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Changes in within-flock prevalence of *Mptb* shedding following vaccination with Gudair® in high & low prevalence flocks

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The University of Sydney

Abstract

Project OJD.033 was a longitudinal observational study examining the efficacy of Gudair™ in decreasing the prevalence of shedding of infectious organisms following the commencement of vaccination in flocks varying in initial OJD prevalence. Twelve flocks were examined over a 6 year period, 3 with high, 5 with medium and 4 with low OJD prevalence at the commencement of the study. Changes in the prevalence of shedding as the proportion of vaccinates in the flock increased were estimated and it was found that vaccinates had significantly lower prevalence of shedding than unvaccinated sheep (0.63% versus 1.66%; $P<0.001$). However a significant observation was that at the final sampling when all adult sheep had been vaccinated as lambs, a total of 10 of the 11 flocks still in the study had sheep with detectable shedding, ranging from 0.13% to 1.29%. The persistence of shedding for an extended period following onset of vaccination presents a risk for spread and recrudescence of OJD. It is recommended that the study be continued for a further 3 rounds of testing to provide additional data on the rate of decline of shedding in flocks now composed entirely of 'second generation vaccinates'. This will assist sheep producers to assess the risks of ceasing vaccination and purchasing vaccinated re-stocker sheep.

Executive Summary

Ovine Johne's disease (OJD) is a fatal enteric infection of sheep by *Mycobacterium avium* subspecies *paratuberculosis* 'S' strain (*Mptb*) that has proven difficult to both diagnose and control. Collaborative research in project OJD.009 demonstrated the efficacy of vaccinating lambs between 1 and 4 months of age with Gudair^{TM}, a killed whole cell vaccine imported from Spain, for controlling OJD in high prevalence Australian sheep flocks. Vaccination reduced the prevalence of mortality by 90%, delayed the onset of faecal shedding of *Mptb* by 12 months, and reduced the prevalence of shedders by 90% compared to unvaccinated lambs. This study led to the registration of Gudair™ and it is now established as the key strategy to control the disease and manage the risk of transmission of OJD both within and between flocks.

The original vaccine research in Australia (OJD.009) was conducted in the first cohort of vaccinates from 3 flocks that were considered to be heavily infected, with presumed exposure of intra-uterine and neonatal lambs of infected ewes to significant *Mptb* challenge. However following registration of Gudair™ many lower prevalence flocks also commenced vaccination as a precaution against increased mortalities and as a means to improve their ability to sell re-stocker sheep through the risk based trading Assurance Based Credit (ABC) point scheme. It was suggested that the efficacy of the vaccine might be superior in flocks with low OJD prevalence where later drops of vaccinated lambs were exposed to sheep of a much lower risk of developing OJD than in the original study (OJD.009). Modelling work suggested that the prevalence of mortalities and shedding would fall rapidly after the commencement of a vaccination control program depending on disease prevalence at the time of commencing vaccination. Validation of this work by field research was required.

In this study (OJD.033) we report on the changes in the prevalence of shedding over the first 6 cohorts of vaccinates in 12 infected flocks of variable prevalence when they commenced vaccinating. The study determined the changes in shedding of *Mptb* in the 3-4year and 5-6year old cohorts in 2003-4, 2005-6 and 2007-8 following initiation of vaccination with Gudair™ in 1-4 month old lambs in 2002.

A significant decrease in shedding rates of *Mptb* were observed in the majority of flocks in the study. However we identified that shedding was detectable in 10 of the 11 flocks that remained in the study until 2008. The study provides evidence that Gudair™ vaccination is a valuable but imperfect tool in managing OJD and it is recommended that the study be continued for a further 3 rounds of testing to provide more accurate data on the decline of shedding rates in flocks composed entirely of 'second generation vaccinates' (sheep that are progeny of accredited vaccinates). The outcomes from the extension of these studies will greatly assist sheep producers to assess the risk of ceasing vaccination in their flocks and the risk of purchasing vaccinated re-stocker sheep.

Acknowledgements

This project required collaboration between the University of Sydney (UOS) and the Tablelands Livestock Health and Pest Authority (LHPA). The significant inputs of Dr Jeff Eppleston and his staff from the Tablelands LHPA were essential to the completion of the studies. Professor Richard Whittington and Dr Navneet Dhand of the UOS provided important contributions including advice on pooling of samples and calculation of prevalence estimates, and Dr Evan Sergeant of Ausvet Animal Health Services was involved in initiating and monitoring of the project and assisted in epidemiological design. Thanks are due to Anna Waldron and staff for laboratory support and to MLA for provision of project resources. The co-operation of the 12 property owners involved in the study is gratefully acknowledged.

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1 Background and Industry Context

1.1 Introduction

Johne's disease is an important disease of ruminants worldwide that is difficult to control and usually fatal. It is caused by enteric infection with *Mycobacterium avium* subspecies *paratuberculosis* (*Mptb*). Production losses through wasting and mortality due to Johne's disease have been significant in sheep in Australia. In addition, speculation of a potential zoonotic link with Crohn's disease of humans and hence food safety issues has further enhanced interest in both Bovine (BJD) and Ovine Johne's Disease (OJD). A National OJD Control and Evaluation Program (NOJDCEP) commencing in 1998, was completed in 2004. This program funded the collaborative research project OJD.009 that demonstrated the efficacy of vaccinating lambs between 1 and 4 months of age with Gudair™, a killed whole cell vaccine imported from Spain (Reddacliff *et al*, 2006). Vaccination reduced the prevalence of mortality by 90%, delayed the onset of faecal shedding of *Mptb* by 12 months, and reduced the prevalence of shedders by 90% compared to unvaccinated lambs.

