x sex interaction effects. The adjusted mean proportion of survivors to the end of the trial for BACT18M negative sheep was 97.9%, compared with 22.7% for BACT18M positive sheep.

Association between BACT18M and HISTO

GLMM binomial analyses were performed on the individual HISTO lesion types with a model comprising the fixed effects of sex, treatment contrasts T1 and T2 and BACT18M (as a two-level factor) and the random effects of replicate, replicate x sex and replicate x treatment (as a seven-level factor). There was a significant effect of BACT18M on HISTO (i.e. a significant association between faecal culture BACT18M result and HISTO lesion type) for lesion types 0 (P<0.001) and 3b (P<0.001) but not for the other scores (0.05<P<0.10 for lesion type 1, P>0.20 for the rest)

[d] The infection rates quoted in this paragraph are adjusted for fixed and random effects in the model. The fixed effects were sex, T1 and T2 and the random effect was replicate.

Section F.

Liveweight change in surviving OJD cases

The effect of the WT4 (first adult weight) covariate (1.09 ± 0.04 kg) was very highly significant (P<0.001). The adjusted effects of T1 and T2 were non-significant (P>0.20). The adjusted effect of sex was highly significant (means: Female 40.4 ± 1.27 kg, Male 42.0 ± 1.3 kg, SED = 0.48 kg, P<0.01), but there was a weakly significant sex x T2 interaction (0.05<P<0.10) (Single high exposure: Female 40.6 ± 1.6 kg, Male 41.4 ± 1.6 kg, SED = 0.61 kg; multiple high exposure: Female 40.2 ± 1.6 kg, Male 42.5 ± 1.6 kg, SED = 0.62 kg).

The T2 means were: single high exposure 41.0 ± 1.5 kg, multiple high exposure 41.4 ± 1.5 kg, SED = 1.8 kg; and the T1 means were: ULL 38.7 ± 2.3 kg, rest 41.2 ± 1.3 kg, SED = 2.34 kg. The random replicate x treatment interaction was very highly significant (P<0.001) and there were some moderate random effects of replicate and replicate x sex. For the sheep included in this analysis the WT4 mean was 36.5 kg.

The linear mixed model for WT16 reported above (fixed effects of WT4, T1, T2, sex and sex x T2 and random effects of replicate, replicate x sex and replicate x treatment (as a seven-level factor)) was extended by adding HISTO (as a six-level factor of lesion types). Sheep with OJD pathology of type 3a or 3b were predicted to be 3.48 or 3.76 kg respectively lower in liveweight than would have been predicted from their 15 month liveweight, sex and paddock, if they were free of these types of OJD lesions

Appendix 3

Final Report on Part 2 of OJD.002A

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Background

There is evidence from studies with cattle that neonates are more susceptible to infection with *Mycobacterium paratuberculosis* than adult cattle. There is less clear evidence in the case of sheep and, although a declining susceptibility with age is suspected in sheep, the extent of the relative susceptibility is not understood.

Part 1 of this experiment (OJD.002A) was designed to examine factors associated with infection of lambs. In Part 1, a group of uninfected adult ewes was allowed to lamb on pastures which had been contaminated with faeces from OJD-infected sheep in the preceding three weeks and for the first three weeks after the uninfected ewes were added. The experiment was controlled (so there was one 'treatment' in which ewes lambed on non-contaminated pastures) and replicated (so there were two replicates of each treatment).

While Part 1 included other treatment groups as well, there was an opportunity to compare the susceptibility to OJD of the adult ewes and their off-spring, because both ewes and offspring had been exoposed to similar levels of *M ptb* over similar periods of time. In addition, this comparison could be conducted using physical infrastructure which was no longer required in Part 1 after lambing had finished. These opportunities gave rise to Part 2 of the study.

Knowledge of the relative susceptibility of sheep of different ages to OJD infection is important to the development of OJD-control strategies on infected farms based on grazing management. With this knowledge, producers with affected flocks can make decisions about the way they graze OJD-contaminated pastures so that they minimise the economic impact of the disease in their flocks. There are many ways in which this information can be applied. For example, owners of affected flocks can use purchased flocks of OJD-free sheep to reduce pasture infectivity and, with the knowledge generated from this study, predict the time before OJD is clinically evident in the sheep, and predict the prevalence of infection. As another example, flock owners could take steps to avoid exposing young animals to heavily infected pastures until they are adult if there is a significant reduction in susceptibility in adult sheep.

Project objectives

1. To quantify the difference in OJD prevalence between sheep which were first exposed to OJD infection at birth or sheep first exposed at three and four years of age.

2. To provide an early indication of the relative susceptibility of adult sheep and lambs prior to commencing a comprehensive experiment to examine issues of age susceptibility and size of challenging dose, which may facilitate the design of that experiment.

Methodology

Experimental design

A flock of 230 adult ewes which were previously free of *M ptb* infection were placed on either a *M ptb*-contaminated pasture or a *M ptb*- free pasture for 90 days, during which time they lambed and reared their lambs. After a further 56 days the two groups of ewes were mixed and run together for a further 641days before they were slaughtered and examined for evidence of *M ptb* infection. The infection rates in the two groups of ewes were compared and also compared to the infection rates in their lambs when they were slaughterd 316 days later.

Sheep

In Part 1, 480 pregnant ewes were purchased from a commercial flock in August 1999. Serological testing of these ewes before purchase indicated that they were free of OJD infection. Subsequent faecal culture of these ewes confirmed that they were free of infection on arrival at the experimental site. The ewes were aged three, four, five and six years at the time of purchase and they lambed in September. These ewes formed Flock U in Part 1 of the study.

When the ewes and lambs were weaned, on Day 175 of Part 1, about half of Flock U were sold but 230 ewes were retained and became the experimental sheep in Part 2.

Also in Part 1, 360 pregnant ewes were purchased from a second flock and brought to the experimental site. These ewes were known to be infected with OJD. They had a major role in Part 1 but their contribution to Part 2 was limited to their legacy of *M ptb*-contaminated paddocks.

More detailed information about Flock U and Flock I ewes is contained in the main body of the report.

Study dates

The same timeline is used for Part 2 as in Part 1. This report briefly summarises the history of the ewes and the paddocks before Day 175, when Part 2 became independent of Part 1 and provides more detail of the period after Day 175. Part 2 concluded on Day 816, when the ewes were killed at an abattoir and specimens were collected for histopathology and bacteriological culture of faeces.

Paddocks used in the experiment

The experiment was replicated once. Within each replicate, ewes were grazed in a series of 10-ha paddocks. These paddocks were numbered 2, 3, 6, 7, 8 and 9 and the paddock numbers were, if necessary, prefixed by the Replicate number; *eg*, 1.2, 2.2, 1.3, 2.3, *etc*. More detailed information about the paddocks and the experimental site is contained in the main body of the report. The paddock numbering system is consistent in both parts of the study and the same number refers to the same paddock in both the main body of the report and in this appendix.

Preparation of OJD-contaminated pasture

Paddocks 2, 3, 8 and 9 in each replicate were contaminated with *M ptb* by Flock I ewes before and, in the case of Paddocks 8 and 9, partly contemporaneously with, grazing of the previously unexposed ewes from Flock U.

