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Prepared by: Joan Lloyd, Sam Beckett, Tony Radford, Keith Walker

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Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

A Review of MLA-funded research into Johne's Disease in Australia

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Executive Summary

From 1 July 1999 until 1 March 2016, MLA will have invested approximately $21.5 million in 46 research projects on ovine Johne's disease (OJD) and, to a lesser extent, bovine Johne's disease (BJD). These research projects have investigated the epidemiology, economic impact and pathogenesis of the disease; control and management options; and new diagnostic tests. Half of the research investment has been resourced from matching R&D dollars from the Commonwealth Government. The balance of the research investment has been made from MLA producer levies, levy reserves held by Animal Health Australia and through cash contributions from private enterprise. The last two of these investment streams have been made through the MLA Donor Company.

In 2006, MLA's investment in OJD research as part of the National OJD Control and Evaluation Program 1998-2004 was analysed by Agtrans Research. Based on the assumptions made, the investment returns were positive, with a net present value of $18.7 million at a 5 per cent discount rate, a benefit-cost ratio of 1.8 to 1 and an internal rate of return of just less than 14 per cent. In his report, Dr Chudleigh concluded:

> *"There is no question that the results of the R&D investment provided valuable direction to all concerned and that the vaccine and grazing management technologies that were developed have given sheep producers options for management that they would not have had without the R&D investment. These management tools also underpinned the policy developments that have been made in the area of risk management and reduced regulation."*

In 2014, MLA decided it would be advantageous to analyse its JD research investments further so as to determine whether additional research investment is justifiable, and, if so, which research should receive priority investment.

This report provides an *ex-post* review of MLA's JD research investments between 1998 and 2015 and attempts to identify knowledge gaps that warrant further research investment. Our terms of reference were to review the body of on-farm MLA-funded JD research 1998 to 2015, drawing conclusions as to the:

a) Significant JD knowledge gaps when the research started.
b) Scientific significance of MLA-funded research discoveries to date.
c) Degree of adoption/implementation of completed R&D deliverables; their impact on practices (both in laboratories and on-farm).
d) Time to market of proposed R&D deliverables from current projects.
e) Significant remaining JD knowledge gaps, their researchability (likelihood of achievement) and potential industry impact.

As a MLA-directed component of this review, opinion was sought from Cattle Council of Australia, Sheepmeat Council of Australia and Animal Health Australia as to their respective stakeholders' views about the body of research into OJD and BJD that MLA has funded since 1998.

In consultation with the MLA project manager, our approach to the first term (a) was to accept, with minor additional discussion, the position put forward by Hussey and Morris in their 1998 report to the (then) Minister for Primary Industries and Energy, Canberra. This report included an analysis of the gaps in information available to government and industry as to the epidemiology, detection and control of (specifically) OJD.

Qualitative scales were developed to evaluate each research project in terms of its scientific significance, adoption and impact, and time to market of proposed deliverables. These scales provided a means by which to rank the funded projects against each other according to the criteria requested by MLA for this review. The scales do not have validity external to this review and, because the review was focussed on MLA project reports, do not utilise scientific publication citation rates or impact factors. As such, they should not be read as providing a *de facto* quantitative assessment or as being an absolute measure against an external standard.

There is no doubt that the early research commissioned by MLA and industry as part of the National OJD Control and Evaluation Program 1998-2004 to address the knowledge gaps identified by Hussey and Morris has made a valuable contribution to the management of both OJD and BJD in Australia.

Key deliverables from this research programme for the sheep industry have included abattoir surveillance and confirmation of the efficacy of the Gudair® vaccine in Australian sheep. In recent years, abattoir surveillance has been expanded to become a valuable endemic disease surveillance tool for the industry. The Gudair® vaccine has been widely adopted as a control tool for OJD in Australian sheep flocks.

The research programme has delivered a cost-effective test to detect infection within a flock, the pooled faecal culture test. Subsequent to the adoption and acceptance of this test for use in sheep, MLA-managed research has resulted in the test being approved for use in cattle and goats.

OJD has spread inexorably over 30+ years in Australia, principally by between-flock sheep trading prior to the recognition of clinical disease. However, addressing other industry concerns, a valuable finding from the early research programme was that wildlife (kangaroos and rabbits) are not important reservoirs for JD in Australia. This finding, along with research projects which revealed that de-stocking was not a viable means of eradication in endemic areas, and that cattle and sheep in Australia are most commonly infected with different strains of *Mycobacterium avium* subsp. *paratuberculosis* (Mptb), was fundamental in shaping Australia's JD control and management programmes over the past 15 years.

The epidemiology of the disease was closely studied in a small number of infected sheep flocks over several years. This revealed information about the age-related susceptibility of infection, and the potential for lateral spread between paddocks and between infected farms and neighbours. This information, combined with the results of studies on the survivability of Mptb in the environment, has helped producers to manage risks and has provided grazing management options for the within-flock control of OJD.

In response to the research needs identified to MLA by Sheepmeat Council of Australia and WoolProducers in 2010, the long-term performance of the Gudair® vaccine has been investigated, with results continuing to demonstrate declining prevalence of disease with

continued use of the vaccine in most vaccinated flocks. Although Mptb shedding was still detectable in many of the vaccinated flocks in these studies, in a small number of flocks detectable shedding had ceased. Breed susceptibility of sheep has been investigated, with all of the breeds examined (Merino, Merino cross White Suffolk, Border Leicester, Poll Dorset) proving to be susceptible to infection, although some breeds (Merino, Merino cross White Suffolk) appear to develop clinical OJD at a younger age. Adoption drivers and blockers affecting the Sheep Health Statement have been investigated in a MLA project (Taylor, et al. 2011), while the reasons for OJD prevalence areas changes, the application of the Assurance Based Credit scheme in managing risk, and sheep movement volumes and patterns have been investigated by others (East and Foreman, 2011; Lloyd, 2011).

With the exception of the pooled faecal culture test, diagnostic test development has been an area with variable outcomes from the JD research program. DNA typing methodologies have allowed strains to be characterised, which has strong epidemiological value, if the need arises, with recent sheep strain infection in cattle an example of such a need. More recently, some success has been achieved in developing a direct polymerase chain reaction (PCR) for Johne's disease (HT-J PCR). While demonstrated as a quick, accurate test and comparable to international methods, the HT-J PCR remains labour-intensive. The relatively low level of uptake (in absolute numbers) of this test makes it an unattractive area for serious commercial involvement. M7H9C, a new liquid culture medium and application strategy to replace radiometric BACTEC culture, was developed for use by laboratories throughout Australia.

Project investigations examining cellular immunity were well below the scientific standard seen in current commercial tests. The need for Mptb antigens or epitopes that provide a level of specificity is well established, and has not as yet been examined thoroughly. The use of such specific antigens in whole blood interferon (IFN) testing has been an Australian innovation that has largely transformed human tuberculosis infection diagnosis worldwide since 2004, allowing diagnosis in the presence of vaccination. Such antigens are not available for Mptb despite sustained efforts by many teams, including Australian investigators.

Regarding vaccine research, our review has found a lack of clarity in focus and consensus in expected outcome between producer groups, funding bodies, and research groups. No fixed program goals have been set for vaccine research, and the general understanding has been an expressed desire for a 'better, safer and cheaper' vaccine than Gudair® and presumably Silirum®, for which less information is currently available. Working to produce a vaccine without quantified, written and agreed objectives of vaccine efficacy will inevitably lead to wasted effort and resources.

We also believe that the expectations of the vaccine research may be overly optimistic. Producers accustomed to the efficacious and economic virtues of other vaccines may underestimate the difficulties of vaccine development against mycobacterial disease, which has yet to be effectively achieved in humans, where aggressive reactive adjuvants are not tenable. The review team has no expectation of a deliverable vaccine from current efforts, and the researchers confirm this expectation.

The three phases of the laboratory basic research program that aimed to increase understanding of the pathogenesis of JD, to develop innovative diagnostic tests, to

investigate safer and more efficacious vaccine options, and to investigate methods to identify cattle and sheep genetically resistant to clinical expression of the disease and/or bacterial shedding have received approximately $AU14m in total funds, but have delivered proportionately fewer concrete deliverables than the generally lower-cost observational studies that helped to clarify important aspects of the epidemiology, management or control of JD (in particular, OJD) in Australia. The result was not unexpected, however, as basic research is by nature high-cost and exploratory, and is usually associated with a lower certainty of absolute scientific success and the delivery of tangible industry outcomes/tools.

Because of the high cost and inherent risk of basic research, we propose that in the future it should follow a strategic plan that extends beyond the outcomes of primary experiments to the raft of practical considerations that are likely to determine how easily or cost-effectively these can be implemented to industries' advantage. This will often include considerations around the manufacture and/or widespread application of products and, as relevant, their outlook for successful commercialisation.

Further, wider consultation by MLA to obtain specialist advice in the scientific field prior to funding, with a specific view of seeking the best collaborative teams, would likely enhance project outcomes. We recommend that any future studies in diagnostics, genomics or proteomics, or in any field requiring specialised skills and intensive capital, be structured to include additional external advice and collaboration, not only from academic sources. Within the laboratory based research there has been a heavy dependence on the resources and in-depth JD knowledge at the University of Sydney. Leveraging this knowledge resource by ensuring future projects have effective collaboration with specialist expertise, including that residing in commercial areas, will likely yield more practical outcomes in less time and with less wasted effort. Basic research should also be carefully prioritised, with the perceived needs of industry subject to a rigorous analysis of their technical and practical underpinning. We repeatedly heard, for example, a call for a 'better, safer and cheaper' vaccine for OJD. However, it is our assessment that the currently available alternative (Gudair®) is: (a) overall, far more efficacious than any other purported mycobacterial vaccine; (b) associated with virtually no reported self-injection injuries, following the advent of the shrouded vaccinator; (c) acceptable to the sheepmeat processing sector; and (d) very unlikely to be made cheaper by any basic research, given the relatively small population of potential users. We are not aware of an assessment that systematically addressed these criteria and that differs from our conclusion.

We also recommend to MLA the value of critical, independent review before, during and after any larger, higher-risk research effort. The value of recruiting specialists with appropriate expertise to project manage these studies on behalf of industry should not be underestimated.

Several potential researchable knowledge gaps are suggested to MLA for consideration. However, before embarking on any future JD research effort, we strongly recommend that MLA negotiate a clear vision with and for industry as to what future JD research will seek to deliver.

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1 Background

Johne's disease (JD) is a chronic disease of cattle, sheep and other ruminants caused by infection with the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (Mptb).

Bovine Johne's disease (BJD) was first diagnosed in Australia in Victoria in 1925 (Department of the Environment and Primary Industries, 2013) and is now endemic in New South Wales, South Australia, Tasmania and Victoria. The disease is largely compartmentalised within dairy herds, with lower prevalence in beef herds.

Ovine Johne's disease (OJD) was first detected in Australia on the Central Tablelands of New South Wales in 1980 (Seaman et al., 1981). It was thought that sheep imported from New Zealand, where the disease had been detected in the Canterbury region of the South Island in 1952, had brought the disease to Australia. Since 1980, OJD has progressively spread or been independently detected in all states of Australia except Queensland. There was and is clear evidence of spread by sheep movements from undetected and detected infected flocks.

In the late 1990s MLA embarked on a series of research investments on JD and, from 1 July 1999 until 1 March 2016, will have invested approximately $21.5 million in 46 research projects on BJD and OJD (Appendix 1). These research projects have investigated the epidemiology, economic impact and pathogenesis of the disease; control and management options for the disease; and the development of new diagnostic tests. Half of the research investment has been resourced from matching R&D dollars from the Commonwealth Government. The balance of the research investment has been made from MLA producer levies, levy reserves held by Animal Health Australia or through cash contributions from private enterprise. The last two of these investment streams have been made through the MLA Donor Company.

At the end of the National OJD Control and Evaluation Program 1998-2004, Harvest Year conferences were held in North Sydney (MLA, 2004) and Adelaide (MLA, 2005) to showcase the findings of the MLA-funded research programme. In 2011 MLA prepared a third Harvest Year report to showcase the research results obtained 2005-2011 (Sergeant, 2011).

These Harvest Year reviews provide a synopsis and overview of the JD research achievements.

An important early finding from the research program in the late 1990s and early 2000s was that eradication of OJD was not practical in regions where the disease was already endemic at much higher than expected within-flock prevalence. Another important early research finding was that wildlife reservoirs (kangaroos and rabbits) did not have an important role in the transmission of the JD in Australia. These early research findings helped shape the control programmes for BJD and OJD in Australia.

The early research programme also confirmed of the efficacy of vaccination in Australian sheep. Vaccination was demonstrated to slow and ameliorate the clinical expression of the disease, reduce the number of clinical cases within a flock and reduce the faecal shedding of Mptb.

Early research on diagnostic tests resulted in the validation and widespread adoption of pooled faecal culture methods for cattle, sheep and goats, and the later widespread adoption of abattoir surveillance for the detection of OJD and other diseases of sheep. More recently, a high throughput JD polymerase chain reaction test (HT-J PCR), also used on pooled faecal samples, has been developed, along with a liquid culture method to replace the commercial source of BACTEC medium when the provider ceased to market this product.

Epidemiological investigations have provided valuable information on the environmental survival of Mptb, the effect of risk factors such as soil pH and organic matter, cross-species infections between cattle and sheep, and age differences in susceptibility and clinical expression of the disease.

The economic impact of the disease on- and off-farm has been evaluated.

Investigation of the epidemiology of JD in beef cattle has highlighted the sporadic nature of the disease in these cattle and the important role of dairy cattle as a source of infection. Although early work showed the biochemical delineation of cattle and sheep strains of Mptb, there was later some accumulated field observation evidence for the infection of beef cattle with sheep strains of Mptb when co-grazing with sheep. In collaboration with a commercial partner, research has investigated the role of vaccination with a commercial vaccine (Silirum®) now registered as a control measure for cattle.

Since 2002, MLA has invested in a programme of basic research at the University of Sydney. The aim for this research is to increase understanding of the pathogenesis of JD, to develop innovative diagnostic tests, investigate safer and more efficacious vaccine options, and to investigate methods to identify cattle and sheep genetically resistant to clinical expression of the disease and/or bacterial shedding.

In 2006, MLA's investment in OJD research as part of the National OJD Control and Evaluation Program 1998-2004 was analysed by Peter Chudleigh of Agtrans Research (Chudleigh, 2006). The principal research outputs included in the analysis were lowered death rates and production losses, reduced trading losses, saved costs of destocking and/or spelling, and reduced anxiety of producers and others in rural communities. Based on the assumptions made, the investment returns were positive, with a net present value of $18.7 million at a 5 per cent discount rate, a benefit-cost ratio of 1.8 to 1 and an internal rate of return of just less than 14 per cent. However, these results were thought to be an under-estimate because the environmental benefits associated with control were not valued and the potential benefits of the basic research programme then underway at the University of Sydney were not included. In his report, Dr Chudleigh concluded:

> *"There is no question that the results of the R&D investment provided valuable direction to all concerned and that the vaccine and grazing management technologies that were developed have given sheep producers options for management that they would not have had without the R&D investment. These management tools also underpinned the policy developments that have been made in the area of risk management and reduced regulation."*

In 2014 MLA decided it would be advantageous to further analyse its JD research investments to date, to determine whether further research investment is justifiable and, if so, which research should receive priority investment.

This report provides an *ex-post* review of MLA's JD research investments between 1998 and 2015 and attempts to identify knowledge gaps that warrant further research investment.

2 Projective objectives

The objective of this project is to provide an *ex-post* review of MLA's JD research investments to April 2015 and identify significant knowledge gaps that warrant significant investment.

The areas to be covered in the review included:

a) Significant JD knowledge gaps when the research started
b) Scientific significance of MLA-funded research discoveries to date
c) Degree of adoption of completed R&D deliverables and their impact on practices
d) Degree of duplication of R&D done elsewhere
e) Time to market of proposed deliverables from current projects
f) Significant remaining JD knowledge gaps, their researchability and potential industry impact.

