final report

Project code: P.PSH.0309

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Date published: October 2011

ISBN: 9781741916577

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Evaluation of the effectiveness of Gudair™ vaccination for the control of OJD in flocks vaccinating for at least 5 years

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Project P.PSH.0309 was an observational study examining the efficacy of Gudair™ vaccine in decreasing the prevalence of shedding of infectious organisms 5 years after the commencement of vaccination in flocks varying in initial OJD prevalence. The study was conducted in 2009 and 2010, when pooled faecal culture of 350 sheep (PFC350; 7 pools of 50) was conducted on 40 selected trial flocks from the southeast of NSW and Victoria. This was approximately 5 years or more after commencement of vaccination of lambs with Gudair™ in these flocks. The prevalence data on shedding of *Mycobacterium paratuberculosis* was compared with available data at, or prior to, the commencement of vaccination in these flocks, on which basis they were categorised as High, Medium or Low OJD prevalence flocks. The data indicated there had been a noticeable decline from 14 to 8 flocks categorised as High prevalence flocks. Seven flocks had no detectable shedders.

Examination of combined prevalence data including all the various sources of surveillance information, including serology, faecal culture, histopathology and abattoir surveillance, enabled estimates of prior and current OJD prevalence to be determined. Bayesian modelling estimated a significant decline in OJD prevalence from a pre-vaccination median prevalence of 2.99% (95% Probability Intervals (PI) 1.50, 7.92%) to a post-vaccination median prevalence of 0.74% (95% PI 0.42, 1.29%). However 33 of the 40 flocks were still shedding and half of the flocks (20/40) were still shedding at a sufficient rate to classify them as having a medium or high prevalence for OJD. It was also noted that 7 of the 14 flocks that were classified as having an initial low prevalence had increased to medium or high prevalence after 5 years of vaccination. A risk factor study was conducted by a questionnaire survey of management factors in 36 of the 40 flocks sampled by PFC350. Flocks with OJD prevalence exceeding 1% following vaccinating with Gudair™ were associated with managers reporting having sheep stray and the introduction of new sheep on farms. Having a concurrent cattle enterprise was shown to be protective.

To examine the sensitivity of the initial PFC350 for the detection of low-prevalence flocks, follow-up sampling was conducted in 2010, on 600 sheep (PFC600; 12 pools of 50) from 4 of the 7 flocks in NSW and Victoria where shedding had not been detected in 2009. This study also included examination of 16 flocks on Kangaroo Island in South Australia that were examined by the same protocol, including 6 flocks that had been recently found not to be shedding by PFC350. Only one of the 4 NSW flocks and 2 of the KI flocks was found to be shedding in the 2010 study. As all 3 flocks had at least one positive pool detected, in the first 7 pools, it is suggested that the current protocol of PFC350 is of acceptable sensitivity to detect flocks at very low prevalence. It was noted that the positive flock in NSW had ceased to vaccinate wethers and both positive flocks on Kangaroo Island were found to have introduced unvaccinated sheep in recent years. Of note on Kangaroo Island, there was a significant decline in within-flock OJD prevalence ($P<0.001$) from initial cohort OJD prev. range (0.24-1.94%) prior to vaccination, to the current cohort OJD prev. range (0.00-2.17%). However in addition to subsidised whole flock vaccination, a number of other changes to farm management had been introduced to minimize the spread of OJD on KI and were likely to have contributed to this finding.

This project concluded that despite a rapid decrease in OJD mortality in flocks following the commencement of a vaccination program, shedding persisted for at least 5 years in a majority of flocks other than on KI. These findings are of concern if sheep are to be traded from these flocks or vaccination ceases. The association of flocks with OJD prevalence greater than 1% and sheep straying, or the introduction of new sheep, suggests that whilst Gudair™ vaccine does decrease the OJD prevalence levels in vaccinating flocks, it does not negate the continuing need for farm biosecurity in the control of OJD. However the data from Kangaroo Island is encouraging and suggest that second generation vaccinates are likely to have greater protection from shedding than first generation vaccinates.

Executive Summary

Ovine Johne's disease (OJD) is an enteric infection of sheep by *Mycobacterium avium* subspecies *paratuberculosis* 'S' strain (*Mptb.*) that has proven difficult to both diagnose and control in Australia. Collaborative research in project OJD.009 demonstrated the efficacy of vaccinating lambs between 1 and 4 months of age with Gudair™, a killed whole cell vaccine imported from Spain, for controlling OJD in high prevalence Australian sheep flocks. Vaccination reduced 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™. This vaccine and the Assurance Based Credit (ABC) Scheme are now established as the key strategic intervention to control the disease in Australia. Computer modelling suggested that the occurrence 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. However it was acknowledged at the time that validation of this modelling by field research was required, particularly as points for vaccination became incorporated into the ABC risk based trading scheme.

Following registration of Gudair™ many flocks with an apparent low prevalence also commenced vaccination as a precaution against increased mortalities and as a means to improve their ability to sell re-stocker sheep through the ABC scheme. In project OJD.033, we reported on the changes in the prevalence of shedding of *Mptb.* in the 3-4year and 5-6year old cohorts in 2003-4, 2005-6 and 2007-8 following initiation of vaccination of 1-4 month old lambs in 2002. The study found a significant decrease (1.66% to 0.63%; $p<0.001$) in shedding rates of *Mptb.* in the majority of flocks in the study. However we also identified that shedding was detectable in 10 of the 11 flocks that remained in the study until 2008 (range 0.13% to 1.29%) and it was recommended that a broader study of the current prevalence of OJD in flocks that had been vaccinating for 5 years or more was required. This led to project P.PSH.0309 as reported here. More recently it was also decided that evaluation of shedding in the remaining flocks in Project OJD.033 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). This study has commenced as Project P.PSH.0565. Appropriate extension of the outcomes from 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.

Project P.PSH.0309 was an observational cross-sectional study conducted in 40 selected trial flocks of varying initial OJD prevalence from the southeast of NSW and Victoria, examining the efficacy of Gudair™ vaccine in decreasing the prevalence of shedding of *Mptb* 5 years after the commencement of vaccination. OJD prevalence in these flocks after 5 years was determined by pooled faecal culture of 350 sheep (PFC350; 7 pools of 50) and was compared with estimates of OJD prevalence from various data estimating prevalence (including serology and culture) recorded prior to commencement of vaccination. Results show that 5 years or more after the commencement of vaccination with Gudair™ there has been a noticeable decline from 14 to 4 flocks categorised as high prevalence flocks, with 7 flocks having no detectable shedders. However 82.5% of the 40 flocks still contained sheep that were shedding. By combining shedding data with other prevalence information by Bayesian modelling (resulting in elimination from analysis of 2 flocks with inadequate initial prevalence data), the results identified a significant decline in the median OJD prevalence pre-vaccination of 2.99% to 0.74% post-vaccination (95% PI 0.42, 1.29%). Despite 18.4% (7/38) of the flocks apparently not shedding currently, 47.4% (18/38) flocks had a cohort prevalence of >0 and ≤1% and 34.2% (13/38) had a

cohort prevalence of >1% Seven of the 14 flocks with initial low prevalence had increased prevalence (medium or high) after 5 years vaccination.

To examine the sensitivity of the initial PFC350 and better understand infection in low-prevalence flocks, sampling was conducted on 600 sheep (PFC600; 12 pools of 50) from 4 of the 7 negative flocks from the initial survey and one was found to be shedding. This flock had ceased to vaccinate wethers. When the PFC 600 was conducted on 16 known infected flocks on Kangaroo Island in South Australia, including 6 flocks that had been recently found not to be shedding by PFC350, no subsequent shedding was found in 14 of the flocks. This indicates a decline in proportion of these 16 flocks as positive for shedding from 100% to a proportion of 12.5% of flocks shedding currently. It was later identified that both positive flocks on Kangaroo Island had introduced unvaccinated sheep in recent years. Further, it was noted that in addition to whole flock vaccination, a number of other farm management factors designed to minimize the spread of OJD had been introduced on KI.

These data indicate that despite a rapid decrease in OJD mortality in flocks following the commencement of a vaccination program, shedding is likely to have persisted for at least 5 years in a majority of infected flocks in NSW and Victoria and is of concern if sheep are to be traded from these flocks or vaccination ceases. However the data from Kangaroo Island are encouraging and suggest that the second generation vaccinates have greater protection from shedding than the first generation vaccinates and will likely present a substantially lower risk of transmission of the disease.

Acknowledgements

This project was the results of an extensive collaboration between staff at the University of Sydney (UOS) and the Tablelands Livestock Health and Pest Authority (LHPA), with staff in NSW Industry and Investment (NSWI&I), the Victorian Department of Primary Industries (VDPI) and Primary Industries of South Australia (PIRSA). There were many significant inputs, ranging from the willing producers in 3 states who cooperated in this work, to those staff who collected the samples and performed the laboratory tests. In particular, we acknowledge the support of Mr John Seaman of NSWI&I and Peter Whyte of PIRSA for assistance in coordinating the sampling. Thanks are due to Anna Waldron and staff for laboratory support and Ms Marion Saddington for administrative support at the UOS.

The efforts of the two UOS final year Honours veterinary students, Ms Josie Gollan with support from Mr Tony Morton of the Livestock Health and Pest Authority (LHPA) in Wagga, plus Ms Amy Masters with support from Ms Deb Lehmann of the Kangaroo Island Veterinary Hospital and Andrew Ewers from PIRSA on Kangaroo Island, is gratefully acknowledged, as is their funding support provided by the Australian Wool Education Trust.

Thanks also to Mr Ian Links supplied the abattoir surveillance data. Critical to this work is the ongoing support of Dr Evan Sergeant of Ausvet Animal Health Services who was involved in initiation and monitoring of the project and assisted in epidemiological design and interpretation. Thanks also to Dr Keith Walker of Meat and Livestock Australia and Ms Lorna Citer of Animal Health Australia for provision of project resources and facilitating the industry partnership program that enabled the contributions of WoolProducers Australia and the Sheepmeat Council of Australia through Animal Health Australia.

Contents

Abbreviations

ABC - Assurance Based Credit Scheme

AGID – agar gel immunodiffusion serological test

AS - abattoir surveillance

CPREV - cohort OJD prevalence

dse- dry sheep equivalent

ELISA – enzyme linked immunosorbent assay serological test

KI – Kangaroo Island

LHPA - Livestock Health and Pest Authority

MAP - Sheep Market Assurance Program (MAP)

Mptb.- Mycobacterium avium subspecies *paratuberculosis*

NSW I&I - NSW Industry and Investment

OJD – ovine Johne's disease

PIC - Property Identification Code

PIRSA - Primary Industries and Resources South Australia

PFC350 – pooled faecal culture test on 7 pools of 50 samples

PFC600 – pooled faecal culture test on 12 pools of 50 samples

SASAG - South Australian Sheep Advisory Group

VDPI - Victorian Department of Primary Industries

1 Background

1.1 Background from previous studies

Johne's disease is an important global disease of ruminants caused by enteric infection with *Mycobacterium avium* subspecies *paratuberculosis* (*Mptb.*). It is difficult to control and is usually fatal in animals that develop clinical signs of the disease. Severe production losses through wasting and mortality and the continued spread of OJD has been of considerable concern to the sheep industries of Australia for over a decade. A National OJD Control and Evaluation Program (NOJDCEP) commencing in 1998 and completed in 2004, initially 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. This OJD.009 study led to the registration of Gudair™ for the control of OJD in Australia.

Vaccination with Gudair™ and risk based trading (via the Assurance Based Credit or ABC point scheme) are now well established as the key strategies to control the disease and manage the risk of transmission of OJD both within and between flocks in Australia. The original vaccine research in sheep in Australia (OJD.009) was conducted in single cohorts 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. Further, 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).

Computer modelling of OJD vaccine outcomes used assumptions on the efficacy of vaccination. It was 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, et al, 2002). 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 the modelling by field research that quantified the rate of disease reduction following the commencement of vaccination has been seen as an important component of OJD control research as it provides important insights into the risk of transmission of the disease.

Following the registration of Gudair™ vaccine in April 2002, OJD control has largely relied on voluntary vaccination and promotion of assurance using a self-declaration system for OJD risk. A risk based trading approach was adopted and the National Sheep Health Statement identifies how credits may be allocated using the Assurance Based Credit (ABC) Scheme which identifies the key risk factors in the spread of OJD. Each credit represents an approximate 4 fold decrease in the risk that the sheep are infected, with up to 4 of the 12 ABC points available being allocated for approved vaccination. This program potentially encourages flock owners in Medium and High OJD prevalence areas to commence vaccination programs as a precaution against increased mortalities and to improve their ability to sell re-stocker sheep through the risk based trading ABC scheme.

Determining the efficacy of vaccine in flocks with variable OJD prevalence has been considered important, as many producers have been encouraged to commence vaccination in flocks where the prevalence is suspected to be low, such as infected flocks with few mortalities or flocks in

Low and Medium Prevalence Areas. Although it was considered reasonable to expect that early intervention with vaccine would also rapidly reduce prevalence of disease in low prevalence flocks, data on the effect of vaccination in these flocks is lacking. As the effect of vaccination will become more evident over an extended period, particularly following vaccination of a number of successive lamb cohorts, 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. 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 (Windsor, 2006). 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%. It was 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'.

Many NSW producers commenced vaccination around the time of registration of Gudair™ in April 2002. Mortalities are now rarely reported in vaccinating flocks in NSW. The persistence of shedding for an extended period following onset of vaccination as determined by Project OJD.033, presents a risk for spread and recrudescence of OJD from vaccinating flocks. It was agreed there was a need for a broader study on the impact of vaccination on shedding rates in flocks of varying initial prevalence. Investigations in a larger number of flocks were required to provide an indication of long-term effects of vaccination in flocks of known infection and vaccination history. In addition, as the results from Project OJD.033 indicated that the significant decrease in shedding over time was not being achieved in all infected flocks, the reasons for this need to be investigated. Project PSH. 0.0309 was initiated to assess the impact of vaccination on shedding in a range of infected flocks that have been vaccinating since the registration of the vaccine.

It was considered timely to utilise commercial flocks that have been vaccinating for 5 years or more to provide information on the rate of decline in OJD risk following the introduction of a vaccination strategy in flocks with varying disease prevalence. A study of the management procedures used in these flocks was conducted to identify risk factors that may have influenced the results. This information is of particular importance in assisting determination of the risk of trading in vaccinated animals.

The initial project brief was to design a project that evaluated shedding in flocks in NSW, Victoria and South Australia (SA). However as OJD was still regulated in SA and there were a number of disease management approaches used in that state that would have confounded direct comparisons of observations, such as the widespread adoption of sponsored whole flock vaccination strategies, it was decided to initially confine the study to 40 flocks in NSW and Victoria. Presentation of the preliminary results to sheep industry veterinarians did raise considerable discussion and interest in the way Gudair™ vaccine had been used in SA, particularly when we became aware that flocks were being released from quarantine on the basis of a negative test in the PFC350. It was decided to request a variation to the contract for Project PSH. 0.0309 to permit a study of shedding rates in vaccinated flocks on Kangaroo Island and this was approved.

OJD was first diagnosed on Kangaroo Island in June 1998 (Sergeant, 2001). It is estimated that more than 35 properties were infected at the time of initial detection and pathological lesions consistent with the disease were reported in 13.85% of flocks when abattoir surveillance commenced (Whyte 2009, pers. comm.). Primary Industries and Resources South Australia (PIRSA) established the Kangaroo Island OJD Control Program (KIOJDCP) in 1998 and whole of island PFC350 testing was conducted within the program from 2001 to 2004. Ongoing tracing and abattoir surveillance for OJD led to the diagnosis of OJD in 92 of 330 flocks on Kangaroo Island (Whyte 2009, pers. comm.). Although eradication of OJD by destocking of sheep had limited success in Australia with the disease frequently recurring in flocks when restocked (Taylor, 2004), destocking for two consecutive summers was initially advocated by the KIOJDCP to reduce residual pasture contamination. Reappearance of infection on a significant number of the 49 properties that completely destocked led to abandonment of this strategy on Kangaroo Island. However it is considered likely that destocking may have contributed significantly to reduced initial OJD prevalence on Kangaroo Island even though it did not eradicate the disease from infected flocks.

Subsequently the South Australian Sheep Advisory Group (SASAG) enabled whole flock vaccination by subsidising 100% of the cost of Gudair™ vaccine for infected properties on KI from June 2002 when Gudair™ became commercially available. With continued vaccination, an increasing number of properties have been released from quarantine in recent years and industry has encouraged continuation of vaccination on released properties by providing a partial subsidy for half the purchase price of Gudair™ vaccine (priced at $1.70 per head in South Australia). Subsidised vaccine expenditure represents approximately 70% of the $750,000 that industry currently allocates to the control of OJD in SA. Although the uptake of unsubsidised vaccine recorded in SA was greater than expected in 2009, it is considered that the complete phase out of the subsidy as has been suggested, would undoubtedly negatively impact the continued use of Gudair™ on Kangaroo Island (Whyte 2009, pers. comm.).

During the first 7 years of the KIOJDCP, an average of 12 flocks per annum were diagnosed with OJD, compared to an average of 1.4 flocks per annum during the last 5 years. This suggests that the control strategies implemented on Kangaroo Island are likely to have been successful in decreasing the prevalence of OJD. Current abattoir surveillance indicating that only 3.4% of flocks are now infected on KI and further evidence of the decline in OJD was recently provided when 14 of 17 flocks eligible for clearance produced a negative PFC350 test and were released from quarantine (Whyte 2009, pers. comm.). Note that a PFC 350 test is classified as negative when *Mycobacterium avium* subspecies *paratuberculosis* (*Mptb.*) is not cultured from any of the 7 pools where each pool is comprised of one faecal pellet collected from each of 50 eligible sheep.

The variation to Project PSH.0.0309 examined the efficacy of Gudair™ vaccination and other on-farm control strategies on KI by comparing past and current flock OJD prevalence as determined by PFC. The project proposed to determine if the high rate of clearance of properties on Kangaroo Island is likely to reflect an absence of OJD in these flocks or is a consequence of a testing regimen that has insufficient sensitivity to detect persistent infection at the current levels in these flocks. The project variation examined the *Mptb.* shedding levels in Kangaroo Island flocks under regulatory order to test in early 2010, or already recently released from quarantine. The testing protocol used the PFC600 test, with faecal samples from 600 sheep collected into 12 pools of 50 sheep, and then cultured. It was also decided to re-test with the PFC600, as many of the 7 negative flocks from the initial round of testing of the 40 NSW and Victorian flocks that were tested by PFC350 in 2009. Four of these 7 flocks agreed to be re-sampled.