Contamination of Paddocks 8 and 9 was achieved by grazing with Flock I ewes for seven weeks (Table 1). They grazed the paddocks from 6 August 1999 (Day 1 of the study) until 24 September 1999 (Day 50)

at a stocking density of 3.6 per hectare[a]. Subsequent faecal culture of the infected sheep indicated that 13 – 15% (Replicate 1) or 12 – 14% (Replicate 2) of the ewes were excreting *M ptb* at the time.

Contamination of Paddock 2 occurred when Flock I ewes grazed for a period of 25 days (from Day 25 – 100) and contamination of Paddock 3 occurred when the same sheep grazed from Day 101 – 175 inclusive (Table 1). For both paddocks, Flock I ewes grazed at a density of six sheep per ha and the indicative prevalence of faecal shedding was the same as given in the previous paragraph.

Table 1 History of contamination of paddocks 2, 3, 8 and 9. Contamination occurred by grazing with sheep from an infected flock (Flock I) at the stocking densities indicated in the table.

Day of study:	1 to 50	51 - 74	75 - 100	101 - 175
Paddock 2	Ungrazed		6 sheep ha^{-1} x 25 days	Ungrazed
Paddock 3	Ungrazed			6 sheep ha^{-1} x 74 days
Paddocks 8 & 9	3.6 sheep ha^{-1} x 49 days	Ungrazed by infected sheep		

Formation of a group of high-exposure ewes (Group H)

The ewes from Flock U were placed in Paddocks 8 and 9 on 1 September (Day 27) and starting lambing on the same day. Lambing continued until Day 67. The lambs born in these paddocks were weaned and removed on 1 December 1999 (Day 118). In January (Day 175), the oldest two age groups of the ewes were removed from the site and sold. Approximately 115 ewes, aged three and four years remained and these formed Group H (high exposure).

Contamination had been occurring for 26 days before they were introduced and continued for 24 days after they were introduced into the paddock (Table 2). Approximately two thirds of the contamination (in sheep-days per ha) had occurred in the 26 days before the ewes were introduced and the balance occurred in the subsequent 24 days. A total of 181 sheep-days per ha of contamination occurred in these paddocks and the Group H ewes grazed them until Day 175 (a total of 147 days). No further contamination occurred from Day 50 to Day 175 – the last 125 days of their occupation of the paddock.

Group H ewes were then (Day 175) placed in Paddock 2 which had received 150 sheep-days per ha of contamination between Days 75 and 100. No further contamination occurred in the 75 days before Group H ewes entered the paddock.

Formation of a group of low-exposure ewes (Group L)

Ewes which lambed in non-contaminated paddocks (Paddocks 6 and 7) lambed between Days 11 and 67 and were weaned at the same time as ewes lambing in contaminated paddocks (Day 118). In January the oldest two age groups were removed from the site and sold and the remainder (approximately 115 ewes, aged three and four years) formed Group L (low exposure).

Group L ewes were not exposed to contaminated pasture before Day 119, when they were placed in Paddock 2 (Table 2). No contamination had occurred in the 19 days before they entered the paddock and no further contamination occurred after they were introduced.

[a] This figure represents an average stocking density because the ewes were, initially, grazed on each pasture in large groups and alternated between paddocks.

Management of Group H ewes and Group L ewes together

After 112 days of grazing in Paddock 2, the ewes were divided into two equal groups at random, after stratification on age. One group continued to graze Paddock 2 for the duration of the study (a further 527 days) while the other group was placed in Paddock 3 and remained there for the next 527 days. At the time of introduction into Paddock 3, it had been ungrazed for 112 days, before which it had received a total of 456 sheep-days per ha of contamination over a continuous 74 day period. From Day 175 onwards, both groups of ewes grazed the same pastures.

Table 2 History of exposure of previously unexposed ewes to OJD-contaminated paddocks. Paddocks are numbered 2, 3, 6, 7, 8 and 9.

Day of study:	2 - 27	28[b] - 118	119 - 175	176 – 288	289 - 816
High exposure ewes (H)	Clean pastures	Contaminated pastures (paddocks 8 and 9)	8 and 9	2	2 and 3
Low exposure ewes (L)	Clean pastures (paddocks 6 and 7)		2	2	2 and 3

Collection of specimens

Faecal samples were collected from the ewes in October 2000 (Day 440 – 450) and at slaughter in October 2001 (Day 816). Tissues of intestinal tissue and lymph nodes were collected at slaughter as described for the experimental sheep in Part 1 of the study.

Husbandry procedures

Husbandry procedures for the ewes before Day 175 are described in the main body of the report. After that time, the ewes were wormed once (with ivermectin on Day 711). Shearing was performed in April of 2000 and 2001 in the mobile shearing plant.

Statistical analysis

Logistic regression (SPSS version 11.5 for Windows) was used to examine the significance of replicate, ewe age and exposure history (high or low) on the likelihood of positive histopathology at slaughter. Infection rates between Group H ewes and UHL lambs were compared using the chi-squared test and Fisher's Exact test.

Estimation of M ptb exposure

The use of decay rates for estimating the survival of *M ptb* bacteria on pasture and, therefore, the availability of viable *M ptb* to grazing sheep, was discussed in the main body of the report.

Briefly, we used three different decay rates - one, three and five logarithms per month (28 days) – to predict the highest, most-likely and lowest level of survival of the organisms, respectively.

M ptb availability was expressed in terms of an index of pasture contamination related to the number of known-infected Flock I ewes that had grazed, and were grazing, the pasture. The index was in units of faecal culture-positive ewes per ha per day, abbreviated to E/D.

[b] The first day in each series represents the day after the sheep were introduced; ie, in this case the sheep grazed from Day 28 to Day 118 *inclusive*.

Results

The predicted *M ptb* availabilities for the paddocks and grazing periods described in Table 1 and Table 2 are indicated in Table 3.

Table 3 Areas under the curve (E/D) for M ptb availability during the grazing periods for Group H ewes (top), Group L ewes (middle) and ewes of both groups (bottom). Results are shown for decay rates of one, three or five logarithms every 28 days.

High exposure (Group H) ewes

Paddock	Days	Replicate 1			Replicate 2		
		1 log	3 logs	5 logs	1 log	3 logs	5 logs
8	28-118	205	50	26	175	42	19
9	28-118	114	26	14	274	82	44
8	119-175	0.3	$<10^{-6}$	$<10^{-14}$	0.1	$<10^{-6}$	$<10^{-14}$
9	119-175	0.2	$<10^{-6}$	$<10^{-14}$	0.3	$<10^{-6}$	$<10^{-14}$

Low exposure (Group L) ewes

Paddock	Days	Replicate 1			Replicate 2		
		1 log	3 logs	5 logs	1 log	3 logs	5 logs
2	119-175	18.9	0.12	$<10^{-3}$	23.4	0.2	$<10^{-3}$

Both Group H and Group L ewes

Paddock	Days	Replicate 1			Replicate 2		
		1 log	3 logs	5 logs	1 log	3 logs	5 logs
2	176-288	0.2	$<10^{-6}$	$<10^{-15}$	0.2	$<10^{-6}$	$<10^{-15}$
2	289-816	$<10^{-4}$	$<10^{-15}$	$<10^{-15}$	$<10^{-4}$	$<10^{-15}$	$<10^{-15}$
3	289-816	$<10^{-1}$	$<10^{-10}$	$<10^{-15}$	$<10^{-1}$	$<10^{-10}$	$<10^{-15}$

Incidence of OJD infection in the ewes

Group H vs Group L

A total of 204 sheep were examined at death or slaughter by histopathology, faecal culture or both. Twenty six sheep were lost to follow up because data of their exposure history was lost – 21 because their ear tags were lost and five because their exposure histories were unknown.