In consultation with the MLA Project Manager, we decided not to devote time to the fourth term (d) 'Degree of duplication of R&D done elsewhere'. It is the opinion of the panel that if researchers are aware of and collaborate with other researchers, then duplication can be beneficial, not detrimental. This has been the case with the JD basic research program funded by MLA and industry at the University of Sydney. For example, the high throughput JD PCR (HT-J PCR) test now validated for use in Australian cattle and sheep had its origins in collaborative research between the University of Sydney and the Japanese Institute of Animal Health, and the University of Sydney is providing standardised Mptb inoculum for use in vaccine trials being conducted at Massey University in New Zealand.

Following negotiation with MLA management, no consideration has been given in this review to the existence or absence of human health effects due to Mptb exposure, or to matters of off-farm research directed towards food safety, product quality and zoo-sanitary issues.

3 Methodology

3.1 Terms of Reference

Our terms of reference were to the review the body of on-farm MLA-funded JD research, drawing conclusions as to the following key areas:

a. Significant JD knowledge gaps when the research started.
b. Scientific significance of MLA-funded research discoveries to date.
c. Degree of adoption/implementation of completed R&D deliverables; their impact on practices (both in laboratories and on-farm).
d. Time to market of proposed R&D deliverables from current projects.

e. Significant remaining JD knowledge gaps, their researchability (likelihood of achievement) and potential industry impact.

The terms of reference for the review are provided in Appendix 2.

In consultation with the MLA project manager, our approach to the first term (a) was to accept with minor additional discussion, the position put forward by Hussey and Morris in their 1998 report to the (then) Minister for Primary Industries and Energy, Canberra (Appendix 3). This report included an analysis of the gaps in information available to government and industry as to the epidemiology, detection and control of (specifically) OJD. The report then drew on this analysis to present immediate steps for OJD research in Australia.

The National OJD Control and Evaluation Program 1998-2004 was established based on the recommendation in the Hussey and Morris Report that "*a nationally coordinated program of combative and research action be pursued*". The Program was jointly funded by the sheep industry and the Commonwealth and the State Governments (50 per cent sheep industry, 20 per cent Commonwealth and 30 per cent State Governments), with $40.1million provided over the six years of the Program). Animal Health Australia (formerly Animal Health Council Limited) was given a leadership role in managing and coordinating the Program on behalf of industry and government. The aims of the Program, as stated in the Deed of Agreement between Animal Health Australia, the Commonwealth, State and Australian Capital Territory governments, Sheepmeat Council of Australia and the Wool Council of Australia (now WoolProducers Australia) were to:

- Provide, through an effective research and operations program, sufficient information to allow an informed decision to be made on the national management of OJD, and especially on the feasibility and cost-effectiveness of eradication; and to
- Control OJD during the research period (Chudleigh et al. 2001).

Initially the Program included four key subprograms:

- A research and development program;
- An operations program to contain OJD;
- A management program to ensure appropriate funding and achievement of objectives; and
- A communications program.

The research subprogram built on the gap analysis provided by Hussey and Morris in 1998 and provided the first steps toward the body of work that we have addressed in our review.

3.2 Materials

The body of work that we reviewed extends from the commencement of the National OJD Control and Evaluation Program 1998-2004 through to 2015. A complete list of research project numeric identifiers, titles, costing, start- and end-dates, and research providers, is given in Appendix 1. In Appendix 4 are listed the particular reports, proposals and other supporting documents that the review was based on. A small number of the most recent projects were incomplete at the time of the review (the first half of 2015) and, in these cases, our judgements were based on (as available) project proposals and milestone reports only.

3.3 Ratings

Qualitative scales were developed to evaluate each research project against term of reference 'b' (scientific significance), term of reference 'c' (adoption and impact) and term of reference ‘d’ (time to market of proposed deliverables from projects) (Section3.1). These scales provided a means by which to rank the funded projects against each other according to the criteria requested by MLA in the terms of reference for this review. The scales do not, however, have validity external to this review and should not be read as providing a *de facto* quantitative assessment or as being an absolute measure against an external standard.

Qualitative scale for scientific significance:

Very High	Very substantially improved understanding of the pathogenesis, diagnostics, epidemiology, impact or control of JD, or has the potential to do so
High	Substantially improved understanding of the pathogenesis, diagnostics, epidemiology, impact or control of JD, or has the potential to do so
Moderate	Provided some improvement in the understanding of the pathogenesis, diagnostics, epidemiology, impact or control of JD, or has the potential to do so
Low	Led to no noticeable improvement in the understanding of the pathogenesis, diagnostics, epidemiology, impact or control of JD, and is unlikely to do so

Qualitative scale for adoption and impact:

Very High	Very substantially adopted and has made a very high contribution to the diagnosis or control of JD or to reducing the on-farm impact of the disease
High	Substantially adopted and has made a high contribution to the diagnosis and/or control of JD or to reducing the on-farm impact of the disease
Moderate	Moderately adopted and has made some contribution to the diagnosis and/or control of JD or reducing the on-farm impact of the disease
Low	Has not been adopted and has not contributed to the diagnosis or control of JD or to reducing the on-farm impact of the disease

Qualitative scale for categories for time to market of proposed deliverables:

Not Applicable	Project was never intended to provide a deliverable outcome
No Deliverable	Project was unsuccessful in obtaining a deliverable outcome
Immediately Applicable	Applicable outcome was manifest in the completion of the project; applies largely to knowledge basis improvements
Deliverable; Assessment	There was/is a deliverable outcome and the time and the realistic chance of it becoming manifest is considered

3.4 Consultation

As a MLA-directed component of this review, opinion was sought from Cattle Council of Australia, Sheepmeat Council of Australia and Animal Health Australia as to their respective stakeholders' views about the body of research into OJD and BJD that MLA has funded since 1998. The questions put to the Councils and to AHA are listed in Appendix 5. We also asked Professor Richard Whittington to provide us with a presentation of the University of Sydney's ongoing basic research into the pathogenesis, diagnosis and control of JD, and sought the opinion of Dr Evan Sergeant as to the implementation and adoption of research outcomes since 1998.

State Veterinary laboratories in New South Wales, Queensland, South Australia, Victoria and Western Australia were contacted through the JD Technical Working Group (Dr Ian Marsh) and asked to provide JD laboratory test numbers for the 2014 calendar year.

The responses gained from this process of consultation helped to inform our understanding of the context of specific projects and their objectives and outputs. The responses also helped to guide us in the broader discussion of the body of MLA-funded research, its merits and effectiveness and the conclusions and recommendations that might be drawn from it.

4 Results

4.1 Johne's Research: A Gap Analysis

4.1.1 Knowledge gaps in 1998

In their 1998 report to the (then) Minister for Primary Industries and Energy, Canberra Hussey and Morris concluded that:

> "*It is not feasible to attempt to eradicate OJD nationally* [then the preferred approach to managing the disease] *while a substantial number of key pieces of information which would be essential to the process remain unknown or uncertain, and crucial tools are as yet unavailable.*"

A program of appropriate research was recommended to fill knowledge deficits in the following five main areas:

a) Methods to detect infected flocks
b) Methods to demonstrate freedom of flocks from infection
c) Epidemiological research on OJD
d) Methods of sheep identification and traceback
e) The potential role of vaccination in the control of OJD

4.1.1.1 Methods to detect infected flocks

In 1998 a reliable test to detect flock infection reliably did not exist. The serological test (agar gel immunodiffusion test) had high specificity, but low sensitivity, and required substantial numbers of animals per flock (all up to 450 head) to be tested to detect the presence of disease. Hussey and Morris recommended the more sensitive faecal culture and PCR as the

most promising test methods to detect infection, and abattoir surveillance based on histopathology as a method of identifying OJD-infected and -free flocks/areas/regions.

4.1.1.2 Methods to demonstrate freedom of flocks from infection

In 1998 (as in 2015), proving flocks free of infection from OJD was even more difficult than reliably detecting infection. Hussey and Morris recommended evaluation of the interferon gamma (IFN) test being developed by CSIRO for BJD, as well as other approaches as appropriate.

4.1.1.3 Epidemiological research on OJD

In 1998, most of the basic epidemiological information regarding OJD in Australia was not known. Many of the measures being taken against the disease were based on inference from general principles or extrapolation from other diseases, including BJD. For example, it wasn't known if eradication from a flock was possible, yet this was being attempted. Hussey and Morris recommended studying a small number of infected flocks to determine how the disease spreads, the dynamics of infection in various classes of animals and the likely success of various control options.

4.1.1.4 Methods of sheep identification and traceback

Hussey and Morris were of the opinion that, in the future, Australia would need to be able to operate a tracing system for sheep to remain competitive on international markets. They also saw value in such a system for managing OJD and recommended that the development of such a system form part of the research program for OJD. Although research on sheep identification was conducted, it was not funded from the OJD Research Program budget (Chudleigh et al., 2001).

4.1.1.5 Review the potential role of vaccination in OJD control

Hussey and Morris recommended research on the then internationally available commercial vaccine (Gudair®), as well as research on alternative vaccine options.

4.1.2 OJD knowledge gaps in 2010

In 2010 WoolProducers Australia and Sheepmeat Council of Australia used the midpoint of the OJD Management Plan 2007-12 to review past research and development achievements, to consider possible new research projects and to develop a policy position for post-2012. As part of this review, an *"Ovine Johne's Disease Research Needs Position Paper"* was prepared for the Program Manager Animal Health and Welfare at MLA (Appendix 6). This paper identified several knowledge gaps of importance to industry that were considered to impact on the accurate diagnosis and successful control of JD. These included, in no particular order:

- Long-term vaccination performance (validating models and predicting)
- Breed resistance/susceptibility
- Crohn's disease risk management
- Sheep movement volume/patterns
- Sheep Health Statement drivers and blockers
- Vaccination adoption drivers and blockers

- Reasons for prevalence changes
- Application of the Assurance Based Credit scheme in managing risk

Two additional outcomes were also considered important:

- Improved/safe vaccine or vaccine formulation
- Improved flock and individual tests

4.2 Scientific significance, degree of adoption and impact on practices, and time to market of deliverables from completed projects

Wherever possible, the 'research summaries' that appear in this section were taken directly from the text of the MLA project reports provided to us. This approach was taken in order to minimise the possibility of translation errors or imprecision. The summaries are included only to assist with the readability of our report. Our assessment of the underlying research was based on the MLA project reports.

4.2.1 Projects that investigated the epidemiology of Johne's disease

4.2.1.1 OJD.003: Survival of JD in the environment

Full Title: Survival of Johne's disease in the environment

Value: $167,257

Period: November 1999 to March 2001

Research Provider: R Whittington (NSW Agriculture)

Research Summary: The report we reviewed for this work related to three MLA projects that investigated the duration of survival of the sheep strain of Mptb (projects TR.055, TR.055A and OJD.003). Using plot and box experiments, the authors found that survival exceeded 12 months in faecal pellets in a shaded location, but was much less in faecal pellets in an unshaded location unless vegetation was not grazed. Sunlight (including UV, visible and infra-red radiation) was a significant factor influencing bacterial survival. However, because it was thought UV-visible and infra-red radiation would not have penetrated the faecal pellets, temperature flux was proposed as the key reason that shade was so important. Moisture levels and lime application did not appear to influence survival. Decay rates for the organism were found to be inversely proportional to the period of observation. There was a rapid decay phase lasting about six weeks during which the majority of viable bacteria died. This was followed by a period of dormancy of variable duration during which the organism could not be cultured, but its DNA could still be detected. This was sometimes followed by a period of apparent replication during which bacterial numbers increased. Finally, there was a slow decline phase lasting many months.

Liming pasture did not reduce survival and moisture did not increase it. Shade was the most significant factor favouring survival. The organism moved from faecal pellets into the surface litter as pellets broke down. It also entered the soil profile and the authors found that Mptb could be cultured readily from the surface layers of the soil. Pasture emerging through contaminated faeces could be contaminated with relatively high concentrations of the organism. The organism was also found associated with the infective third stage larvae of

gastro-intestinal nematodes that developed in the faeces of sheep. These larvae may be found on pasture and may contribute to infectivity of pasture. The organism tended to move away from infected sites in run-off water and to survive for prolonged periods in water. It was noted that the duration of survival in water was longer than that in soil in the same environment. Based on studies of other bacteria including the related organism *M. avium*, the authors considered that there was some potential for interactions between Mptb and single-celled aquatic life.

Significance of science outcomes: The research provided industry with critical and novel baseline information about the ability of Mptb to survive in the Australian environment. This information was not otherwise available.

The scientific significance of this project is rated as Very High.

Degree of adoption and impact on practices: This was a key project that framed much of the policy associated with de-stocking. The project also informed decision-making for management factors to control OJD, in particular paddock spelling based on the finding that 90 per cent of contamination was gone within three months. This information was critical to the sheep industries prior to the widespread use of vaccination.

Information from the project is currently informing policy associated with the de-stocking of beef herds, for example on the north coast of New South Wales and in the Gippsland region of Victoria.

The degree of adoption and impact on practices of this project is rated as Very High.

Time to market of deliverables: Immediately applicable.

4.2.1.2 TR.050: Prevalence of JD in rabbits and kangaroos

Full title: Prevalence of Johne’s disease in rabbits and kangaroos

Value: $4,545

Period: July 2001 to June 2002

Research provider: K Abbott (University of Sydney)

Research summary: A study of rabbits and kangaroos on OJD-infected farms in New South Wales was implemented following reports from Scotland that rabbits on JD-infected farms in the Tayside region were infected with Mptb. Between 1996 and 2000, 300 rabbits and 300 eastern grey kangaroos from 10 farms grazing OJD-affected sheep flocks were killed and examined for evidence of JD. Of the 300 rabbits tested, 253 were tested by radiometric culture of their faeces and 47 by smear and Ziehl-Neelsen stain of tissues combined with histopathology of the lower small intestine and regional lymph nodes. No evidence of JD or Mptb was detected in any rabbit. Of the 300 kangaroos, 206 were examined primarily by faecal culture and 94 by smear and histopathology. Some animals were examined by faecal culture and histopathology. One kangaroo specimen produced evidence of low numbers of Mptb in faeces; however, histopathological examination revealed no evidence of active infection. It was concluded that the bacteria identified in this animal had been ingested from pasture contaminated by infected sheep and had survived passage through the gut.

Significance of science outcomes: This study, in conjunction with study TR.054, showed that rabbits and kangaroos are unlikely to play a significant role in the epidemiology of JD in Australia. This potentially important aspect of the epidemiology of JD in Australia was previously unknown.

The scientific significance of this project is rated as High.

Degree of adoption and impact on practices: In concert with project TR.054, the results of this project had a very substantial impact on policy at the time. Overseas rabbits had been considered important carriers of Mptb. Likewise, the importance of badgers and other wildlife to the epidemiology of bovine tuberculosis had been established and comparisons to JD had been postulated. The results of the study enabled policy that treated wildlife rationally and without undue concern. This was a key outcome, as the converse would have resulted in significant complications as regards pest management and wildlife protection and a completely different approach to the management of JD in Australia.

The degree of adoption and impact on practices of this project is rated as Very High.

Time to market of deliverables: Immediately applicable

4.2.1.3 TR.054: Potential wildlife reservoirs for Mptb

Full title: A survey of potential wildlife reservoirs for Mycobacterium paratuberculosis

Value: $5,000

Period: November 1997 to June 1998

Research provider: Department of Natural Resources and Environment

Research summary: This project included separate studies that investigated whether Mptb could be harboured in populations of rabbits or eastern grey kangaroos. One hundred rabbits were caught on three properties in Victoria, one Merino enterprise with endemic OJD, one beef enterprise with BJD and one dairy enterprise with BJD. In total, 310 rabbits, four hares and three feral goats were examined and Mptb was not found in any of the animals. Between April 1997 and November 1998, 100 eastern grey kangaroos that were being culled for pest management purposes in central Victoria were necropsied and examined. None had evidence of Mptb in their gut.