The OJD.009 study led to the registration of Gudair™ for the control of OJD in Australian sheep flocks and it is now established as the key strategy to control the disease and manage the risk of transmission of OJD both within and between flocks. However as the original vaccine research in Australia (OJD.009) was conducted in a single cohort of vaccinates from 3 flocks that were considered to be heavily infected, with presumed exposure of intra-uterine and neonatal lambs of infected ewes to significant *Mptb* challenge, many questions have been raised regarding the likely efficacy of the vaccine in flocks of lower prevalence. It became apparent that many producers commenced vaccination in flocks of low OJD prevalence flocks and it was unclear what the impact of vaccination will be in these flocks.

From the OJD.009 study it was considered reasonable to expect that early intervention with vaccine will rapidly reduce prevalence of disease in flocks of high OJD prevalence. Preliminary observations on the prevalence of shedding in 2 year old sheep vaccinated as lambs on seven heavily OJD-infected farms demonstrated persistence of shedding but with a significant decline in the mean flock prevalence (0.55 +/- 0.006 versus 0.28 +/- 0.005%; $P<0.01$) between the 1999 and 2001 drop lambs (Eppleston *et al*, 2005). However data on the efficacy of vaccination in low prevalence flocks is lacking. It was suggested that the efficacy of the vaccine might be superior in flocks with low OJD prevalence where later drops of vaccinated lambs were exposed to sheep of at a much lower risk of developing OJD than in the original study (OJD.009). However is it possible that the effectiveness of vaccination is diminished in the absence of continual environmental challenge? The issue of the risk of disease occurring in vaccinates being dependent on the disease prevalence at the time of commencing a vaccination control program has became of increasing importance as OJD has continued to spread. As the efficacy of vaccination will become more evident over an extended period following vaccination of a number of successive lamb crops, it became apparent that a longitudinal study on the shedding rates of vaccinates in flocks of varying prevalence at the time of initiation of the vaccination program, was required.

Computer modelling of OJD vaccine outcomes uses assumptions on the efficacy of vaccination. It is assumed that the rate of disease reduction in vaccinated flocks will depend on both the efficacy of the vaccine (assumed 80%) and on the prevalence of disease at the commencement of vaccination (Sergeant, 2002). There has been a pressing need to test the conclusions from modelling by quantifying the rate of disease reduction following the commencement of vaccination. The modelling studies suggested that the prevalence of mortalities and shedding would fall rapidly after the commencement of a vaccination control program depending on disease prevalence at the time of commencing vaccination. Validation of this work by field research was required.

Following the registration of Gudiar™ vaccination in April 2002, most states have adopted limited regulation of the disease and promoted a self-declaration system for the risk of OJD spreading from their flocks via a risk based trading approach. The national Sheep Health Statement allocates Assurance Based Credit (ABC) points for the key risk factors in the spread of OJD, with up to 4 of the 12 points available, allocated for accredited vaccination. This led to flocks of lower OJD prevalence commencing vaccination programs as a precaution against increased mortalities and as a means to improve their ability to sell re-stocker sheep through the risk based trading Assurance Based Credit (ABC) point scheme.

These concerns led in late 2002 to the conclusion that it was timely to initiate a longitudinal study of *Mptb* shedding rates in 12 commercial flocks that had recently started a vaccination program. The aim was to provide data on the rate of decline in OJD risk over an extended period of time, hopefully demonstrating the elimination of detectable shedding of *Mptb* in a proportion of the vaccinating flocks. Project OJD.033 was initiated to investigate the *Mptb* shedding rates in 12 flocks of initial varying OJD prevalence over a 6 year period, by examining the changes in shedding of *Mptb* in the 3-4year and 5-6year old cohorts in 2004, 2006 and 2008 following initiation of vaccination with Gudair™ in 1-4 month old lambs in 2002. It was expected that the outcomes from this study would also assist sheep producers in assessing both the risk of cessation of vaccination in their flocks and the risk of purchasing vaccinated re-stocker sheep.

1.2 Project objectives

The project examined the use of Gudair™ to elicit changes over time in the prevalence of *Mptb* shedding following the commencement of vaccination in flocks varying in initial OJD prevalence. Up to 4 flocks each with high, medium or low OJD prevalence at the commencement of a vaccination program will be sampled over a 6-year period to estimate changes in the prevalence of shedding as the proportion of vaccinates in the flock increases. By December 2008 the project will have measured changes in the prevalence of shedding in OJD infected flocks and determined any effect of initial flock prevalence on the efficacy of vaccination with Gudair™. An expected outcome from the project will include prediction of the infectivity of flocks over time following the commencement of a vaccination program. It was considered that this information may assist decisions on the risks of trading vaccinated sheep, the timing of cessation of vaccination and the removal of a risk status from a flock.

2 Methods

2.1 Design

In the initial trial protocol up to 4 flocks in each of 3 initial disease prevalence categories (high, medium & low) were selected for monitoring changes in faecal shedding over the following 4-year period. Flocks were required to contain sufficient sheep in each age class up to 6 years of age as illustrated in Figure 1.