Of the 204 for which exposure history and post-mortem information existed, three died before the trial ended. All three were faecal culture-negative for *M ptb*. Histopathology was performed on one only and it was negative for OJD. The remaining 201 sheep were examined at slaughter and histopathology was performed on all but seven. A faecal culture result was obtained for all except 19. Prevalence of infection at death or slaughter within groups was calculated from the population (194) which were examined with histopathology (**Error! Reference source not found.**). Seven animals (3.6%) were positive. Only one positive faecal culture (0.5%) was detected – from an animal which was also positive at histopathology.

Of the 26 cases lost to follow up, none was faecal culture positive and one (3.8%) was histopathology positive – with a type 1 lesion.

There was no statistically significant difference in the prevalence of sheep with positive histopathology between replicates (3.4% and 3.7% for type 1 and type 2 respectively) and no difference between three year old and four year old sheep (4.0% and 3.2% respectively). The odds ratio for infection in high-exposure sheep compared to low exposure sheep was 5.87 (95% confidence intervals 0.7, 50.0) The probability that exposure to high levels of infection was a significant predictor of infection approached significance at the .05 level (P=.054).

There was only one sheep in the low exposure group which had a lesion of OJD and the lesion was mild (type 1). By contrast, the six sheep with lesions in the high exposure groups included three with type 1 lesions, and one each with a type 2, 3a and 3b lesion. The sheep with a type 3b lesion had positive faecal culture. One sheep with a type 1 lesion had no faecal culture result; the other five animals were faecal culture negative.

Table 4. OJD infection rates in sheep exposed at high or low levels to OJD contamination on pastures.

Replicate	Age	Exposure level	N	N with +ve faecal culture	N with +ve histo-pathology[¶]	N with -ve histo-pathology	Prevalence[§] (%)
1	3	High	27	1	1 (3b)	24	4.0[¥]
1	4	High	26	0	2 (1,2)	22	8.3
2	3	High	30	0	2 (1, 3a)	26	7.1
2	4	High	24	0	1 (1)	23	4.2
Sub-total			107	1	6	95	5.9%
1	3	Low	21	0	0	20	0
1	4	Low	21	0	0	18	0
2	3	Low	27	0	1 (1)	26	3.7
2	4	Low	28	0	0	28	0
Sub-total			97	0	1	92	1.1%

[¶]The lesion types are shown in parentheses (Perez et al, 1996). [§]Prevalence is calculated from the numbers of animals for which histopathology was performed. [¥]The sheep with positive histopathology (type 3b) was also faecal culture positive.

Discussion

Group H vs Group L

It was not possible in Part 2 of this study to provide zero-contamination paddocks for the ewes after weaning. If that had been possible, there would have been a near-perfect match of *M ptb*-exposure between the Group H ewes in Part 2 and the UHL lambs in Part 1. Knowing that was not possible, Group L ewes were retained and run with the Group H ewes so that it was possible to account for the effects of low levels of *M ptb* exposure in the post-weaning period.

Thus, from Day 175 to Day 816, Group L ewes and Group H ewes were run together in Paddocks 2 and 3 in each replicate. The predicted levels of exposure to *M ptb* on pasture in that period were exceedingly low (Table 3 , bottom).

There was a period, however, between Days 118 and 175 when Group H ewes were run in pastures which were predicted to be of a higher level of contamination than the post-weaning pastures of UHL lambs, albeit the levels were still extremely low in comparison to the levels experienced before weaning.

Over this same period, Group L ewes were run in paddocks which were predicted to be of higher levels of *M ptb* availability than those paddocks containing Group H ewes (Table 3, top vs middle). Although the levels were low, this period probably still represented the greatest *M ptb* challenge to Group L ewes during their entire period on the site.

One other possible source of exposure for Group L ewes requires acknowledgement. As discussed in the main body of the report, Paddocks 1.6b and 2.6b were both possibly contaminated by stray sheep in the pre-weaning period. The only infected ewe identified in Group L had been grazing 2.6b before weaning.

Consequently, the Group L ewes were not a perfect control for the Group H ewes for it is likely that they experienced a higher level of exposure in the pre-weaning period (Paddocks 1.6b and 2.6b) and the immediate post-weaning period than Group H. The low level of infection (1/97) in Group L is, therefore, an interesting result. Because it represents the outcome of exposure in common with Group H for most of the post-weaning period, plus some additional exposure, it does infer that the post-weaning exposure of Group H was likely to have been responsible for no more than one of the six cases observed in that group (**Error! Reference source not found.**).

We can conclude, therefore, that the period of exposure of Group H ewes to Paddocks 8 and 9 during the pre-weaning period was responsible for at least five cases of OJD (the difference between the number infected in Group H and Group L) and, at most, six. In conclusion, it can be stated that exposure of ewes aged three and four years to *M ptb*-contaminated pasture for a period of 90 – 150 days resulted in infection rates of 4.7% to 5.6% when slaughtered 21 months later.

Incidence of infection in Group H ewes vs infection in lambs

Ewes in Group H were the dams of lambs which formed Group UHL and UHH for Part 1 of the study. While Group H ewes grazed Paddocks 8 and 9 in each replicate starting on Days 25 – 28 (they were added progressively over three days) and finishing on Day 118, their lambs were born and raised to weaning in the same paddocks over the same time period. Lambing occurred in these paddocks between Days 25 and 67. While the majority of lambs were born in the first week of that period, some lambs had less exposure to the contaminated pasture than their dams did. Nevertheless, for consistency with the main experiment, the median lamb was considered to have been born on Day 28. In Paddocks 8 and 9, however, the median lamb may have been a few days later than that.

Group H ewes remained in Paddocks 8 and 9 for a further 56 days after weaning (Days 119 – 175). This was predicted to add to their *M ptb* exposure history by only very small amounts (Table 3).

The UHL lambs grazed in paddocks which were considered to be free of *M ptb* infection from day 119 onwards at least until shedding of *M ptb* bacteria from some infected animals commenced some months after weaning.

Of Group H, 5.6% had OJD lesions, compared to 6.0% of UHL (Table 4). There was one year between the post-mortem examinations of the two groups so the possibility exists that more Group H ewes may have either developed infections or resolved infections had they been left alive for a further year. Given the data that are available, however, it appears that the incidence of persistent OJD histopathological lesions is similar in both groups. Similarly for severe lesions (types 3a and 3b) two adults and one lamb were affected. Although numbers are small, there is nothing to suggest that sheep exposed only as lambs in the pre-weaning are at particular risk of severe lesions compared to sheep exposed as adults.

There is an overall difference in infection rates between the two groups which is statistically significant (Group H 5.6%, UHL 16.4%, P=0.02). This difference, however, resulted from the diagnosis of seven (10.4%) UHL animals based on positive tissue culture in the absence of histopathological lesions. Tissues from ewes were not cultured (only faeces were cultured) so cases of ewes with negative HISTO and positive tissue culture may have been missed. It is safer to conclude that there is no difference in infection rates between the two groups.