Results from these studies may enable improved understanding of the period of vaccination required to reduce the risk of spreading OJD through the trading of vaccinates. This information is important in determining the number of ABC points that should be allocated for flocks using Gudair™ when trading sheep under the national system of vendor declaration (Animal Health Australia, 2010).

1.2 Project Objectives

The original objectives of this project were to:

1. Estimate prevalence and shedding levels in up to 40 flocks from 3 states with known OJD testing and vaccination history for at least 5 years, including high, medium and low-prevalence flocks;

2. Compare current prevalence and shedding levels in these flocks with estimates from prior to commencement of vaccination;

3. Investigate the relationship between estimated prevalence/shedding and abattoir surveillance results in project flocks;

4. Identify and discuss factors which may have affected the effectiveness or otherwise of vaccination on changes in levels of infection; and

5. Make recommendations on the effectiveness of medium-term vaccination in high, medium and low prevalence flocks and the role of vaccination in risk management as part of the ABC.

On 16th May, 2008, a variation to OJD Project P.PSH.0309 project was requested and approved. The objectives of the variation were to:

1. Estimate OJD prevalence using 12 pools of 50 to determine *Mptb*. shedding levels in up to 7 flocks from 2 states with known OJD testing and lamb only vaccination history for at least 5 years, where no shedding was detected on the most recent PFC using 7 pools of 50.

2. Estimate OJD prevalence using 12 pools of 50 to determine *Mptb*. shedding levels in 15 flocks on KI with known OJD testing and whole flock vaccination history for at least 5 years, where no evidence of pathological infection has been detected by recent Abattoir Surveillance (AS) and the flock is being assessed for clearance from quarantine.

3. Identify and discuss factors which may have affected the effectiveness of the testing regimen and vaccination strategy on changes in levels of infection on these properties.

4. Evaluate whether the additional data from this project variation can assist in assessing the risk of transmission of OJD from 2nd generation vaccinates.

2 Methodology

2.1 Selection and sampling of 40 vaccinating flocks in NSW and Victoria

A planning meeting of lead project collaborators was conducted on 13th June 2008 at NSW Industry and Investment Head Office in Orange to discuss progress on recruiting flocks and more accurately define the most suitable pre-project prevalence data for enrolment of flocks in the project. Potential flocks were identified using official data including disease surveillance and laboratory testing information held in the databases at the University of Sydney, NSW I&I, LHPA's and VDPI. A list of potential flocks from most of the appropriate LHPA's (those in High and Medium OJD Prevalence Areas) and Victoria were assembled and farm owners or managers were then contacted to determine whether they would give permission for their participation in the study. A total of 40 flocks from New South Wales and Victoria were selected to take part in P.PSH0309. The list included known OJD-infected flocks in the former RLPB's (Rural Lands Protection Boards) of Central Tablelands, Hume, Wagga, Young, Yass, Goulburn, Braidwood and Gundagai. Following assessment of initial prevalence data from a range of flocks, using where possible, either 450 AGID or ELISA tests or preferably, results of the PFC350, it was concluded that sufficient numbers of flocks were available to meet the project objective of testing 40 flocks.

Note that each flock was categorised according to the following prevalence levels where 7 pools of 30-50 were available for PFC or 450 AGID tests were available:

- High prevalence flock: ≥4 +PFC or ≥10 AGID positives
- Medium prevalence flock: 2-3 +PFC or 4-9 AGID positives
- Low prevalence flock: ≤1 +PFC or ≤3 AGID positives

The historical data on flock testing as used to determine prevalence category is summarized in Appendix 1.

Due to delays in settling of the project contract, access to the appropriate sheep was not available until many flocks were close to the point of lambing in 2008, requiring that faecal sampling was delayed in a number of flocks until after weaning in late spring/early summer of 2008-2009. A total of 32 of the 40 flocks were sampled in 2008 and sampling in the remaining 8 flocks was completed in early 2009. Producers were remunerated for labour associated with mustering and sampling.

2.2 Comparison of pre- and post-vaccination ordered prevalence

Comparison of current prevalence (i.e. post-vaccination) was made with the estimates from PFC and AGID done prior to vaccination (i.e. pre-vaccination) for 38 flocks using ordinal generalised linear mixed models, with flocks as a random effect and the estimation time (pre- or post-vaccination) as a fixed effect. Prevalence estimates were ordered into three categories and constituted the ordinal outcome variable

2.3 Comparison of true prevalence using a Bayesian approach

Comparison of ordered prevalence was a crude approach as orders were subjectively defined. Since it was not possible to compare the actual pre- and post-vaccination prevalence estimates due to use of different diagnostic tests (with different sensitivities and specificities), we developed a Bayesian approach to estimate true pre-vaccination prevalence after accounting for sensitivities and specificities of diagnostic tests. This prevalence was then compared to the true

post-vaccination prevalence estimated adopting the Dhand et al (2010) approach in a cohesive Bayesian model. The methods and results for this section are presented in Appendix 2.

2.4 Analysis of PFC data and risk factor study from vaccinating flocks in NSW and Victoria

2.4.1 Selection and sampling of sheep flocks:

The reference population for P.PSH0309 was OJD infected sheep flocks in NSW and Victoria comprising a range of estimated OJD prevalence levels and included flocks that met the selection criteria as described in 2.1.

A cohort represented a group of sheep in each flock selected for sampling. Ideally, each cohort had 7 pools of 50 sheep to total 350 animals. However occasionally the size and/or number of pools varied, thus the total number of sheep in each cohort varied, resulting in cohorts containing: 213 (1 cohort); 308 (1); 321 (1); 342 (1); 343 (1); 361 (1); 374 (1); 350 (29); 400 (3); and 1050 (1) sheep. Pooled faecal culture (PFC) was performed on 7 pools in 33 of the flocks, 8 pools in 6 of the flocks and 1 flock with 21 pools. Faecal sample collection was performed by local district veterinarians and involved collecting one faecal pellet per rectum from each sheep selected, with gloves changed between pools. Samples were submitted to the University of Sydney Camden laboratories where they were stored at -80°C until cultured using a modified BACTEC radiometric method (Whittington et al., 2000). Pools were considered positive if there was growth in the culture that tested positive for IS*900* by PCR and REA (Cousins et al., 1999). Individual animal OJD prevalence within each flock/sex cohort was estimated using the on-line Pooled Prevalence Calculator (Sergeant, 2009) employing the variable pool size option and the mean for each sex/vaccination group was compared using a paired T-test.

The PFC350 is estimated to detect of a minimum prevalence of 2% with 98% confidence. Each cohort aimed to have sheep the same sex and age. However due to factors such as drought and de-stocking, this was sometimes difficult and cohorts of mixed age and sex were included in the sampling. Thirty-five flocks had cohorts of ewes only while the remaining 5 flocks had a mixed cohort of ewes and wethers. Sheep in individual pools within a cohort were always the same sex. Thirteen flocks had cohorts of 3 year old sheep only; 5 flocks had cohorts of 4 year old sheep only; 21 flocks had cohorts of sheep both 3 and 4 years of age; 1 flock had a cohort of sheep 3, 4 and 5 years of age. Sheep in individual pools within a cohort were always the same age.

2.4.2 Questionnaire design and implementation

A questionnaire of 43 questions was developed to collect data from the 40 farms. Five of these were closed-ended questions with 2 to 8 choices available. Three questions were semi-closed with 2 or 3 choices available followed by a description. Twenty-nine were open-ended questions requiring quantitative information. These questions obtained information about farm management, farm enterprises, OJD infection history, Gudair™ vaccination history and other OJD control strategies. The remaining 6 questions regarded personal information, locality, Property Identification Code (PIC) and the date of the interview. No formal methods were employed to assess the reliability or repeatability of questionnaire responses.

Questionnaire administration was performed between April 2009 and May 2009 by telephone interview by a University of Sydney Bachelor Veterinary Science Honours student. The farm owners/managers were sent a cover letter and a copy of the questionnaire in March/April 2009 and they were then phoned to complete the survey within 3 weeks of receiving the letter. The mail out was staggered in two groups of approximately 20 farms, two weeks apart to allow time

to complete the survey. The telephone interviews were performed by one interviewer to help reduce bias. The survey was piloted on 2 farmers prior to the first interview.

2.4.3 Data management and statistical analysis

All data from the questionnaires was managed in a spreadsheet created in MS Excel 2007. The PIC numbers were used to identify the information pertaining to each of the 40 flocks. Thirty variables and subsequent categories were derived from the data obtained. Once calculated, cohort OJD prevalence and pool status was added to the spreadsheet.

2.4.3.1 Outcome variables:

Cohort OJD prevalence level (CPREV). The OJD prevalence of each cohort was calculated from the results from the PFC. Since many of the pools were of varying size, a variable pool size method described by Williams and Moffitt (2001) was used. The online program Pooled Prevalence Calculator enabled the calculation of OJD prevalence as found via the e-link: http://epitools.ausvet.com.au/content.php?page=PPVariablePoolSize.

The OJD prevalence levels of the cohorts allowed for categorisation into 2 categories, <1% prevalence or ≥1% prevalence. This was based on the statistical data and allowed for a more even spread of flocks than 3 categories. Univariable and multivariable binomial logistic regression analyses were performed to identify explanatory variables associated with higher levels of cohort OJD prevalence.

Pool OJD status (PSTATUS). The PFC results identified the number of positive and negative pools in each cohort and thus allowed the creation of a binary outcome variable. Univariable binomial logistic regression analyses were performed followed by a multivariable binomial logistic regression analyses. A generalised linear mixed model was finally built to identify factors statistically associated with positive pool status.

2.4.3.2 Explanatory variables:

From the questionnaire data, 30 explanatory variables were investigated. All variables were categorical with 24 comprised of discrete data and the remaining 6 were continuous data categorised based on the median and quartiles where necessary.

Univariable analyses were performed to investigate the associations between each explanatory variable and both outcome variables using UniLogistic macro (Dhand, 2010). Screening of variables was performed using the likelihood-ratio χ^2 – test. The explanatory variables unconditionally associated with the outcome variables at P <0.25 were selected for inclusion in the relevant multivariable model.

Multivariable analyses were performed on two models, being:

Model for cohort OJD prevalence level (CPREV). The variables statistically associated with CPREV were submitted for multivariate analyses, performed with a macro (http://sydney.edu.au/vetscience/biostat/macros/). Given the small study size, the level of significance for an association between an explanatory variable and the outcome was increased from *P* <0.05 to *P* <0.1. Age and sex were not included in this model because they were pool-level variables and therefore not suitable for inclusion at cohort level.

Model for pool OJD status (PSTATUS). Multivariable logistic regression analyses were conducted and significant explanatory variables were then offered to the multivariable generalised linear mixed model. Due to the larger number of observations, the level of significance remained at $P<0.05$. Pool age and pool sex were forced into the model as fixed effects and clustering of pools within flocks was accounted for in the model.

2.5 Additional data from vaccinating flocks in NSW and Victoria

2.5.1 Assessment of the sensitivity of the PFC

To determine whether the failure to detect shedding in the 7 negative flocks from the initial round of testing of the 40 NSW and Victorian flocks that were tested by PFC350 in 2009 was likely to be a reflection of the absence of OJD, or a consequence of a testing regimen that is too insensitive to detect persistent infection at the current levels in these flocks, we sought to re-test these flocks with a more sensitive PFC. This was done under the Variation to project P.PSH0309 using a PFC600 test where 12 pools of 50 sheep were sampled. The 7 negative flocks were initially identified from official databases of disease surveillance, sampled by PFC350 in 2009, and approached again in 2010 for re-testing by PFC600. Four of the flocks only had sufficient sheep remaining and agreed to be re-tested. The sampling of the 4 flocks was performed by the same veterinarians who carried out faecal collection for PFC350 in 2009. Flock OJD status (positive or negative) was determined from the most recent PFC results and the online program Pooled Prevalence Calculator enabled calculation of initial and current cohort OJD prevalence.

2.5.2 Abattoir surveillance data

The Property Identification Code (PIC) and LHPA (Livestock Health and Pest Authority) assessment number for each of the NSW flocks, was forwarded to the NSW Industry and Investment AS database manager to enable collection of the AS records for each flock. Similar information was also sought from the Victorian DPI. Where available the AS OJD information was compared with the OJD prevalence estimates determined at the initial and current PFC sampling stages for each flock.

2.6 Case Study of OJD on Kangaroo Island

2.6.1 Selection of sheep flocks

The reference population for the variation for Project P.PSH0309 was 16 flocks from Kangaroo Island in SA. To have been eligible for OJD clearance testing, Kangaroo Island flocks must have fulfilled the following criteria outlined by PIRSA:

1. The flock must be comprised only of approved vaccinate sheep. An approved vaccinate complies with one of the following definitions:
   a. Vaccinated with Gudair™as a lamb between 3 and 16 weeks of age
   b. Vaccinated with Gudair™ as an adult prior to exposure to *Mptb.* as determined by an accredited Sheep Market Assurance Program (MAP) veterinarian
2. At least 2 years must have elapsed since the last known OJD-infected sheep left the property

In order to be released from quarantine, flocks eligible for clearance must demonstrate a negative PFC350 test, that is, an absence of detectable shedding of *Mptb.* by a PFC test in which 7 pools of 50 sheep at least 2 years of age are sampled (i.e. 98% confidence of detecting at least 2% infection). Of the 16 Kangaroo Island flocks included in the study population, PFC was performed in 2 flocks for the purpose of clearance. A further 8 flocks were subjected to PFC

for ongoing monitoring of *Mptb.* shedding levels whilst their eligibility for clearance was assessed by PIRSA. The remaining 6 Kangaroo Island flocks had previously been released from quarantine and agreed to be included in the study population for repeat PFC testing. Potential flocks were identified from official records including disease surveillance and laboratory testing information held in the databases at PIRSA. The selected Kangaroo Island flocks were owned or managed by individuals willing to complete a face-to-face interview regarding farm management, OJD infection history, Gudair™ vaccination history and other OJD control strategies.

Producers were remunerated for labour associated with mustering and sampling.

2.6.2 Sampling of sheep cohorts in flocks

The current sample size of 350 sheep per flock for surveillance and market assurance testing in Australia using PFC should provide the required 98% flock-sensitivity in flocks with a prevalence of 2% or greater and a reduced, but still acceptable, flock-sensitivity in lower prevalence flocks (Sergeant et al. 2002). However it has been acknowledged that for very low prevalence flocks, a gain in flock-sensitivity of PFC could be made by increasing the number of pools tested (Dhand et al, 2010). It is considered possible that false negatives or underestimated prevalence levels could result where cohort OJD prevalence exists at < 2% (Whittington et al., 2000; Whittington and Sergeant, 2001). It was estimated that the sampling of 12 pools of 50 sheep would increase the flock-sensitivity of PFC to provide 95% confidence of detecting a cohort prevalence of 1% (Sergeant 2009, pers. comm.). This protocol was used for sampling the flocks included in the study population.

A cohort represented a group of sheep in each flock selected for sampling and in most cases included 12 pools of 50 sheep to total 600 animals. Occasionally the number of pools and therefore the total number of sheep in each cohort varied due to limits to availability of sheep on Kangaroo Island, with only 400 animals available from 2 flocks, 450 animals from one flock and 600 sheep available for the remaining 13 flocks. Thus the PFC was performed on 8 pools in 2 flocks, 9 pools in 1 flock and 12 pools in 13 flocks. Mixed aged cohorts were formed by sampling a variety of sheep > 2 years of age. Nine flocks had cohorts of ewes only and 7 flocks had mixed cohorts of ewes and wethers. Sheep in individual pools within a cohort were always the same age and sex. Sheep with a body condition score <2 or lower than the flock average were preferentially sampled. The local private veterinarian and a University of Sydney Bachelor of Veterinary Science Honours student performed faecal sample collection by collecting one faecal pellet per rectum from each sheep selected.

Given that eligible flocks on Kangaroo Island are released from quarantine on the basis of a negative PFC350 test, PIRSA approved clearance provided an absence of detectable shedding of *Mptb.* was demonstrated in pools 1 to 7. Pools 8 to 12 were de-identified prior to submission to the laboratory to ensure that only University of Sydney researchers could determine the flocks from which the samples were collected. De-identification of pools 1 to 12 was performed prior to submission of samples collected from flocks previously released from quarantine.

2.6.3 Questionnaire design and implementation

A questionnaire of 65 questions was developed to collect data from the 16 Kangaroo Island flocks. Twenty questions were closed with 2 choices provided and 3 questions were semi-closed with three choices provided. Eight questions were semi-closed and requested a description. Twenty-eight questions were open and required quantitative information. These questions obtained information about property management, property enterprises, OJD infection history, Gudair™ vaccination history and other OJD control strategies. The remaining 6 questions

collected personal information, locality, Property Identification Code (PIC) and the date of the interview. No formal methods were implemented to assess the reliability or repeatability of questionnaire responses. The questionnaire was administered by face-to-face interview with the property owner/manager during January 2010 and February 2010. Due to recent relocation to the South Australian mainland one producer was interviewed by telephone. The University of Sydney BVSc Honours student conducted all interviews to eliminate bias associated with misinterpretation of questions. Interview duration ranged from 40 to 150 minutes.

2.6.4 Data management and statistical analysis

Questionnaire data were managed in a spreadsheet created in Microsoft Excel® 2008 for Mac. Information pertaining to each of the 16 KI flocks was identified by the PIC. Sixty-one variables and subsequent categories were derived from the data obtained. Flock OJD status, initial cohort OJD prevalence, current cohort OJD prevalence and cohort OJD prevalence difference were calculated and later added to the spreadsheet.

2.6.5 Outcome variables

2.6.5.1 Cohort OJD prevalence level and flock status in Kangaroo Island flocks

Initial cohort OJD prevalence was calculated from the results of PFC performed at the time OJD was first diagnosed in each Kangaroo Island flock. Current cohort OJD prevalence was calculated for each flock from the most recent PFC results in which 12 pools of 50 sheep were sampled by the aforementioned protocol. The Pooled Prevalence Calculator enabled calculation of cohort OJD prevalence (http://epitools.ausvet.com.au/content.php?page=PPFreq1). Cohort OJD prevalence difference was calculated for the 16 KI flocks from initial and current cohort OJD prevalence. The difference in prevalence variable was log transformed to make the distribution approximately normal. Although univariable and multivariable linear regression analyses were attempted to identify explanatory variables associated with cohort OJD prevalence difference, as data could be obtained only from 16 flocks, it was subsequently shown that only 2 of these flocks were positive in the PFC600 and any statistical analysis is likely to be testing for risk-factors associated with initial prevalence, it was decided that only a descriptive analysis of the data could be performed.