	Age in year:										
Year born	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
1996	4	5	6								
1997	3	4	5	6							
1998	2	3	4	5	6						
1999	1	2	3	4	5	6					
2000		1	2	3	4	5	6				
2001			1	2	3	4	5	6			
2002				1	2	3	4	5	6		
2003					1	2	3	4	5	6	
2004						1	2	3	4	5	6
2005							1	2	3	4	5
2006								1	2	3	4
2007									1	2	3
2008											

Figure 1. Design of sampling to determine changes in OJD prevalence following vaccination for flocks commencing vaccination in 2001 (grey area denotes Gudair™ vaccination).

As it proved difficult to find adequate numbers of flocks to place 4 flocks in each of the categories of high, medium and low prevalence (particularly in recruiting sufficient flocks of low prevalence), the sampling schedule was modified to enable each sampling to occur over a 2 year period. Sampling 1 occurred in 2003-4, Sampling 2 in 2005-6, and Sampling 3 in 2007-8. This strategy enabled a comparison of the shedding rates of *MPtb* from vaccinated and unvaccinated sheep as vaccinates moved through the flock, as per Figure 2.

Sampling Number		Age cohort (years of age at sampling)			
	Year	3	4	5	6
1	2003-4	Not vaccinated	Not vaccinated	Not vaccinated	Not vaccinated
2	2005-6	Vaccinated	Vaccinated	Not vaccinated	Not vaccinated
3	2007-8	Vaccinated	Vaccinated	Vaccinated	Vaccinated

Figure 2. Project sampling schedule demonstrating vaccination status as vaccinates move through the flock over time.

2.2 Selection and survey of flocks

An estimate of initial disease prevalence was carried out by Pooled Faecal Culture (PFC, using 7 pools of faeces from 50 sheep cultured by Bactec™ for *Mptb*) on more than the required 12 flocks to ensure sufficient flocks with appropriate prevalence without clustering of disease within age groups. A crude classification of initial prevalence was assessed as:

High: >5% mortalities, >4/7 positive pools
Medium: <5% mortalities, 2-4/7 positive pools
Low: mortalities not recognized, <2/7 positive pools

As the proportion of positive pools was used to determine the selection of farms, this data is shown below in the results, although the major analyses were conducted on animal prevalence derived from the pool data (described below). In addition participating flocks were selected on the following criteria:

1. Vaccination program has commenced in 2000 or 2001.
2. Only lambs were vaccinated in the first year.
3. High level of willingness and managerial ability in flock owner.
4. Self-replacing Merino flock lambing in excess of 500 ewes per year.
5. Ability to accurately identify age groups of sheep

As mentioned, due to difficulties in finding suitable flocks within the vaccination area, we were unable to find and complete sampling of the low prevalence flocks until 2004. This resulted in sampling from only 8 of the 12 flocks in 2003 and necessitated a delay by a year for completion of each round of testing, to enable the low prevalence flocks to be sampled. A variation to the initial contract was proposed and accepted to allow this alteration in sampling times and reporting.

In 2008 a survey of the 12 flocks was undertaken to record flock health management and subjectively assess the possibility that management practices in addition to vaccination may have contributed to the rate of *Mptb* shedding. This mail and phone survey compiled responses to a list of 20 questions relating to farm management factors. Key data collected included farm area, rainfall, percentage improved pasture, soil type, carrying capacity, micron, lambing patterns (autumn versus spring or both), peak OJD losses, risk of lateral spread, introductions, vaccination of wethers, age wethers sold, terminal lambs sold and use of vaccination contractors.

2.3 Faecal excretion prevalence and statistical analysis

Faecal samples for PFC were collected on 3 occasions from 11 flocks and on 2 occasions from one flock (Flock L3 withdrew from the trial in 2007). The samples were collected randomly from 3, 4, 5 and 6 year old female sheep from each flock and stored at –80°C until cultured. Faecal culture was by modified BACTEC 12B radiometric culture and direct confirmation by IS900 PCR using published methods (Whittington *et al*, 1998, 2000). The prevalence of sheep shedding *Mptb* in faeces was estimated from the total number of pools, number of pools positive and number of samples per pool using the pooled prevalence calculator (Sergeant, 2004). The sensitivity and specificity of the PFC for OJD in Australian sheep has been previously established (Sergeant *et al*, 2001) and calculations assessed using published modifications (Williams and Moffitt, 2001). The weeks of incubation required for cultures to develop peak growth index was also recorded, being inversely related to the number of viable organisms inoculated into the culture media, and so to shedding levels in sheep (Reddacliff *et al*, 2003).

As identified in the project proposal, statistical analyses of these data will be complicated by the lack of controls. Inclusion of control sheep, by either leaving some of the flock unvaccinated, or selecting non-vaccinating control flocks was discounted for practical and design reasons. The problem of not having an experimental control requires that the project is promoted as a longitudinal observational study only, with publication of results in an epidemiology or veterinary journal. Two main types of analyses were undertaken.