It was decided to test faeces from Group H at slaughter, rather than culture tissues, as both a cost-saving measure and to enable a comparison of faecal culture positive rates between Group H and UHL from faeces collected in the same month. Faeces were collected from UHL in October 2001 (FS12) but they have been stored and have not been cultured. Faeces collected in February 2001 (FS8) were cultured and provided BACT18M results. In UHL, three sheep, all in Replicate 1, were BACT18M-positive. The difference in proportion was not statistically significant (P=0.15). If subsequent testing of FS12 indicated that a higher proportion of UHL sheep were positive at FS12 than were at FS8, the comparison may indicate a statistically significantly higher risk of faecal shedding amongst sheep exposed first as lambs, compaed to those first exposed as adults. It would require that approximately 10% of UHL were positive at FS12 before statistical significance could be achieved.

Table 4 Comparison of OJD infection rates in sheep first exposed to *M ptb* as lambs (UHL group from Part 1) and sheep exposed as adults (Part 2). The UHL sheep were examined at slaughter one year later than the adult sheep.

			Lesion type						
	Replicate	**N**	**0/1[¶]**	**1**	**2**	**3a**	**3b**	**3c**	**Neg**
Group H ewes	1	53	0	1	1	0	1	0	50
	2	54	0	2	0	1	0	0	51
	Both (%)		**0%**	**2.8%**	**0.9%**	**0.9%**	**0.9%**	**0%**	**94.4%**
UHL sheep	1	32	4	1	1	0	0	0	26
	2	35	3	1	0	0	1	0	30
	Both (%)		**10.4%**	**3.0%**	**1.5%**	**0%**	**1.5%**	**0%**	**83.6%**

[¶] 0/1 refers to animals with negative HISTO results but positive culture. In the case of Group H ewes, culture was performed on faeces collected at slaughter while, in the case of UHL sheep, it was performed on intestinal and lymph node tissues.

Conclusions

The evidence from Part 2 for any significant level of age resistance in Merino sheep is weak. While there was a general trend towards lower levels of infection in sheep exposed as adults compared to sheep exposed as lambs, the differences were small and not statistically significant and not sufficiently large to be of practical benefit in the management of OJD.

Appendix 4

Plates

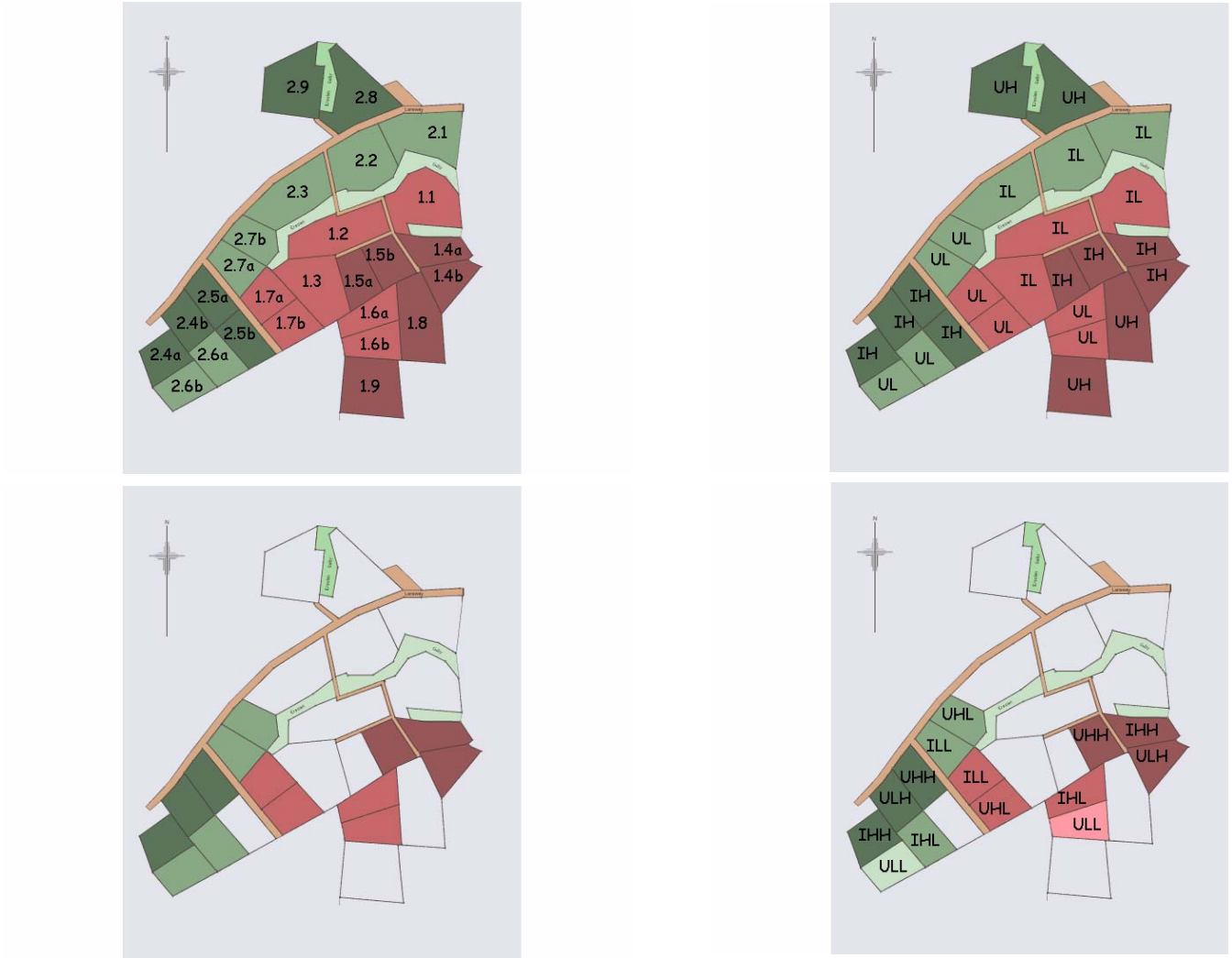

Plate 1. The experimental site. The site consisted of 18 10-ha paddocks, some of which were sub-divided into 5-ha paddocks. **Top left**; replicate 1 is in brown, replicate 2 is in green. **Top right**; pre-weaning paddock allocation. Light-shaded area was free of contamination to weaning while dark-shaded area was contaminated by infected ewes before weaning. **Bottom left**; the 14 coloured 5-ha paddocks are the only ones grazed after weaning. **Bottom right**; post-weaning paddock allocation. Only two paddocks held unexposed sheep (ULL) post-weaning.