Current PFC results enabled identification of the number of OJD-positive and OJD-negative KI flocks. The likelihood-ratio χ^2-test was implemented to investigate the association between each explanatory variable and flock OJD status. Given the small number of flocks and observations, no statistically significant associations between flock OJD status and each of the explanatory variables was obtainable using the χ^2-test.

2.6.6 Explanatory variables

Sixty-one explanatory variables were identified from the questionnaire data for further investigation. All variables were categorical with 44 comprised of discrete data and 17 comprised of continuous data. Descriptive analyses were then conducted for all the questions in the questionnaire.

3 Results

3.1 PFC results from 40 vaccinating flocks in NSW and Victoria

A summary of flocks by district, initial prevalence category and sampling progress is presented (Table 1). Note that each flock was categorised according to the following prevalence levels where 7 pools of 30-50 were available for PFC or 450 AGID tests were available:

- High prevalence: ≥4 +PFC or ≥10 AGID positives
- Medium prevalence: 2-3 +PFC or 4-9 AGID positives
- Low prevalence: ≤1 +PFC or ≤3 AGID positives

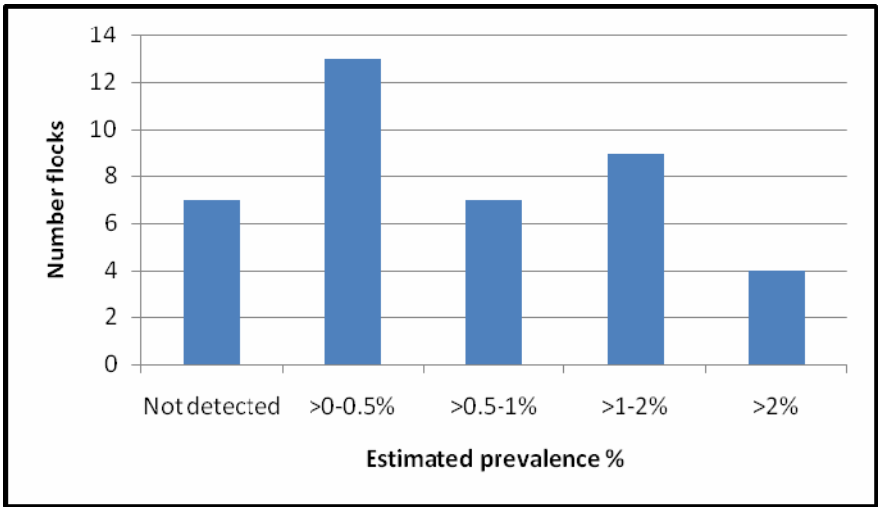
Table 1. Classification of P.PSH.0309 flocks by location and initial prevalence.

District	*Initial Prevalence estimate**			*Total*
	High	Low	Medium	
Braidwood		1		1
Central Tablelands	1	3	1	5
Goulburn			3	3
Hume	4	2	3	9
Moss Vale			1	1
Victoria	4	4		8
Wagga	3	1	3	7
Young	2	3	1	6
Total	14	14	12	40

* categorised using 7 pools of 30-50 for PFC or 450 AGID tests as above

The estimated OJD prevalence in 2009 for the 40 NSW and Victorian flocks as based on the proportion of positive pools and then submitted to the pooled prevalence calculator, is presented in Figure 1. As can be seen, no shedding was detected in 7 flocks and the prevalence exceeded 2% in 4 flocks.

Figure 1. Distribution of estimated prevalence of sheep shedding *Mptb.* in 40 infected flocks, 5 years after commencing vaccination.



The change from the initial prevalence categories as documented in Table 1 when re-examined with current PFC data, is presented in Table 2, representing the change in prevalence category after 5 years of vaccinating with Gudair™. Two flocks were excluded from analyses because of failure to meet selection criteria due to unreliable data for correctly categorizing their initial OJD prevalence. There was a reduction in number of flocks in the high prevalence category from 14 to 8 over the 5 years since commencement of vaccination. Despite 18.4% (7/38) of the flocks apparently not shedding currently, 47.4% (18/38) flocks had a cohort prevalence of >0 and ≤1% and 34.2% (13/38) had a cohort prevalence of >1% (Table 2).

Table 2. Changes in estimated prevalence of sheep shedding *Mptb.* in 38 infected flocks, after 5 years of vaccination

Initial prevalence*	Current Prevalence				
	Not Detected	Low >0 to 1%	Medium >1% to 2%	High >2%	Total
Low (4)	5	4	3	1	13
Medium (10)	0	8	2	1	11
High (14)	2	6	4	2	14
Total	7	18	9	4	38

* Initial number of farms in each category is provided in brackets

Comparison of current prevalence (i.e. post-vaccination) was made with the estimates from PFC and AGID done prior to vaccination (i.e. pre-vaccination) for 38 flocks using ordinal generalised linear mixed models. The results of the model are presented in Table 3 and indicate that the prevalence after vaccination was significantly lower than the prevalence before vaccination, with flocks before vaccination approximately 6 times more likely to have higher ordered prevalence compared to after vaccination.

Table 3. Summary results for an ordinal generalised linear mixed model to compare distribution of OJD prevalence among prevalence categories prior to and post vaccination in 38 study flocks.

Effect	Prevalence	*b*	*SE*	Odds ratios (95% CI)	P-value
Intercept	3	-2.42	0.47		
Intercept	2	-0.90	0.39		
Intercept	1	1.92	0.47		
Pre vaccination		1.78	0.47	5.92 (2.28, 15.39)	0.0002
Post vaccination		0		1	

Results for comparison of pre- and post-vaccination prevalence using Bayesian approach are presented in Appendix 2. These analyses estimated that the median OJD prevalence pre-vaccination was 2.99%, declining to 0.74% post-vaccination.

3.2 Analysis of PFC data and risk factor study from vaccinating flocks in NSW and Victoria

3.2.1 Questionnaire completion rates

Although the study was designed to have 40 farms, only 36 farms were included in the final risk factor analysis. Of the 4 missing farms, 2 were not included because during analysis they were deemed not to have met the selection criteria. The remaining 2 farms were non-responders, deemed so after 4 telephone calls. Of the 36 questionnaires used, the interviewer collected 32, whilst 3 were collected by telephone interview by the local district veterinarians due to difficulty in contacting the owners. The remaining questionnaire was faxed by the farm owner who did not wish to complete it over the phone.

3.2.2 Final study flocks for analysis

The majority of the 36 farms included in the analysis were from NSW (28) of which 5 were in the Central Tablelands, 3 near Goulburn, 6 near Hume, 6 near Wagga Wagga, 6 near Young and 1 near Braidwood. The remaining 8 farms where located in Victoria. The median size of the farms was 1007 hectares (range 202-2600) and the median altitude and rainfall was 332 metres (range 151-1036) and 620 millimetres (range 500-965) respectively. All 36 farms were self replacing merino flocks and the median adult fleece micron was 19 (range 17.5-21). The median farm sheep stocking rate was 3.8dse/h (range 0.4-16.6) and most farms lambed in spring (20) while 8 lambed in autumn and 8 in winter. All farms ran multiple enterprises, namely cattle (21), cropping (22), alpacas (1), pigs (1) or other sheep (28).

Included in the study was the index OJD property, diagnosed in 1980 as well as farms diagnosed with OJD as late as 2003. The estimated mortality rate from OJD on the properties in the last 12 months ranged from 0-4% with a median value of <1%. The mortality rates were based on farmer estimates and rarely involved post mortem confirmation so this data should be interpreted with care.

3.2.3 Vaccination protocols:

At the time of sample collection, the farms had been vaccinating the merino lamb drops at marking for 5 years (2), 6 years (16), 7 years (9), 8 years (7), 9 years (1) and 10 years (1). Wethers were left unvaccinated in some or all of the drops on 10 farms and of these, 7 farms sold the wethers at > 10 months of age. This age was of significance as sheep as young as 8 months have been shown to shed *Mptb.* (Reddacliff et al, 2006). Terminal and cross-bred lambs were not vaccinated on 20 farms and were sold at > 10 months of age on 8 of the farms. Professional vaccinators were routinely employed on 10 farms to administer Gudair™.

3.2.4 OJD management protocols:

None of the study farms shared facilities or rams. However 7 shared roads used to walk sheep and sheep strayed between neighbours on 22 of the farms. In the last 5 years, 25 farms used ≥ 2 ram sources and 17 farms introduced ≥ 30 individual rams. The introduction of replacement stock in the last 5 years occurred on 13 farms. The introduced rams and stock had varying vaccination histories. However only 4 of the owners of the farms suspected that the introduced rams may have been infected with OJD.

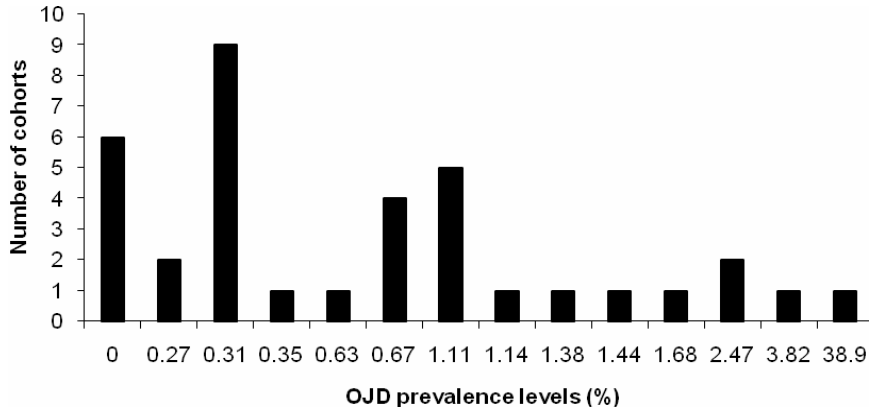
High loss mobs were identified and sold from 14 farms as a means to manage OJD flock prevalence. However, not all farms had high loss mobs. The sheep were run in age groups on nearly all the farms (32) and sheep lambed and lambs were weaned onto clean paddocks on 22

and 30 farms respectively. In this analysis, pastures were deemed 'clean' if they were spelled or alternated with cattle for at least one month as the drought made spelling for 3 months very difficult. Ideally, pastures should be spelled for 3 months to reduce *Mptb.* contamination by 90% and this is particularly important for lambs and weaners, the age groups most susceptible to infection (Abbott et al., 2004). Clinically affected sheep were culled on 31 of the farms with the methods of disposal including putting them in a pit (28) to leaving them in the paddock (3).

3.2.5 Outcome Variables:

The PFC results from the 36 cohorts were used to calculate the cohort OJD prevalence (CPREV). The cohort OJD prevalence level ranged from 0-38.9% and is presented in Figure 2. A total of 272 pools were tested and of these, 78 (28%) were positive and 194 (72%) were negative for OJD. A contingency table of the variables for CPREV and PSTATUS is presented as Appendix 1.

Figure 2. Distribution of current cohort (farm) OJD prevalence levels calculated from PFC results



3.2.6 Binomial logistic regression analyses for cohort OJD prevalence

Of the 30 variables analysed, 7 were unconditionally associated with cohort OJD prevalence at *P <0.25* presented in Appendix 2*.* After deletion of 1 variable which would not converge with the model, 6 were offered to the multivariate models. Final model results based on 36 sheep cohorts are presented in Table 5.

Table 5. Summary results for a binomial logistic regression model evaluating risk factors for cohort (farm) OJD prevalence (CPREV) on 36 study farms.

Parameters	*b*	SE(*b*)	Odds-ratios	LCL (OR)[a]	UCL (OR)[a]	*P value*[b]
Sheep stray between neighbours	-	-	-	-	-	0.001
No	0	-	1	-	-	-
Yes	4.16	1.61	64.24	4.61	>999.999	-
Concurrent cattle enterprise	-	-	-	-	-	0.065
No	0	-	1	-	-	-
Yes	-1.92	1.12	0.15	0.01	1.12	-
Professional Contractor used for	-	-	-	-	-	0.096

Gudair™ vaccination						
No	0	-	1	-	-	-
Yes	2.07	1.36	7.91	0.71	216.12	-
High loss mobs sold	-	-	-	-	-	0.008
No	0	-	1	-	-	-
Yes	2.56	1.10	12.98	1.87	154.43	-

[a] Profile likelihood 95% confidence intervals for odds ratio
[b] Based on likelihood-ratio χ^2- test of significance

3.2.6.1 Generalised linear mixed modelling for pool OJD status (PSTATUS)

Of the 30 variables investigated, 16 (including both confounders) were unconditionally associated with pool OJD status at *P<0.25* and are presented in Appendix 3. These were then offered to the generalised linear mixed models. Parameter estimates and odds ratios for the unconditional association between the explanatory variables and the cohort OJD prevalence (CPREV) being ≥ 1% are presented in Appendix 4. Parameter estimates and odds ratios for the unconditional association between the explanatory variables and the pool OJD status (PSTATUS) being positive are presented in Appendix 5. Final model results (*P>0.05)* based on culture results of 272 faecal pools (positive or negative) collected from 36 flocks are presented in Table 6.

Table 6. Summary results for a generalized linear mixed model evaluating risk factors for pool OJD status (PSTATUS) on 36 study farms.

Parameters	*B*	SE(*b*)	Odds-ratios	LCL (OR)[a]	UCL (OR)[a]	*P value*[b]
Confounders						
Age of sheep in pool	-	-	-	-	-	0.89
3	0.23	1.21	1.26	0.12	13.78	-
4	0.38	1.22	1.46	0.13	16.20	-
5	0	-	1	-	-	-
Sex of sheep in pool	-	-	-	-	-	0.65
Ewe	0	-	1	-	-	-
Wether	-0.35	0.75	0.71	0.16	3.11	-
Fixed effects						
Sheep moved on shared roads	-	-	-	-	-	0.014
No	0	-	1	-	-	-
Yes	-1.48	0.60	0.23	0.07	0.75	-
Other sheep introduced in last 5 years	-	-	-	-	-	0.0043
No	0	-	1	-	-	-
Yes	1.18	0.41	3.25	1.44	7.33	-

[a] Profile likelihood confidence intervals for odds ratio
[b] Based on likelihood-ratio χ^2- test of significance

3.3 Analysis of additional data from vaccinating flocks in NSW and Victoria

3.3.1 Assessment of the sensitivity of the PFC

As previously discussed, an objective of the project variation was to seek to determine if the current sample size of 350 sheep per flock for surveillance and market assurance testing in Australia using PFC to provide the required 95% flock-sensitivity in flocks with a prevalence of 2% or greater, has acceptable flock-sensitivity when used in lower prevalence flocks (Sergeant et al. 2002). It was estimated that the sampling of 12 pools of 50 sheep (PFC600) would increase the flock-sensitivity of PFC to provide 95% confidence of detecting a cohort prevalence of 1% (Sergeant 2009, pers. comm.). This protocol was used for re-sampling of those flocks in the study of prevalence in 40 flocks from NSW and Victoria that were found to have a clear PFC350. However only 4 of the 7 negative flocks agreed to be re-tested, all were from NSW and the sampling was performed in mid-2010.

The results from this small study using the PFC600 identified only one of the 4 negative flocks as having one positive pool. The positive culture was found in the second of the 12 pools.

3.3.2 Abattoir surveillance data

Results from interrogation of the abattoir surveillance (AS) database are presented in Appendix 6. Only 22 of the 40 NSW and Victorian flocks were identified on the AS database as having been detected with OJD by AS. There were no records for 15 flocks and 3 flocks had only negative lines, despite all 40 flocks having had a previous positive diagnosis of OJD and 33 currently having an OJD estimated prevalence exceeding zero. Of interest are the historical AS records of the first 7 flocks in this dataset, with a current estimated prevalence from PFC350 of zero and 5 of these flocks recorded as positive on the AS database (Appendix 6). The historical AS records from these flocks are presented in Table 7.

Table 7. Historical Abattoir surveillance records of flocks with current estimated prevalence of zero

Name initial	Year*	Age**	Lesions %	Line Result***	Initial Prevalence Category	Current Estimated Prevalencefrom PFC
D	2007	4T+	1.43	P	L	0
S	2001	3-5	16	P	L	0
S	2003	Aged	5.56	P	L	0
W	2001	5+	7.78	P	H	0
W	2002	5-Aged	10.80	P	H	0
W	2002	2T+	0.67	P	H	0
K	2004	3-5	0.77	P	L	0
C	NR				H	0
G	1999	4-5	0.91	P	L	0
G	2004	5+	0	N	L	0
G	2007	3-5	0.18	P	L	0
G	2008	5-Aged	0	N	L	0
G	2008	4-Aged	0.67	P	L	0
G	2009	5-6	0	N	L	0
M	NR				L	0

- *NR = no record
- **Age recorded as years or by T (teeth) with + indicating likely older cohorts
- *** P=positive, N=negative

Examination of the AS data for the University of Sydney 'Arthursleigh' flock that was placed in the Medium prevalence category on pre-vaccination estimates is of interest as there has been close annual monitoring of the 'tail' of this flock due to annual necropsies of at least 50 sheep since 2002. These necropsy studies of the 'tail' have shown a very slow decline in diagnoses of OJD at necropsy between 2002 and 2006 from 50% to 30% of sheep examined. Since then there has been a rapid decline in the OJD diagnoses in the 'tail' with approximately 5% OJD affected in 2007 and 2008 and 2% in 2009 and 2010. The current OJD prevalence as estimated by PFC350 in 2009 in 3-4yr olds was 0.67% and the AS data for 'Arthursleigh' is as follows (Table 8).

Table 8. Historical Abattoir surveillance records of Arthursleigh flock; current estimated prevalence of 0.67%

Year	Age	Lesions %
2006	4-A	1.11
2006	3-5	2.19
2006	3-A	1.27
2007	3-4	0.69
2009	6T+	0

Also of interest is the last 13 flocks in the AS dataset. With a current estimated prevalence from PFC350 of >1% placing them in the H category, it is noted that 6 of these flocks are yet to be recorded on the AS database (Appendix 6). The historical AS records from these flocks are presented in Table 9. Note that of these 13 flocks now in the H category, 6 were placed in the H, 3 in the M category and 4 in the L category on the initial prevalence data provided.