Significance of science outcomes: Refer to Project TR.050.

Degree of adoption and impact on practices: Refer to Project TR.050.

Time to market of deliverables: Refer to Project TR.050.

4.2.1.4 OJD.027: Computer models to describe epidemiology of OJD

Full title: Development of computer models to describe epidemiology of Johne’s disease in sheep

Value: $103,300

Period: September 2001 to April 2003

Research provider: AusVet Animal Health Services

Research summary: The pathogenesis, epidemiology and control options for OJD were reviewed and mathematical models developed to simulate the spread of the disease within infected flocks and between flocks on a regional basis. The models provided a method of investigating the potential rate of spread of infection, the likely costs of the disease and the effectiveness and cost-benefit of proposed control strategies, particularly at the farm level. As more precise estimates of the values of key parameters became available, the models allowed for a rapid assessment of the likely impact of these on disease spread and informed the policy and control programmes.

Significance of science outcomes: This study cannot be said to have produced scientific outcomes *per se*, and scientific significance is thus rated as Low.

Degree of adoption and impact on practices: The modelling results were used to underpin the Assurance Based Credits scheme, the South Australian approach to OJD and the recommendations for use of Gudair®. The results also drew attention to the long-term timeframes for the management of OJD.

The degree of adoption and impact on practices of the study is rated as High.

Time to market of deliverables: Immediately applicable

4.2.1.5 OJD.002A: Exposure factors – OJD infection and clinical disease

Full title: Exposure factors leading to establishment of OJD infection and clinical disease: epidemiology of OJD-1

Value: $782,197

Period: July 1999 to December 2002

Research provider: K Abbott, R Whittington and H McGregor (University of Sydney)

Research summary: The aim of this project was to develop grazing management strategies to reduce the impact of OJD on affected flocks. The benefits of low contamination environments for Merino lambs were investigated over a three-year period in an experimental flock in an endemic area near Goulburn, New South Wales. In the first part of the project (the main experiment), sheep were exposed to different levels of Mptb from birth to weaning, or from weaning onwards. The level of exposure in the groups with high prevalence was about 10 times higher than in the groups with medium prevalence. The groups with low prevalence were not deliberately exposed to Mptb, although accidental contamination at very low levels did occur. In the second part of the experiment, previously unexposed sheep were exposed to Mptb at three to four years of age, simultaneously with their lambs, and then kept for two years before slaughter and post-mortem examination.

Significance of science outcomes: The study demonstrated that steps taken to limit the degree of exposure of pre-weaned lambs to infection from pastures will lead to reduced rates of severe infection in those sheep later in their life. Weaned lambs and adult ewes remain susceptible to infection and there is little evidence for an age-related resistance to OJD.

The scientific significance of this project is rated as Moderate.

Degree of adoption and impact on practices: The findings of this project were important to policy and the management approach to OJD prior to the availability of Gudair®.

The degree of adoption and impact on practices of the study is rated as Moderate.

Time to market of deliverables: Immediately applicable

4.2.1.6 OJD.028: Pasture contamination level, age susceptibility and tests

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

Value: $721,602

Period: October 2001 to Jun 2005

Research provider: R Whittington and H McGregor (University of Sydney)

Research summary: A flock of 840 sheep was studied to determine whether the age of infection and pasture contamination levels affect the severity OJD. Key findings were that post-weaning lambs (median age five and a half months at start of trial) are highly susceptible to infection with Mptb and that, if exposed to high levels of contamination, a proportion will develop severe infection leading to clinical disease and death. Hoggets and adult ewes are less likely than lambs to develop clinical disease after exposure. Sheep in all age classes could be infected and shed the organism in faeces, acting as a source of transmission for the disease, but this happened more often in lambs than in older age classes. It was not considered necessary for infected sheep to be present in a paddock for transmission of infection to occur. When infected sheep were present in neighbouring paddocks, a conventional wire-strand fence did not prevent the spread of infection.

Significance of science outcomes: This study helped to clarify aspects of the on-farm epidemiology of OJD. This was an intensive single-flock study that had limited application to flocks outside the same geographical area and flock demographic. However, it is noted that in their 1998 report Hussey and Morris recommended that the disease be studied intensively in a small number of infected flocks. One of the study's material outcomes also appeared to contradict 0JD.002A. Specifically, OJD.028 (this study) reported that, "*Hoggets and adult ewes are less likely than lambs to develop clinical disease after exposure. Sheep in all age classes may become infected and shed the organism in faeces, but this will happen more often in lambs than in older age classes*". OJD.002A, however, stated that, "*Weaned lambs and adult ewes remain susceptible to infection and that there is little evidence for an age-related resistance to OJD*". There was no discussion within OJD.028 about this apparent turnaround in thinking about the age-resistance of sheep, and yet this was one of the study's important conclusions.

Overall, the scientific significance of the study is rated as Moderate.

Degree of adoption and impact on practices: The findings of this project were important to policy and the management approach to OJD prior to the availability of Gudair®.

The degree of adoption and impact on practices of the study is rated as Moderate.

Time to market of deliverables: Immediately applicable: note caveats above

4.2.1.7 TR.022: DNA Typing of JD organisms

Full title: DNA typing of Johne's disease organisms

Value: $18,808

Period: July 1998 to June 1999

Research provider: R Whittington (NSW Agriculture)

Research summary: This study was undertaken to determine whether JD in sheep and cattle in Australia could be considered separate diseases, and thus be subject to independent control programs. About 350 separate isolates of Mptb from about 100 farms in New South Wales, Victoria, Tasmania and South Australia were evaluated. A genetic test was developed to enable the bacterium to be typed quickly and accurately. The study found that JD in sheep was almost always due to sheep strains of Mptb, whereas cattle were almost always infected with cattle strains. However, it was concluded that JD had occasionally spread from sheep to cattle in New South Wales, probably under unusual circumstances. JD also appeared to have spread from sheep to goats.

Significance of science outcomes: The key findings of this study were that: (a) JD in sheep was almost always due to sheep strains of Mptb while cattle were almost always infected with cattle strains; and (b) JD has occasionally spread from sheep to cattle.

On balance, the scientific significance of project is rated as Very High.

Degree of adoption and impact on practices: This was very important early work which indicated that, under Australian conditions, sheep tend to be infected with the sheep strain and cattle with the cattle strain, and that infection of cattle with the sheep strain is uncommon. The DNA typing method developed provided an easier method of differentiating the two strains than was previously available i.e. three month subculture following BACTEC culture. The PCR method developed facilitated subsequent projects on cross species transfer (OJD.005, OJD.016. P.PSH.0206, P.PSH.0301).

The degree of adoption and impact on practices of the project is rated as High.

Time to market of deliverables: Deliverable Assessment: Typing of strains is a key aspect of understanding disease epidemiology. The method is in current use but at very low levels, and is unlikely to be adopted commercially. Given the observation of sheep type infections in cattle, and concern on strain behaviour, resourcing future strain typing needs are a consideration.

4.2.1.8 OJD.005: Cross species transmission of OJD - Phase 1

Full title: Cross species transmission of ovine Johne’s disease - phase 1

Value: $34,738

Period: September 1999 to September 2000

Research provider: R Whittington and C Taragel (NSW Agriculture)

Research summary: This project investigated JD in goats on farms where the infection was acquired from sheep. By summarising the available information about JD in goats, and conducting surveys on several farms, a picture of the disease in fibre goats was developed. Infected goats were usually detected using laboratory tests, not because they had obvious disease, and it appeared that the tendency to develop severe disease was less in goats than sheep. In addition, on two farms where the disease was established, the proportion of goats infected was less than the proportion of sheep infected. The reasons for the different disease pattern in sheep and goats were uncertain, but were thought to include lower doses of the organisms being acquired from the environment by goats due to their browsing behaviour, a relative resistance to infection on the part of goats or a degree of adaptation of the sheep strain of Mptb to sheep rather than goats. The circumstances that resulted in OJD spreading from sheep to goats on two farms appeared to include high stocking rates and prolonged or continuous direct and indirect contact between sheep and goats.

Significance of science outcomes: The study yielded basic parameters for the epidemiology of JD in goats, which was largely unknown at the time.

The scientific significance of this project study is rated as High.

Degree of adoption and impact on practices: The results obtained from this study helped to clarify the role of goats in the epidemiology of OJD. The study informed the decision to include goats in both the BJD and OJD control programmes, resolved confusion over the movement of goats that was occurring at the time and helped farmers understand the importance of managing sheep and goats separately.

The degree of adoption and impact on practices of this project is rated as High.

Time to market of deliverables: Immediately applicable

4.2.1.9 OJD.016: Cross Species transmission of OJD - phase 2 cattle

Full title: Cross species transmission of ovine Johne’s disease - phase 2 cattle

Value: $71,229

Period: October 2000 to March 2002

Research provider: B Moloney and R Whittington (NSW Agriculture)

Research summary: In this project, 1,764 cattle from 12 properties with a known significant history of JD in sheep were tested by ELISA and faecal culture. All animals were negative on serology. One animal from a herd of 349 had a single positive faecal culture result, with all follow-up investigations being negative, suggesting passive transfer of the organism. Due to the small size of some of the herds tested, and the fact that no confirmed infected animals or herds were detected, it was not possible to give maximum estimate of the prevalence of the sheep strain of Mptb in exposed susceptible cattle. However, using information derived from previous investigations and some additional results in the addendum to this project report, it was concluded that there were at least six cattle herds in New South Wales infected with sheep strain of Mptb at the time the report was written (2002).

Significance of science outcomes: This study, when viewed with other studies on the same topic (in particular, P.PSH.0206 and P.PSH.0301), provided early evidence for the very low rate of infection of (or passive transfer by) cattle with the sheep strain of Mptb.

The scientific significance of this project is rated as Moderate.

Degree of adoption and impact on practices: This study was very influential in framing policy, specifically the exclusion of the sheep strain from BJD control programmes. It was also the basis for recommendations to producers that, while infection of cattle with the sheep strain can happen, it is uncommon.

The degree of adoption and impact on practices of this project is rated as High.

Time to market of deliverables: Immediately applicable

4.2.1.10 P.PSH.0206: OJD in cattle – case study 1

Full title: Ovine strain of *Mycobacterium paratuberculosis* in beef cattle: a case study

Value: $39,995

Period: March 2006 to June 2007

Research provider: L Fahy and S Ridge (DPI, Victoria)

Research summary: In May 2005, a single beef cattle herd in the Ballarat region of Victoria was found to be infected with sheep strain Mptb. The property had a history of clinical JD in sheep from 1998 to 2002. The owner of the herd elected to remove (for slaughter) all the cattle on the property. In total, 73 head of cattle were sampled before and after slaughter. Fifteen animals (20.5 per cent of the herd) returned positive results to at least one of the non-serological tests and 14 animals (19.2 per cent of the entire herd) returned positive results to at least one of the definitive, post-mortem tests (histology or tissue culture). The positive animals ranged in age from fifteen months to six years. Co-grazing of cattle (in particular calves) with infected sheep, direct exposure of cattle to infected pastures (cattle following infected sheep) and indirect exposure of cattle to infected pastures (cattle grazing paddocks contaminated by run-off from infected neighbouring flocks) were all occurring on the study property from at least 2002.

Significance of science outcomes: This small case study provided definitive evidence of the possibility of widespread infection of beef cattle with the sheep strain of Mptb. Although this scenario has not been replicated (in fact, the second case study showed no transmission at all) it remains an important example of what could be considered a worst case scenario, in that in appropriate circumstances sheep strain can adapt and become widespread in a cattle herd.

On balance, the scientific significance of the outcomes from this project is rated as Moderate.

Degree of adoption and impact on practices: This study did not further inform policy, but was influential in ensuring that the role of sheep strain in the epidemiology of JD in beef cattle remained in focus within Australia.

The degree of adoption and impact on practices of the study is rated as Moderate.

Time to market of deliverables: Not applicable

4.2.1.11 P.PSH.0301: OJD in Cattle – case study 2

Full title: Ovine strain of *Mycobacterium paratuberculosis* in beef cattle: a case study #2

Value: $33,287

Period: October 2007 to September 2008

Research provider: N Stone and I McLaren (DPI, Victoria)

Research summary: In 2007, a Monitored-Negative 2 Cattle Market Assurance Program accredited stud beef herd in the Ballarat region of Victoria was identified as infected with a sheep strain of Mptb. There was no history of exposure to an infected sheep flock, either on or adjacent to the affected property. To determine the within herd distribution and prevalence of infection, and in an attempt to identify risk factors for infection and transmission, samples from the 55 head herd were examined. No further infection with Mptb was detected. The authors concluded that further testing of cattle enterprises in OJD prevalent areas was required to evaluate the significance of sheep strain infection in cattle, although believed it likely to be rare.

Significance of science outcomes: This second case study offset the first, inasmuch as its results were virtually opposite. These opposing results suggest that transmission of Mptb between sheep and cattle can occur, but is not easy. This fits with the basic science of evolution i.e. that filling a niche entails adequate numbers of organisms having enough replicative cycles for adaptive mutations to occur, and the requirement for these adapted organisms to find a host. The implications of these findings are that some practices to reduce soil burden of Mptb and cattle exposure should occur if co-grazing sheep and cattle.

The scientific significance of the study is rated as Moderate.

Degree of adoption and impact on practices: The results of this study did not further inform policy.

The degree of adoption and impact on practices of the study is rated as Low.

Time to market of deliverables: Not applicable

4.2.1.12 OJD.012: Risks of transmitting OJD in semen by artificial insemination

Full title: Assessing the risks of transmitting OJD in the semen of rams by artificial insemination

Value: $13,471

Period: July 1999 to November 2000

Research provider: Central Tablelands Rural Lands Protection Board

Research summary: This study was conducted to determine the risk of transferring Mptb in the semen of rams. Semen and other reproductive samples were collected from 11 clinically affected rams. Mptb was cultured from the semen from three rams and from the seminal vesicles of a fourth ram, suggesting that the bacterium could be transferred to the reproductive tract of ewes at mating or by artificial insemination.

Significance of science outcomes: The research showed for the first time that semen collected from rams clinically infected with OJD may contain Mptb, and that the bacteria may be transferred to the reproductive tract of ewes by mating or artificial insemination.

The scientific significance of this project is rated as Moderate.

Degree of adoption and impact on practices: This project demonstrated that the majority of the risk of transferring OJD by semen was associated with clinical animals, which simplified the implementation of risk-minimisation policy. It helped producers to understand the direct risk of transmitting OJD through the purchase or borrowing of rams that might have been in early clinical stages of the disease, and helped artificial insemination and embryo transfer centres and technicians to better understand this risk.

The degree of adoption and impact on practices of this project is rated as Moderate.

Time to market of deliverables: Immediately applicable

4.2.1.13 OJD.024: Maternal transmission of OJD

Full title: Ewe-lamb transmission of ovine Johne’s disease

Value: $60,679

Period: August 2001 to June 2001

Research provider: NSW Agriculture

Research summary: This study investigated the likelihood of intrauterine or trans-mammary transmission of Mptb in sheep, a potentially important factor in the design of control programs that had received little attention at the time of the project. Studies in cattle had found that up to 25 per cent of foetuses from clinically affected cows could be infected. In this project, 151 ewes from heavily infected flocks and their late term foetuses were examined using a range of available antemortem and necropsy tests. Five of six ewes with clinical OJD had infected foetuses. One of 54 sub-clinically affected ewes and none of 16 apparently uninfected ewes had infected foetuses. Only two ewes (both clinical cases that also had infected foetuses) had detectable Mptb in their milk or mammary glands. The authors concluded that although intrauterine or trans-mammary transmission may occur frequently in clinically affected sheep, it is infrequent in sub-clinically infected ewes or in ewes not detectably infected (even if from a heavily infected flock). The authors concluded that intrauterine or trans-mammary transmission of Mptb was unlikely to significantly affect existing OJD control programs.