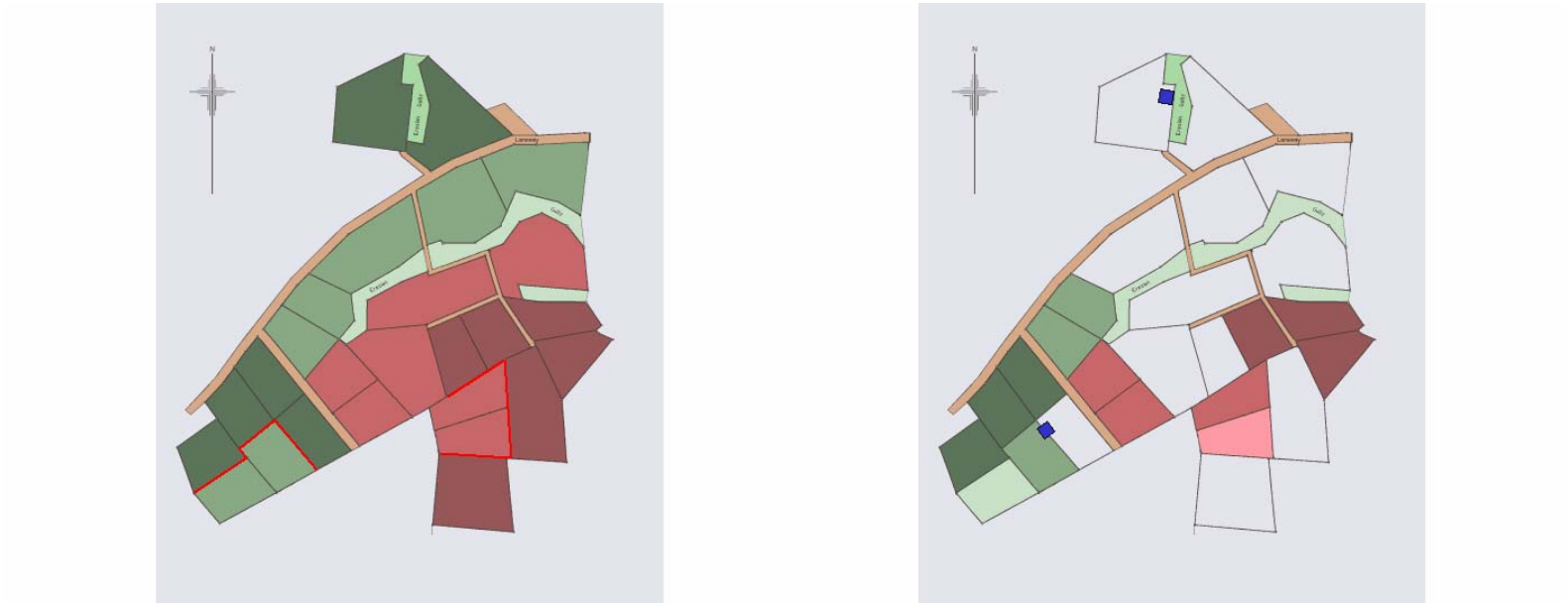

Plate 2. The experimental site. **Left**; shade cloth (red line) was used to separate high-contamination paddocks from low-contamination paddocks if they were contiguous. **Right**; two dams (blue boxes) provided water which was reticulated to all paddocks on the site.

Plate 3. Movement of Flock I ewes on the site. **Left**; ewes were delivered in groups to three points on the site (solid blue circles) then dispersed from those points during the contamination phase prior to lambing. **Right**; Some flock I ewes were moved into paddock 1 in each replicate immediately before lambing and then moved twice during the pre-weaning phase. Movements between paddocks occurred either through adjacent gateways, under fences or by trailer.

Plate 4. **Top left**; Shade-cloth was placed on the fences of high risk paddocks to reduce the movement of wind-borne faeces onto low contamination pastures. **Top right**; 180 Flock I ewes were unloaded over the fence into Paddock 9, Replicate 1, on their arrival on day 0. **Bottom left**; when supplementary feed was required it was fed onto the ground through the fence. Paddocks 4a (foreground) and 6b, Replicate 2, at the south-west end of the site illustrated. **Bottom right**; Shearing was performed in a mobile trailer taken to each paddock.

Appendix 5

Milestone 15 – Final report detailing subclinical effects of OJD

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Abstract

Wool growth and serum biochemical parameters were measured over time in sheep naturally exposed to OJD at pasture. Only serum albumin levels were consistently associated with the development of clinical signs of OJD. There were inconsistent changes in other serum chemistry parameters in groups of sheep that later developed severe intestinal lesions likely to lead to clinical OJD, compared to sheep with mild lesions that were unlikely to lead to clinical OJD by 3 years of age. Rates of wool growth, and greasy fleece weights were not related consistently to increasing severities of OJD lesion. These findings suggest that the biochemical disturbances which must underlie body weight loss in clinical Johne's disease cannot be predicted usefully in sheep with sub-clinical OJD using conventional measures. Low serum albumin levels can be the result either of reduced synthesis of albumin for example due to reduced protein intake, or increased loss from the body, for example through gastrointestinal tract lesions, but the cause remains elusive in OJD. The pathogenesis of OJD is more complicated than hitherto suspected. OJD is a systemic disease with manifestations beyond the intestinal tract.

Project objectives

Measure and describe the changes in protein and calcium metabolism and productivity in animals affected with OJD during the pre-clinical phase of the disease and, in those where the disease progresses, during the progression from sub-clinical to the terminal phase.

Develop a model for disease progression using biochemical indicators of pathogenesis to indicate the stage and severity of infection.

Develop a better understanding of effects on animals of the pre-clinical disease process; both the physiological and production effects.

Investigate and determine the application of this information as a predictive tool in research and disease management.

Success in achieving milestone:

The milestone was achieved. The lack of significant alteration in serum biochemistry apart from albumin precluded development of a model for disease progression. Low serum albumin concentrations alone were associated with development of severe intestinal lesions.

Overall progress of the project

Materials and methods

Classification of sheep

Sheep were selected from a larger group in trial OJD.002 which were exposed naturally at pasture to OJD-infected sheep. All sheep with positive histopathology and culture results were included in this study together with a random sample of control animals selected from the animals that had a histopathology score 0 and that were negative for Mptb on tissue culture. No animals with a histopathology score of 0 but positive on tissue culture were included in the study.

For the purposes of analysis of sub-clinical effects, sheep were grouped according to histopathological findings at the time of slaughter (when they were approximately 3 yr-old; based on lambing in Sept/Oct 99 and slaughter in Sept 02). Ear tags by group are shown in the Appendix while a summary of the groups is provided in Table 1. Histopathology was graded according to the scheme of Perez. Category 1 sheep with 0 scores for histopathology and culture of Mptb acted as controls. Sheep with sub-clinical lesions were in categories 2-4. It was assumed that sheep with histopathological lesion scores of 3b or 3c at slaughter would have developed clinical Johne's disease in the near future . Therefore these sheep were combined in categories 5 and 6 with sheep that developed clinical disease and died during the study. Many sheep in categories 5 and 6 had sub-clinical disease at the time of slaughter. All category 6 animals survived to the end of the trial and were slaughtered. Out of the original 24 category 5 animals only 9

survived to the end of the trial. Of these deaths, 9 occurred in 2002 (June-September) and 6 in 2001 (April-December).

Table 1. Classification of sheep

Category	Histopathology score	Culture of M. paratuberculosis from ileum and mesenteric lymph node	*No. of sheep
1	0	0	15
2	1	11	15
3	2	5	12
4	3a	11	12
5	3b	18	24
6	3c	5	5

***Numbers of sheep are the maximum available for selection based on inclusion criteria. Actual numbers of samples collected at each time point changed over time due to deaths and incomplete muster.**

Productivity

Body weight changes of sheep over time were reported in the report for milestone 14.