Table 9. Historical Abattoir surveillance records of flocks with initial and current estimated prevalence of >1%

Name	Year	Age	Lesions%	Line Result	PreTrial Prevalence	Est Prevalence from PFC
Ha					M	1.11
Th					H	1.11
McH					L	1.11
Br	2001	4th+	0.00	P	H	1.11
Br	2002	FM	0.01	P	H	1.11
Br	2002	FM	0.01	P	H	1.11
Br	2004	U	0.00	P	H	1.11
Br	2005	5	0.01	P	H	1.11
Br	2006	3-5	0.16	P	H	1.11
Wa	2003	3	0.04	P	H	1.11
Wa	2004	FM	0.04	P	H	1.11
Wa	2004	3/5	0.02	P	H	1.11
Wa	2006	4T+	0.01	P	H	1.11
Wa	2006	4T+	0.01	P	H	1.11
Wa	2007	6T+	0.02	P	H	1.11
Wa	2008	4T+	0.03	P	H	1.11
Wa	2009	6T+	0	N	H	1.11
McG	2006	4-A	0.02	P	M	1.68
McG	2006	4-A	0	N	M	1.68
McG	2009	5-6	0.01	P	M	1.68
McG	2009	3-5	0	N	M	1.68
Cr	NR				L	1.68
Ba	2000	5	0	N	H	1.68
Ba	2000	Aged	0	N	H	1.68
Ba	2000	6	0	N	H	1.68
Ba	2000	6	0	N	H	1.68
Ba	2000	4	0	N	H	1.68
Ba	2001	5+	0.00	N	H	1.68
Ba	2001	5+	0.20	P	H	1.68
Ba	2001	5+	0.01	I	H	1.68
Ba	2003	4-6	0	N	H	1.68
Ba	2003	H-3	0.02	P	H	1.68
Ba	2004	H-3	0	N	H	1.68
Ba	2004	3-5	0.03	P	H	1.68
Ba	2006	6T+	0.05	P	H	1.68
Ba	2008	6-A	0	N	H	1.68
War	2008	6T+	0.01	P	L	1.68
Mu	2001	2+	0.01	P	M	2.47
Mu	2001	3-5	0.03	P	M	2.47
Wha	NR				L	2.47
Me	NR				H	3.81
We	2001	FM	0.40	P	H	>4*
We	2002	4/6	0.07	P	H	>4*
We	2004	3-5	0.00	P	H	>4*

>4* all 7 pools positive in PFC350 so prevalence unable to be calculated

3.4 Case Study of OJD flocks on Kangaroo Island

3.4.1 OJD on Kangaroo Island

Data describing property type, management and enterprise on KI are tabulated in Appendix 7 (appendices 7a, 7b and 7c respectively) with the sheep enterprise information provided in Table 10. The median property area was 590 hectares (range 117-1012) and the median annual rainfall was 537.5 millimetres (range 450-750). Thirteen flocks were self-replacing and the median adult fleece micron was 21 (range 19-30). Increasing prime lamb production has occurred on KI over the last 5 to 10 years and 14 flocks currently run crossbred sheep. Most flocks lambed in winter regardless of whether progeny were Merino (11) or British breed (10) sired. The absolute sheep density, median sheep equivalent density and total stock density were calculated for each property using formulae described previously (Lugton 2004). The median sheep equivalent density was 7.35 dse/ha (range 4.53-11.05). Six properties ran cattle enterprises and the median total stock density for these farms was 9.96 dse/ha (range 7.61-11.62).

Table 10. Descriptive statistics for sheep enterprise and management on KI.

Variable and Categories	Frequency	Percent
Ewe breed		
Fine wool Merino	1	6.3
Medium wool Merino	8	50.0
Fine wool Merino and F_1	1	6.3
Medium wool Merino and F_1	6	37.5
Ram breed		
Medium wool Merino	2	12.5
Medium wool Merino, Border Leicester and Poll Dorset	1	6.3
Medium wool Merino, Border Leicester and White Suffolk	2	12.5
Medium wool Merino, Border Leicester, Poll Dorset and White Suffolk	2	12.5
	1	6.3
Medium wool Merino and Poll Dorset	1	6.3
Medium wool Merino and White Suffolk	3	18.8
Medium wool Merino, Poll Dorset and White Suffolk	1	6.3
Border Leicester and Black Suffolk	1	6.3
Border Leicester, Poll Dorset and White Suffolk	1	6.3
Poll Dorset and White Suffolk	1	6.3
Fine wool Merino, Poll Dorset and White Suffolk		
Self replacing flock		
No	3	18.8
Yes	13	81.3
Lambing month (Merino sire)		
Not applicable	2	12.5
May	1	6.3
May-June	2	12.5
June	2	12.5
June-July	1	6.3
July	3	18.8
July-August	3	18.8
August	1	6.3
August-September	1	6.3
Lambing month (British breed sire)		
Not applicable	2	12.5
May	1	6.3
May-June	3	18.8
June	5	31.3
June-July	3	18.8
July-August	1	6.3
August-September	1	6.3
Cattle currently present		
No	10	62.5
Yes	6	37.5

3.4.2 OJD infection history

The history of OJD infection in 16 flocks on Kangaroo Island is presented in Table 11. OJD was first diagnosed in the Kangaroo Island flocks included in the study population in 1998 (2), 2001 (4), 2002 (6), 2003 (2) and 2004 (2). The 6 flocks previously released from quarantine achieved clearance in 2007 (4), 2008 (1) and 2009 (1). Three flocks had previously destocked in accordance with the KIOJDCP recommendations. Only two producers were suspicious that OJD was present in their flock prior to official diagnosis and only three producers reported mortalities due to the disease.

Table 11. Descriptive statistics for property OJD infection history for 16 flocks on Kangaroo Island

Variable and Categories	Frequency	Percent
Property previously destocked		
No	13	81.3
Yes	3	18.8
Year OJD diagnosed		
1998	2	12.5
2001	4	25.0
2002	6	37.5
2003	2	12.5
2004	2	12.5
Flock released from quarantine		
No	3	18.8
Yes	13	81.3
Year flock released from quarantine		
Not applicable	3	18.8
2007	4	25.0
2008	1	6.3
2009	1	6.3
2010	7	43.6
Producer suspected OJD present in flock	14	87.5
No	2	12.5
Yes		
Producer reported mortalities due to OJD	13	81.3
No	3	18.8
Yes		

3.4.3 Gudair™ vaccination protocols

The history of Gudair™ vaccination usage in flocks on Kangaroo Island that were included in the study population in 2002 (7), 2003 (8) or 2004 (1) are presented in Table 12. The first drop of lambs vaccinated in each flock was the 2002 (6), 2003 (8) or 2004 (2) drop. Whole flock vaccination was completed on 13 properties and at the time of sampling 9 flocks were comprised only of approved vaccinates.

Sheep that are born into an already approved vaccinate flock and vaccinated with Gudair™ at 3 to 16 weeks of age are defined by PIRSA as second generation vaccinates. Second generation

vaccinates were present in 6 flocks included in the study population. Adult vaccinated sheep were identified in 5 flocks. Ten flocks currently receive free Gudair™ while 6 flocks purchase partially subsidised vaccine. Twelve property owners/managers indicated they would continue vaccinating all animals if the subsidised vaccine were completely phased out. However the remaining 4 producers indicated they would reduce vaccination costs by excluding particular mobs such as crossbred lambs. Professional contractors were not employed by any of the 16 KI flocks included in the study population.

Table 12. Descriptive statistics for property Gudair™ vaccination history for 16 flocks on Kangaroo Island.

Variable and Categories	Frequency	Percent
Year vaccination commenced		
2002	7	43.8
2003	8	50.0
2004	1	6.3
First drop lamb vaccinated		
2002	6	37.5
2003	8	50.0
2004	2	12.5
Whole flock vaccination completed		
No	3	18.8
Yes	13	81.3
Approved vaccinate flock		
No	7	43.8
Yes	9	56.3
Second generation vaccinates present		
No	10	62.5
Yes	6	37.5
Adult vaccinated sheep present		
No	11	68.8
Yes	5	31.3
Free vaccine currently received		
No	6	37.5
Yes	10	62.5
Partially subsidised vaccine currently received		
No	10	62.5
Yes	6	37.5
Continue vaccination if subsidy phased out		
No	4	25.0
Yes	12	75.0
Professional contractor used for Gudair™ vaccination		
No	16	100.0
Yes	0	0.0

3.4.4 OJD management protocols

Data on OJD management is presented in Tables 13, 14 and 15. Fifteen producers reported sheep straying between neighbouring flocks and 2 producers shared roads with adjacent properties to relocate stock. None of the flocks included in the study population shared rams or facilities. Eight properties were surrounded entirely by flocks currently using Gudair™. However 2

producers were aware of at least one neighbouring sheep or goat grazing property not vaccinating against OJD. The vaccination status of adjoining sheep grazing properties was unknown by the remaining 6 producers (Table 10).

The median number of ram sources used by KI flocks since vaccination commenced on their properties was 4 (range 1-7) and the median number of rams introduced was 35 (range 4-117). Ten flocks purchased other sheep and the median number of animals introduced was 800 (range 200-2000). None of the 16 KI property owners/managers suspected that OJD was introduced to their flocks by purchased sheep. Four producer's culled mobs in which OJD was detected and one producer reported disposing of two rams suspected to have been clinically infected (Table 11).

Table 13. Descriptive statistics for property OJD biosecurity data for 16 flocks on Kangaroo Island.

Variable and Categories	Frequency	Percent
Neighbouring flocks Gudair™ vaccinated		
No	2	12.5
Yes	8	50.0
Unknown	6	37.5
Rams, facilities and/or roads shared between neighbours	14	87.5
No	2	12.5
Yes		
Straying reported by producer		
No	1	6.3
Yes	15	93.8
Sheep purchased and introduced to flock		
No	6	37.5
Yes	10	62.5
Producer suspected OJD introduced by purchased sheep	16	100.0
No	0	0.0
Yes		

Table 14. Descriptive statistics for property OJD biosecurity data relating to rams for 16 flocks on Kangaroo Island.

Variable	n	Minimum	Q1	Median	Q3	Maximum
Number of ram sources	16	1	2	4	6	7
Total number of rams introduced	16	4	19.5	35	62.5	117
Total number of sheep introduced	16	200	375	800.	1425	2000

Table 15. Descriptive statistics for property OJD control strategies relating to culling for 16 flocks on Kangaroo Island.

Variable	N	Minimum	Q1	Median	Q3	Maximum
Average ewe culling age (years)	16	5.50	5.75	6.50	7.50	9.5
Average wether culling age (years)	16	4.50	4.50	5.50	6.50	7.50
Oldest sheep currently on property (years)	16	5.00	5.50	6.50	7.50	9.00

Weaners and hoggets were run separately in 14 flocks and 6 producers ran single-age group mobs. A 'clean' paddock was reserved for lambing and weaning by 4 and 14 producers respectively. Due to the limitations imposed by ongoing drought, pastures were deemed 'clean' in this study if spelled or alternately grazed by cattle for at least one month. Terminal sires were used in 14 flocks included in the study population and joined to culls, poor wool or body type ewes or ewes > 4.5 years of age in most cases. The median culling age of ewes and wethers was 6.5 (range 5.5-9.5) and 5.5 (range 4.5-7.5) years respectively. Two producers decreased the culling age of sheep on their property as an OJD control strategy (Table 16).

Table 16. Descriptive statistics for property OJD control strategies data for 16 flocks on Kangaroo Island.

Variable and Categories	Frequency	Percent
High prevalence mobs culled		
No	12	75.0
Yes	4	25.0
Separate young sheep		
No	2	12.5
Yes	14	87.5
Single age group mobs present		
No	10	62.5
Yes	6	37.5
'Clean' paddock reserved for lambing		
No	12	75.0
Yes	4	25.0
'Clean' paddock reserved for weaning		
No	2	12.5
Yes	14	87.5
Terminal sires used in flock		
No	2	12.5
Yes	14	87.5
Year terminal sires introduced to flock		
Not applicable	2	12.5
1997	1	6.3
2000	1	6.3
2002	2	12.5
2005	2	12.5
Terminal sires always used in flock	8	50.0
Ewe groups joined to terminal sires		
Not applicable	2	12.5

Poor wool or body type ewes and culls	3	18.8
Poor wool or body type ewes and ewes >4.5 years of age	3	18.8
Poor wool or body type ewes and ewes >5.5 years of age	2	12.5
Ewes >3.5 years of age	1	6.3
Ewes >4.5 years of age	1	6.3
All ewes	4	25.0
Disposed of clinical OJD cases		
No	15	93.8
Yes	1	6.3
Decreased average culling age in flock		
No	14	87.5
Yes	2	12.5

3.4.5 Initial cohort OJD prevalence in Kangaroo Island flocks

Initial cohort OJD prevalence level ranged from 0.24 to 1.94%. PFC was performed on 5 pools in 2 flocks, 7 pools in 9 flocks, 8 pools in 3 flocks and 9 pools in 2 flocks. The total number of sheep in each cohort therefore varied: 250 (2), 350 (9), 400 (3) and 450 (2). A total of 115 pools were tested of which 26 (23%) were positive and 89 (77%) were negative for OJD. The number of positive pools produced by each KI flock varied: 1 (11), 2 (2), 3 (2) and 5 (1).

3.4.6 Current cohort OJD prevalence in Kangaroo Island flocks

Current cohort OJD prevalence level ranged from 0.00 to 2.17%. PFC was performed on 8 pools in 2 flocks, 9 pools in 1 flock and 12 pools in 13 flocks. The total number of sheep in each cohort therefore varied: 400 (2), 450 (1) and 600 (13). A total of 181 pools were tested of which 12 (7%) were positive and 169 (93%) were negative for OJD. The number of positive pools produced by each KI flock varied: 0 (14), 4 (1) and 8 (1).

3.4.7 Cohort OJD prevalence difference in Kangaroo Island flocks

Cohort OJD prevalence difference was calculated for the 16 Kangaroo Island flocks from initial and current cohort OJD prevalence. Cohort OJD prevalence difference values were right skewed. The data approximated a normal distribution after log transformation. Cohort OJD prevalence difference was statistically different from zero ($P<0.001$).

3.4.8 Flock OJD status for Kangaroo Island flocks

The 2 flocks that produced a positive PFC result had been subjected to PFC for ongoing monitoring of *Mptb.* shedding levels whilst their eligibility for clearance was assessed by PIRSA. Pools 1, 2, 3, 4, 7, 8, 9 and 11 were positive in the first infected flock and represented 4.5 year old ewes, 5.5 year old ewes, 3.5 year old wethers, 4.5 year old wethers, 6.5 year old ewes, 2.5 year old ewes, 3.5 year old wethers and 4.5 year old wethers respectively. Pools 4, 6, 9 and 10 were positive in the second infected flock and represented 5.5 year old ewes, 3.5 year old ewes, 3.5 year old ewes and 5.5 year old ewes respectively. No statistically significant associations between the explanatory variables and flock OJD status were identified by the likelihood-ratio χ^2-test. This result was expected given the small number of OJD-positive flocks detected by PFC.

4 Discussion

The work of this project has considerably advanced our knowledge of the long term impacts of vaccinating with Gudair™ in flocks of varying initial prevalence of OJD. The key findings are discussed under the headings of the work conducted, as follows:

4.1 Selection and sampling of 40 vaccinating flocks in NSW and Victoria

After 5 year of vaccinating with Gudair™, the estimated OJD prevalence in 2009 for the 40 NSW and Victorian flocks as based on the proportion of positive pools and then submitted to the pooled prevalence calculator (as presented in Figure 1) indicated that currently, 17.5% (7/40) of the flocks were apparently not shedding, 50.0% (20/40) flocks had a cohort prevalence of >0-1% and 32.5% (13/40) had a cohort prevalence of >1%. Subsequent analysis led to a change in the initial prevalence categories (as documented in Table 1) with a reduction in flocks in the H category from 14 to 4, with 12 and 18 flocks remaining in the M and L categories respectively. Comparison of current prevalence from PFC350 with the estimates from PFC and gel tests done prior to vaccination for 38 flocks, using ordinal generalised linear mixed models (with flocks as random effect and the estimation time of pre- or post-vaccination as a fixed effect) indicated that the prevalence after vaccination was significantly lower than the prevalence before vaccination, with flocks before vaccination approximately six times more likely to have higher ordered prevalence compared to after vaccination. Similarly, Bayesian analyses indicated that the median prevalence reduced from a pre-vaccination level of 2.99% (95% PI 1.50, 7.92%) to a post-vaccination level of 0.74% (95% PI 0.42, 1.29%). Moreover, 90% of the flocks had prevalence less than 3.09% (95% PI 1.57, 10.01) post-vaccination compared to 19.90% (95% PI 6.79, 65.47) pre-vaccination.

4.2 Analysis of PFC data and risk factor study of vaccinating flocks in NSW and Victoria

This risk factor study that examined management factors on 36 of the 40 properties that were sampled in Project P.PSH.0565 sought to identify factors that may be affecting the efficacy of the Gudair™ vaccine in flocks vaccinating for at least 5 years. The results of the PFC, with 72% of the pools tested negative and only 28% positive, confirmed that the majority of the flocks had a current cohort OJD prevalence of <1% with 6 of the 36 flocks having undetectable levels. This correlates with the literature that Gudair™ vaccine is effective in reducing the shedding rate of *Mptb.* over time (Reddacliff, 2006). However, as indicated above, after 5 years of vaccination with Gudair™ 13 flocks still had a cohort OJD prevalence of ≥1% and it is likely that sheep from these flocks would present a high level risk of transmission of OJD. The PFC350 is currently the most sensitive and economically viable test available for the detection of OJD in live animals, estimated to have 98% sensitivity for detecting flocks with a ≥ 2% prevalence (Sergeant et al, 2002). Since 32 of the farms had a cohort OJD prevalence of <2% the sensitivity of the test on these flocks may be lower and false negatives could have resulted. As some of the test-negative flocks could in fact be infected, the findings of this study should be interpreted with caution.

4.2.1 Farm biosecurity

Farms where sheep stray between neighbours were significantly more likely to have a cohort OJD prevalence of ≥1% (*P =0.001)*. This could be due to an increase in pasture contamination if neighbouring sheep were shedding high numbers of *Mptb.* and strayed onto vaccinating farms. The contribution of one heavily infected sheep can be as high as 10^8 bacilli per gram of faeces and doses $>10^3$ and $<10^7$ bacilli have been shown to be infective (Whittington et al., 2000; Lugton, 2004). Of particular importance would be if shedding sheep strayed onto pasture that was used for lambing or weaning as these age groups are highly susceptible to infection with

Mptb. (Whittington and McGregor, 2005). Gudair™ vaccine reduces *Mptb.* pasture contamination and thus infectious challenge for subsequent lamb drops, by decreasing the shedding rates of infected sheep by up to 90% (Eppleston et al., 2004). If pasture contamination remains high due to straying sheep, the efficacy of Gudair™ vaccine is likely to be reduced. This could also occur if young sheep strayed to neighbouring farms with higher levels of *Mptb.* contaminated pasture. However there was no relationship between cohort OJD prevalence or pool status and having unvaccinated neighbours. This may be explained by many owners not knowing the vaccination history of their neighbours, especially where multiple neighbours were concerned. Alternatively, farms where sheep stray may have an increased number of lambs that miss marking and consequently are not vaccinated with Gudair™ before the recommended 16 weeks of age. If these lambs were then infected with *Mptb.* the reduced shedding effects seen with Gudair™ would be negated and there would be increased pasture contamination potentially leading to a higher OJD prevalence level within the flock.