Significance of science outcomes: The study yielded evidence for the low rate of intrauterine or trans-mammary transmission of Mptb infection in sheep, except in clinically affected animals. This information was not previously known.

The scientific significance of this project is rated as High.

Degree of adoption and impact on practices: Although scientifically important, this project did not lead to any specific policy decisions because sheep are largely managed as a group, and not as individual animals. Dam-offspring transmission is more important for the cattle industry, but this was already known.

The degree of adoption and impact on practices of this project is rated as Low.

Time to market of deliverables: Immediately applicable

4.2.1.14 OJD.038: Identification of risk factors for OJD in sheep flocks

Full title: Identification of risk factors for OJD infection-level in sheep flocks

Value: $237,475

Period: January 2004 to June 2005

Research provider: JA Toribio, N Dhand and R Whittington (University of Sydney)

Research summary: This project was a cross-sectional study on 92 infected properties located in New South Wales, Victoria, Tasmania and Western Australia. The information obtained from each property included the disease prevalence in specific groups of adult sheep measured using pooled faecal culture, details of farm and flock management, and soil analyses from paddocks on which the sheep sampled had grazed. Risk factors identified included farming practices such as fertiliser application, as well as aspects of flock management and soil type. In particular, weaner management and nutrition of sheep to hogget stage were important. High soil fertility, organic matter and clay content were also associated with higher levels of OJD; conversely, there was less OJD associated with sandy soils.

Significance of science outcomes: This large observational study examined a wide range of putative risk factors for the prevalence of OJD in infected Australian flocks. Because of this generalist approach, its conclusions were heavily couched in consideration for possible confounding and other artefacts of design or analysis. The study provided some evidence in support of prevailing hypotheses about the importance of soil type and key aspects of management.

The scientific significance of this project is rated as Low.

Degree of adoption and impact on practices: The findings of this project further informed management practices; however, it had less impact because the vaccine was already available and the possible impact of soil type had already been identified.

The degree of adoption and impact on practices of the study is rated as Low.

Time to market of deliverables: No deliverable

4.2.1.15 P.PSH.0204: Epidemiology and control of BJD in beef cattle herds

Full title: Epidemiology and control of bovine Johne’s disease (BJD) in beef cattle herds

Value: $141,349

Period: March 2006 to June 2008

Research provider: P Kluver, J Webb Ware and J Larsen (Mackinnon Project, University of Melbourne)

Research summary: This study examined 49 herds from Victoria, 59 from New South Wales and one from South Australia. The study's objectives were to: (a) identify likely risk factors for the introduction and establishment of BJD ; (b) describe the occurrence of clustering of BJD, in either age or family cohorts, and likely risk factors for the establishment of infection; (c) describe methods used to control and eradicate BJD and the factors that influence the decision to eradicate the disease from a beef herd; (d) identify factors contributing to the success or failure of eradication programs; and (e) assess the cost-effectiveness of different program. The study found that association with dairy breeds was the most important risk factor for the introduction of BJD. In addition, some beef breeds (Murray Grey and Shorthorn) were over-represented in herds know to be infected with BJD and the report authors concluded that these breeds should also be considered an important secondary risk for introduction of the disease. Index cases were most likely detected by veterinarians investigating clinical cases, emphasising their important role for disease monitoring. Of the herds reporting clinical cases, 80 per cent had a single case with only one high prevalence herd. The authors maintained that this showed that BJD is self-limiting in many beef herds, with management strategies to control the disease and environmental conditions limiting spread.

Significance of science outcomes: This study identified aspects of the epidemiology of JD in beef cattle herds. The association between the purchase of dairy animals and the development of BJD and the increased rate of infection associated with some beef breeds were important risk factors for industry to be aware of. The study was constrained to affected herds, limiting any inference that could have been drawn about risk factors. The authors devoted substantial attention to the economic aspects of their terms of reference, with comparatively less detail about epidemiology.

The scientific significance of the study is rated as Moderate.

Degree of adoption and impact on practices: The findings of this project reinforced the direction of the BJD control program. It also confirmed the sporadic nature of the disease in beef cattle, and highlighted the over-arching importance of dairy cattle in general as a source of infection for beef cattle and the historical associations of BJD with some less numerous beef seed-stock.

The degree of adoption and impact on practices of the study is rated as High.

Time to market of deliverables: Immediately applicable

4.2.1.16 B.AHE.0089: Role of soil in OJD

Full title: Systematic literature review: association between soil and clinical expression of Johne’s disease

Value: $36,275

Period: June 2012 to December 2012

Research provider: B Cowled and R Burns (AusVet Animal Health Services)

Research summary: The conclusion of this literature review was that, because the evidence found was inconclusive, further study is required to assess the role of soil type in the clinical expression of JD. However, the review authors advised that the need for further study should be balanced against the practicality of applying any future research results.

Significance of science outcomes: This was a review paper, and without specific scientific outcomes *per se*. Its importance in this regard is consequently rated as Low.

Degree of adoption and impact on practices: This study reinforced the contemporary view that, while soil type may have a role in the epidemiology of OJD in Australia, it is "*difficult to justify a program of complex, costly and high-risk research which may not provide any real benefit to the producers who ultimately provide the funding*"[1]. Based on the finding of this review, MLA and the JD Research Advisory Group decided against funding further research on this issue.[2]

The degree of adoption and impact on practices of the study is rated as Moderate.

Time to market of deliverables: No deliverable

4.2.2 Projects to develop new diagnostic tests, including basic research

4.2.2.1 OJD.006: Pooled faecal culture workshop proceedings

Full title: Pooled faecal culture workshop proceedings

Value: $13,927

Period: July 1999 to June 2000

Research provider: R Whittington (NSW Agriculture)

Research summary: The objective of this workshop was to facilitate the transfer of the pooled faecal culture test technology to laboratories around Australia. The pooled faecal culture test was developed by New South Wales Agriculture as a flock test for assessing flocks for OJD. It was shown to be more sensitive than serological screening and to offer potential costs savings through reduced collection and laboratory costs. The (former) Sub-Committee on Animal Health Laboratory Standards (SCAHLS), the Pooled Faecal Culture Working Group and Veterinary Committee (now Animal Health Committee) all recognised, in reviewing this test, the importance of adequate transfer of this technology to participating laboratories.

Significance of science outcomes: Workshop only – not applicable.

[1] http://www.ojd.com.au/wp-content/uploads/2013/02/OJD-and-soils-research-26-6-13.pdf accessed 11 May 2015.
[2] http://www.ojd.com.au/wp-content/uploads/2013/02/OJD-and-soils-research-26-6-13.pdf accessed 11 May 2015.

Degree of adoption and impact on practices: This project facilitated the transfer of the pooled faecal culture method from New South Wales Agriculture to other Australian laboratories

The degree of adoption and impact on practices of this workshop is rated as High.

Time to market of deliverables: Not applicable

4.2.2.2 OJD.011: National field validation of pooled faecal culture

Full title: National field validation of pooled faecal culture - Interim report 24

Value: $38,995

Period: July 2001 to June 2003

Research provider: No research organisation

Research summary: The objectives of this work were: (a) to transfer the technology of pooled faecal culture to relevant laboratories servicing the Australian sheep industry; (b) to provide wider field experience and finalise the methodology for the pooled faecal culture; (c) for relevant authorities to approve the pooled faecal culture test and protocols for use nationally and in States/Territories; (d) for the pooled faecal culture to be implemented in the National OJD Control and Evaluation Program 1998-2004 in relevant States; and (e) to provide further information on the relevant sensitivity and specificity of the pooled faecal culture. In the five States in which the test was to be applied, 900 pools were sampled. In each State, the pools were selected to reflect known infected and preferably Monitored Negative or Non-Assessed flocks across a wide variety of environments (dry/wet and hot/cold). Where infection was present in a State, the number of infected and non-infected flocks was balanced. Infected flocks were also chosen across the range of available environments taking account of mobs with suspected high and low prevalence of the disease. Field personnel were instructed in the collection of faecal samples. Estimates of sensitivity and specificity were obtained.

Significance of science outcomes: Not applicable - this study did not produce scientific results *per se*.

Degree of adoption and impact on practices: This project provided further facilitation for the transfer of the pooled faecal culture method to Australian laboratories and the approval of the test method by SCAHLS.

The degree of adoption and impact on practices of this project is rated as High.

Time to market of deliverables: Immediately applicable

4.2.2.3 OJD.022: Pooled faecal culture and agar gel immunodiffusion as flock screening tests for OJD

Full title: Evaluation and comparison of pooled faecal culture and agar gel immunodiffusion as flock screening tests for OJD

Value: $10,450

Period: August 2001 to October 2001

Research provider: E Sergeant (AusVet Animal Health Services)

Research summary: A Monte Carlo simulation model was used to estimate the sensitivity of pooled faecal culture and agar gel immunodiffusion as flock screening tests for OJD under a range of scenarios. In this context, the flock-sensitivity of a test was the level of confidence of detecting a specified prevalence of infection. The mean flock-sensitivities for a Check Test (sample size = 100) were 67 per cent and 42 per cent for pooled faecal culture and agar gel immunodiffusion, respectively, and for a Sample Test (sample size = 350 for pooled faecal culture; 500 for agar gel immunodiffusion) were 98 per cent and 93 per cent respectively. When large flocks were sampled, sample sizes of 300, 350 and 450 provided flock sensitivities for pooled faecal culture of about 95 per cent, 98 per cent and 99 per cent respectively, to detect infection if present at a prevalence of two per cent in the sampled population. The author found that although the agar gel immunodiffusion appeared to perform reasonably well in higher prevalence flocks, its flock-sensitivity in low prevalence or recently infected flocks was likely to be very low, unless sample sizes two to three times those used for pooled faecal culture were used. This result was important because, at the time, the majority of flocks being investigated outside endemic areas were relatively recently infected and still had only a low prevalence of infection. In these circumstances, the report author recommended that pooled faecal culture was the preferred test, and that larger sample sizes or whole-flock testing considered to maximise flock-sensitivity.

Significance of science outcomes: This was a small analytic study that did not appear to apply any primary research. Consequently its scientific significance is rated as Low.

Degree of adoption and impact on practices: This study was a continuation of the work that underpinned the acceptance of the pooled faecal culture method for the diagnosis of OJD. Based on the findings of this and other related projects, the pooled faecal culture method became a preferred screening test for surveillance and market assurance testing for OJD in Australia.

The degree of adoption and impact on practices of this project is rated as High.

Time to market of deliverables: Immediately applicable

4.2.2.4 AHW.080: Pooled faecal culture for caprine JD

Full title: Validation of pooled faecal culture for caprine Johne’s disease

Value: Not supplied (project not included in TOR appendix).

Period: Start date not supplied – project completed March 2006

Research provider: G Eamens (NSW DPI)

Research summary: In this study, a procedure for pooled faecal culture based on radiometric (BACTEC) culture and IS900 PCR/REA confirmation was used to examine the faeces of 21 goats that had returned a prior positive culture result for Mptb. Fourteen of the 21 samples (stored for up to four years at -80°C) were found to yield Mptb on subsequent culture. This included a range of evaluation studies, where samples were mixed with normal goat faeces at pooling rates from 1:5 to 1:50. A further two samples contained very low numbers of organisms ($< 2 \times 10^3$/g) and were only culture-positive from undiluted faeces on re-culture.

Three-quarters of the 16 culture positive goats were considered to be shedding low to moderate levels of Mptb at an estimated rate of less than 10^6/g of faeces. At pooling rates of up to 1:25, pooled faecal culture was able to detect 13 of the 16 culture-positive goats. An incubation period of at least 10 weeks at the 1:25 rate was needed to detect all 13 goats shedding Mptb at greater than 4 x 10^4/g of faeces, representing an estimated inoculum per vial of fewer than six organisms. These data supported a pooling rate of 1:25 for the application of the pooled faecal culture as a diagnostic tool in the management of caprine JD.

Significance of science outcomes: Whole herd faecal culture, based on individual culture of samples, was recognised as a sensitive but expensive diagnostic tool to evaluate herd infection rates of Mptb in goats. Pooled faecal culture based on radiometric culture procedures, with confirmation by IS900 PCR/REA, was shown to offer considerable cost savings. The validity of this approach when applied to goats had not been investigated prior to the study.

The scientific significance of the study is rated as Moderate.

Degree of adoption and impact on practices: This project identified the appropriate pooling rate for use of the pooled faecal culture in goats and allowed the pooled faecal culture to become a SCAHLS-approved test for use in goats in 2007[3]. Based on the laboratory fees cited at the time of the study's release, pooled faecal culture applied to whole-herd testing reduced the cost of goat herd diagnosis by approximately 40 per cent relative to serology and 75 to 90 per cent relative to individual faecal culture.

The degree of adoption and impact on practices of this project is rated as High.

Time to market of deliverables: Immediately applicable

4.2.2.5 P.PSH.184: Pooled faecal culture for low-shedding cattle

Full title: Validation of pooled faecal culture for bovine Johne's disease with low level shedder cattle

Value: $17,122

Period: May 2005 to March 2006

Research provider: G Eamens (NSW DPI)

Research summary: A sensitive procedure for pooled faecal culture, based on radiometric culture and IS900 PCR/REA confirmation, was used to examine pooling rates in low-shedding cattle. Appropriate samples were defined by slow growth of Mptb on initial radiometric culture, that is, first growth index at five weeks or later. Eight samples (stored for up to 17 months at -80°C) of 14 selected were found to yield Mptb on subsequent culture, including evaluation studies when samples were mixed with normal cattle faeces at pooling rates from 1:5 to 1:50. All were considered to be shedding relatively low levels of Mptb, estimated at less than 10^5/g of faeces in seven of the eight cases and less than 5 x 10^5/g in the remaining animal. At pooling rates of more than 1:5, the sensitivity of the pooled faecal

[3] www.scahls.org.au/LabTests/Pages/SCAHLS-approved-tests.aspx accessed 28 April 2015

culture was found to be low. An incubation period of at least 10 weeks at the 1:5 pooling rate was needed to detect cattle shedding <10^4 Mptb organisms/g of faeces, representing an estimated inoculum per vial of fewer than 20 organisms.

Significance of science outcomes: This study helped to establish acceptable pooling rates for pooled faecal culture and clarify the test's performance in the identification of (in particular) low-shedding cattle herds. The scientific significance of this outcome is rated as Moderate.

Degree of adoption and impact on practices: This project identified the appropriate pooling rate for use of the pooled faecal culture in cattle and allowed the pooled faecal culture to become a SCAHLS-approved test for use in cattle in 2009[4].

The degree of adoption and impact on practices of this project is rated as High.

Time to market of deliverables: Immediately applicable

4.2.2.6 OJD.007: Two methods of abattoir surveillance for OJD

Full title: Evaluation and comparison of two methods of abattoir surveillance for detection of ovine Johne's disease

Value: $103,580

Period: October 1999 to June 2000

Research provider: L Denholm, M Ryan and I Lugton (NSW Agriculture)

Research summary: This project was designed to determine whether properties infected with OJD could be identified at abattoirs by routine monitoring of cull sheep for lesions of OJD or the presence of Mptb.

Thirty-five lines of sheep were obtained from 17 known infected properties (average line 330, range 50 to 683). In most cases the trial lines were heavily infected. Over a period of six months, trial lines were delivered to abattoirs throughout New South Wales for slaughter, but mostly to the two major export works. One line was killed in Victoria. Trained inspectors were stationed in abattoirs to examine not less than 50 per cent and up to 95 per cent of the abdominal viscera from all lines of adult sheep slaughtered during each kill shift (about 10 to 15 lines per shift at the two major export abattoirs). Where visible lesions suggestive of OJD were observed, fixed tissue samples were taken for confirmatory histopathology from up to three suspect sheep per line. Inspectors were not told the identity of the trial lines, but were aware that there was a trial line to be killed during the particular shift. The inspectors detected gross lesions suggestive of OJD in 34 (97 per cent) of the 35 eligible trial lines. Whether this abattoir screening technique was sufficiently sensitive for the routine detection of flocks recently infected with OJD or with persistent low prevalence infection was uncertain. The only trial line in which lesions were not detected by inspectors was a line of cross-bred ewes introduced as adults to a property on which OJD had only been reported at low prevalence in Merinos.