In the first set of analyses, animal level prevalence was calculated by the method described (William and Moffit, 2001) given that there were pools of variable size. Then using the following linear mixed model approach, the effect of age and year on prevalence was determined as:

- Outcome: Animal level prevalence
- Fixed effects: age group of sheep (3, 4, 5 or 6 years of age), year of sampling (1st, 2nd or 3rd) and their interaction [+/- sex was considered not significant but can be a confounder]
- Random effects: Flock and Sampling year

In the second set of analyses, the pool status was used as an outcome (0 or 1) and generalised linear mixed models were used to evaluate the trends in likelihood of a pool to be positive over the years:

- Outcome: Pool positive or negative
- Fixed effects: same as above + log pool size to account for variable pool sizes.
- Random effects: Flock and Sampling year and Vaccination Status

3 Results

3.1 Farm selection and survey

Of the 18 flocks involved in the initial round of sampling, 12 were chosen that best met the criteria, with the proportion of positive pools displayed in Figure 3. Consideration of mortality data resulted in designation of 3 High prevalence farms K, L, and I, 4 medium prevalence, farms B, G, E and C, and 5 low prevalence and farms J, F, M, A and D. Note that Flock D (L3) was included despite nil pools being positive in the PFC and this flock withdrew from the trial after sampling 2, preventing a complete set of data to be collected at Sampling 3.

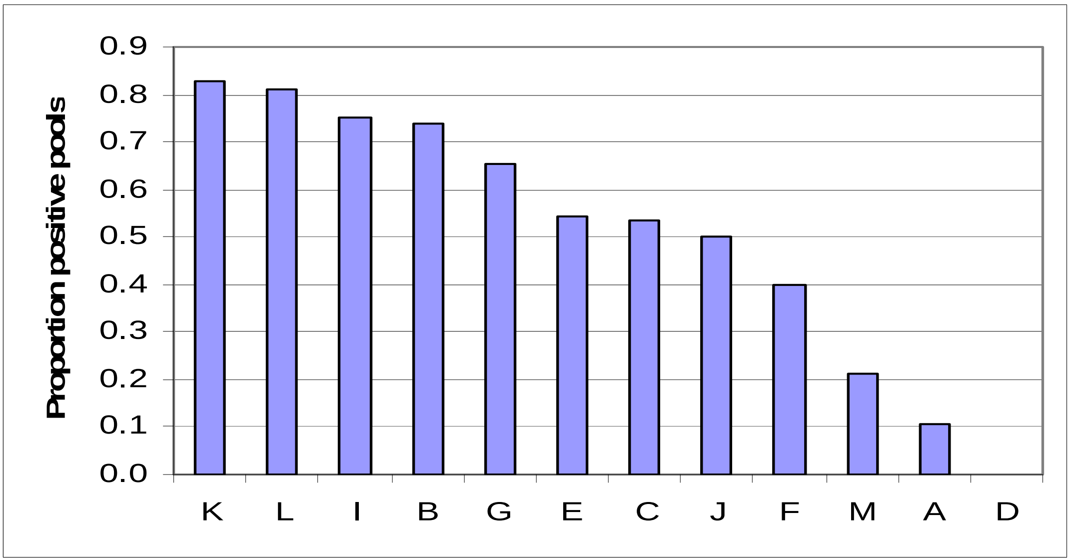

Figure 3. Proportion of positive pools at the first round of sampling for 12 farms (both 3-4 and 5-6year olds)

The results of the second round of sampling demonstrated the decline in proportion of positive pools for the 3-4year old cohorts in 10 of the 12 farms at both samplings. However note that proportion of positive pools increased in flocks F and D (Figure 4).

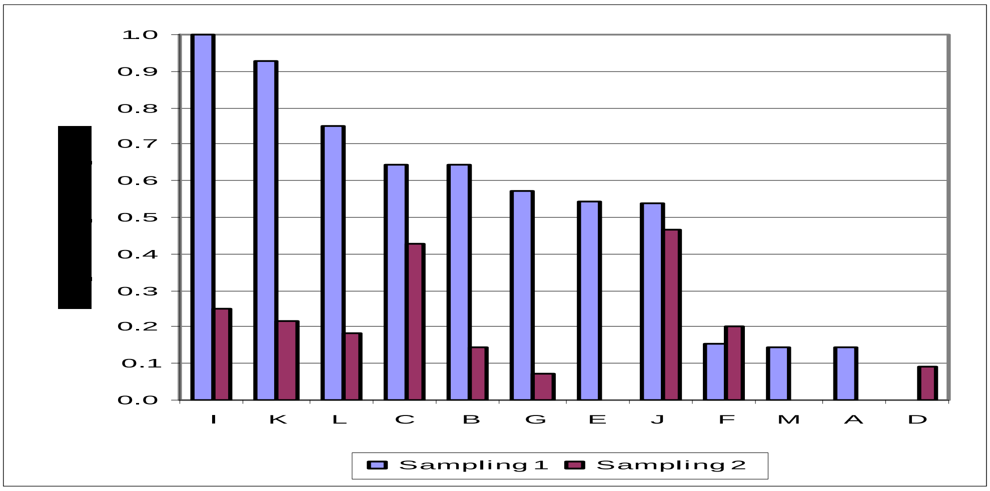

Figure 4. Proportion of positive pools at the first and second round of sampling for 12 farms (3-4year old)

Results from the survey of farm management practices are summarised in Appendix 3. Of interest was that peak OJD losses ranged from 0 (L2, L5) to 5% (H1, H2, H3, and M3). Number of years infected at start of the study ranged from 1 (L1, L2) to 8 years (M3). Only 2 farms were not vaccinating wethers (H1, H3). However both these farms sold their wethers at 1 year of age, as did 4 additional farms (M2, L4, H2, M3). Half of the farms used a contract vaccinator and this did not appear to be correlated with other risk factors.