Fleece weights and dye-banding. Greasy fleece weights were measured on three occasions at shearing in September 2000, 2001 and 2002 after the fleeces had been skirted, using a standard weigh-scale. Side-samples of shoulder wool were taken at shearing in 2001 and 2002. Samples were sent for analysis at a commercial laboratory (Riverina Wool Testers Pty Ltd). The parameters measured were micron, fibre diameter relative to flock mean, spinning fineness % break, yield, curvature, % fibres >30 micron and comfort factor

Wool growth was measured by dye-banding at 4 times (Table 2), which enabled the change in staple length over time to be monitored. The dye banding procedure used was as described by Chapman RE and Wheeler, JL., 1963.(Dyebanding: A technique for fleece growth studies. Austr .J. Sci. 26, p.53). Dyeband sections of shoulder wool were removed from fleeces at shearings in 2001 and 2002. Following collection, the dye band samples were examined for a representative group of staples. The staple length between dye bands was measured with a ruler after straightening the staple but maintaining the crimp. Three measurements were taken and averaged to account for slight differences in uptake of dye and cut of wool at shearings. To account for slight differences in age and genetic differences in wool growth potential for each animal, the ratio of wool growth in periods 2, 3 and 4 to that grown in period 1 was calculated as a measure of relative wool growth for each sheep in each period. Absolute staple length was also measured for each growth period and included in the analyses.

Table 2. Dye banding periods to estimate changes in wool growth

Period	Date of dye banding	No days growth	Time period
1	14.06.01-30.08.01	77	Jun 2001 – Aug 2001
2	12.10.01-16.01.02	96	Oct 2001 – Jan 2002
3	16.01.02-12.06.02	148	Jan 2002 – June2002
4	12.06.02-28.08.02	79	June 2002 – Aug 2002

Data sets were examined for outliers and none were found. For periods 2, 3 and 4, a Kruskal- Wallis analysis of variance was used to determine whether there were differences between categories of sheep in the amounts of wool growth relative to period 1. The other wool parameters were also analysed using this test.

Blood collection

Serum samples were collected at intervals from the sheep as described in the report for milestone 14. Briefly, blood was collected from all sheep present at 6 week to 3 month intervals from lamb marking until euthanasia of all sheep. For biochemical analysis, blood was collected into serum and lithium heparin tubes. Following collection, blood was stored on wet ice in the shade and transported in cool conditions to the laboratory as soon as possible. Serum samples were left to stand for 3 hours to allow formation of a clot prior to centrifugation. Samples were centrifuged at 2095 x g for 30 minutes. Sera and plasma were aspirated and stored at -20°C until required for analysis.

Serum biochemistry

Samples were submitted to a commercial laboratory (Mayne Veterinary Diagnostics) for multiple biochemical analyses. Data sets were examined for outliers but none were found. For each serum collection time point, a Kruskal-Wallis analysis of variance was used to determine whether there were differences between categories of sheep in the levels of serum biochemical constituents.

Results

Wool data

Sheep were 1 year old at the first shearing and 3 years old at the third. There were no significant differences in greasy fleece weight between sheep in the different categories at these times (Table 3). There were no significant differences between categories of sheep in rate of wool growth measured by dye bands (Table 4). There were some significant differences in wool measurements between sheep in different categories but these did not correlate with the severity of infection and for this reason are not regarded as having biological significance (Tables 5, 6).

Table 3. Greasy fleece weight of sheep in each category

Category	Shearing date	Shearing timepoint	Mean weight/kg	s.d.
0	13.09.00	1	3.191	0.821
1	13.09.00	1	3.177	0.795
2	13.09.00	1	3.158	0.763
3a	13.09.00	1	3.214	0.856
3b	13.09.00	1	3.119	0.687
3c	13.09.00	1	3.190	0.691
0	12.10.01	2	4.677	0.653
1	12.10.01	2	4.670	0.593
2	12.10.01	2	4.682	1.037
3a	12.10.01	2	4.895	0.760
3b	12.10.01	2	4.731	0.987
3c	12.10.01	2	4.870	0.634
0	15.09.02	3	4.840	0.986
1	15.09.02	3	5.440	1.075
2	15.09.02	3	5.240	1.265
3a	15.09.02	3	5.050	0.986
3b	15.09.02	3	4.944	0.975
3c	15.09.02	3	5.275	1.066

Table 4. Mean rate of wool growth of sheep in all categories

Dyeband period	Days growth	Mean growth/mm	Mean growth rate mm/day	s.d.
1	77	21.676	0.282	0.095
2	96	34.100	0.355	0.038
3	148	38.971	0.259	0.054
4	79	10.299	0.128	0.059

Table 5. Wool measurements for the flock

Category	Shearing date	Mean	s.d.
Micron	12.10.01	18.305	1.568
Fibre Diam	12.10.01	-0.735	1.568
Spinning Fineness	12.10.01	17.887	1.451
% Break	12.10.01	62.89	11.68
Yield	12.10.01	75.163	4.175
% fibres > 30 micron	12.10.01	1.220	0.964
Curvature	12.10.01	96.734	6.637
Comfort factor	12.10.01	98.780	0.964
Number of sheep		72	
Micron	15.09.02	19.665	1.858
Fibre Diam	15.09.02	0.625	1.858
Spinning Fineness	15.09.02	19.002	1.609
% Break	15.09.02	57.98	12.47
Yield	15.09.02	74.228	4.004
% fibres > 30 micron	15.09.02	1.579	1.647
Curvature	15.09.02	93.483	6.752
Comfort factor	15.09.02	98.421	1.647
Number of sheep		60	

Table 6. Median values for wool parameters that differed significantly between categories of sheep, with Kruskal-Wallis P values.

Parameter	Shearing timepoint	P	Histological category					
			0	1	2	3a	3b	3c
Micron	1	0.015	18.1	19.41	17.17	17.94	18.75	17.39
Fibre Diam	1	0.015	-0.95	0.37	-1.87	-1.1	-0.29	-1.65
Spinning Fineness	1	0.029	17.69	18.66	16.88	17.53	18.48	16.72
% Break	1	0.095	68	65	62	62	65	57
Number of sheep	1		14	13	12	11	8	5
% Break	2	0.024	64	45	56	62	58	71
Number of sheep	2		14	13	10	11	8	5

Note: fibre diam measurements are calculated in relation to flock average micon and so can include negative values

Serum biochemical data

Normal values for serum biochemical data provided by the commercial laboratory are provided in Table 7 together with the range observed for each parameter in this study. The results from stored samples from sheep in this study in general were close to the normal ranges, confirming that sample storage and handling was adequate and did not compromise the analyses. Selenium and zinc concentrations overall appeared to be lower than the normal ranges provided by Radostits et al. (Table 7) but additional fresh samples from sheep from the property will need to be tested to determine whether the levels are truly low overall or whether they are the result of sample storage.

Only one serum constituent differed significantly between categories of sheep in two or more periods, serum albumin (Table 8). Serum albumin levels were significantly lower in sheep with severe lesions that would lead to clinical disease. This was apparent before the onset of clinical disease (Figure 1). Suppressed albuimin levels occurred well before death. In the lower panel of Figure 1 sheep that died with clinical OJD have been removed from the data, and albumin levels are shown only for animals with subclinical lesions. Albumin levels in sheep with severe lesions that survived to the end of the trial were significantly lower than those in controls at the last time point ($P<0.001$), and were trending lower than those in controls at earlier time points (Figure 1).