[4] www.scahls.org.au/LabTests/Pages/SCAHLS-approved-tests.aspx accessed 28 April 2015

Significance of science outcomes: This project provided industry with an estimate for the confidence that could be placed in the detection of high-prevalence flocks through routine abattoir inspections. The fact that the single undetected flock was of relatively lower prevalence was noted as a point of concern and an issue for follow-up research. It would have been preferable to have included a wide range of flock-prevalences in the lines included for this study, so as to have been able to answer the question without need for further research.

On balance, the scientific significance of the outcomes from this project is rated as High.

Degree of adoption and impact on practices: The outcomes of this study were extremely important to the management of OJD in Australia. Under the current Standard Definitions, Rules and Guidelines for the management of OJD in sheep and goats, abattoir testing can be used as the primary form of surveillance: (a) as a means to monitor a broad cross section of a region's sheep flocks for evidence of OJD infection, to demonstrate low prevalence or freedom; (b) as a means to detect individual infected flocks; (c) to screen large numbers of sheep flocks in a region to determine the prevalence of infection at a flock level; and (d) as a means of assessing flock status for assurance purposes. Abattoir surveillance for OJD and other endemic diseases continues today under the National Sheep Health Monitoring Program.

The degree of adoption and impact on practices of this workshop is rated as Very High.

Time to market of deliverables: Immediately applicable

4.2.2.7 OJD.029: Animal-level sensitivity of abattoir surveillance for OJD

Full title: Determination of individual animal-level sensitivity of abattoir surveillance for ovine Johne’s disease

Value: $124,989

Period: April to October 2002

Research provider: T Bradley (Department of Primary Industries, Victoria)

Research summary: The aim of this project was to establish the animal-level sensitivity of abattoir surveillance for OJD under conditions encountered in a meat works. Three inspectors from different OJD prevalence areas in Australia were involved in the trial. Approximately 1,200 sheep from infected farms were examined by the inspectors and subsequently with follow-up histopathology. The ability of inspectors to detect gross lesions varied from 53 to 87 per cent. The best performing two inspectors had a high level of agreement between their diagnoses (74 per cent and 87 per cent) despite coming from very different prevalence areas (New South Wales and Western Australia). The third inspector had not undergone the formal OJD inspector training, which may have contributed considerably to the lower level of sensitivity. The results suggested that abattoir inspection could detect 70 to 75 per cent of histologically positive animals. However, for the purposes of setting a sensitivity level for a negative assurance scheme, it was thought reasonable to use a sensitivity of 50 per cent to account for the lower level of heavily infected animals in low prevalence flocks. A small control line of presumed negative sheep was inspected at the abattoir without confirmatory histopathology. These two hundred sheep yielded an extremely

low false-positive rate with only one inspector misclassifying one sheep. The false-positive rate was higher for the infected lines. Since confirmatory histopathology is performed on the most diagnostic lesions in such circumstances, it is extremely unlikely that a flock of sheep would be falsely labelled as infected because of gross pathology.

Significance of science outcomes: This study showed that the individual-level sensitivity of abattoir inspection is in the order of 70 to 75 per cent. The scientific significance of this outcome is rated as Moderate. This project built on OJD.007, which focussed on the flock-level sensitivity of abattoir inspection.

Degree of adoption and impact on practices: This study was very important to industry, as it quantified at an animal level the value of abattoir surveillance. It helped to underpin abattoir surveillance as the main method of surveillance for OJD and, by establishing an estimate of sensitivity in negative flocks, allowed abattoir surveillance to be use for flock negative assurance under the Assurance Based Credit scheme. Abattoir surveillance can still be used for flock negative assurance on the current Sheep Health Statement[5].

The degree of adoption and impact on practices of this project is rated as Very High.

Time to market of deliverables: Immediately applicable

4.2.2.8 OJD.014: Field Evaluation of the tracer weaner model

Full title: Field evaluation of the tracer weaner model: early detection of natural infection of sheep with *Mycobacterium avium* subsp. *paratuberculosis*

Value: $60,328.

Period: July 2000 to June 2002

Research provider: Unknown author (NSW Agriculture)

Research summary: This study sought to determine whether histopathology of samples from weaner sheep could be used as a surrogate test for the contamination of pasture with Mptb. Two farms in the endemic area of New South Wales were included in the study. In the most heavily contaminated environment, 43 to 75 per cent of sheep sampled at more than eight months post-exposure were culture-positive. Infection of introduced naïve sheep was first detected by culture of tissues five to six months after exposure. In environments with lower levels of contamination, infection was detected six to twelve months post-exposure. In the lowest contamination scenario, where pasture had been grazed by infected ewes for two months only, then left un-grazed for a month before the introduction of tracers, a single infected sheep was detected 12 months after first exposure.

Significance of science outcomes: The study showed that groups of naïve sheep of any age, tested at slaughter by culture of pooled tissues from individual sheep after six to twelve months exposure, could be used to assess the level of pasture contamination on infected farms. The study maintained that groups of weaners could be utilised as practical and economical tracers to assess pasture infectivity within on-farm disease control programs.

[5] http://www.ojd.com.au/wp-content/uploads/2013/02/National-Sheep-Health-Statement-16-July-2013-FINAL1.pdf accessed 23 May 2015

The scientific significance of the results from this project is rated as Low.

Degree of adoption and impact on practices: This testing strategy was never adopted by Australian sheep producers because it is impractical and uneconomic. It is also unacceptable from an animal welfare perspective.

The degree of adoption and impact on practices of this project is rated as Low.

Time to market of deliverables: Immediately applicable: note low utility

4.2.2.9 OJD.020: Individual animal tests for OJD

Full title: Individual animal tests for OJD

Value: $185,213

Period: June 2001 to April 2004

Research provider: L Reddacliff (NSW Agriculture)

Research summary: On a heavily infected farm, 77 sheep were examined at 12, 18 and 24 months of age by histopathology and culture of biopsied ileal and mesenteric lymph nodes. Results from the biopsies were compared to those from routine tests (serology, IFN, skin testing, faecal culture and direct PCR (D-PCR)) applied at six-monthly intervals, and to necropsy findings at three years of age. A total of 170 biopsies were performed. Overall, 36 per cent of sheep were uninfected at three years of age. Of these, 16 were uninfected at all sampling times and 11 had recovered (i.e. they had been infected at an earlier sampling). The remaining 64 per cent of sheep were classified at necropsy as infected, including 36 with subclinical OJD (six had severe diffuse lesions and thirty had milder lesions) and 11 that had died of OJD. Biopsy was consistently the most sensitive nonlethal technique for identification of infected sheep, although even at 36 months it detected only two thirds of infected sheep. Because some sheep recover from early infection, testing at 12 months of age was not predictive of later infection status. By 18 to 30 months of age, positive test results for cell mediated immunity, humoral immunity or faecal excretion were highly associated with later death from OJD, although not often with infection status at three years. The lack of statistical association with final infection status reflects the low sensitivity of these tests at this early stage of the disease.

Significance of science outcomes: This detailed prospective examination of a heavily infected flock to three years of age provided a unique perspective on the disease. Insights gained include that: (a) a proportion of infected sheep recover from detectable infection, thus any early test and cull program will remove some possibly genetically resistant sheep; (b) even using every available antemortem test (including biopsy) on repeated occasions, many infected sheep remain undetected, therefore there can be no guarantees that a particular sheep is clean; (c) results for most tests (in particular those for cell mediated immunity) are not consistent over time; (d) culture from tissues is superior to histopathology at necropsy in the early stages of the disease. Routine diagnostic tests for OJD have poor sensitivity in the early stages of the disease and transmission can occur before detection. At the time of the report's publication, there were no tests to accurately confirm early infection in individual sheep. Such tests were required to provide trading opportunities for producers who might

have valuable stock at low risk of infection. Surgical biopsy is one means of disease detection using relatively sensitive laboratory procedures, but was unproven.

On balance, the scientific significance of the outcomes from this project is rated as High.

Degree of adoption and impact on practices: This study had no impact on policy and control programmes, although the biopsy method has been useful from a scientific perspective in subsequent research projects.

The degree of adoption and impact on practices of this project is rated as Low.

Time to market of deliverables: No deliverable

4.2.2.10 OJD.025: IFN test for the diagnosis of OJD

Full title: Validation of the gamma interferon test for diagnosis of ovine Johne's disease

Value: $155,054

Period: September 2001 to July 2005

Research provider: D Stewart (CSIRO Livestock Industries)

Research summary: This study was carried out to further assess the sensitivity and specificity of an IFN test using whole Mptb antigens. Because of non-specific IFN responses, raised cut-points were required to achieve high specificity (≥98 per cent). This resulted in reduction of sensitivity to below 50 per cent, limiting the application of the test for early detection or certification from disease freedom. The authors concluded that the assay may have application in a test and cull program as a surrogate test for faecal shedding and the removal of sheep with severe disease, but stressed that this approach would require further confirmation. A major limitation for adoption identified was that widespread use of vaccination would preclude the use of immunological tests for diagnosis. Other limitations, apart from relatively low sensitivity, included the cost of the test as well as the narrow window of approximately six hours between blood sample collection and laboratory initiation of the assay so that test result validity is not compromised. Thus, in its current format, the authors concluded that it was unlikely that the IFN assay (apart from possibly stud flocks) would have wide application in the sheep industry.

Significance of science outcomes: This study confirmed what was known at the time about the generally poor performance of an IFN test based on Mptb PPD antigens. The scientific significance of the study is rated as Low.

Degree of adoption and impact on practices: This project demonstrated that an IFN test based on Mptb PPD antigens was not suitable for the diagnosis of OJD in the format then available. Work to improve the test continues as part of the University of Sydney research project, despite the almost certainty that the test will not meaningfully differentiate between true positives (infected) and false positives (vaccinated) unless a new antigen is found.

The degree of adoption and impact on practices of this project is rated as Low.

Time to market of deliverables: No deliverable

4.2.2.11 TR.060: Rapid test for OJD based on pooled faeces

Full title: Development of a rapid cost effective test for ovine Johne's disease based on testing of pooled faeces

Value: $30,000

Period: July 1999 to June 2000

Research provider: I Marsh and R Whittington (NSW Agriculture)

Research summary: This project was undertaken to develop and evaluate a rapid, cost-effective, flock test for Mptb in pooled faecal samples, based on hybridisation-capture polymerase chain reaction (HC-PCR). However, a simpler direct technique (D-PCR) was found to be more sensitive than HC-PCR. About 67 per cent of culture positive pooled faecal samples were positive when tested using D-PCR. In a blind trial, 83 per cent of 12 farms identified by culture of pooled faecal samples were detected using D-PCR. The cost of D-PCR was considered to be no greater than that of other flock-detection strategies. The test was considered to be suitable for use in the National OJD Control and Evaluation Program 1998-2004; however, a constraint existed in that Veterinary Committee did not recognise the results of DNA-based tests for Mptb as being definitive, and the costs of follow-up testing to confirm infection were high. Recommendations were made to improve the test and reduce its cost.

Significance of science outcomes: This study was an early step in the eventual development of a sensitive, rapid, cost-effective, PCR test for Mptb in pooled faecal samples. The scientific significance of this project's findings is considered Moderate.

Degree of adoption and impact on practices: This study did not successfully develop a rapid PCR test for Mptb in pooled faecal samples that was as sensitive as the pooled faecal culture.

The degree of adoption and impact on practices of this project is rated as Low.

Time to market of deliverables: No deliverable

4.2.2.12 B.AHW.079: Improving the D-PCR test for Mptb from faeces

Full title: Possibilities for improving the D-PCR test for M. a. paratuberculosis from faeces

Value: Not supplied (project not included in TOR appendix).

Period: Start date not supplied – project completed December 2007

Research provider: I Marsh (NSW Agriculture)

Research summary: The objective of this project was to increase the sensitivity of the D-PCR to equal to or greater than that of faecal culture, while maintaining the technical practicalities of the test and keeping the cost low. A series of experiments was undertaken with the intention of enhancing the D-PCR procedure: (a) a new Mptb isolation and DNA extraction procedure was to be implemented; (b) an internal PCR control was to be included to more accurately interpret negative results; and (c) a new multiplex PCR was to be

developed to overcome the need for restriction endonuclease analysis for the confirmation of Mptb. The authors succeeded only with parts (a) and (b), and concluded that the refined D-PCR failed to even achieve the sensitivity of the original D-PCR and substantially less than faecal culture.

Although not part of the original research plan, the authors then focussed on a real-time PCR methodology that had been trialled at the Japanese National Institute of Animal Health and a Japanese faecal extraction technique (JohnePrep™, Shimadzu Corporation). This test was not available in Australia, but was being developed for use in sheep by the Farm Animal Health Unit of the Faculty of Veterinary Science at the University of Sydney. The Japanese test identified 86 per cent of faecal culture positive samples, including all five recent faecal culture-positive samples. The authors concluded that a more thorough examination of the Japanese test was warranted, including the new faecal extraction method. They recommended that future evaluation should include both real-time and conventional PCR using the new multiplex PCR and long internal control developed as part of this project. They also maintained that an internal control should also be considered for the real-time PCR.

Significance of science outcomes: Although this project did not result in improvements to the D-PCR test, it did include some initial evaluations of the PCR method developed at the Japanese National Institute of Animal Health and of the Japanese faecal extraction technique. These methods were eventually improved and validated as the HT-J PCR test. The scientific significance of this project is considered Moderate.

Degree of adoption and impact on practices: This project did not successfully develop a D-PCR test for Mptb.

The degree of adoption and impact on practices of this project is rated as Low.

Time to market of deliverables: No deliverable

4.2.2.13 OJD.031V3: Strategic research for diagnosis and prevention

Full title: Pathogenesis of OJD: strategic research for diagnosis and prevention

Value: $3,202,000

Period: September 2002 to March 2008

Research provider: R Whittington, D Begg, K Bosward, K de Silva and D Taylor (University of Sydney)

Research summary: This project included nine separate subprograms.

Subprogram 1a was established to adapt the existing IFN tests for tuberculosis (Pfizer-CSL) (Bovigam®) to JD. This subprogram attempted improvements to physical and chemical aspects of the current test protocol to enhance its practicality and efficacy. The research suffered from lack of effective consultation with the two Australian companies who were commercially providing IFN tests, as most of the knowledge deemed discovered was already available. Little of relevance to an effective test was found. The subprogram also attempted to evaluate the cells responsible for IFN production, but was unable to do so because the cells could not be found in sufficient numbers in the peripheral blood of research sheep.

Subprogram 1b was established to evaluate alternative assay methods for detecting IFN or other cytokines, and to compare the results of this with other tests for detection of OJD. It is the opinion of the reviewers that more consultation with commercial groups would have been beneficial, as many of the starting assumptions on IFN testing were incorrect. The reviewers also consider that, although they can provide research insight, none of the methods attempted had potential for field test applications, as they are laborious, specialised, and/or add no practical information.

As an addition to the core objectives, the study considered the antigen-specific secretion of IL-10. An elevated IL-10 response was observed in blood and lymph node cells associated with sites of infection (ileal lymph node and jejunal lymph node) of sheep that have been exposed to Mptb, but not in a peripheral lymph node from these animals. The authors concluded that the ability to detect an IL-10 response in blood cells makes it a potentially useful diagnostic test. This possibility did not appear to have been examined further.