3.2 Faecal excretion prevalence

The mean prevalence estimates from the raw PFC culture data for each age group for each sampling were calculated using the approach described (Williams and Mofitt 2001) and are presented in Table 1. Although a decreasing rate of excretion was not consistently observed in all age cohorts, as was expected, an observable decrease in the mean prevalence during the across the 6 years of the study, declining from 2.05% to 0.87%.

Sampling number	3yr olds	4yr olds	5yr olds	6yr olds	Total
1 (2004)	1.43%	2.74%	1.93%	2.28%	2.05%
2 (2006)	0.72%	0.99%	2.55%	1.59%	1.58%
3 (2008)	1.03%	1.05%	0.58%	0.90%	0.87%
Total	1.07%	1.69%	1.75%	1.73%	1.55%

Table 1. Mean prevalence estimates for each age group for each sampling (note shading demonstrates appearance of vaccinated cohorts)

An evaluation of the effects of vaccination on prevalence of shedding of *Mptb* was conducted. Vaccinates had significantly lower prevalence (0.63%) than non-vaccinates (1.66%) as would be expected (P<0.001) from comparison of the data for vaccinates (shaded) with unvaccinated sheep (unshaded) in Table 1. No other variable was significant when added to the model of prevalence on vaccination status.

A model of prevalence on age by sampling year was used to determine the changes in prevalence over the years in different age groups. There were significant differences in prevalence over the years and in different age groups (P<0.001; as expected from visual examination of Table 1). Further, there was a significant reduction in prevalence across the three samplings, with the table of predicted means for year being 1.54%, 1.08% and 0.71% for samplings 1, 2 and 3 respectively. In general, prevalence was higher in older age groups, with the table of predicted means for age being 0.72% for 3year olds, 1.14% for 4year olds, 1.135 for 5year olds and 1.33% for 6year olds. Interestingly, the prevalence was not significantly lower in the same cohort at the next sampling (such as 5year olds at Sampling 2 compared to 3year olds at Sampling 1).

The results of the analyses conducted with pool status as an outcome, were also similar. Non-vaccinates were 7.92 times more likely to be pool positive compared to vaccinates. The analyses of age and sampling year for pool status determined that there was not a significant reduction in the likelihood of a pool being positive in Sampling 2 compared to Sampling 1. However there was a significant reduction in the likelihood of a pool being positive in Sampling 3 compared to Sampling 2, plus a significant reduction in the likelihood of a pool to be positive in Sampling 3 compared to the Sampling 1. From the table of predicted means for year by age, there was a significant increase in the likelihood of a pool being positive for a 3year old in Sampling 1 compared to 5year old in Sampling 2 (despite both being unvaccinated). However there was a non-significant decrease in the likelihood of a pool to be positive in: a 4year old in Sampling 1 compared to 6year old in Sampling 2; a 3year old in Sampling 2 compared to 5year old in Sampling 3; and a 4year old in Sampling 2 compared to 6 year old in Sampling 3. Presumably this finding is because all these sampling comparisons have the same vaccination status.

The animal prevalence estimates for each sampling are shown in Figure 5. Note that data from Sampling 3 (Year 4) was not available from Farm L3 as it withdrew from the study.

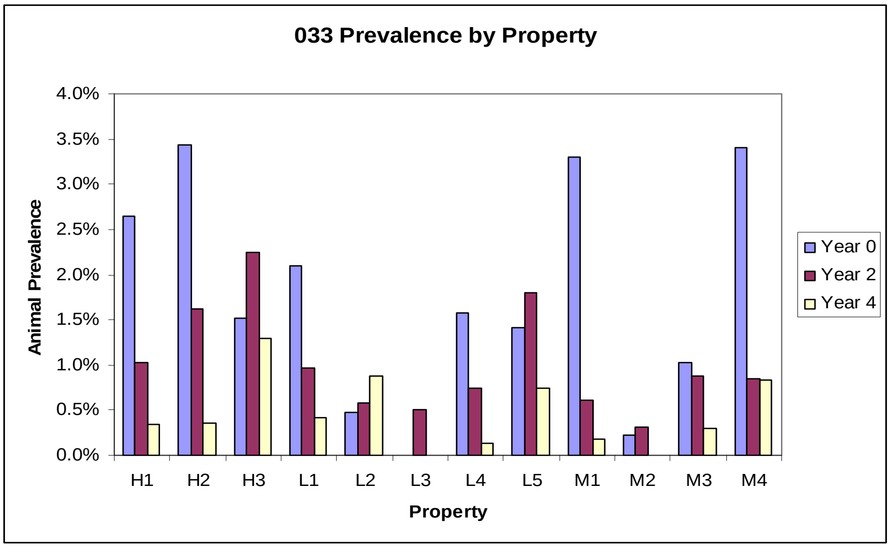

Figure 5. Animal Prevalence by Farm (property) and Year sampled (Sampling 1=Yr0, Sampling 2=Yr2, Sampling 3=Yr4).

4 Discussion

Analysis of the pooled faecal culture results from this longitudinal observational study on 12 OJD-infected farms over a 6 year period provides important information on the impact of Gudair™ vaccination on the prevalence of OJD as determined by faecal shedding of *Mptb*. A previous study (OJD.009) had identified that the onset of shedding of *Mptb* in vaccinates was delayed by 12months and the prevalence of shedders amongst vaccinates was reduced by 90% (Reddacliff *et al*, 2006). However this pivotal study did identify high levels of excretion by vaccinates on some occasions, the presence of multibacillary lesions of OJD in the few vaccinates that died, plus persistence of subclinical infection in a significant proportion of vaccinates.