Farms where sheep were introduced onto the property in last 5 years were about 3 times more likely to have pools test positive for OJD (*P<0.05).* The relationship between the introduction of OJD infected or unvaccinated sheep and increased risk of disease due to lateral spread and pasture contamination is clear and well documented (Sergeant, 2001). However there is also a risk associated with introducing Gudair™ vaccinated sheep that are shedding onto an uninfected property. As Gudair™ does not prevent OJD infection, any vaccinated sheep can still be infected with OJD and consequently be a source of *Mptb.* pasture contamination (Reddacliff et al., 2006). As vaccinates that develop clinical OJD may have multibacillary lesions and shed *Mptb.* at levels likely to be infective, these sheep can significantly contribute to pasture load (Reddacliff et al., 2006; Windsor, 2006) and would be of great concern if they were traded. These findings indicate that biosecurity is of importance in reducing pasture contamination and thus controlling OJD in Gudair™ vaccinating flocks.

4.2.2 High loss mobs sold

The sale of high loss mobs is a common recommendation for the control of OJD. However in this study there was a positive association between the farms selling high loss mobs and having a cohort OJD prevalence of ≥1% (P <0.1), presumably reflecting a management response on farms still experiencing high losses or high percentages of clinically affected sheep. Other studies have found similar associations which suggest that this practice is a response to and not a cause of higher cohort OJD prevalence (Lugton, 2004; Dhand et al, 2007).

4.2.3 The use of professional contractors for Gudair™ vaccination

Interestingly, an association between higher cohort prevalence levels and the use of professional contractors in the administration of Gudair™ vaccine was found (*P <0.1)* although there is a 10% probability of this result being due to chance. Despite being an unexpected finding, the association is weak and does not warrant recommendations on the cessation of professional contract vaccinators. However the observation may provide an interesting study if research into the role of professional contractors in disease control should occur.

4.2.4 Protective factors

Cattle may have a protective value in reducing prevalence levels in vaccinating flocks as farms that had a concurrent cattle enterprise were found to be less likely to have a cohort OJD prevalence of ≥1% (*P <0.1).* The presence of cattle on the farms could result in a greater ability for producers to spell paddocks or rotationally graze, thereby reducing the *Mptb.* contamination of pastures. Spelling or grazing with cattle can lead to a reduction in *Mptb.* contamination levels,

which can survive in pasture for up to 7 months (Abbott et al., 2004; Whittington and McGregor, 2005). In the drought conditions that persisted throughout this study, many producers had limited capacity to effectively spell pasture and thus the lambs and weaners may have been exposed to higher levels of infection than would occur in non-drought years and the cumulative effect of long term Gudair™ vaccination on decreasing pasture contamination may have been diminished. However having a concurrent cropping enterprise was not found to significantly affect the cohort OJD prevalence even though this presumably may have similar effects on *Mptb.* pasture decontamination as grazing cattle (Abbott et al., 2004).

Moving sheep on shared roads was also found to be associated with OJD prevalence, with farms moving sheep on shared roads less likely to have positive pool status (*P<0.05).* This contradicts other studies which have found moving sheep on shared roads was associated with an increased risk of lateral spread of OJD (Dhand et al., 2007). This finding could potentially be explained by farms only moving stock on roads where the risk of lateral spread from neighbours was very low, as may occur where there is knowledge of the OJD status of neighbours and vaccination history. Alternatively, these producers may choose to only move older vaccinated sheep that are less susceptible to OJD infection than young sheep or the roads may be reserved for moving sheep younger than 11months of age which have not yet begun to shed *Mptb.* (Whittington and McGregor, 2005). It is also possible that producers with higher prevalence of OJD are more biosecurity conscious and in not wanting to spread disease to neighbours, are less likely to use shared roads.

4.2.5 Control of confounding:

To reduce confounding bias, known potential confounders were forced into the multivariable models for the associations with the outcome variable positive pool status (PSTATUS). Neither sex nor age of the sheep, were shown to be associated with OJD infection.

4.2.6 Study validity, strengths and limitations:

The objective measurement of the outcome variables contributed significantly to the validity of this study. The use of the PFC350 to detect OJD in sheep has markedly better sensitivity than serology and very high specificity, thus reducing the risk of false-positive samples (Sergeant et al., 2002). The risk of false negatives due to imperfect sensitivity remains with the PFC, with a sensitivity estimated at 98% probability for detecting flocks with an OJD prevalence of ≥2%. Since 33 of the flocks had a prevalence of <2%, 7 of which were undetectable, the sensitivity of PFC may not have been as high in this study and false negatives may have resulted. This may have affected the outcome variable CPREV as some test-negative flocks might have been included in the <1% category, when in fact true prevalence was ≥1%. The flock-sensitivity could have been increased by whole flock testing or increasing the number of pools cultured. However this would be prohibitively expensive.

Bias of the explanatory variables was unavoidable as this was a cross-sectional study using data from questionnaires that are highly reliant on farmer recall. Efforts were made to minimise the bias by conducting the questionnaires by telephone interview. One person conducted 32 of the questionnaires in an effort to standardise the way the questions were asked and to avoid misinterpretation of the questions by the farmers. Unfortunately 3 of the questionnaires had to be conducted by the local district veterinarians and the remaining questionnaire was faxed by the farmer. Furthermore, many of the flock management questions assumed that the practices had not changed over the 5 year period of interest. If they had changed this may also have biased the results. In addition, the small study size of only 36 of the 40 farms sampled could have reduced the number of associations found between the explanatory and outcome variables. A larger study

size would have been ideal but it was cost prohibitive as both the manpower and PFC tests required for such a study would exceed the budget available. Two outcome variables were investigated to try and maximise the chance of finding significant associations.

A large number of explanatory variables were tested in this study, which though not uncommon in observational studies, does introduce a possibility of identification of spurious associations. For example, with a 5% level of significance, one variable out of every 20 tested could be identified to be significant just due to chance. Therefore, the results of significant associations should be interpreted with due caution. The associations should at best be considered only indicative and further research should be conducted to confirm the results.

4.3 Analysis of additional data from vaccinating flocks in NSW, Victoria and Kangaroo Island

4.3.1 Assessment of the sensitivity of the PFC

As national surveillance and market assurance programs in Australia sample 350 sheep per flock by PFC to detect a minimum OJD prevalence of 2% with 98% confidence (Sergeant et al., 2002) it may be important to assess the sensitivity of this test in flocks with a prevalence of infection <2%, particularly given the potential for underestimated prevalence levels or false negative results (Whittington et al., 2000; Whittington and Sergeant, 2001). The PFC600 protocol used in this study population was estimated to detect a minimum OJD prevalence of 1% with 95% confidence and involved sampling 12 pools of 50 sheep for PFC. Unfortunately only 4 of the 7 flocks from NSW and Victoria and 6 flocks on Kangaroo Island that were negative on the PFC350 agreed to be retested.

The results of this small study interestingly showed that the flock OJD status would have remained unchanged whether based on the results of pools 1 to 7 or pools 1 to 12 for both of the 2 of 16 Kangaroo Island flocks sampled that had positive pools and the one of 4 NSW flocks re-sampled that was positive. It was observed that regardless of pool order, for a flock with 8 of 12 pools positive, there is a 100% probability of detection by sampling any 7 pools at random. This drops to 99% for 4 of 12 and 58% for 1 of 12 i.e for a low prevalence flock (<0.2%), there is still a 58% chance of detection with 7 pools regardless of order of sampling. These results suggest that the standard sampling protocol of 7 pools of 50 sheep is likely to be adequate where cohort OJD prevalence ranges from 0.00 to 0.57%. However the interpretation of these preliminary findings is constrained by small sample size and may require further investigation.

4.3.2 Abattoir surveillance data

It is noted that only 22 of the 40 NSW and Victorian flocks were identified on the AS database as having been diagnosed with OJD, despite all 40 flocks having had a previous positive diagnosis of OJD and 33 currently having an OJD estimated prevalence exceeding zero when submitted to PFC350.However as AS only screens a relatively small proportion of sheep killed and that the majority are from low-prevalence areas, it is not unexpected that some heavily infected flocks might remain unscreened and hence undetected. And example in this study was flock ‘C’ with an initial H category that has a current estimated prevalence of zero and has yet to appear on the AS database. In addition flocks ‘Ha, Th and Me’ that were initially in the H category and with a current estimated prevalence from PFC350 of >1% also placing them in the H category, are yet to be recorded on the AS database.

Other interesting observations were that in flock ‘Ba’ with 14 records in the AS database indicating examination for OJD, only 4 of these examinations resulted in a positive diagnosis of

OJD, with 9 negative and one inconclusive, in a flock that was initially placed in and remains in the H category. Although this may be interpreted as consistent with previously published reservations regarding the sensitivity of AS, especially in low prevalence flocks (Abbott and Whittington, 2003), it is noted that the AS program screens several thousand lines of sheep per year with the vast majority from low prevalence areas. Advice is that in 2009, only about 400 PIC's from high and medium prevalence areas of NSW and 270 PIC's from the medium prevalence area of Victoria were examined in 2009 (E Sergeant, pers. comm.)

Regardless of these interpretations, AS does appear to be doing a very useful job in detecting OJD in many flocks. In the example of flock 'Br' that was initially and is currently placed in the H category and sampled on 6 occasions between 2001 and 2006, a positive diagnosis was found on each occasion. Almost as consistent has been the AS results as seen with flocks 'Wa' initially and currently placed in the H category and sampled on 8 occasions between 2003 and 2009, with a positive diagnosis on each occasion with the exception of the 2009 sampling. Perhaps the most compelling record of the decline in OJD prevalence following vaccination is provided by examination of necropsy data on the tail of the mob at 'Arthursleigh'. The initial serological results placed this flock in the M category prior to vaccination and necropsy records enabled confirmation of OJD prevalence consistent with the AS records from lesion % suggesting the 2006 OJD prevalence as high as 2.2%. There has been a significant decline on OJD diagnosed at necropsy correlated with a decline in AS diagnoses by lesion % to less than 1% for the first time in 2007 and a current prevalence by PFC350 of 0.67%.

4.3.3 Incorporation of information from AS and multiple sources using a Bayesian approach

One of the design considerations in this project was the difficulty of comparing current OJD prevalence as estimated by PFC with the variable quality and quantity of information available on prevalence that was available at the time of commencement of vaccination. This was approached in two ways. Firstly we used the serological and pooled faecal culture data to develop a qualitative category of flock prevalence as High, Medium or Low prevalence. Secondly, we utilized multiple sources of information on OJD prevalence that were available at the commencement of vaccination, including serology, culture, abattoir surveillance and histopathology, to develop a Bayesian model that incorporated information from previous studies on the sensitivities of these tests. This enabled an estimated median prevalence pre- and post-vaccination in the selected flocks to be calculated and demonstrated a decline in prevalence confirming that the post-vaccination prevalence estimate was significantly lower compared to the pre-vaccination prevalence ($P<0.001$). It is noted that the current mean prevalence of 0.74% does likely present a significant risk in the trading of vaccinates currently.

4.4 Case Study of OJD on Kangaroo Island

4.4.1 Vaccine efficacy in Kangaroo Island flocks

Although the objective of this study was to examine the efficacy of Gudair™ vaccination and other on-farm control strategies by comparing initial and current cohort OJD prevalence, the extent to which vaccination has decreased disease prevalence in KI flocks was not able to be quantified independently of other on-farm OJD control strategies. Current OJD prevalence was significantly different from the initial prevalence by both non-parametric tests and a one-sample t-test ($P<0.001$), verifying a decrease in *Mptb.* shedding levels from the time OJD was first diagnosed in each flock. Given that the national sheep industry awards assurance based credits to flocks vaccinating against OJD, it is important to confirm a continued reduction in disease prevalence within flocks as vaccination control programs in Australia progress. The significance

reduction in prevalence in this study provides preliminary evidence supporting the allocation of increasing credits as successive generations of sheep are vaccinated (Eppleston et al., 2005).

Vaccination reduces faecal shedding of *Mptb.* by approximately 90% in addition to delaying the onset and decreasing the incidence of OJD-associated mortalities (Reddacliff et al., 2006; Windsor, 2006). However it is important to remember that vaccinates may continue to shed *Mptb.* as vaccination does not appear to decrease the proportion of subclinically infected sheep that have multibacillary lesions. Sheep with multibacillary lesions may excrete in excess of 10^8 organisms per gram of faeces, potentially equivalent to the amount excreted by many hundreds of sheep in the early stages of the disease or with paucibacillary infections (Reddacliff et al., 2006). This means that the "breakdown" of a single animal with clinical OJD can have a disproportionate effect on potential transmission of infection and the persistence of disease in the flock. This is particularly important if the breakdown in OJD occurs at a critical time such as lambing when a whole cohort of young susceptible animals may be exposed to high numbers of *Mptb.* (Reddacliff et al., 2006; Whittington and McGregor, 2005).

Previous studies indicate a similar efficacy of vaccination when Gudair™ is administered to sheep at 3 months and 8 months of age, with a lower prevalence of *Mptb.* shedding detected in both groups compared to unvaccinated controls (Windsor, 2006). Vaccination in the post-weaning period may therefore be beneficial even when sheep have been exposed to a heavily contaminated environment since birth. It is likely that the level of pasture contamination with *Mptb.* was initially limited on 13 of the KI properties by implementation of whole flock vaccination and has since been minimised by vaccination of successive annual crops of lambs.

Twelve KI producers reported that they would continue vaccination if subsidies were completely phased out. However cessation of industry support for vaccination would undoubtedly impact future uptake of Gudair™ on KI. Research on the financial impact of OJD suggests that vaccination at the current cost is unlikely to be economically viable in the KI study population flocks (Bush et al., 2008). KI flocks would be categorised as 'at-risk' according to the model described by Bush et al. (2008) and therefore unlikely to reach a vaccination breakeven point within a 20-year time frame regardless of the enterprise type (Merino, first-cross or second-cross). There is a negative return to vaccination because the cost of Gudair™ is not compensated by a reduced OJD mortality rate (Bush et al., 2006; Bush et al., 2008). The benefit from vaccination in these flocks is likely to be associated with any market advantage 'at-risk' vaccinated animals could command. The extent of trading losses associated with the lost opportunity to sell live sheep was not addressed by the model and largely depends on the disease status, enterprise mix and production system of individual farms (Bush et al., 2008). Of note is the financial impact of trading losses is minimal for most KI flocks as they primarily sell sheep direct to slaughter.

As discussed, vaccination does not prevent all sheep from becoming infected with *Mptb.* and there remains a risk that some vaccinated sheep will transmit the disease (Eppleston et al., 2005; Reddacliff et al., 2006). Accurate quantification of the risk that vaccinated sheep will transmit OJD is currently challenging given that Gudair™ is less effective in reducing subclinical infection and because subclinical disease is more difficult to detect by routine diagnostic testing (Eppleston, 2005). It is considered likely that sustained vaccination will be necessary to avoid recrudescence of OJD on KI. However the findings of this study on KI do provide preliminary evidence suggesting that the risk of transmitting the disease by the trading of vaccinates is significantly decreased after medium-term use of Gudair™ in flocks with a low initial cohort OJD prevalence (range 0.24-1.94%). Vaccination for OJD commenced in these KI flocks in 2002 (7), 2003 (8) and 2004 (1) indicating vaccination for 8 years, 7 years and 6 years respectively.

This study has determined that the high rate of clearance of OJD in KI flocks is more likely to reflect the absence of OJD than a testing regimen that is too insensitive to detect persistent infection at the current levels in these flocks. Whittington and Sergeant (2001) commented that the passage of time and repeated testing are our greatest allies in the detection of OJD given that infected animals progress in the disease process and most tests are more effective in the later stages of the disease. A continued absence of *Mptb.* shedding in the 6 flocks previously released from quarantine in 2007(4), 2008 (1) and 2009 (1) is implied by their negative OJD result when tested by the PFC600 test. The second generation vaccinates, present in only 6 of these flocks, are the most likely cohort to carry a sufficiently low risk of being infected that they may act as a source of 'low OJD risk' sheep for commercial producers and as replacements for infected flocks undergoing eradication.

4.4.2 Sampling protocol for the detection of OJD by PFC

As discussed in 4.3.1, as national surveillance and market assurance programs in Australia sample 350 sheep per flock by PFC to detect a minimum OJD prevalence of 2% with 98% confidence (Sergeant et al., 2002), the existence of flocks with a prevalence of infection <2% appears to be an important factor to consider in accreditation testing given the potential for underestimated prevalence levels or false negative results (Whittington et al., 2000; Whittington and Sergeant, 2001). To address this the PFC600 protocol used in this study population was estimated to detect a minimum OJD prevalence of 1% with 95% confidence and involved sampling 12 pools of 50 sheep for PFC. The results indicate the flock OJD status would have remained unchanged whether based on the results of pools 1 to 7 or pools 1 to 12 for the 16 Kangaroo Island flocks sampled and that the standard sampling protocol of 7 pools of 50 sheep will still detect some flocks where cohort OJD prevalence ranges from 0.00 to 0.57%. For example, flocks with 1/12 pools positive (equivalent to <0.2% prevalence) would be detected with a level of confidence of about 58%.

4.4.3 Risk factors for increased cohort OJD prevalence since OJD diagnosis

Properties that purchased and introduced sheep on KI were found less likely to have a decrease in *Mptb.* shedding levels and this association was exacerbated as the total number of sheep introduced increased. . Of the two properties currently shedding in this study, one infected flock had destocked in three phases between 1998 and 2003. The producer then introduced approximately 1500 sheep from 7 KI sources and commenced vaccination in 2003. The other infected flock has changed ownership three times since OJD was first diagnosed on the property and twice since vaccination commenced in 2002. Approximately 1400 sheep were introduced to this flock from 5 mainland SA sources between 2003 and 2009. Although the mobs purchased and introduced to the two OJD-positive flocks were believed to have been vaccinated prior to delivery and have accreditation with the SheepMAP in some cases, the true vaccination status of these sheep remains uncertain. Although some mobs were vaccinated on arrival, this was an inconsistent protocol on both properties. Neither producer suspected that OJD was introduced to their flock by non-home bred sheep. Given that Gudair™ does not prevent infection with *Mptb.*, there remains a possibility that any vaccinated sheep may be infected with OJD and subsequently contribute to pasture contamination (Eppleston et al., 2005; Reddacliff et al., 2006).