Subprogram 1c was established to develop a novel assay for OJD based on apoptosis, the death of cells that occurs as a normal and controlled part of growth or development. The authors noted that commercially available kits (e.g. the TUNEL assay) could be used to detect apoptosis in ovine tissues *in situ*. In addition, a high throughput flow cytometry method (to detect apoptosis in ovine peripheral blood or tissue mononuclear cells) and a real time quantitative PCR assay (using unique ovine specific primers to detect expression of apoptosis related genes in ovine tissues) were established. The study found that, while a difference in the level of apoptotic cells in the peripheral blood mononuclear cells of exposed and unexposed sheep was detected soon after exposure to Mptb, there was no difference between these two groups at later time points. The study did, however, find that TUNEL counts of apoptotic cells within the terminal ileum were significantly higher in infected animals compared to exposed negative controls. No conclusions were drawn from this observation as to the diagnostic value of the TUNEL assay.

Subprogram 2a was established to develop *in vitro* models to study the interaction between Mptb and host cells. *In vitro* models are used to study the attachment and penetration of Mptb in the early stage of infection, and the responses of the host. Models are also required for genomic and proteomic studies. Options for this work included explant cultures of gut tissue, immortal cell lines from sheep and other species (monocyte, macrophage and epithelial) and primary cell lines from sheep. The study sought to establish: (a) immortal macrophage/monocyte, epithelial and hybridoma cell lines in culture and to determine optimal research conditions for Mptb; and (b) methods for primary culture of blood leukocytes of sheep. The study also sought to investigate a method for ovine explant tissue culture from surgical, post mortem or abattoir material. The murine macrophage cell line RAW264.7 was chosen to study macrophage responses *in vitro*. Due to the low numbers of monocytes isolated from blood, and the inappropriateness of removing large volumes of blood from sheep, it was decided that it would not be possible to supply adequate numbers of ovine monocytes for ongoing Mptb research. Due to the low rate of infection of epithelial cells by Mptb it was decided not to progress with explant cultures.

Subprogram 2b was established to provide experimentally infected young sheep and biological materials for study of the pathogenesis of OJD. Specifically, the objectives of this study were to: (a) make available young lambs twice yearly for on-going studies; (b) develop seed stocks of Mptb; (c) infect young lambs orally with Mptb and examine naturally infected

sheep to compile a collection of biological samples for testing, then characterise the phenotype of these sheep by sequential testing and post mortem examination, for example at about 3 years of age; (d) consider the applicability of surgical models for the study of pathogenesis of OJD, including biopsy, infection delivered at specific intestinal sites in intact lambs, infection delivered into isolated gut loops and lymphatic cannulation models; and (e) produce cytokine-positive control blood samples for assay standardisation by immunising sheep with killed Mptb. Objectives (a) and (b) were met. An experimental infection model (c) was developed using a mild ovine pure clonal strain of Mptb (Telford 9.2) and resulted in repeatable infection outcomes across multiple trials. Regular biological samples were acquired to assess the phenotype of the sheep. Surgical biopsies (d) were not required to study disease pathogenesis, as the regular blood samples and tissues accessed at necropsy provided adequate biological samples to assess the phenotype of the sheep. Throughout the project at least two OJD vaccinated animals were maintained at pasture to provide blood samples for cytokines assay development and positive controls for the assays (e).

Subprogram 3a was established to detect Mptb genes or the gene products that are involved in the pathogenesis of OJD. The authors maintained that 'nothing' was at that point known about how Mptb replicates and spreads within the host. The intent of this study was to: (a) develop methods for studying the genome and proteome of Mptb; and (b) analyse the behaviour of Mptb in cell cultures and ovine samples using genomic and proteomic techniques. A new SYBR Green based real-time quantitative PCR (QPCR) assay based in IS900 was developed to directly detect and quantify Mptb in faeces and validated for use on sheep. Both the cattle and sheep strains of Mptb were detected by the QPCR assay. No cross reactions were detected with 51 other species of mycobacteria and none of the control negative faecal samples were positive. The authors indicated that this was the first report of a direct faecal quantitative PCR assay for sheep that has similar sensitivity to a gold standard radiometric culture assay. Part (b) of the study focussed on the development of an *in vitro* model to evaluate the proteome of Mptb during dormancy, based on prior observations that dormancy occurred as a result of temperature flux in the natural environment. Data were obtained for both sheep and cattle strains of Mptb. The study also examined Mptb proteomic response to hypoxia and starvation, and found this to be similar to other mycobacteria. The authors postulated that differentially expressed proteins are potential screening targets for future diagnosis, prevention and control and that their identification will assist understanding the pathogenesis of diseases. As the final step, the study examined the immunogenicity of recombinant dormancy-associated proteins of Mptb in infected sheep. The authors suggested that this may have practical implications for the development of future diagnostic tests to detect early immune responses in sheep.

Subprogram 3b was established to detect genes or gene products in the host that are involved in the pathogenesis of OJD. Specifically, the study sought to: (a) develop genomic and proteomic tools and methods to analyse host gene expression; and (b) analyse host gene/proteome expression in *in vitro* models and in samples from sheep with OJD. The study resulted in an approach (a) for selection of reference genes, conducting assays with technical replicates in duplicate rather than triplicate, determining decision-limit quality control criteria for technical replicates and assessing the significance of gene expression fold differences. The objective of part (b) was to examine host gene expression during the sub-clinical to clinical stage of Mptb to identify known or novel genes regulating the response to infection and to define genes that can be used to identify infected animals earlier than

currently possible. Four new genes were found to be differentially regulated in the intestine of the sheep in the response to Mptb, although their use in a diagnostic context was not remarked upon. The study also analysed the expression of ten toll-like receptor genes relative to validated reference genes at predilection sites in ileum, jejunum and associated lymph nodes, as well as in peripheral blood, to determine if toll-like receptor gene expression is altered in response to infection with Mptb in out-bred sheep. (Toll-like receptors are part of the innate immune system involved in the initial recognition of pathogens.) They found that there were differences in toll-like receptor gene expression between early paucibacillary and multibacillary groups when compared to uninfected sheep, with most toll-like receptor genes for the paucibacillary group having lower expression levels than the multibacillary group. Further research was recommended. The final part of the study was to use proteomic profiling of serum to determine whether biomarkers could be found for the early diagnosis of OJD. Detailed statistical analyses were provided, but the authors did not appear to draw any conclusions as to the practical output from the work.

Subprogram 4a was established to assess the potential for detection of Mptb in blood as an alternative diagnostic procedure. Mptb has been found in blood during infection. The study sought to: (a) determine whether Mptb could be found in leukocytes during infection; and (b) examine the surface receptors on infected and normal blood leucocytes and evaluate methods to concentrate infected blood-leukocytes using knowledge about receptor expression. Mptb was cultured from the peripheral blood of a small number (not specified) of infected animals. However, no single cell surface marker currently able to be measured in sheep could be used for identification of sheep with JD and feasible methods to concentrate infected cells from blood using antibodies against cell surface markers could not be developed.

Subprogram 4b was established to stimulate infected macrophages to move from intestinal tissue into blood, skin or faeces to increase the sensitivity of diagnostic tests that rely on detection of the organism. More specifically, the objectives of this study were to: (a) analyse the capacity of normal and infected macrophages to migrate, and evaluate methods to induce migration from the intestinal region into blood, skin or faeces of infected sheep; and (b) evaluate the sensitivity of PCR and culture to detect Mptb in blood or other samples with and without stimulation of migration. The authors examined the migration of macrophages (a) but did not appear to develop methods to induce or facilitate it. A quantitative PCR able to detect 10 organisms in 10 mL of blood was developed.

Significance of science outcomes: This body of work examined a broad range of topics and applications. The ELISPOT assay and Cell-ELISA techniques were explored, but remain research techniques because they are laborious and required specific expertise. New antigens were not investigated to improve IFN detection (Subprogram 1b). A difference in the level of apoptotic cells in the peripheral blood mononuclear cells of exposed and unexposed sheep was detected soon after exposure to Mptb, but there was no difference between these two groups at later time points (Subprogram 1c). The murine macrophage cell line RAW264.7 (Subprogram 2a) was chosen to study macrophage responses to *in vitro* infection, but due to the low numbers of monocytes isolated from blood, and the inappropriateness of removing large volumes of blood from sheep, it was not possible to supply adequate numbers of ovine monocytes for ongoing Mptb research. Likewise, due to the low rate of infection of epithelial cells by Mptb, it was decided not to progress with explant cultures. Subprogram 2b was largely concerned with practical objectives, and most

of these were met. A quantitative PCR was developed in Subprogram 3a. Information about the triggers and characteristics of dormancy may eventually lead to practical applications, but this is in the longer term. Subprogram 3b uncovered four new genes that may be differentially regulated in the intestine of the sheep in the response to Mptb, although their use in a diagnostic context was not remarked upon. Further research was considered necessary to investigate observed differences in toll-like receptor gene expression between early paucibacillary and multibacillary groups when compared to uninfected sheep. Subprogram 4a failed to identify a cell surface marker that could be used for identification of sheep with JD and was unable to concentrate infected cells from blood using antibodies against cell surface markers. Subprogram 4b examined migration of macrophages but did not develop methods to induce or facilitate it. A quantitative PCR able to detect 10 organisms in 10 mL of blood was developed, but apparently not validated.

On balance, the significance of these outcomes is rated as Low for cell based assay and Moderate with respect to dormancy and toll-like receptor expression. The large and multifaceted project provided an enormous scope for continuing laboratory-based research, but was configured in a way that was unlikely to result in concrete and immediately redeemable scientific findings.

Degree of adoption and impact on practices: The focus of this project was basic laboratory research and it wasn't designed to result in immediately concrete and adoptable findings for industry.

The degree of adoption and impact on practices of this project is rated as Low.

Time to market of deliverables: No deliverable

4.2.2.14 P.COM.0140: Strategic research for diagnosis and prevention

Full title: Pathogenesis of OJD: Strategic research for diagnosis and prevention

Value: $224,253

Period: November 2007 to July 2011

Research provider: University of Sydney and MLA Commercialisation Manager

Research summary: No report for this study was made available for review. It was explained, however, that P.COM.0140 is the intellectual property file for project OJD.0031. This project resulted in the development of several intellectual property applications, which covered relevant aspects of new understandings of the early pathogenesis of the disease and the use of that understanding and other developments for the production of diagnostic tools and possible vaccines and other therapeutics. Development and preparation of the applications and management of the application process was not covered in the original project B.OJD.0031 budget and this project was designed to cover the shortfall in funding.

Significance of science outcomes: Not applicable

Degree of adoption and impact on practices: Not applicable

Time to market of deliverables: No deliverable. Current status of intellectual property applications not provided.

4.2.2.15 P.PSH.0297: BJD basic research for diagnosis and prevention

Full title: Bovine Johne's disease: basic and applied research for improved diagnosis and prevention

Value: $1,312,200

Period: January 2008 to July 2011

Research provider: R Whittington, D Begg, K de Silva, I Marsh, K Plain, A Purdie and S Thirunavukkarasu (University of Sydney)

Research summary: The research program for this project was structured around three key groups of studies: (a) application of Australian and international advances in diagnostics (b); basic research on immune responses in early stages of BJD; and (c) animal resources and infection models.

Part A - Application of Australian and international advances in diagnostics: This part of the project had two separate objectives. The first objective was to improve diagnosis through enhancement of IFN technology, by continuing the work on ELISPOT and CELL-ELISA undertaken in OJD.031, incorporating developments from Scandinavian and Japanese researchers. The authors postulated that these developments would lead to a test with improved sensitivity, specificity and practicality. However, without the specific antigens known by that time to be critical in tuberculosis testing, and as both ELISPOT and CELL-ELISA are labour intensive methods, how this improvement could be achieved is unclear. The second objective was to improve the detection of Mptb in faeces using D-PCR. This work would again build on the outcomes of OJD.031 and Japanese developments, and would be carried out in conjunction with NSW DPI (I Marsh).

The first part of Part A noted that two blood additives had been trialled. These aided IFN production levels but were found to have (further) reduced the test's specificity. As part of the search for specific antigens in this and linked projects, the investigators looked at antigens that Mptb produces when stressed, and the identification and cloning of Mptb genes with B/T cell epitopes and other proteins. The second part of Part A resulted in the development and validation of the HT-J PCR test for use in cattle, although additional work was still required to allow improved estimates of the sensitivity of the test.

Part B - Basic research on immune responses in early stages of BJD: This part of the project had three separate objectives. First was to develop an improved understanding of cellular immunology in calves, on the assumption that most research to date had focussed on advanced cases in adult cattle. The researchers postulated that an early cellular immune response should be detectable, with diagnostic and prognostic value. The second objective was to improve understanding of antibody responses in early stages of BJD, while the third was to examine the intestinal response during early infection.

Studies examining cellular and humoral responses during early infection did not yield definitive results, although the authors reported that immune suppression and weight loss during BJD may be explained by deregulation of amino acid (tryptophan) metabolism. Although not described in the research proposal, the authors also undertook gene

expression studies into features of the early immune response, opening up possible new avenues for research on diagnosis and prevention.

Part C - Animal resources and infection models: This part of the project focussed on the provision of infected calves and macrophages (from peripheral blood) through the methodology developed in OJD.031. Two trials were undertaken: (a) a short-term pilot study to see if a defined cattle strain of Mptb would result in infection; and (b) a long-term study to evaluate infection outcomes in cattle. The authors found that four of the five inoculated animals from the pilot study were successfully infected with Mptb. One of the animals was also faecal culture positive. This experiment confirmed the validity of the infection methodology, allowing a larger trial to begin. In the second trial, thirty calves aged two to four months were age matched then randomly allocated into a group of 20 to be inoculated and a group of 10 controls. Control animals were housed separately from the inoculated animals, in paddocks where no Mptb infected livestock had been housed in the past. The 20 inoculated animals were infected using the same infection schedule as the pilot trial. At 17 to 18 months post inoculation, there was no histological or tissue culture evidence of infection from the biopsy results. However, the second round of biopsies at 26 months post infection indicated two of the animals had microscopic lesions in sections of ileum. Mean Mptb antigen-specific whole blood IFN ELISA levels increased steadily until 13 months post inoculation at which time the responses began to slowly decrease. Levels also rose in the control group, although the two groups were visually distinct. An isolation technique that provides a high purity and yield of bovine monocytes was described.

Significance of science outcomes: The scientific significance of the study was rated by the reviewers as Low for IFN assays and Moderate for the HT-J PCR test, basic research on immune responses in early stages of BJD and animal resources and models. Although the study provided some useful indicators for future research and developed reliable and repeatable infection models, with the exception of the HT-J PCR test, there were very few finite and measurable outcomes. It is also of concern that the study's findings and its research report were virtually identical to those of project PSH.0311. The latter was focussed on OJD and sheep, but there appeared to be very few material differences between the two projects. There also appeared to be some blurring between the objectives and outcomes of this work and that of OJD.031V3.

Degree of adoption and impact on practices: A PCR test on faeces based on a method from a Japanese research lab was re-developed, validated and transferred to the NSW DPI laboratory at EMAI. The test is mentioned in the Testing for BJD in Beef Herds factsheet on the Animal Health Australia website[6] and the test was used in the recent BJD incident investigations in Queensland. The HT-J PCR test is a much faster screening tool than the pooled faecal culture. However, it requires follow up with other tests in herds not previously known to be infected or suspected of infection. Importantly, the test, as now standardised for sensitivity, is a herd, not an individual animal, test.

Fewer than 4,000 HT-J PCR tests were done in veterinary diagnostic laboratories in Australia (New South Wales, Queensland, South Australia, Victoria and Western Australia) in the 2014 calendar year, including samples from both cattle and sheep.

[6] http://www.farmbiosecurity.com.au/wp-content/uploads/2014/02/Testing-for-BJD-in-beef-herds.pdf, accessed 11 May 2015

The degree of adoption and impact on practices of HT-J PCR test emanating from this project for use in cattle is rated as Moderate.