Findings from a study of shedding rates in 2 year old vaccinates on 7 farms confirmed that shedding persisted in vaccinates although at a declining rate (Eppleston *et al*, 2005). These observations led to the conclusion that there is a risk that some vaccinated sheep will spread the disease and implied that sustained vaccine use will be necessary to avoid recrudescence of infection and clinical disease in infected flocks (Reddacliff *et al*, 2006).

Project OJD.033 was designed to explore this risk and address the issue of vaccine efficacy in flocks of low OJD prevalence, as determined by rates of shedding of *Mptb* in sheep of different ages (3, 4, 5 and 6 year olds) following their vaccination with Gudair™ as lambs. The data confirm as expected, that vaccinates had significantly lower prevalence of shedding than non-vaccinates (0.63% versus 1.66%; P<0.001) with unvaccinated sheep 7.92 times more likely to be pool positive compared to vaccinates. A decrease in the total mean prevalence across the 6 years of the study was confirmed, declining from 2.05% at Sampling 1 in 2003-4 when only unvaccinated sheep were sampled, to 1.58% at Sampling 2 in 2004-5 when the 3-4year olds but not the 5-6year olds were vaccinated, to a final mean prevalence at Sampling 3 in 2007-8 of 0.87% when all the sampled sheep were vaccinated.

A significant finding from this study is that at Sampling 3, 10 of the 11 flocks still in the trial, had detectable shedding, using the current PFC testing technology. The range of prevalence at Sampling 3 on these 11 farms when all sheep were vaccinated was considerable; from 0 (M2) to 1.294% (H3). As can be seen from Figure 5, of the 11 farms that remained in the study, the animal prevalence continued to decline on 6 of the farms (H1, H2, L1, L4, M1, M3), rose then declined on 3 farms (H3, L5, M2), declined then persisted on one farm (M4) and continued to rise on one farm (L2). It is interesting that farms that displayed a continual downward trend in the shedding rate throughout the study included 2 farms from each of the H, M and L initial prevalence categories.

These observations suggest that the response to Gudiar™ vaccination is highly variable between flocks. Subjective scrutiny of the survey data also noted the high variability of management factors between the 12 farms and particular risk factors that may be correlated with the trend in prevalence of shedding were not identified. Although there were insufficient data to draw valid conclusions as to why this occurs, it would appear that an obvious relationship between the trend of animal prevalence following vaccination and the initial prevalence is unlikely to be determined. Even with additional data from surveys of sheep husbandry practices on these farms, the high number of management variables in addition to vaccination suggests that more definitive conclusions as to why some farms have not shown the expected rate of decrease in shedding following implementation of vaccination, is unlikely from this set of data.

The finding that shedding persists in a majority of flocks even when all sheep are vaccinated confirms the importance to OJD control of a better understanding of the risk of shedding in vaccinated sheep over an extended time period. The encouraging trend in the decline of shedding on most farms in the study suggests that important information can be obtained from continuation of this study. We recommended that the study be continued for a further 3 rounds or 6 years of testing to provide more accurate data on the rate of decline of shedding rates in flocks composed entirely of 'second generation vaccinates'. These are sheep that are progeny of accredited vaccinates and currently accrue maximum points for vaccination status in the ABC risk based trading scheme. Data is urgently needed on the risk of spread of OJD from the trade in these sheep. The outcomes from the extension of these studies will greatly assist sheep producers to consider the risk of ceasing vaccination in their flocks and assessing the risk of purchasing vaccinated re-stocker sheep. In addition, it is recommended that the new data be incorporated in computer models for OJD prevalence to enable better prediction of the risks of trading in vaccinates

5 Success in Achieving Objectives

This study has successfully met the objectives described. The project examined the use of Gudair™ to elicit changes over time in the prevalence of *Mptb* shedding following the commencement of vaccination in flocks varying in initial OJD prevalence. Twelve flocks were recruited into the project, 3 with high, 5 with medium and 4 with low OJD prevalence at the commencement of the study. Only one flock withdrew from the study. This was a low prevalence flock (L3) that was sold prior to Sampling 3, with data available for Samplings 1 and 2 from this flock. Sampling was conducted over a 6-year period and changes in the prevalence of shedding as the proportion of vaccinates in the flock increased, were estimated. By December 2008 the project had measured changes in the prevalence of shedding in these OJD infected flocks.

The study demonstrated no clear differences in pattern of response to vaccination among high, medium and low prevalence flocks, without a clear relationship between initial prevalence and vaccine efficacy. An expected outcome from the project was the prediction of the infectivity of flocks over time following the commencement of a vaccination program, to be used to validate computer models. At this stage it appears that 10 of the 11 flocks still contained animals capable of spreading OJD, although the prevalence was variable between flocks and ranged from 0.13% (L4) to 1.29% (H3) at the conclusion of the study.

It was considered that this information may assist decisions on the risks of trading vaccinated sheep, the timing of cessation of vaccination and the removal of a risk status from a flock. These findings provide further support to previous conclusions that there is a risk that some vaccinated sheep will spread the disease and that sustained vaccine use will be necessary to avoid recrudescence of OJD in infected flocks. The findings are an important reminder of the implications from previous studies on the risk of shedding from vaccinates and suggest that further work plus continued use of the vaccination in OJD infected flocks is warranted at this stage.