Low serum albumin levels can be the result either of reduced synthesis of albumin, for example due to reduced protein intake, or increased loss from the body, for example through gastrointestinal tract lesions, but the cause of low albumin levels in OJD is uncertain.

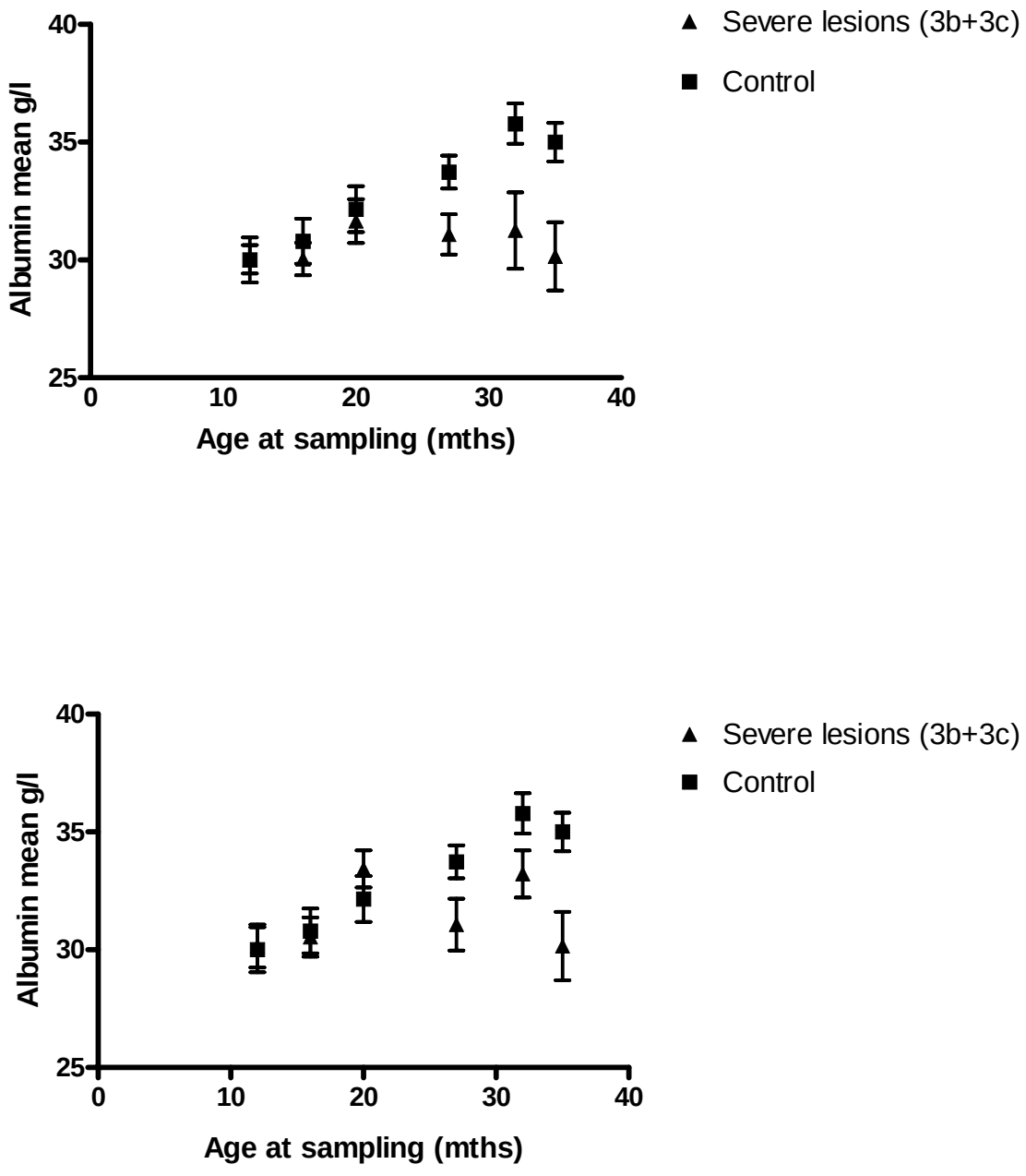

Figure 1. Serum albumin levels (mean and standard deviation) in control sheep and sheep with severe OJD lesion s.(n=13-15, 13-28 respectively). Top panel – all sheep. Bottom panel – sheep that died with clinical OJD removed.

Table 7. Range of means for serum biochemical parameters for sheep in each histological category, and normal values provided by the commercial laboratory unless otherwise indicated in the footnote.

Parameter	Units	Normal range	Histological category											
			0		1		2		3a		3b		3c	
			Min	max	min	max	min	Max	min	max	min	max	min	max
Sodium	mmol/L	130-142	136.50	154.30	142.43	153.92	144.00	157.42	140.30	152.25	141.14	149.95	119.00	154.50
Chloride	mmol/L	97-111	99.80	106.60	99.80	105.90	101.60	109.00	100.00	105.17	99.48	107.78	81.60	106.00
Urea	mmol/L	5.0-9.5	8.37	11.85	7.64	11.77	8.34	10.71	9.11	12.11	8.54	12.74	9.53	13.82
Creatinine	mmol/L	0.07-0.16	0.06	0.09	0.05	0.08	0.05	0.08	0.05	0.08	0.05	0.07	0.06	0.09
Glucose**	mmol/L	2.2–4.4	2.31	4.85	2.38	3.80	2.12	4.11	2.37	4.38	2.35	4.73	2.04	5.12
Bilirubin	mmol/L	1-20	1.00	2.15	1.27	2.25	1.08	1.92	1.00	1.70	1.16	2.11	1.20	1.88
AST**	U/L	60 – 280	72.93	92.07	76.93	109.17	77.58	98.08	70.55	92.92	76.57	93.89	78.20	98.40
GGT**	U/L	20-52	49.07	58.07	46.40	56.25	51.08	57.50	52.58	64.17	45.00	54.26	50.00	57.75
ALP**	U/L	70 - 390	76.07	148.87	54.75	145.53	51.08	135.92	60.67	142.75	48.67	142.32	57.00	128.20
T_Protein	g/L	58-77	67.47	78.07	62.47	73.79	66.41	76.08	65.91	75.22	67.65	76.43	67.40	79.60
Albumin	g/L	24-30	30.00	35.79	30.60	35.41	30.92	37.08	29.17	32.18	29.00	31.19	29.80	33.80
Globulin	g/L	28-50	37.47	45.92	31.87	42.60	35.17	44.83	36.75	44.11	37.57	45.24	37.60	46.00
Calcium	mmol/L	2.00-3.10	2.40	2.60	2.35	2.73	2.52	2.65	2.34	2.54	2.03	2.51	2.28	2.50
Phosphate	mmol/L	1.69-2.49	1.69	2.39	2.10	2.40	2.11	2.54	1.84	2.49	1.81	2.56	1.66	2.10
CPK	U/L	<258	97.80	217.70	150.70	194.50	109.80	472.70	145.50	286.10	100.60	224.20	53.50	173.40
S_Glutamate	U/L	6-10	3.73	14.62	5.08	13.71	5.17	16.33	4.58	15.67	4.00	19.14	3.00	19.00
S_Iron**	μmol/L	18 – 36	18.70	34.30	16.83	35.40	17.92	33.67	20.58	31.83	19.20	29.26	20.75	24.60
Fructosamine	mmol/L	n.a.	266.67	371.70	286.13	432.20	265.17	422.70	253.40	379.50	259.05	418.00	234.20	430.00
S_B_Hydroxybutyrate*	mmol/L	0.2 – 0.6	0.25	0.46	0.26	0.53	0.26	0.48	0.32	0.42	0.32	0.52	0.24	0.44
S_Copper*	μmol/L	>8.5	11.71	14.47	10.58	14.30	11.75	14.77	10.41	12.90	9.37	15.09	8.82	14.24
P_Selenium**	μmol/L	1.5 – 1.9	0.66	1.03	0.45	1.02	0.50	1.04	0.53	0.93	0.62	0.90	0.64	1.12
Zinc**	μmol/L	12 – 18	7.66	12.10	7.81	12.93	7.74	12.58	8.07	12.76	7.07	12.12	6.60	12.14