The following observations are also of interest with respect to the two OJD-positive flocks on KI. The total number of rams introduced and the number of properties from which rams were sourced for the two OJD-positive flocks exceeded the median values for the study population. The first infected property introduced 117 rams from 7 sources while the second infected property introduced 74 rams from 6 sources since vaccination commenced. The introduction of large numbers of rams of uncertain vaccination status may carry similar risks to the introduction

of large numbers of non-home bred sheep. The owner of the first infected flock failed to complete whole flock vaccination when he initially commenced in 2003, resulting in the presence of approximately 100 to 200 ewes between 6.5 and 7.5 years of age that have never been vaccinated. It is likely that these unvaccinated adult sheep would have disproportionately contributed to pasture contamination of the property with *Mptb*. In highly contaminated environments the prevalence of clinical cases would be expected to be higher and to occur at a younger age than in cleaner environments (Dhand et al., 2007; Lugton, 2004; Whittington and Sergeant, 2001). It is likely that the environment of the first infected flock is highly contaminated given that *Mptb.* shedding was detected in ewes only 2.5 years of age at the most recent PFC and the producer reported mortalities attributable to OJD. Diagnosis of OJD by PFC and abattoir surveillance in 2003 and 2008 respectively indicates a high level of pasture infectivity in the first infected flock. Running single age group mobs in addition to reserving a 'clean' paddock for lambing and weaning would seem to have been of limited effectiveness in reducing the transmission of OJD between generations of sheep in the first infected flock.

4.4.4 Study limitations

The findings of this study must be interpreted with caution given the small sample size and reliance on property owner/manager recall. Although a larger study size would have been preferable, difficulties were encountered in the recruitment of Kangaroo Island flocks. Properties previously released from quarantine were reluctant to participate in repeat PFC testing due to apprehension that detection of *Mptb.* would result in the flock again being placed under order. Despite PIRSA approving repeat PFC testing without risk of the property being quarantined subsequent to a positive result and the offer of remuneration for labour associated with mustering and sampling, only 6 cleared flocks agreed to participate. Live sheep trading limitations imposed on flocks currently under regulatory order seem only of significance to stud enterprises on Kangaroo Island. Release from quarantine provides no economic incentive to those producers content to sell direct to slaughter, particularly given the free Gudair™ vaccine available to flocks under order. It is also acknowledged that the prevalence estimates from the PFC600 may be biased because the estimation methods used assume random allocation of pellets to pools. In this study, allocation was often on a non-random basis, so that while the results provide an indication of likely prevalence, there is some potential for error.

Inaccuracies occur when questionnaires rely on producer recall and opinion, although the questionnaire was administered by interviewer in a face-to-face interview to minimise bias associated with misinterpretation of questions. One producer was telephone interviewed due to recent relocation to the SA mainland. The potential for change in management practices from the time of flock infection with a chronic disease such as OJD to the time of questionnaire administration complicated investigation of management factors in this study. Efforts were made to contact multiple producers where properties had changed ownership.

5 Success in Achieving Objectives

The 5 initially contracted objectives and the 4 additional objectives approved in the project variation were combined as 9 objectives and were achieved. Key findings and comments related to each objective are summarised below.

5.1 Objective 1: Estimate prevalence and shedding levels in up to 40 flocks from 3 states with known OJD testing and vaccination history for at least 5 years, including high, medium and low-prevalence flocks.

The initial pre-vaccination estimate of OJD prevalence from historical records of *Mptb*. culture and serological data resulted in an almost equal spread of the 40 selected flocks from NSW and Victoria across the 3 within-flock prevalence ranges of H, M and L (High, Medium and Low prevalence), that is 14,12 and 14 in each category respectively. Results from PFC350 on these 40 flocks following 5 years of vaccination, indicates that currently, there are 8, 12 and 13 flocks in the H, M and L categories with 7 flocks now having no detectable shedders.

5.2 Objective 2: Compare current prevalence and shedding levels in these flocks with estimates from prior to commencement of vaccination.

Comparison of current prevalence estimates from PFC with estimates from prior to commencement of vaccination indicate that 17.5% (7/40) of the flocks have no detectable shedding. However 32.5% (13/40) had a cohort prevalence of >1% and 50% of the flocks (20/40) were found to be shedding at >0-1% cohort prevalence. Importantly, 7 of the 14 flocks in the initial L category had increased prevalence to be now placed in the M or H category (medium or high) after 5 years of vaccination. This data indicate that despite a rapid decrease in OJD mortality in flocks following the commencement of a vaccination program, shedding of *Mptb.* persists for at least 5 years in a majority of flocks and that up to 82.5% of the flocks have shedding at rates that would be of concern if sheep were being traded from these flocks or vaccination ceased. Analysis of the data by Bayesian modelling identified a significant decline in the range of estimated OJD prevalence pre-vaccination to the estimated post-vaccination prevalence, with 90% of the flocks having an OJD prevalence less than 3.09% (95% PI 1.57, 10.01) post-vaccination, compared to 19.90% (95% PI 6.79, 65.47) pre-vaccination.

5.3 Objective 3: Investigate the relationship between estimated prevalence through shedding and abattoir surveillance results in project flocks.

As we see examples of some flocks having several negative records in the AS database as well as some positive records, is likely to indicate that even in a high prevalence flocks, significant differences in OJD prevalence in different mobs is expected to occur within a flock. Perhaps the most compelling record of the decline in OJD prevalence following vaccination is provided by examination of necropsy data on the tail of the mob at Arthursleigh where initial serological results placed this flock in the M category prior to vaccination and necropsy records enabled confirmation of the decline in OJD prevalence that is also reflected in the AS records.

5.4 Objective 4:-Identify and discuss factors which may have affected the effectiveness or otherwise of vaccination on changes in levels of infection.

Management factors found to be associated with OJD prevalence exceeding 1% in flocks vaccinating with Gudair™ include having sheep stray and the introduction of new sheep on farms. The use of professional contractors for the administration of the vaccine also appeared to increase the risk of having higher OJD prevalence levels although this was a weak association and further study of this preliminary finding is required. Having a concurrent cattle enterprise was

shown to be protective. The findings of this study suggest that while Gudair™ vaccine does decrease the OJD prevalence levels in vaccinating flocks, it does not negate the continuing need for farm biosecurity in the control of OJD.

5.5 Objective 5:- Make recommendations on the effectiveness of medium-term vaccination in high, medium and low prevalence flocks and the role of vaccination in risk management as part of the ABC.

The analysis of the data by Bayesian modelling that identified a significant decline in the estimated OJD prevalence in 90% of the NSW and Victorian flocks, from 19.90% (95% PI 6.79, 65.47) pre-vaccination to less than 3.09% (95% PI 1.57, 10.01) post-vaccination, is very encouraging as it provides clear evidence of effectiveness of vaccination in reducing thr risk of disease transmission. However as 82.5% of flocks are still shedding *Mptb.* and thus contain vaccinated sheep able to transmit OJD, this study provides evidence of the importance of recommendations that promote persistence with vaccination beyond 5-6 years.
Further, the apparent declining prevalence of OJD on Kangaroo Island, provides preliminary evidence suggesting that the risk of transmitting the disease by the trading of vaccinates is significantly decreased after medium-term use of Gudair™ in KI flocks. Vaccination for OJD commenced in these KI flocks in 2002 (7), 2003 (8) and 2004 (1) indicating vaccination for 8 years, 7 years and 6 years respectively has achieved an apparent OJD negative status. This preliminary evidence supports the concept of allocating of increasing ABC points as successive generations of sheep are vaccinated with Gudair™.

5.6 Objective 6: Estimate OJD prevalence using 12 pools of 50 to determine *Mptb*. shedding levels in up to 7 flocks from 2 states with known OJD testing and lamb only vaccination history for at least 5 years, where no shedding was detected on the most recent PFC using 7 pools of 50.

Only 4 of the 7 negative flocks in NSW and Victoria following their 2009 PFC350 test agreed to be retested in 2010 by the PFC600 and only one of these 4 flocks was found to have a single positive pool that occurred in the second of the 12 pools on the re-test.

5.7 Objective 7: Estimate OJD prevalence using 12 pools of 50 to determine *Mptb*. shedding levels in flocks on KI with known OJD testing and whole flock vaccination history for at least 5 years, where no evidence of pathological infection has been detected by recent AS and the flock is being assessed for clearance from quarantine.

The PFC600 was used in 6 flocks on Kangaroo Island that were previously tested as negative by the PFC350 and no shedding was detected in these flocks. Only 2 of the remaining 10 flocks tested by the PFC600 on KI were positive The study on Kangaroo Island verified a significant decrease in *Mptb.* shedding levels from the time OJD was first diagnosed in KI flocks providing preliminary evidence to support the allocation of increasing assurance based credits under the national system of vendor declaration as successive generations of sheep are vaccinated with Gudair™.

5.8 Objective 8: Identify and discuss factors which may have affected the effectiveness of the testing regimen and vaccination strategy on changes in levels of infection on these properties.

There were many management factors used in the flocks on Kangaroo Island that are likely to have contributed to a decline in OJD prevalence, including vaccine subsidy, whole flock vaccination, elimination of high risk mobs, terminal sires over higher risk mobs, improved farm

biosecurity etc. Due to small sample size and only 2 flocks found to be OJD positive, the extent to which vaccination has decreased disease prevalence in Kangaroo Island flocks was not able to be quantified independently of other on-farm OJD control strategies.

5.9 Objective 9: Evaluate whether the additional data from this project variation offers can assist in assessing the risk of transmission of OJD from 2nd generation vaccinates.

This study on Kangaroo Island has determined that the high rate of clearance of OJD in these flocks is more likely to reflect the absence of OJD than a testing regimen that is too insensitive to detect persistent infection at the current levels in these flocks. Continued absence of *Mptb.* shedding in the 6 flocks previously released from quarantine in 2007(4), 2008 (1) and 2009 (1) following their negative PFC350 test and then confirmed as negative by the PFC600 test in 2010 implies absence of OJD in these flocks. The second generation vaccinates, present in only 6 of these flocks, are the most likely cohort to carry a sufficiently low risk of being infected that they may act as a source of 'low OJD risk' sheep for commercial producers and as replacements for infected flocks undergoing eradication.

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

Findings from this project are of direct relevance to the sheep industries of Australia as they provide quantitative estimates for the decline in OJD prevalence in flocks in NSW and Victoria that have been vaccinating with Gudair™ for 5 years or more. There has been a significant decrease in estimated OJD flock prevalence by Bayesian modelling from a median prevalence pre-vaccination of 2.99% (95% PI 1.50, 7.92%) to a post-vaccination level of 0.74% (95% PI 0.42, 1.29%). However the results identified that shedding was still detectable in 82.5% of flocks in NSW and Victoria, confirming that there remains a significant risk of spread of OJD through the trading of vaccinates. These data strongly support the need to continue vaccinating in the majority of flocks. Importantly, the PFC350 was shown to be a sound testing strategy and capable of detecting infection in flocks even when the OJD prevalence is less than 2%. However a single negative test was shown not to detect infection in all of the 10 negative flocks on the mainland and Kangaroo Island that agreed to be retested at PFC600, with one NSW flock (10%) found to have single positive pool on the re-test. Repeated PFC350 tests may be necessary to indicate that infection is no longer in a flock, and that persistence with continued use of Gudair™ vaccine is currently necessary on both the mainland and Kangaroo Island to control OJD.

These studies also provide information on the importance of management factors in the control of OJD. In the 3 flocks that were found to be positive on the PFC600, the decision to omit the vaccination of wethers or introduction of sheep that were not vaccinated with Gudair™ into the flock appeared most likely to have been responsible for the persistence of infection (although this was not significant in regression models for cohort prevalence and pool status). The risk factor study also identified that flocks vaccinating for 5 years or more that currently had an OJD prevalence >1% were more likely to have straying sheep between neighbours, have sold high risk mobs, used a professional contractor for the vaccination or have not been grazing with cattle. These findings suggest that improving farm biosecurity to minimise introduction of infection from straying sheep plus the use of cattle to clean up infected pastures, may be strategies worthy of promotion to OJD infected producers.

This study has indicated that there has been a modest but significant improvement in the OJD prevalence of infected flocks in NSW and Victoria following commencement of vaccination with Gudair™ and confirm that the time frame for disease control is necessarily prolonged. However the findings on Kangaroo Island do provide optimism that if vaccination persists, eventually OJD prevalence will decline sufficiently so that some flocks will achieve apparent eradication of the disease. The preliminary findings from Kangaroo Island suggest that the second generation vaccinates are likely to have a much lower prevalence of OJD infection and represent a far lower risk to transmission of the disease than the first generation vaccinates that comprise the majority of this study.

7 Conclusions and Recommendations

The work undertaken in this project has greatly advanced our knowledge of the efficacy of OJD vaccination.

7.1 Extension

This finding supports the need for advisory programs to promote the continued use of Gudair™ vaccine and management interventions that can minimize disease risk. The generally slow decline in OJD flock prevalence as measured in this study is indicative of the insidiousness of OJD and the need for infected flock owners to persist with vaccine and be patient. However the preliminary findings from Kangaroo Island are encouraging that second generation vaccinates are a lower risk of spreading OJD. Future verification of the expected low risk status of second generation vaccinates through current studies is likely to give industry greater confidence that eventually, the trading of vaccinates is less risky than it has been in the first 5 years since the registration of Gudair™. Consideration of the best extension strategy for these results, in addition to that currently occurring through conferences, rural press and an advisory note for distribution by Animal Health Australia, merits further discussion.

7.2 Further research

The longitudinal study that continues monitoring the flocks in Project OJD.033 to more accurately estimate the changes in shedding over at least a decade, now progressing as Project P.PSH.0565, is aimed at achieving this objective and should receive continuing support.

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9 Appendices

9.1 Appendix 1: Initial flock testing results for classification of estimated prevalence at time of vaccination in the 40 trial flocks

State	Prev.	Gel?	Gel no's	Gel +	PFC?	PFC no's	PFC +	Histo?	Histo no's	Histo +
NSW	L	Yes	467	1	No			Yes	1	1
NSW	H	No			Yes	7	6	No		
NSW	M	No			Yes	7	2	No		
NSW	L	Yes	450	0	Yes	1	1	No		
NSW	L	Yes	217	4	Yes	3	3	Yes	3	3
NSW	M	No			Yes	7	3	No		
NSW	H	Yes	450	23	No			No		
NSW	M	Yes	449	8	No			No		
NSW	M	Yes	450	4	No			No		
NSW	M	No			Yes	11	3	No		
NSW	H	No			Yes	7	5	No		
NSW	L	No			Yes	7	1	No		
NSW	H	No			Yes	7	7	No		
NSW	M	No			Yes	7	4	No		
NSW	M	No			Yes	7	3	No		
NSW	H	No			Yes	7	5	No		
VIC	H	Yes	900	29	No			No		
VIC	L	Yes	957	1	Yes	20	6	No		
VIC	L	Yes	678	3	Yes	10	8	Yes	3	1
VIC	H	No			Yes	10	5	No		
VIC	H	No			Yes	10	4	No		
VIC	L	Yes	522	2	No			No		
VIC	H	No			Yes	19	19	No		
VIC	L	Yes	848	2	No			Yes	4	4
NSW	H	Yes	50	0	Yes	7	4	Yes	3	0
NSW	H	No			Yes	10	9	Yes	1	0
NSW	M	No			Yes	7	2	No		
NSW	L	No			Yes	10	2	No		
NSW	H	Yes	1	1	Yes	7	4	Yes	1	1
NSW	H	Yes	34	0	Yes	4	1	No		
NSW	M	No			Yes	14	3	No		
NSW	L	Yes	454	2	No			No		
NSW	M	Yes	130	1	Yes	7	2	Yes	1	1
NSW	L	No			Yes	7	1	No		
NSW	H	No			Yes	7	6	No		
NSW	L	No			Yes	7	0	Yes	2	2
NSW	M	No			Yes	7	3	No		
NSW	L	Yes	50	0	Yes	7	1	Yes	1	1
NSW	H	Yes	644	11	Yes	7	4	No		
NSW	L	No			Yes	7	3	No		
NSW	M	Yes	?100	?20	No			Yes	3	3

Empty cells indicate unavailable data

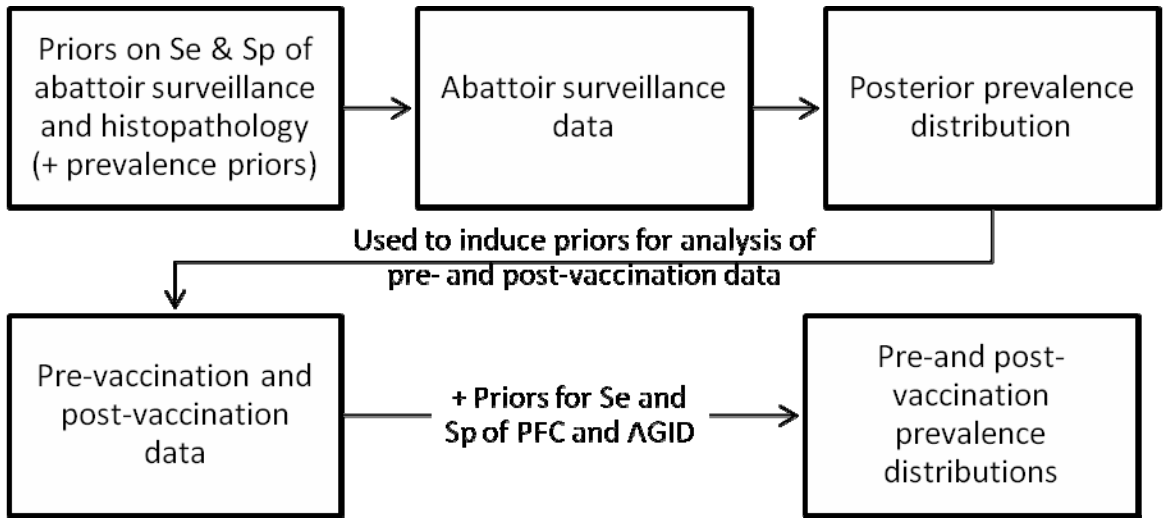
9.2 Appendix 2: Comparing pre- and post-vaccination prevalence using a Bayesian approach

Estimation of pre-vaccination prevalence:

Estimation of pre-vaccination prevalence was based on the data obtained from the respective farmers and regulatory agencies. Unlike a consistent approach employed for determining post-vaccination prevalence (based on PFC results), the diagnostic tests employed to determine the pre-vaccination prevalence varied across flocks: (a) PFC for eight flocks, (b) Agar gel immune diffusion (AGID) test for 22 flocks and (c) both PFC and AGID for 10 flocks.