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

The Merino industry was significantly damaged by OJD for an extended period prior to the registration of Gudair™. The vaccine has been a powerful influence in rescuing many producers who were greatly disadvantaged by the regulatory approach used to control the disease prior to 2004. The original research (OJD.009) suggested 90% efficacy of vaccination against mortalities but warned that persistence of shedding presented a risk to trading. Following registration of the vaccine many high, medium and low prevalence flocks commenced vaccination as a means to protect against OJD mortalities and as a means to improve their ability to sell re-stocker sheep through the risk based trading Assurance Based Credit (ABC) point scheme. Modelling work suggested that the prevalence of mortalities and shedding would fall rapidly after the commencement of a vaccination control program depending on disease prevalence at the time of commencing vaccination, but this required validation by field research. Project OJD.033 now provides further evidence that vaccinates had significantly lower prevalence of shedding than unvaccinated sheep (0.63% versus 1.66%; $P<0.001$).

However this project provides the significant observation that at the final sampling when all adult sheep had been vaccinated as lambs, a total of 10 of the 11 flocks still in the study had sheep with detectable shedding ranging from 0.13% to 1.29%. The conclusion is that although Gudair™ vaccination significantly decreased the shedding of *Mptb* in the majority of flocks in the study, shedding persisted in most flocks for an extended period, presenting a risk for spread and recrudescence of OJD. This has led to recommendations that the study be continued for a further 3 rounds or 6 years of testing to provide more accurate data on the rate of decline of shedding rates in flocks composed entirely of ‘second generation vaccinates’. This will greatly assist computer modelling of OJD prevalence and enable better prediction of the risks of trading in approved vaccinates.

With the detection of newly OJD infected farms increasing in most states and disease control largely dependent on an understanding of the risk of trading in approved vaccinates, additional longitudinal data is urgently needed on the risk of spread of OJD from the trade in these sheep. The outcomes from the extension of these studies will greatly assist sheep producers in their decisions on whether to continue vaccination for OJD in their flocks when visible mortalities have ceased. Without this information, it is likely that use of vaccine will decline, widespread mortalities from OJD will re-emerge, and the disease will continue to spread through the sheep industry at a significantly increased rate.

7 Conclusions and Recommendations

This study reports on the changes in the prevalence of shedding over the first 6 cohorts of vaccinates in 12 infected flocks of variable prevalence when they commenced vaccinating with Gudair™ for OJD. The study determined that despite significant decreases in shedding rates of *Mptb* in the majority of flocks in the study, shedding was detectable in 10 of the 11 flocks that remained in the study until 2008. The study provides evidence that Gudair™ vaccination is a valuable but imperfect tool in managing OJD and that the persistence of shedding in fully vaccinated flocks currently presents a significant risk of recrudescence of infection in these flocks and spread of the disease to flocks through the trade of vaccinated animals. It is recommended that the study be continued for a further 3 rounds of testing to provide accurate data on the rate of decline of shedding rates in flocks composed entirely of 'second generation vaccinates' now that these flocks are composed of sheep that are progeny of accredited vaccinates. This information is of critical importance to OJD disease control in Australia.

8 References

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9 Appendices

9.1 Appendix 1

Table of mean prevalence estimates for Farm (H=high; M=Medium, L=Low), Sampling number (1,2 or 3), and Prevalence (with LCL=lower confidence limits and UCL=upper confidence limits).

Farm	Sampling 1			Sampling 2			Sampling 3		
	Prev	LCL	UCL	Prev	LCL	UCL	Prev	LCL	UCL
H1	0.0265	0.0154	0.0427	0.0103	0.0051	0.0181	0.00349	0.0011	0.0081
H2	0.0344	0.0203	0.0555	0.0163	0.0093	0.0262	0.00354	0.0009	0.0092
H3	0.0152	0.0087	0.0245	0.0224	0.0136	0.0349	0.01294	0.0064	0.0229
L1	0.021	0.0123	0.0333	0.0097	0.0048	0.017	0.00422	0.0013	0.0098
L2	0.0048	0.0019	0.0098	0.0057	0.0023	0.0116	0.0088	0.0044	0.0155
L3	N/A	N/A	N/A	0.0051	0.0016	0.0118	M	M	M
L4	0.0158	0.0086	0.0262	0.0074	0.0026	0.0159	0.00127	7E-05	0.0056
L5	0.0141	0.0073	0.0243	0.018	0.0098	0.0301	0.00741	0.0038	0.0127
M1	0.033	0.0201	0.0517	0.006	0.0026	0.0117	0.00182	0.0003	0.0056
M2	0.0023	0.0006	0.0059	0.0031	0.001	0.0071	N/A	N/A	N/A
M3	0.0103	0.0047	0.0192	0.0088	0.0031	0.019	0.00294	0.0005	0.0091
M4	0.0341	0.0196	0.0559	0.0085	0.003	0.0182	0.00831	0.0033	0.0169

Note: M=Missing data (farm withdrew from trial); N/A=prevalence cannot be estimated as all pools negative

9.2 Appendix 2

Table of prevalence estimates for Farm (H=high; M=Medium, L=Low), Sampling number (1,2 or 3), Age (3,4,5, or 6) and Prevalence