* NSW DPI

** Radostits et al (2000). Vetrerinary Medicine. WB Saunders Co, London.

n.a. not available

Table 8. P values for biochemical parameters that differed significantly in Kruskal Wallis tests. Data are shown for comparisons of all categories of sheep (all), and for comparison where sheep in categories 3b and 3c were combined so that all sheep with severe lesions were in the same group (3b+3c).

Timepoint Histological category Parameter	1 all	2 all	3 all	4 all	4 3b+3c combined	5 all	5 3b+3c combined	6 all	6 3b+3c combined
Creatinine				0.044					
Glucose			0.051						
Albumin							0.05	0.002	0.001
Phosphate								0.016	
CPK				0.023					
S_Iron				0.009	0.004				

Publications

McGregor H, Abbott KA, and Whittington RJ. "Development of grazing management stratergies for the control of ovine Johne's disease." Australian Sheep Veterinary Society Conference Proceedings May 2004; 60-65

Abbott KA, McGregor H, Whittington RJ. " Control of Ovine Johne's disease by management of lambs – preliminary results of a field study." Australian Sheep Veterinary Society Conference Proceedings May 2003; 90-95

Recommendations

A paper to be prepared for publication in a refereed scientific journal.

Appendices

The appendix contains the ear tags and histological findings in each sheep included in this study.

Report prepared by:

R Whittington and H MGregor
23rd December 2004

Appendix

Table A1. Animals selected from trial OJD.002 for inclusion in this study of subclinical effects.

Tag No	Last date known alive	Histopath score	Culture result	Replicate	Group
7761	10/09/2002	ns		2	IHL
1141	16/03/2001	aut		1	UHH
7838	11/12/2001	aut		2	ILL
1577	10/09/2002	3c	MP	2	UHH
7636	10/09/2002	3c	MP	1	IHH
7667	10/09/2002	3c	MP	1	IHH
7748	10/09/2002	3c	MP	2	IHH
7848	10/09/2002	3c	MP	2	ILL
1023	9/05/2002	3b		2	UHH
1044	15/03/2002	3b		1	UHH
1176	22/03/2002	3b	MP	1	ULH
1224	10/09/2002	3b	MP	1	ULH
1334	26/06/2002	3b	MP	2	ULL
1337	10/09/2002	3b	MP	2	ULH
1532	6/12/2001	3b	MP	2	UHH
1539	10/09/2002	3b	MP	2	UHL
1559	3/08/2001	3b	MP	2	UHH
1568	10/05/2002	3b		2	UHH
7601	12/03/2002	3b	MP	1	IHH
7651	5/09/2002	3b		1	IHL
7653	2/10/2001	3b	MP	1	IHL
7723	10/09/2002	3b	MP	1	ILL
7732	31/07/2001	3b	MP	1	ILL
7757	10/09/2002	3b	MP	2	IHH
7759	8/02/2002	3b		2	IHL
7771	10/09/2002	3b	MP	2	IHH
7784	28/06/2002	3b	MP	2	IHH
7796	19/04/2002	3b		2	IHL
7797	15/06/2001	3b	MP	2	IHH
7806	11/04/2001	3b	MP	2	IHH
7828	10/09/2002	3b	MP	2	ILL
7850	10/09/2002	3b	MP	2	ILL
1061	10/09/2002	3a	MP	1	UHH
1173	10/09/2002	3a	MP	1	ULH
1218	10/09/2002	3a	MP	1	ULL
1465	10/09/2002	3a	MP	1	ULL
1558	10/09/2002	3a	MP	2	UHH
7649	10/09/2002	3a	MP	1	IHL

Tag No	Last date known alive	Histopath score	Culture result	Replicate	Group
7705	10/09/2002	3a	MP	1	ILL
7749	10/09/2002	3a	MP	2	IHH
7770	5/09/2002	3a		2	IHL
7800	10/09/2002	3a	MP	2	IHH
7818	10/09/2002	3a	MP	2	ILL
7854	10/09/2002	3a	MP	2	ILL
1063	10/09/2002	2		1	UHL
1193	10/09/2002	2		1	ULL
1556	10/09/2002	2	MP	2	UHH
7613	10/09/2002	2		1	IHL
7616	5/09/2002	2		1	IHL
7617	10/09/2002	2		1	IHH
7619	5/09/2002	2		1	IHH
7635	10/09/2002	2	MP	1	IHL
7673	10/09/2002	2	MP	1	IHL
7750	10/09/2002	2	MP	2	IHH
7758	10/09/2002	2		2	IHH
7843	10/09/2002	2	MP	2	ILL
1010	10/09/2002	1		2	UHH
1043	10/09/2002	1	MP	2	UHH
1076	10/09/2002	1		1	UHL
1425	10/09/2002	1	MP	2	ULL
1496	10/09/2002	1	MP	1	ULL
7607	10/09/2002	1	MP	1	IHL
7608	10/09/2002	1	MP	1	IHH
7625	10/09/2002	1	MP	1	IHL
7629	10/09/2002	1	MP	1	IHL
7647	10/09/2002	1	MP	1	IHL
7659	10/09/2002	1	MP	1	IHL
7665	10/09/2002	1	MP	1	IHL
7683	10/09/2002	1	MP	1	IHL
7690	10/09/2002	1		1	IHL
7823	10/09/2002	1		2	ILL
1051	10/09/2002	0		1	UHL
1096	10/09/2002	0		1	UHL
1144	10/09/2002	0		1	UHH
1228	10/09/2002	0		1	ULH
1367	10/09/2002	0		2	ULH
1412	10/09/2002	0		2	ULH
1491	10/09/2002	0		1	ULH
1538	10/09/2002	0		2	UHL
1595	10/09/2002	0		2	UHL

Tag No	Last date known alive	Histopath score	Culture result	Replicate	Group
7644	10/09/2002	0		1	IHH
7699	05/09/2002	0		1	ILL
7733	10/09/2002	0		1	ILL
7775	10/09/2002	0		2	IHH
7814	10/09/2002	0		2	IHH
7856	10/09/2002	0		2	ILL

Histopathology score according to Perez; MP – mptb isolated