Since it was not possible to compare the apparent pre- and post-vaccination prevalence estimates due to use of different diagnostic tests (with different sensitivities and specificities), we developed a Bayesian approach to estimate true pre-vaccination prevalence after accounting for sensitivities and specificities of diagnostic tests. This prevalence was then compared to the true post-vaccination prevalence estimated adopting the Dhand et al (2010) approach in a cohesive Bayesian model. A schema of the Bayesian approach implemented is presented in Figure 1.

Figure 1. A schema of the models developed for analysis of pre- and post-vaccination data. Se: Sensitivity; Sp: Specificity; PFC: Pooled faecal culture; AGID: Agar gel immunodiffusion test.



First we describe the models developed to estimate true pre- and post-vaccination prevalence and then discuss the priors elicited to build the Bayesian models.

Modelling the data for estimating prevalences among flocks

We assume a sample involving r flocks, each with its own unknown pre-vaccination prevalence. It is assumed that these flocks constitute a random sample from a super population of flocks, all with distinct non-zero prevalences of OJD. The unknown prevalences π_i across flocks are modelled as independent and sampled from a Logit-Normal (LN) distribution:

$$[logit(\pi]_i) = \nu + v_i, \quad \text{where } v_i \sim N(0, \lambda^2), \qquad \ldots(1)$$

which is equivalent to:

$$\pi_i = \frac{\exp(\nu + v_i)}{1 + exp(\nu + v_i)}$$

where ν is the mean and λ the standard deviation of the prevalence distribution on the logit scale. Priors were later induced for ν and λ based on analysis of abattoir surveillance data (see below). Our main goal in what follows will be to estimate the prevalence distribution before and then again after vaccination and to compare those distributions.

The median of the pre-vaccination prevalence distribution is simply:

$$\mu_{pre} = \frac{e^{\nu}}{1 + e^{\nu}} \qquad \ldots(2)$$

Also of interest will be the 90th percentile of pre-vaccination prevalence distribution. This is the values for which 90% of all prevalences will be smaller, and 10% larger. The pre-vaccination 90th percentiles are estimated as:

$$\mu_{pre,0.90} = \frac{e^{\nu+1.28\lambda}}{1 + e^{\nu+1.28\lambda}}$$

Models for pre-vaccination data

The pre-vaccination prevalences will be estimated based on three types of test data since flocks were tested by AGID alone, PFC alone or both AGID and PFC. In each case, we will have a single apparent prevalence or two apparent prevalences, modelled as functions of the true prevalences, and the sensitivity and specificity of the particular tests.

In the case of the AGID test, sheep were tested individually while in the case of the PFC, the faecal samples were pooled. So in the case of pooled testing, the apparent prevalence is in fact the apparent pool prevalence as opposed to the apparent animal-level prevalence. This distinction does not matter for inferences since each is a particular function of the true animal-level prevalence and final inferences are about true animal level prevalence. We note that, while the direct information for the prevalences of flocks in the three groups is different, the above model for the prevalence distribution of all flocks ties the information together in the final inference. We now proceed to describe the three models for the three groups of flocks.

Let $P_{ig} = \Pr(+|AGID)$ be the *apparent* pre-vaccination animal-level OJD prevalence of flock i tested using AGID test. This apparent prevalence is related to the *true* animal-level pre-vaccination OJD prevalence (π_i) through AGID test sensitivity Se_g and specificity Sp_g:

$$P_{ig} = \pi_i * Se_g + (1 - \pi_i) * (1 - Sp_g)$$

The data consist of individual test outcomes on the AGID that are either positive or negative. This is usually summarized as a binomial count of the number of positive outcomes out of the number of sheep tested in the given flock. These outcomes are assumed independent from flock to flock.

Similarly, let $P_{if} = \Pr(+|PFC)$, where positive means that the pool is positive without knowing which pellet or pellets might be infected. Methods for handling pooled testing were described in Dhand et al (2010). Briefly, the probability of a pool of size k having no pellets from infected

sheep is $(1-\pi_i)^k$. Hence, the probability of a pool being positive, that is having pellets from at least one infected sheep is $[1-(1-\pi_i)^k]$. The apparent pool prevalence can thus be specified as:

$$P_{if} = \{1-(1-\pi_i)^k\} * Se_f + (1-\pi_i)^k * (1-Sp_f)$$

where Se_f and Sp_f are, respectively, the (pool level) PFC sensitivity and specificity. An assumption is that the sensitivity is the same regardless of the number of infected pellets in the pool. The data for these flocks consist of the binomial counts of the number of pools that are positive within each flock, and again, counts from flock to flock are assumed independent.

Similarly, for flocks where both AGID and PFC tests were conducted, there will be independent binomial counts for the AGID individual animal testing, and for the PFC pool level testing.

The above modelling results in a likelihood function contribution that incorporates the three contributions from the AGID alone flocks, the PFC alone flocks and the both test flocks with the model for the prevalences.

Models for post-vaccination data

There were only PFC data available for post-vaccination animal-level prevalence estimation. Therefore, the modelling of post-vaccination prevalence data for all the flocks follows a similar procedure as outlined above for pre-vaccination PFC data, except that the pool sizes were variable rather than fixed at k for the post-vaccination PFC data. The only effect this had on the model was that k was replaced by the actual pool size for each of the individual pools as in Dhand et al. (2010). Thus, if pool j in flock i is of size k_{ij}, then the post-vaccination apparent pool prevalence ($PP_{\downarrow}ijf$) is:

$$PP_{ijf} = \left\{1-(1-p\pi_i)^{k_{ij}}\right\} * Se_f + (1-p\pi_i)^{k_{ij}} * (1-Sp_f)$$

where $p\pi_i$ is the true post-vaccination prevalence in flock i. The data again consist of independent binomial contributions with these probabilities for positive pools.

Modelling post-vaccination true prevalence

The true prevalences in post-vaccination flocks are expected to be different from those in the same flocks before vaccination. We model pre-vaccination prevalences using models (1-2) as above and the post-vaccination prevalence using the model:

$$logit(p\pi_i) = \nu + \Delta + w_i$$

which is equivalent to:

$$p\pi_i = \frac{\exp(\nu + \Delta + w_i)}{1 + \exp(\nu + \Delta + w_i)}$$

where Δ defines a shift in the distribution of prevalences from the pre-vaccination level, and where w_i is a random effect for flock i and is modelled with a $N(0, \theta^2)$ distribution. We note that the median of the distribution of post-vaccination prevalences is just:

$$\mu_{post} = \frac{e^{\nu+\Delta}}{1+e^{\nu+\Delta}} \quad \ldots(3)$$

We will be interested in comparing pre- and post-vaccination medians. Note that if $\Delta = 0$, the medians are the same, though the variability in prevalence distributions may be different. If $\Delta < 0$, then the median prevalence in the post-vaccination flocks will be less than for the pre-vaccination flocks, etc.

Similar to pre-vaccination prevalence 90th percentile, the post-vaccination 90th percentiles are estimated as:

$$\mu_{post,\,0.90} = \frac{e^{\nu+\Delta+1.28\times\theta}}{1+e^{\nu+\Delta+1.28\times\theta}}$$

The (augmented data) likelihood function for the entire analysis combines the component from the pre-vaccination data with that obtained from the corresponding binomial contributions from post-vaccination, and the contribution due to the modelled pre- and post-vaccination prevalences.

Elicitation of priors

Elicitation of sensitivity and specificity priors

Beta priors were elicited for sensitivity and specificity of AGID and PFC tests based on previous research (Sergeant et al., 2003; Dhand et al., 2010). Sergeant et al. (2003) estimated average AGID sensitivity to be 24.6% using data from six known infected and 12 assumed uninfected sheep flocks (Table 1). Our concentrated specificity prior reflected a very high specificity for AGID reported in that study. We used the same priors for sensitivity and specificity of PFC as used in Dhand et al. (2010) based on analysis of Whittington et al., (2000) data (Table 1). See Dhand et al. (2010) for further details.

Table 1. Priors for sensitivities and specificities for various tests elicited in the study.

Priors	Input values		Prior distributions[2]				Source of priors
	Mode	Lower/ Upper[1]	a	b	Mean	(95% PI)	
Priors for analysing abattoir surveillance data							
Abattoir surveillance							
Sensitivity	0.70	0.40	6.33	3.28	0.66	(0.35, 90)	Bradley and
Specificity	0.98	0.95	151.7	4.08	0.97	(0.94, 0.99)	Cannon (2005)
Histopathology							
Sensitivity	0.65	0.40	7.98	4.76	0.63	(0.36, 0.86)	Dennis et al (2010)
Specificity	0.995	0.99	1137.5	6.71	0.99	(0.99, 1.00)	(Same as PFC)
Priors for analysing pre- and post-vaccination prevalence data							
Agar gel immuno-diffusion test (AGID)							
Sensitivity	0.25	0.60	2.43	5.30	0.31	(0.06, 0.65)	Sergeant et al. (2003)
Specificity	0.995	0.99	1137.5	6.71	0.99	(0.99, 1.00)	Sergeant et al. (2003)
Pooled faecal culture test (PFC)							
Sensitivity	0.60	0.40	10.9	7.6	0.59	(0.36, 0.80)	Dhand et al (2010)
Specificity	0.995	0.99	1137.5	6.71	0.99	(0.99, 1.00)	Dhand et al (2010)

[1]Lower 5% limits were elicited for sensitivity and specificity estimates except for abattoir surveillance sensitivity for which the upper 95% limit was incorporated because the mode was less than 0.5); [2]*a* and *b* are parameters of the respective beta probability distributions.

Priors for pre-vaccination prevalence distribution

We were in the fortunate situation of having abattoir surveillance data from the same population of flocks as our data. We used these data to obtain prior information for the pre-vaccination prevalence distribution. First we will discuss analysis of abattoir surveillance data and then the approach used to induce pre-vaccination prevalence priors based on the results of abattoir surveillance.

Model for analysis of abattoir surveillance data

Data about abattoir surveillance were available from 25 of the 40 flocks in the current study. The abattoir surveillance involves determination of lesion status of sheep at slaughter for samples of animals from given flocks. Thus for each flock, there is a binomial count of the number of animals with detected lesions. These counts have a probability of being positive that is formulaically just like our previous ones, namely:

$$P_{il} = \pi_i * Se_i + (1 - \pi_i) * (1 - Sp_i)$$

where P_{il} is the apparent animal-level OJD prevalence of lesions in flock i based on abattoir surveillance, π_i is again the true animal-level OJD prevalence, now for the i^{th} flock in this sample, and Se_i is the sensitivity and Sp_i the specificity of lesion detection.

In addition, for each flock, a certain proportion, *c*, of the observed lesions among those that were lesion positive (L+) were re-tested using histopathology. The counts of histopathology positive (H+) results are again binomially distributed, with probability:

$$\Pr(H+|L+) = \pi_i * Se_l * \frac{Se_h}{P_{il}}$$

where we have used the facts that $H+$ implies OJD+, and that $H+$ is conditionally independent of $L+$ given OJD+. The (augmented data) likelihood function for this analysis combines binomial contributions from the lesion surveillance and binomial contributions from the histopathology results. The true animal-level prevalences (π_i) across flocks are modelled as independent and sampled from a $Beta(\alpha,\beta)$ distribution, as was done in (Hanson et al., 2003). Briefly, the mean, μ and variance σ^2 for this distribution are related to α and β as:

$$\alpha = \mu\psi \qquad \beta = (1-\mu)\psi$$

where $\psi = \alpha + \beta$. So the mean, μ , is the average prevalence among the super population of flocks, and the standard deviation, $\sigma = \sqrt{\frac{\mu(1-\mu)}{\psi(\psi+1)}}$ is large if ψ is small, and is small if ψ is large. For example, if $\mu = 0.2$ and $\psi = 10$, then $\alpha = 2$ and $\beta = 8$, we obtain a distribution that has 95% of the prevalences between 3% and 48% and a standard deviation of 0.12, whereas if we leave the mean alone and let $\psi = 400$, we obtain a prevalence distribution with 95% of the prevalences between 16% and 24% and a standard deviation of 0.02.

Priors for analysis of abattoir surveillance data

Analysis of the abattoir data was performed in WinBUGS using priors for the mean of the prevalence distribution μ from previous work (Dhand et al., 2010), and using a non-informative prior for ψ (Table 2).

Table 2. Priors for prevalence distributions for analysis of abattoir surveillance and for pre- and post-vaccination data.

Parameters	Input values		Priors	Source of priors
	Mode	Upper[1]		
For abattoir surveillance OJD prevalence model: $Beta(\mu\psi,(1-\mu)\psi)$				
μ	0.16	0.70	$Beta(2.89,0.32)$	Dhand et al (2010)
ψ	-	-	$Uniform(1,1000)$	Non-informative
For pre- and post-vaccination OJD prevalence model: $\pi_i \sim Logit$-$Normal(\nu,\lambda^2)$				
ν	0.08	0.14	$Normal(-0.246,0.97^2)$	Based on abattoir results
λ	-	0.8[2]	$Uniform(1,3)$	Based on abattoir results

[1]Upper 95% limit for prevalence; [2]100% sure that the 90th percentile of c is less than 0.8

Uncertainty about sensitivity and specificity estimates for abattoir surveillance and histopathology was modelled with independent Beta distributions which were elicited from previous published research (Bradley and Cannon, 2005; Dennis et al., 2010). Bradley and Cannon (2005) estimated sensitivity of abattoir surveillance to be 52.5%, 74.1%, and 87.3%, for three inspectors.

We calculated an average value of sensitivity and adopted it as a mode for the Beta prior distribution. Our estimate for the lowest value of sensitivity (5th percentile = 0.4) was based on the lowest 95% confidence interval for sensitivity reported in the paper (0.44). Similarly, our prior for surveillance specificity was based on the reported specificity of 97 to 100% in the paper (Table 1).

Dennis et al. (2010) recently reported results of an investigation conducted to describe changes in infection status and enteric lesions of sheep naturally exposed to *Mycobacterium avium* subspecies *paratubercuolosis*. In this study, histopathological lesions could be detected only from 30 of the 46 infected sheep indicating a sensitivity of about 65%, which was used as a mode in forming a prior Beta distribution prior for histopathology sensitivity (Table 1). We assumed the lower 5% value of sensitivity to be the same as for abattoir surveillance (Table 1).

We note that these priors were used for analysis of abattoir surveillance data, where the output, in particular the mean and 97.5th percentile of the estimated prevalence distribution, was then used to form priors for the pre-vaccination OJD prevalence.

Elicitation of prevalence priors based on analysis of abattoir data
Analysis of abattoir surveillance data indicated that our best guess of mean of the Logit prevalence distribution of the flocks 0.08 (μ_0) and that we are 95% sure that the average prevalences was less than 0.14 $(\mu_{0.95})$. We used these estimates to induce priors for v and λ in our logit-normal model for true prevalences ($\pi_i \sim Logit\text{-}Normal(v, \lambda^2)$).

We first focus on eliciting a prior for v, the mean of the logit-prevalence distribution in the selected flocks, specified as a normal distribution, $v \sim N(a, b^2)$, where a is the mean and b is the standard deviation of v. We substitute $logit(0.08) = -0.246$ for a as our best guess for the average prevalence from analysis of abattoir surveillance data is 0.08. Similarly, we find the value of b that corresponds to the specified value $\mu_{0.95}$ = 0.14, which is obtained as $b = \frac{[logit(\mu_{0.95}) - logit(\mu_0)]}{1.645} = 0.97$.

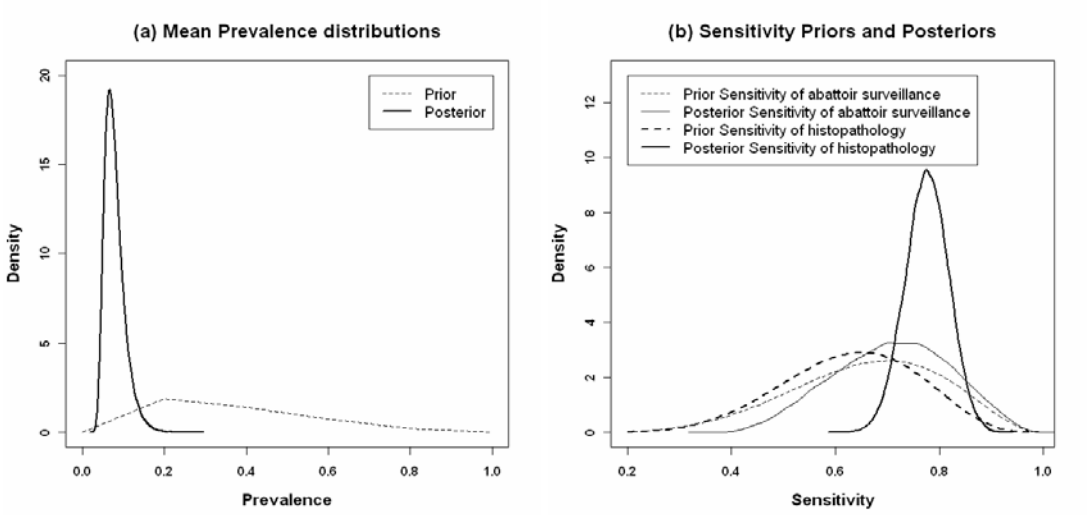
A Uniform $(0, c)$ prior was placed on λ. The value of c was determined by thinking about 90th percentile of the prevalence distribution and was obtained as $[logit(u) - v_0)]/1.28$, where u is the value such that we are virtually 100% certain that this value cannot exceed, given our prior guess v_0.

Implementation
The models were implemented in WinBUGS (Lunn et al., 2000). Convergence was checked by monitoring histories and running quantiles. All models were run for 40,000 iterations for each of the two chains with distinct starting values; the initial 5000 iterations were discarded.

Results

Abattoir surveillance data
The mean for the posterior distribution for μ based on abattoir surveillance was estimated to be 0.08, and the corresponding 95% probability interval (PI) was (0.044, 0.14), meaning we are 95% sure, after seeing these data, that the mean prevalence is in this interval. Prior and posterior distributions for μ, shown in Figure 2a, indicate that the posterior μ was well supported by the prior.

Figure 2. Comparison of prior and posterior distributions for analysis of abattoir surveillance data. (a) Mean prevalence (b) Sensitivity and specificity of abattoir surveillance and histopathology.



Priors and posteriors for sensitivity of abattoir surveillance and histopathology shown in Figure 2b suggest that posterior sensitivity of abattoir surveillance was similar to the prior sensitivity but posterior sensitivity of histopathology was inferred to be higher than under the posterior than under the prior. Posterior estimates of sensitivities and specificities are summarised in Table 3.

Table 3. Posterior estimates for sensitivities and specificities of abattoir surveillance and histopathology based on analysis of abattoir surveillance data.