Observation	Farm	Sampling	Age	Prevalence
1	H1	1	3	0.01113
2	H1	1	4	0.038171
3	H1	1	5	0.038171
4	H1	2	3	0.003078
5	H1	2	4	0.003078
6	H1	2	5	0.038313
7	H1	2	6	0.014606
8	H1	3	3	0.003078
9	H1	3	4	0.006707
10	H1	3	6	0.005804
11	H2	1	4	0.038171
12	H2	1	5	0.016803
13	H2	2	4	0.01113
14	H2	2	6	0.037625
15	H2	3	3	0.005394
16	H2	3	5	0.004038
17	H2	3	6	0.006127
18	H3	1	3	0.01113
19	H3	1	4	0.038171
20	H3	1	6	0.038171
21	H3	2	3	0.006707
22	H3	2	4	0.016803
23	H3	2	5	0.038171
24	H3	3	3	0.038171
25	H3	3	5	0.016803
26	L1	1	3	0.016803
27	L1	1	4	0.016803
28	L1	1	5	0.024744
29	L1	1	6	0.031676
30	L1	2	3	0.003078
31	L1	2	5	0.038171
32	L1	2	6	0.018159

33	L1	3	4	0.01113
34	L1	3	5	0.003078
35	L2	1	3	0.003078
36	L2	1	4	0.003078
37	L2	1	5	0.003078
38	L2	1	6	0.01113
39	L2	2	5	0.021733
40	L2	2	6	0.008077
41	L2	3	3	0.003078
42	L2	3	4	0.01113
43	L2	3	5	0.006707
44	L2	3	6	0.016803
45	L3	2	3	0.004453
46	L3	2	5	0.012598
47	L3	2	6	0.008077
48	L4	1	3	0.038171
49	L4	1	5	0.00364
50	L4	1	6	0.041896
51	L4	2	5	0.035201
52	L4	3	5	0.004939
53	L5	1	3	0.011853
54	L5	1	4	0.021733
55	L5	1	5	0.013767
56	L5	1	6	0.008077
57	L5	2	3	0.019783
58	L5	2	4	0.007194
59	L5	2	6	0.0181
60	L5	3	3	0.009083
61	L5	3	4	0.008249
62	L5	3	5	0.003197
63	L5	3	6	0.00741
64	M1	1	5	0.014104
65	M1	1	6	0.026299
66	M1	2	3	0.003205
67	M1	2	4	0.011346
68	M1	2	5	0.009356
69	M1	3	3	0.002866

70	M1	3	5	0.003324
71	M2	1	3	0.006707
72	M2	1	6	0.003078
73	M2	2	5	0.01113
74	M2	2	6	0.003078
75	M3	1	3	0.006707
76	M3	1	5	0.039929
77	M3	2	3	0.010165
78	M3	2	5	0.027345
79	M3	3	4	0.011829
80	M4	1	3	0.022694
81	M4	1	4	0.035352
82	M4	1	6	0.021733
83	M4	2	3	0.006822
84	M4	2	5	0.023265
85	M4	2	6	0.019501
86	M4	3	3	0.010639
87	M4	3	4	0.014174
88	M4	3	5	0.004513

9.3 Appendix 3

Table of summarised survey data from 12 farms in Study OJD.033

Flock	Area (ha)	Alt (m)	Rain (mm)	Percent impro-ved pasture	Main Soil Type	Sheep dse	Cattle dse	Total se	Stock-ing Rate (dse/ha)	Mic-on	Lambing Month	Number Years Infected @ start	Peak Losses (%)	Risk Lateral spread	Rams introd-uced last 5 yrs	Other Sheep purch-ases	Vaccinate wethers?	Age Wethers Sold (yrs)	Terminal lambs? Age sold (mths)	Contract vaccin-ator?
M4	2600	850	800	40	Granite	6500	4500	11000	4.2	21	May&Sep	5	0.1	Med	35	0	Yes	4	12	No
L3	1100	700	600	10	Granite	5400	0	5400	4.9	18	May&Sep	2	0.0	Med	12	2000	Yes			Yes
M2	4000	800	650	100	Basalt	7800	9000	16800	4.2	21	May	3	0.1	Med	100	750	Yes	1	10	Yes
L2	4000	400	600	100	Granite	10200	4900	15100	3.8	21	Sep	1	0.0	Low	8	0	Yes		10	No
L4	1400	400	650	90	Loam	2000	2000	4000	2.9	20	Apr	7	1.0	Low	30	0	Yes	1	12	No
H1	1275	450	675	95	Gran/Bas	5040	0	5040	4.0	19	Sep	3	5.0	Low	60	0	No	1	6-10	Yes
M1	1300	750	600	40	Granite	5921	0	5921	4.6	17	Mar&Sep	7	4.0	Med	40	220	yes	5	7	No
H2	1200	530	600	95	Granite	3000	2500	5500	4.6	20	Sep	2	5.0	Low	50	0	Yes	1	9	Yes
H3	3200	460	600	100	Bas/Gran	14000	1000	15000	4.7	20	Sep	2	5.0	Med	120	0	No	1	8	No
L5	800	550	670	20	Granite	2400	0	2400	3.0	19	Sep	4	0.0	Med	16	0	Yes	5	na	Yes
L1	800	500	650	70	Granite	2676	0	2676	3.3	21	Sep	1	0.7	Low	50	0	Yes	2	10	Yes
M3	1060	840	800	0	Granite	4300	500	4800	4.5	19	Sep	8	5.0	Med	50	0	Yes	1	12	No