Time to market of deliverables: Deliverable. Much of the basic, animal and infection work is to facilitate methods and knowledge in further research. The utility of the HT-J PCR test may become more apparent in future. The IFN research has no deliverable.

4.2.2.16 P.PSH.0311: OJD basic research for diagnosis and prevention

Full title: Ovine Johne's disease: applications of basic research on enhanced diagnosis and prevention

Value: $2,415,320

Period: January 2008 to July 2011

Research provider: R Whittington, D Begg, K Bower, K de Silva, RB Gurung, I Marsh, K Plain and A Purdie (University of Sydney)

Research summary: Refer to project P.PSH.0297.

Significance of science outcomes: Refer to project P.PSH.0297.

Degree of adoption and impact on practices: A PCR test on faeces based on a method from a Japanese research lab was re-developed, validated and transferred to the NSW DPI laboratory at EMAI. To date the test has not been widely used in the sheep industry and the test is not mentioned in the Testing for OJD factsheet currently endorsed by the National OJD programme, Sheepmeat Council of Australia and Woolproducers Australia[7]. It is possible that the test could be more widely used, for example in Market Assurance Flocks, because it is a faster screening test than the pooled faecal culture.

As noted above, fewer than 4,000 HT-J PCR tests were done in veterinary diagnostic laboratories in Australia (New South Wales, Queensland, South Australia, Victoria and Western Australia) in the 2014 calendar year, including samples from both cattle and sheep.

The degree of adoption and impact on practices of HT-J PCR test emanating from this project for use in sheep is rated as Low.

Time to market of deliverables: Deliverable. The PCR method exists but uptake has been low, as noted. The utility of this test may become more apparent in future.

4.2.3 Projects that investigated the impact of Johne's disease

4.2.3.1 OJD.023: Biological and Economic Impacts of OJD

Full title: A study of the biological and economic impacts of OJD in affected sheep flocks in NSW

Value: $249,636

[7] http://www.ojd.com.au/wp-content/uploads/2013/02/Testing-for-OJD-fact-sheet-final.pdf, accessed 11 May 2015

Period: September 2001 to September 2003

Research provider: JA Toribio, R Bush and P Windsor (University of Sydney)

Research summary: This study was conducted on 12 farms in southern New South Wales. It provided information about the impact of OJD on sheep mortality and farm profitability. The average OJD mortality rate based on inventory records was 6.2 per cent (median 5.8 per cent, range 2.1 per cent to 17.5 per cent), which was similar to the average OJD mortality rate based on necropsy inspections (6.7 per cent, median 4.4 per cent, range 1.1 per cent to 15.0 per cent), but more than twice that which would be considered broadly acceptable in southern Australia. Mortality due to OJD increased from one year of age (10.4 per cent) to peak at four years of age (35.6 per cent), and then fell at over four years of age (19.2 per cent), and was similar between wethers (49.6 per cent) and breeding ewes (50.4 per cent). The disease prevalence in two year old sheep ranged from 0.7 per cent to > 23 per cent and was found to be associated with the disease mortality rate (P=0.02). The association between various environmental, management and disease factors and the quarterly OJD mortality rate was analysed, and several factors (including flock size, stocking rate, area of improved pasture and weaning age) were identified as being important for further investigation. The average estimated cost of OJD losses on the 12 farms over the 12-month study period was $64,100 (median $44,942, range $15,569 to $154,083). The average estimated cost of annual OJD losses/DSE was $7.68 (median $4.11, range $0.84 to $20.51) and of annual OJD losses/ha was $65.92 (median $25.09, range $6.75 to $244.80).

Significance of science outcomes: This analysis did not yield any discrete scientific findings, and its significance is rated as Low.

Degree of adoption and impact on practices: This project, whilst restricted to New South Wales, provided industry with further evidence about the economic impact of OJD. The findings were integrated into policy and control programmes, helping industry remain focussed on the importance of control programmes to limit the spread of the disease.

The degree of adoption and impact on practices of this project is rated as High.

Time to market of deliverables: Not applicable

4.2.3.2 OJD.032: Cost at Slaughter of Vaccination Lesions

Full title: A preliminary study on the potential cost at slaughter of OJD vaccination site lesions to the Australian sheep industry

Value: $35,234

Period: January 2003 to March 2004

Research provider: J Eppleston (Central Tablelands RLPB)

Research summary: This project investigated potential issues around the application of discounts due to Gudair® injection site lesions as the early use of the vaccine expanded. The project included a review of discounting in New Zealand, as well as a preliminary survey of the prevalence of lesions and actual discounts applied to vaccinated sheep in Australia. Information from New Zealand suggested that the most significant discounts there would be

applied to trimmed high-value lamb carcases destined for export in whole carcase form. In Australia, the greatest risk of discounting was considered to be Merino lambs sold into the prime lamb market, but that the low proportion exported as carcases would limit the discount applied. The prevalence of lesions observed was 18 per cent for mutton and 65 per cent for lamb carcases. The value of the trim removed was insignificant, the labour cost of its removal was nil and no carcase was downgraded to a lower value grade.

Significance of science outcomes: This study did not yield any discrete scientific outcomes. It provided some analysis of the issues around discounting, but was of minor scope and its scientific significance is consequently rated as Low.

Degree of adoption and impact on practices: This study was important at the time to demonstrate to industry that vaccination lesions were not a significant issue for the processing sector. The findings from the project helped encourage the uptake of the vaccine

The degree of adoption and impact on practices of this project is rated as High.

Time to market of deliverables: Immediately applicable

4.2.3.3 B.AHE.0041: Impact of OJD on the processing sector

Full title: Financial impact of ovine Johne’s disease on the processing sector

Value: $49,892

Period: May 2012 to September 2013

Research provider: M Hernandez-Jover and G Ramsay (Charles Sturt University), I Links and T Nordblom (NSW DPI), B Jackson and R Bell (Tas DPIW)

Research summary: The objective of this study was to estimate the financial loss from OJD to producers and processors in Tasmania. Data from 358 consignments and 31,858 individual carcases were collected. Six mutton consignments were OJD positive, with a median apparent within-consignment prevalence of 4.6 per cent. Forty-seven consignments had Gudair® vaccination site lesions. The median proportion of carcases with lesions was 3.0 per cent. The mean carcase weight, value, fat class, slaughtering time and skin price per consignment were not associated with the disease, with vaccination site lesions or the proportion of lesions within a consignment. The median proportion of total consignment weight trimmed due to vaccination lesions was 0.03 per cent (5 to 95 per cent confidence interval, 0 to 0.73 per cent).

Significance of science outcomes: This study did not yield any discrete scientific findings, and is given a rating of Low. The authors' conclusion that "*The data collected in this study were not appropriate to examine the impacts of the presence of OJD on processing, due to the limited number of OJD positive consignments*" was disappointing, as this was the objective of the study. However, it reflects the fact that the OJD prevalence in the examined animals was too low for a meaningful cost estimate.

Degree of adoption and impact on practices: The project study failed to demonstrate the expected result seen in a pilot study (almost $6 per sheep loss in a line of sheep due to clinical and subclinical OJD), possibly because of widespread uptake of the vaccine.

The degree of adoption and impact on practices of this project is rated as Low.

Time to market of deliverables: No deliverable

4.2.4 Projects that investigated control options for Johne's disease

4.2.4.1 OJD.001: Evaluation of eradication strategies for ovine Johne's disease

Full title: Evaluation of eradication strategies for OJD.

Value: $1,143,817

Period: September 1999 to October 2004.

Research provider: Pat Taylor and Stewart Webster (NSW DPI)

Research summary: This study was a field evaluation of the biological efficacy and economic viability of destocking for 15 to 21 months, including two summers and restocking as a strategy to eradicate OJD from farms in south-eastern Australia. Three years after re-introduction to decontaminated sites, 28 of 41 flocks (68 per cent) in South Australia, Victoria and New South Wales presented with evidence of OJD. Thirty-nine (39) of 40 Mptb positive faecal cultures collected from these flocks were identified as sheep strain. There was a 20-fold reduction in the mean apparent prevalence between destocking (18 per cent) and three years after restocking (0.9 per cent) on the 28 farms. Eradication failures were considered to have been primarily of local origin (i.e. from neighbouring flocks or from incomplete decontamination). Efforts to identify infectious refugia in the summer prior to restocking yielded only one Mptb-positive culture from 279 samples of topsoil, dam water and dam sediment. Twenty-year simulations of net farm income based on data derived from farms participating in the field evaluation indicated that destocking and restocking was less profitable than vaccination as an OJD management option.

Significance of science outcomes: This early field study provided farmers with evidence to suggest that destocking and restocking was unlikely (32 per cent) to result in a clean flock. The information ratified the more specific experiments of Whittington (OJD.003) who had shown that Mptb could survive for 13 months on shaded pasture. The researchers noted that the study had been carried out under 'worst-case' conditions, as most of the farms selected had a high on-farm prevalence of infection and a likely high level of Mptb in the environment. The authors also noted that under these conditions, neighbouring properties were also likely to be infected. Eradication failures were recorded across the geographic range of enrolled farms, including those in South Australia, Victoria and New South Wales. In closing, the authors remarked that, "*It is unfortunate that more thought was not given during the planning stages to design an evaluation that shed light on the reasons for potential eradication failures. One important concern is that of infected neighbouring flocks. With the benefit of hindsight the results would have been more informative if a proportion of sites had been deliberately selected that had no neighbouring sheep flocks*".

Overall, the scientific significance of this project is considered to have been High.

Degree of adoption and impact on practices: In 1998, steering committees representing the sheep industries and state and federal governments agreed on a national program of regulation, education and research to contain the spread of OJD and to improve the

understanding of the epidemiology and pathology of OJD under Australian conditions. Whole-of-flock destocking and restocking was considered to be one potential strategy to prevent further spread of the disease and underpin systematic regional eradication campaigns. This project was pivotal because it demonstrated that de-stocking was not a viable option in endemic areas. The results of the project helped move the control of OJD towards vaccination and risk-based trading.

The degree of adoption and impact on practices of this project is rated as Very High.

Time to market of deliverables: Immediately applicable

4.2.4.2 OJD.009: Field evaluation of OJD control using Gudair®

Full title: Field evaluation of OJD control using Gudair®

Value: $458,020

Period: November 1999 to April 2005

Research provider: Leslie Reddacliff (NSW DPI)

Research summary: On each of three farms in New South Wales that were experiencing significant losses due to OJD (5 to 15 per cent per annum), 200 Merino lambs aged one to four months were vaccinated with Gudair® and a further 200 lambs were sham-vaccinated with saline. Animal assessments and sample collections were carried out twice yearly until the animals were four or five years of age.

The vaccine stimulated cell-mediated and humoral immune responses. It reduced mortalities due to OJD by 90 per cent and delayed faecal shedding for the first year post-vaccination. Thereafter, the prevalence of shedders among vaccinates was also reduced by 90 per cent, as was the number of Mptb excreted by the vaccinated groups. However, significant levels of excretion by vaccinates did occur on some occasions and although only seven of 600 vaccinates died from OJD, all had multibacillary disease. The authors concluded that there remained a risk that some vaccinated sheep could transfer the disease. Small reductions in liveweight gain were found in vaccinated lambs in the first year after vaccination, although there was little effect on condition score or wool production. Vaccine injection site lesions were detected in almost 50 per cent of sheep two months after vaccination, and these persisted for at least four years in 20 to 25 per cent of vaccinates.

Significance of science outcomes: This study provided a detailed analysis of the effect of vaccination on flocks with a significant prevalence of OJD. The study showed that vaccination could significantly reduce mortality due to OJD, as well as the amount of shedding within the flock. The study also demonstrated a cellular and humoral immune response to vaccination and was able to correlate this with the reduction in mortality. The study demonstrated the field safety of Gudair®, although there was a minor impact on the growth of lambs and a significant incidence of injection-site lesions. The study was very carefully designed and carried out, and was able to provide the data that substantially underpinned registration of the Gudair® vaccine in Australia in 2002. The study also provided useful practical advice to producers as to the beneficial and side effects of vaccination and the dangers of assuming that all vaccinated animals pose little or no risk of carrying and transmitting the disease.

On balance, the scientific significance of the outcomes from this project is rated as High.

Degree of adoption and impact on practices: This study provided the Australian data required to register the Gudair® vaccine.

The degree of adoption and impact on practices of this project is rated as Very High.

4.2.4.3 Time to market of deliverables: Immediately applicable

4.2.4.4 OJD.015: Longitudinal study of whole flock vaccination with Gudair®

Full title: A longitudinal study of OJD and the effects of whole flock vaccination with Gudair®: whole of flock vaccination at Merrill

Value: $103,478

Period: April 2000 to March 2005

Research provider: Peter Windsor (University of Sydney).

Research summary: This was a four year, longitudinal study of vaccine (Gudair®) efficacy in a large self-replacing Merino flock in the Gunning area of New South Wales. The flock had estimated an annual mortality rate due to OJD of around 25 per cent.

Over the study period, adult sheep mortality declined significantly from 24.2 to 2.8 per cent, and this was mostly attributed to a decline in the OJD attributable mortality risk from 19.0 to 1.4 per cent. Significant mortalities due to the disease did, however, continue. The prevalence of faecal shedders also declined from a high to a moderate level following vaccination, although the number of animals (in particular, wethers) that continued to shed was considered to be sufficient to maintain a within-flock transmission cycle. Vaccination was thought to be similarly efficacious when sheep were vaccinated at three or eight months of age. Interpretation of the study was complicated to some extent by a decline in the prevalence of shedders within the unvaccinated group. This may have reflected a series of management changes that the owner put in place at the start of the study. These included the culling of clinical cases, the sale of at-risk stock and a reduction in stocking rate. It may also reflect the reduced challenge of infection arising within the flock as a whole, given that a portion of the animals were vaccinated.

Significance of science outcomes: The intent of this small study was sensible, and it provided some further data about the efficacy of the Gudair® vaccine. The study was constrained, however, by design issues, primarily that: (a) it was limited to a single farm; and (b) it was not possible to separate the impacts of vaccination from those of concurrent management changes. The study provided some further evidence that vaccination does not completely negate infection or shedding, nor completely eradicate it from the flock over a four-year period. It was also valuable to demonstrate that vaccination and management changes can reduce the challenge of infection to unvaccinated animals.

On balance, the scientific significance of the outcomes from this project is rated as Moderate.

Degree of adoption and impact on practices: This project reinforced the value of vaccination, particularly the importance of vaccinating young stock.

The degree of adoption and impact on practices of this project is rated as High.

Time to market of deliverables: Immediately applicable

4.2.4.5 OJD.033V1: Changes in prevalence following vaccination with Gudair®

Full title: Changes in within-flock prevalence of Mptb shedding following vaccination with Gudair® in high and low prevalence flocks

Value: $237,639

Period: January 2003 to April 2009

Research provider: Peter Windsor (University of Sydney)

Research summary: This was a longitudinal observational study examining the efficacy of Gudair® in decreasing the prevalence of shedding in flocks with varying initial prevalence of OJD. Twelve flocks were examined over a six year period: three were categorised as high prevalence, four as medium prevalence and five as low prevalence.

Vaccinated sheep had a significantly lower prevalence of shedding than unvaccinated sheep (0.63 per cent versus 1.66 per cent; P<0.001), although at the final sampling 10 of the 11 flocks still in the study had sheep with detectable shedding (0.13 per cent to 1.29 per cent). The authors noted that persistence of shedding for an extended period following commencement of flock vaccination presents a risk for spread and recrudescence of OJD. It was recommended that the study be continued to provide both additional data on the rate of decline of shedding in flocks composed entirely of ‘second generation vaccinates’ and to identify factors that might explain differences in shedding rates between flocks. It was thought this would assist sheep producers to assess the risks of ceasing vaccination and purchasing vaccinated re-stocker sheep.