Test	Median	95% PI
Abattoir surveillance		
Sensitivity	0.71	(0.49, 0.91)
Specificity	0.998	(0.997, 0.999)
Histopathology		
Sensitivity	0.78	(0.69, 0.86)
Specificity	0.998	(0.997, 0.999)

Pre- and post-vaccination prevalence estimates

The pre- and post-vaccination prevalence distribution characteristics are presented in Table 4 and Figure 3a. The median prevalence pre-vaccination was 2.99% which declined to 0.74% post-vaccination. The posterior probability that the difference in pre- and post-vaccination prevalence was almost 1 indicating that we could be virtually certain that the median post-vaccination prevalence was lower than the median pre-vaccination prevalence. This is similar to saying (using the frequentist terminology) that the differences in pre- and post-vaccination prevalences were significant.

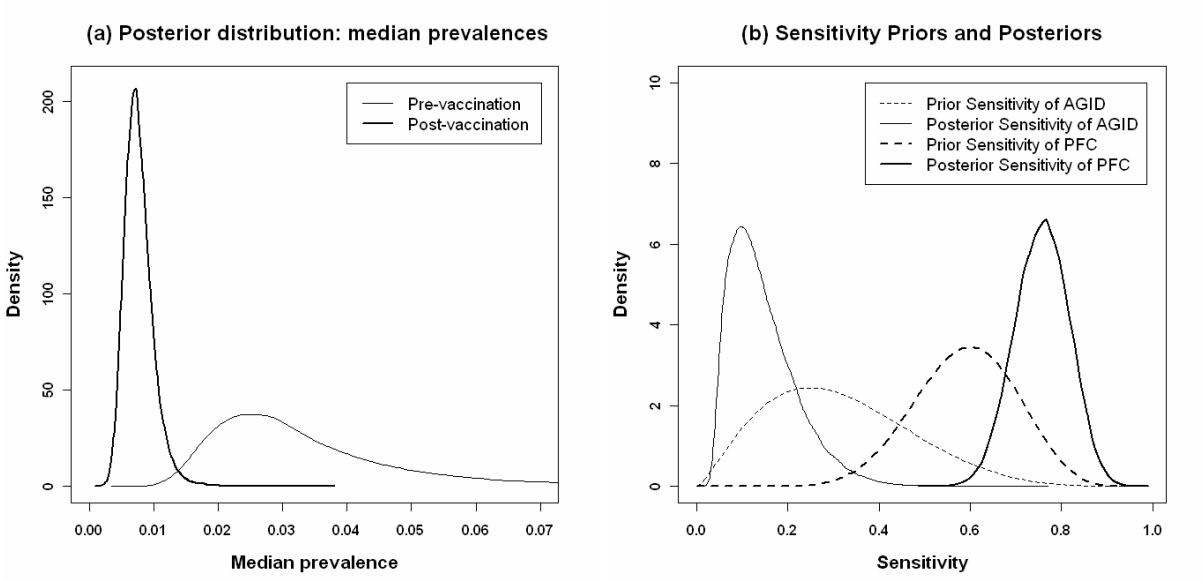
To get an idea of the spread of the prevalence distribution we estimated 90th percentiles of the pre- and post-vaccination prevalences. The results suggest that 90% of the flocks had prevalences lower than 3.09% after vaccination whereas the same proportion was lower than 19.9% before vaccination.

Table 4. Posterior estimates for pre- and post-vaccination prevalences, from a Bayesian model for prevalence estimation.

Parameters	Median	95% PI	Probability[1]
Median Prevalence			
Pre-vaccination	2.99%	(1.50, 7.92)	
Post-vaccination	0.74%	(0.42, 1.29)	
Difference	2.23%	(0.75, 7.07)	0.9998
90th Percentile of prevalence			
Pre-vaccination	19.90%	(6.79, 65.47)	
Post-vaccination	3.09%	(1.57, 10.01)	
Difference	16.28%	(3.12, 61.10)	0.9948

[1]Posterior probability that the difference is positive

Figure 3. (a) Pre-and post-vaccination median prevalences. (b) Priors and posteriors for AGID and PFC sensitivities used for analysis of pre- and post-vaccination data.



Priors and posteriors for sensitivity of AGID and PFC are shown in Figure 3b. The results suggest that posterior sensitivity of PFC was higher than the inputted prior PFC sensitivity but posterior sensitivity of AGID was lower than the prior. Posterior estimates of sensitivities and specificities are summarised in Table 5.

Table 5. Posterior estimates for sensitivities and specificities of AGID and PFC based on the Bayesian model.

Parameters	Median	95% PI
Agar gel Immuno-Diffusion test (AGID)		
Sensitivity	0.13	(0.05, 0.33)
Specificity	0.997	(0.996, 0.999)
Pooled Faecal culture test (PFC)		
Sensitivity	0.76	(0.64,87)
Specificity	0.994	(0.989, 0.998)

9.3 Appendix 3: Risk factor study in NSW & Victorian flocks; contingency table for cohort OJD prevalence level (CPREV) and pool OJD status (PSTATUS) for all 30 explanatory variables

Variables and Categories	Cohort OJD prevalence level: CPREV				Pool OJD status: PSTATUS		
	<1%	≥1%	Total		Positive	Negative	Total
Age of cohort							
3	6	6	12		57	130	187
4	3	0	3		20	58	78
Mixed age [a] (CPREV)/5[b](PSTATUS)	14	7	21		1	6	7
Sex of cohort							
Ewes	20	11	31		75	183	258
Mixed (CPREV)/Wethers (PSTATUS)	3	2	5		3	11	14
Introduced pasture grazed [c]							
< 50%	7	2	9		18	46	64
≥ 50%	16	11	27		60	148	208
Grazed Pasture supered regularly [d]							
<50%	13	4	17		37	101	138
≥50%	10	9	19		41	93	134
Soil fertility							
Low	2	1	3		4	17	21
Low/Medium	7	1	8		11	45	56
Medium	10	7	17		40	98	138
Medium/High	3	3	6		19	24	43
High	1	1	2		4	10	14
Sheep DSE/hectare							
<2	8	2	10		14	59	73
≥ 2 <6	11	6	17		39	97	136
≥ 6	4	5	9		25	38	63
Concurrent cattle enterprise							
No	8	7	15		36	73	109
Yes	15	6	21		42	121	163
Cropping enterprise run							
No	9	7	16		33	94	127
Yes	14	6	20		45	100	145
Other animal enterprise run							
No	19	12	31		70	166	236
Yes	4	1	5		8	28	36
Adult micron ≥ 19							
No	10	5	15		31	88	119
Yes	13	8	21		47	106	153
Other sheep enterprise run							
No	6	2	8		16	57	71
Yes	17	11	28		64	137	201
Merino lambing month							
Autumn	7	1	8		12	46	58
Spring	12	8	20		49	106	155
Winter	4	4	8		17	42	59
Neighbours with unvaccinated sheep							
No	9	7	16		42	73	115
Yes	14	6	20		36	121	157
Sheep moved on shared roads							

No	16	13	29		72	151	223
Yes	7	0	7		6	43	49
Sheep stray between neighbours							
No	12	2	14		26	88	114
Yes	11	11	22		52	106	158
Number of ram sources used in last 5 years							
< 2	7	4	11		24	68	92
≥ 2	16	9	25		54	126	180
Number of rams introduced in last 5 years							
< 30	13	6	19		30	107	137
≥ 30	10	7	17		48	87	135
Other sheep introduced in last 5 years							
No	16	7	23		38	141	179
Yes	7	6	13		40	53	93
Any introduced sheep suspect OJD							
No	20	12	32		68	175	243
Yes	3	1	4		10	19	29
Vaccinating lamb drops continuously for >6 years							
No	13	6	19		41	108	149
Yes	10	7	17		37	86	123
Wethers ever left unvaccinated							
No	15	11	26		62	138	200
Yes	8	2	10		16	56	72
Unvaccinated wethers sold > 10months old							
No	18	11	29		64	157	221
Yes	5	2	7		14	37	51
Other unvaccinated sheep on farm							
No	12	4	16		24	106	130
Yes	11	9	20		54	88	142
Unvaccinated sheep sold > 10months old							
No	17	11	28		61	155	216
Yes	6	2	8		17	39	56
Professional contractor used for Gudair™ vaccination							
No	18	8	26		53	149	202
Yes	5	5	10		25	45	70
High loss mobs sold							
No	17	5	22		40	132	172
Yes	6	8	14		38	62	100
Young sheep separated							
No	3	1	4		6	23	29
Yes	20	12	32		72	171	243
Lambed onto clean paddocks [e]							
No	8	6	14		36	78	114
Yes	15	7	22		42	116	158
Weaned onto clean paddocks							
No	4	2	6		13	29	42
Yes	19	11	30		65	165	230
Dispose of clinically affected OJD sheep							
No	4	1	5		7	29	36
Yes	19	12	31		71	165	236

CPREV is based on the cohort data set (36 cohorts)
PSTATUS is based on the pool-level data set (272 pools)
[a] Mixed age cohorts comprised cohorts with 3 and 4 year old pools and one cohort of 3, 4 and 5 year old pools.
[b] Pools that were comprised of sheep 5 years old

[c] Percentage of total pasture grazed by sheep
[d] Pasture supered at least every 2 years
[e] Clean paddocks – spelled or had cattle run for at least 1 month

9.4 Appendix 4: Risk factor study in NSW & Victorian flocks; parameter estimates and odds ratios for the unconditional association between the explanatory variables and the cohort OJD prevalence (CPREV) being ≥ 1%. Only variables with a *P value* <0.25 are *includ*ed in the table.

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Parameters	*b*	SE(*b*)	Odds-ratios	LCL (OR)[b]	UCL (OR)[b]	*P value*[c]
Age of cohort [a]	-	-	-	-	-	0.156
3	0	-	1	-	-	-
4	-12.31	272.50	<0.001		1.12	-
Mixed	-0.69	0.74	0.50	0.11	2.14	-
Grazed Pasture supered regularly	-	-	-	-	-	0.133
<50%	0	-	1	-	-	-
≥50%	1.07	0.73	2.93	0.73	13.5	-
Merino lambing month	-	-	-	-	-	0.217
Autumn	0	-	1	-	-	-
Spring	1.54	1.16	4.67	0.65	96.06	-
Winter	1.95	1.28	7.00	0.72	165.51	-
Sheep stray between neighbours	-	-	-	-	-	0.024
No	0	-	1	-	-	-
Yes	1.79	0.87	6.00	1.25	44.69	-
Wethers ever left unvaccinated	-	-	-	-	-	0.198
No	0	-	1	-	-	-
Yes	-1.08	0.88	0.34	0.05	1.70	-
Other unvaccinated sheep on farm	-	-	-	-	-	0.210
No	0	-	1	-	-	-
Yes	0.90	0.73	2.46	0.61	11.26	-
High loss mobs sold	-	-	-	-	-	0.036
No	0	-	1	-	-	-
Yes	1.51	0.74	4.53	1.10	20.93	-

[a] The variable of Cohort Age did not converge for the model and was therefore not included in the multivariable analysis model.
[b] Profile likelihood confidence intervals for odds ratios
[c] Based on likelihood-ratio χ^2- test of significance

9.5 Appendix 5: Risk factor study in NSW & Victorian flocks; parameter estimates and odds ratios for the unconditional association between the explanatory variables and the pool OJD status (PSTATUS) being positive. Only variables with a *P value* <0.25 are included in the table.

Parameters	*b*	SE(*b*)	Odds-ratios	LCL (OR)[a]	UCL (OR)[a]	*P value*[b]
Confounders						
Age of pool	-	-	-	-	-	0.481
3	0	-	1	-	-	-
4	-0.24	0.30	0.79	0.43	1.41	-
5	-0.97	1.09	0.38	0.02	2.30	-
Sex of pool	-	-	-	-	-	0.527
Ewe	0.41	0.67	1.50	0.45	6.78	-
Wether	0	-	1	-	-	-
Fixed effects						
Soil Fertility	-	-	-	-	-	0.088
Low	-0.53	0.81	0.59	0.12	2.99	-
Low/Medium	-0.49	0.68	0.61	0.17	2.55	-
Medium	0.02	0.62	1.02	0.32	3.89	-
Medium/High	0.68	0.67	1.98	0.56	8.13	-
High	0	-	1	-	-	-
Sheep DSE/hectare	-	-	-	-	-	0.030
<2	0	-	1	-	-	-
≥ 2 <6	0.53	0.35	1.69	0.86	3.47	-
≥ 6	1.02	0.39	2.77	1.30	6.12	-
Concurrent cattle enterprise	-	-	-	-	-	0.196
No	0	-	1	-	-	-
Yes	-0.35	0.27	0.70	0.41	1.20	-
Other sheep enterprise run	-	-	-	-	-	0.047
No	0	-	1	-	-	-
Yes	0.64	0.33	1.90	1.01	3.78	-
Neighbours with unvaccinated sheep	-	-	-	-	-	0.015
No	0	-	1	-	-	-
Yes	-0.66	0.27	0.52	0.30	0.88	-
Sheep stray between neighbours	-	-	-	-	-	0.067
No	0	-	1	-	-	-
Yes	0.51	0.28	1.66	0.97	2.91	-
Sheep moved on shared roads	-	-	-	-	-	0.003
No	0	-	1	-	-	-
Yes	-1.23	0.46	0.29	0.11	0.67	-
Number of rams introduced in last 5 years	-	-	-	-	-	0.013

< 30	0	-	1	-	-	-
≥ 30	0.68	0.27	1.97	1.16	3.39	-
Other sheep introduced in last 5 years	-	-	-	-	-	0.00
No	0	-	1	-	-	-
Yes	1.03	0.28	2.80	1.63	4.85	-
Wethers ever left unvaccinated	-	-	-	-	-	0.151
No	0	-	1	-	-	-
Yes	-0.45	0.32	0.64	0.33	1.18	-
Other unvaccinated sheep on farm	-	-	-	-	-	0.00
No	0	-	1	-	-	-
Yes	1.00	0.28	2.71	1.57	4.80	-
Professional contractor used for Gudair™ vaccination	-	-	-	-	-	0.136
No	0	-	1	-	-	-
Yes	0.45	0.30	1.56	0.87	2.78	-
High loss mobs sold	-	-	-	-	-	0.01
No	0	-	1	-	-	-
Yes	0.70	0.27	2.02	1.18	3.47	-
Dispose of clinically affected OJD sheep	-	-	-	-	-	0.174
No	0	-	1	-	-	-
Yes	0.58	0.44	1.78	0.79	4.59	-

[a] Profile likelihood confidence intervals for odds ratio

[b] Based on likelihood-ratio χ^2- test of significance

9.6 Appendix 6: Initial flock category, current estimated PFC prevalence and abattoir surveillance data for 40 flocks

Name initial	Init. Prev. Cat.	Pools +ve	Pools tested	Current Est. Prev	Incon.	Negative	Positive
D	L	0	7	0			1
S	L	0	7	0			2
W	H	0	8	0			3
K	L	0	7	0			1
C	H	0	7	0			
G	L	0	7	0		3	3
M	L	0	7	0			
B	M	1	7	0.3078		1	
M	H	1	7	0.3078		6	
L	M	1	7	0.3078		1	3
F	H	1	7	0.3078			2
P	M	1	8	0.3078			
K	H	1	7	0.3078			2
H	L	1	8	0.3078			
G	H	1	7	0.3078			
Sh	H	1	7	0.3078			
A	H	1	7	0.3078			
Lo	M	1	7	0.3078	2	2	2
Go	M	1	7	0.3078			
Mi	L	2	7	0.6707	1	1	1
Sm	M	2	7	0.6707		1	1
Wh	M	2	7	0.6707			2
H	L	2	7	0.6707			
Mi	M	2	7	0.6707		2	
Wo	L	2	7	0.6707		1	1
Wi	M	2	7	0.6707		1	4
Su	H	3	21	0.31			3
Ha	M	3	7	1.113			
Th	H	3	7	1.113			
McH	L	3	7	1.113			
Br	H	3	7	1.113			6
Wa	H	3	7	1.113		1	7
McG	M	4	8	1.6803		2	2
Cr	L	4	7	1.6803			
Ba	H	4	8	1.6803	1	9	4
War	L	4	8	1.6803			1
Mu	M	5	7	2.4744			2
Wha	L	5	7	2.4744			
Me	H	6	7	3.8171			
We	H	7	7	>4*			3

Note >4* unable to be determined as all 7 pools positive

9.7 Appendix 7: Descriptive statistics for property management data on KI.

9.7.1 Appendix 7a. Descriptive statistics for property type on KI.

Variable	*n*	Minimum	Q_1	Median	Q_3	Maximum
Years property owned	16	6.00	13.25	34.00	55.50	74.00
Total property area (ha)	16	117.00	483.75	590.00	914.75	1012.00
Average annual rainfall (mm)	16	450.00	492.50	537.50	618.75	750.00
Perennial pasture species (%)	16	0.00	0.00	1.00	8.75	10.00
Annual pasture species (%)	16	75.00	90.00	98.00	100.00	100.00
Native pasture species (%)	16	0.00	0.00	0.00	0.00	20.00

9.7.2 Appendix 7b. Descriptive statistics for property management on KI.

Variable and Categories	Frequency	Percent
Property supered regularly		
No	1	6.3
Yes	15	93.8
Supplementary feed provided		
No	0	0.0
Yes	16	100.0
Supplementary feed provided on the ground		
No	0	0.0
Yes	16	100.0
Supplementary feed provided in troughs		
No	14	87.5
Yes	2	12.5
Soil type		
Ironstone	4	25.0
Sand over clay	5	31.3
Ironstone and sand over clay	2	12.5
Sand over clay and deep sand	2	12.5
Sandy loam over gravel over clay	1	6.3
Limestone and sand over clay	1	6.3
Bay of Biscui and sandy loam over clay	1	6.3
Soil pH		
Acidic	15	93.8
Neutral	0	0.0
Alkaline	1	6.3
Soil fertility[a]		
Low	0	0.0
Medium	10	62.5
High	6	37.5

[a] Soil fertility was classified on the basis of soil treatment and management not natural fertility

9.7.3 Appendix 7c. Descriptive statistics for property enterprise on KI.

Variable	n	Minimum	Q1	Median	Q3	Maximum
Adult fleece micron (µm)	16	19.00	20.50	21.00	21.00	30.00
Absolute sheep density (dse/ha)	16	2.85	4.12	4.56	5.94	8.05
Sheep equivalent density (dse/ha)	16	4.53	6.07	7.35	8.97	11.05
Total stock density (dse/ha)	16	7.61	7.96	9.96	11.28	11.62
Area cropped annually (ha)	16	0.00	22.50	32.50	97.75	500.00