Significance of science outcomes: The study's findings were broadly consistent with prior research, although derived from farms with a slightly different infection profile. The study did not produce any novel scientific outcomes, but did reinforce existing knowledge about the behaviour of Gudair®. The study provided important data in support of the results of OJD.009 - specifically, that whilst highly efficacious the Gudair® vaccine does not completely halt the shedding of Mptb in all infected flocks within a six-year period . The original vaccine research in Australia (OJD.009) was conducted on the first cohort of vaccinates from three flocks that were considered to be heavily infected, with presumed exposure of intra-uterine and neonatal lambs of infected ewes to significant Mptb challenge. However, following registration of Gudair® many lower prevalence flocks also commenced vaccination as a precaution against increased mortalities and as a means to improve their ability to sell re-stocker sheep through the risk-based trading Assurance Based Credit scheme. Modelling work had suggested that the prevalence of mortalities and shedding would fall rapidly after the commencement of a vaccination program depending on disease prevalence at the time of commencing vaccination. Validation of this work by field research was required.

On balance, the scientific significance of this project is rated as High.

Degree of adoption and impact on practices: This project reinforced the value of vaccination, particularly in the medium term. In addition to demonstrating the efficacy of the vaccine in

flocks with varying initial prevalence, it also demonstrated that in one of the eleven flocks monitored, shedding could not be detected at the conclusion of the study.

The degree of adoption and impact on practices of this project is rated as High.

Time to market of deliverables: Immediately applicable

4.2.4.6 P.PSH.0309: Gudair® in flocks vaccinating for at least five years

Full title: Evaluation of the effectiveness of Gudair® vaccination for the control of OJD in flocks vaccinating for at least five years

Value: $297,244

Period: January 2008 to November 2010

Research provider: Peter Windsor, Jeff Eppleston, Navneet Dhand and Richard Whittington (University of Sydney).

Research summary: This large multi-centre observational study conducted in south-eastern New South Wales and Victoria examined the efficacy of Gudair® at decreasing the shedding of Mptb within flocks of varying prevalence. Pooled faecal cultures from 350 sheep (pooled faecal culture350, seven pools of 50 sheep) were obtained from 40 flocks five years or more after they commenced vaccinating lambs with Gudair®. The flocks had been categorised as High, Medium or Low shedding prior to the start of vaccination. Categorisation was based on a combination of serology, faecal culture, histopathology and abattoir surveillance. A further 16 flocks from Kangaroo Island in South Australia were included to validate the pooled faecal culture 350 test.

The study found a significant decline in prevalence from approximately 2.99 per cent pre-vaccination to 0.74 per cent post vaccination. In seven of the 40 flocks shedding was no longer detectable; however, 33 of the 40 flocks were still shedding and 20 of these at a rate indicative of a medium to high prevalence. It was also noted that seven of the 14 flocks that were classified as having an initial low prevalence had increased to medium or high prevalence after five years of vaccination. Flocks with disease prevalence greater than one per cent after five years of vaccination were associated with sheep straying and the introduction of new sheep. Having a concurrent cattle enterprise was shown to be protective. The authors concluded that the persistence of shedding in vaccinated flocks was of concern if sheep were to be traded or if vaccination was to cease. They also maintained that whilst Gudair® does decrease the prevalence of OJD, it does not negate the continuing need for on-farm biosecurity. Some additional work was undertaken to demonstrate the sensitivity of the pooled faecal culture for detecting flocks with a low prevalence of Mptb shedders.

Significance of science outcomes: This study provided important data on the medium term use of Gudair®, specifically, that whilst highly efficacious, the Gudair® vaccine does not completely halt the shedding of Mptb in all infected flocks within a five year time-frame, or completely protect them from the infection introduced through purchased or straying sheep.

On balance, the scientific significance of this project is rated as High.

Degree of adoption and impact on practices: Similar to project OJD.033V1, this project reinforced the value of vaccination in the medium term. It demonstrated that the risk of transmitting the disease by trading vaccinates may be significantly reduced after medium-term use of Gudair® in a flock with initial low prevalence. The length of time a flock has been continuously vaccinated is included as a question on the new Sheep Health Statement.

In addition, the project demonstrated that in seven of the forty vaccinated flocks, shedding could not be detected, building on the results demonstrated in OJD.033V1.

The degree of adoption and impact on practices of this project is rated as High.

Time to market of deliverables: Immediately applicable

4.2.4.7 P.PSH.0296: Efficacy of Silirum® vaccine in two cattle herds

Full title: Field study to assess the efficacy of Silirum® vaccine in two cattle herds infected with BJD.

Value: $972,549

Period: July 2007 to December 2012

Research provider: Zoetis (then Pfizer Animal Health)

Research summary: Approximately 1,350 animals of varying ages (including calves and adults) were initially recruited into this study. On each property, further new season calves and replacements were recruited in subsequent years. At approximately 36 months there were no notable differences detected between the vaccinated animals and the control animals with respect to rate of faecal shedding, new clinical cases of BJD or individual cow milk production parameters. Comparison of production parameters, faecal shedding rates and incidences of clinically suspected or histopathologically confirmed cases of JD revealed largely similar outcomes for both the control and the vaccinated groups. There were no appreciable differences between vaccinates and non-vaccinates in the mean antibody level.

Significance of science outcomes: This study was incomplete at the time of last reporting to MLA. The study was terminated by Zoetis in February 2012, and MLA did not receive a final report. Because of this, the scientific significance of the findings to date was not rated.

The underlying project continued in-house. The results available at the time of this review are fragmented, with the analysis of the results apparently incomplete. However, interim five-year results (e.g. Figure 1) are substantially more encouraging than those included in the last report to MLA (and cited above). Early examination of culture data suggests a reduction in clinical cases of around 60 per cent and a delay in the progression of disease (10 per cent older at first positive faecal culture). The proportion of shedders amongst vaccinated cattle is between about 15 per cent and 45 per cent that of unvaccinated cattle.

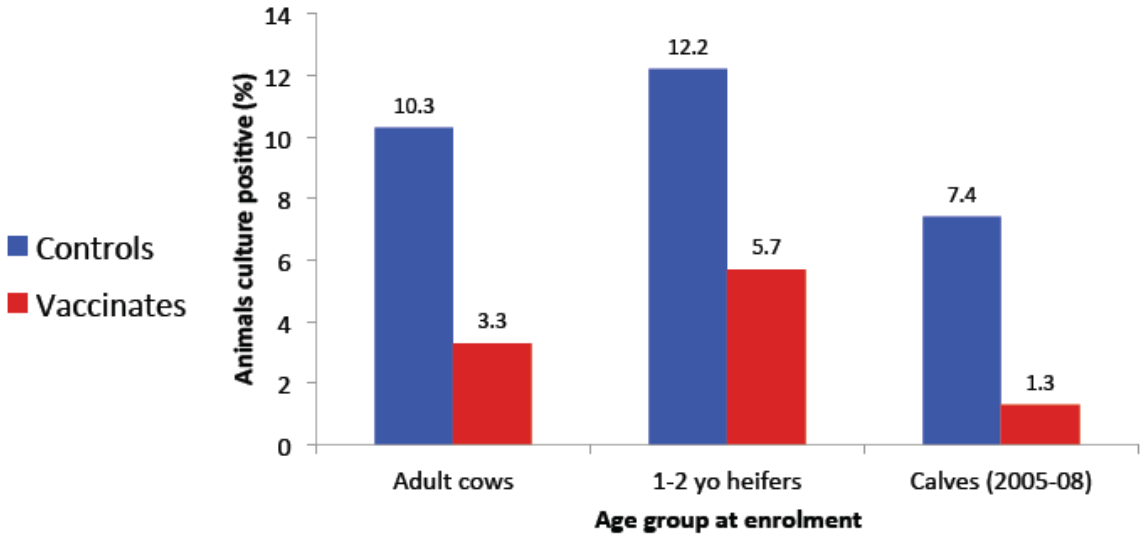

Figure 1: Interim 5-year results of Zoetis Silirum® trial

Source: Peter Little (Pfizer Animal Health, Parkville, Victoria), Jack Winterbottom, Mirta Aranda, Andrew Hodge – undated presentation titled, Field Efficacy of Silirum Vaccine in Two Australian Dairy Herds: 5-Year Interim Summary

Degree of adoption and impact on practices: This study supported registration of Silirum® vaccine for use in cattle in Australia[8]. To date the uptake of the vaccine appears to have been limited.

The degree of adoption and impact on practices of this project is rated as Moderate.

Time to market of deliverables: Likely immediately applicable

4.3 Scientific significance, degree of adoption and impact of practices, and time to market of proposed deliverables from current projects

4.3.1.1 P.PSH.0565: Within-flock prevalence following vaccination with Gudair®

Full title: Extended examination of changes in within-flock prevalence of Mptb shedding following vaccination with Gudair® in high medium and low initial prevalence flocks

Value: $261,108

Period: July 2010 to July 2015

Research provider: P Windsor (University of Sydney) and J Eppleston (Tablelands Livestock Health and Pest Authority)

Research summary: This project continues from OJD.033 as an epidemiologic assessment of the long-term efficacy of Gudair® in reducing the shedding of Mptb in Merino flocks of initial low, medium or high OJD prevalence in the central tablelands of New South Wales.

[8] http://archive.apvma.gov.au/advice_summaries/54408.rtf accessed 27 April 2015

Project OJD.033V1 commenced in 2003 with 12 flocks, 11 of which yielded complete data. The study demonstrated a significant decline in shedding on most farms, although there was a persistently low rate of shedding (range 0.127 per cent to 1.294 per cent) on 10 of the 11 farms at the completion of the sampling in 2008. Industry recommended extending the study to determine the rate of decline and degree of shedding when all animals in these 11 flocks were second generation vaccinates, that is, the progeny of lambs born when the entire flock consisted of approved vaccinates. The 11 flocks thus continued to be assessed for an additional three rounds of sampling at two-yearly intervals. Milestone six, completed in December 2014, was the most recent report available for review. Seven of the eight farms remaining in the trial had been sampled, with the final farm due for sampling in March 2015. The data will be analysed and the project report compiled after the completion of the trial.

Significance of science outcomes: The study was incomplete at the time of our review, and the significance of its final findings could not be determined. However, it is noted that although shedding had ceased in three flocks by Sampling 5, it was detected in seven of the ten flocks despite long-term vaccination.

Degree of adoption and impact on practices: This project continues to reinforce the value of vaccination in the longer term and its findings are likely to significantly impact on policy and practices.

Time to market of proposed deliverables: Likely immediately applicable.

4.3.1.2 P.PSH.0576: Tools for Johne's disease in sheep and cattle

Full title: Diagnostic, predictive and preventative tools for Johne's disease in sheep and cattle

Value: $6,887,783

Period: July 2011 to December 2015 (ongoing)

Research provider: University of Sydney

Research summary: This large, multi-faceted and currently ongoing project aims for the following outcomes:

Vaccines for JD*:* (a) New vaccine adjuvant formulation that does not elicit severe tissue reactions tested in sheep; (b) Prototype new vaccine concept tested in sheep; (c) Indirect measures of vaccine efficacy applied to new prototype vaccine in trials with sheep and cattle; (d) New strategy for vaccine trials enables short term studies in the relevant species

Genomics for JD*:* (a) Breed susceptibility to OJD compared (Merino versus British breeds); (b) Early disease markers evaluated in sheep and cattle; (c) Possible resistance/susceptibility gene markers identified for sheep and cattle and applied in validation studies

Predictive tools and diagnostics*:* (a) A new culture medium and application strategy devised for OJD to replace BACTEC, and available for BJD; (b) A live-dead mycobacteriophage assay available to support diagnoses using D-PCR testing of faeces in cattle and sheep; (c) Immunological and genomic tools used singly or in combination to

predict the susceptibility and resistance to JD at breed, herd/flock and individual animal levels, including early prediction of high faecal shedding for application in control programs.

Significance of science outcomes: The study was incomplete at the time of our review, and the significance of its final findings could not be determined. Some preliminary results from the predictive tools and diagnostics sub-program are précised below.

Predictive tools and diagnostics:

(a) A new culture medium and application strategy devised for OJD to replace BACTEC, and available for BJD. M7H9C liquid culture media has replaced BACTEC 12B medium and is now being used by laboratories throughout Australia. The new culture medium has been published in the scientific literature and is included in the revised Australia and New Zealand Standard Diagnostic Test Procedures which was submitted to SCAHLS for review[9] (. A new method for the processing of cultures to detect Mptb growth has been developed and validated. This was due to the need to perform DNA extractions and PCR on all M7H9C cultures as a confirmation of growth, as M7H9C does not currently incorporate a growth indicator. The existing protocol involved a simple DNA extraction method by ethanol precipitation, however this step was laborious and batch size was limited by processing time and microcentrifuge capacity. The new processing method involves magnetic bead DNA isolation technology and quantitative PCR and was submitted to SCAHLS for consideration in October. A scientific paper reporting the validation of this method is approved by MLA for publication and will be submitted for peer-review.

(b) Assessed a mycobacteriophage assay available to support diagnoses using D-PCR testing in cattle and sheep. This was ineffective.

(c) Immunological and genomic tools used singly or in combination to predict the susceptibility and resistance to JD at breed, herd/flock and individual animal levels, including early prediction of high faecal shedding for application in control programs

Ovine and bovine array studies are ongoing.

Degree of adoption and impact on practices: The new culture medium is available and in use in veterinary laboratories in Australia. The degree of adoption and impact on practices for other deliverables could not be assessed because the project is not yet complete.

Time to market of proposed deliverables:

***Vaccines for JD**: No Deliverable.* The program is not designed to deliver a vaccine within this project (R. Whittington) and the review panel agree. Elaboration of the basis of immunity will likely require more detailed investigation than currently underway. The need for a safer vaccine is moot considering better vaccination techniques and the fact that no human adverse reactions associated with Gudair® were reported by the Australian Pesticides and Veterinary Medicines Authority between 2009 and 2013[10] (Australian Pesticides and Veterinary Medicines Authority reports since 2013 were not yet available at the time of this

[9] This sub-committee has since been disbanded, so the outcome is uncertain.
[10] http://apvma.gov.au/node/10946, accessed 15 May 2015

review) In contrast, there were 14,425 Agriculture workers compensation claims for other injuries during the same time period[11].

Genomics for JD: *No Deliverable whilst work still to be completed.*

Predictive tools and diagnostics: Deliverable; Culture methods and medium have been applied in veterinary laboratories within the timeframe of the project. Processing of culture by PCR will greatly reduce diagnostic timeframes if approved. No other deliverables have derived from this research project or are likely within the current timeframe of the project.

4.3.1.3 B.AHE.0237: Survivability of Mptb on northern properties

Full title: Environmental survivability of *Mycobacterium avium* subsp. *paratuberculosis* (Mptb) on northern grazing properties

Value: $477,539

Period: January 2014 to March 2016

Research provider: W Hein (James Cook University School of Veterinary and Biomedical Sciences)

Research summary: The key objective of this project is to determine the survival times of Mptb bison strain in spiked faecal plots in various northern environments, taking account of seasonality and aggregation points where cattle congregate on farms. The project will also assess the possible role of environmental mycobacteria in the interpretation of HT-J PCR test results from beef herds currently under movement restrictions in Queensland, with the ultimate aim of increasing the test's reliability in determining the BJD status of a herd. The researchers aim to determine the feasibility of using a molecular assay to assist with interpretation of the HT-J PCR when applied to both animals and samples collected from the environment.

Significance of science outcomes: The study was incomplete at the time of our review, and the significance of its findings could not be determined.

Degree of adoption and impact on practices: The data from this project are expected to enhance performance and adoption of the previously developed HT-J PCR test and to better inform recommendations on destocking in northern Australia.

Time to market of proposed deliverables: As noted above.

4.4 Ranking of research outputs

An analysis of the distribution of rankings for scientific significance and for adoption and impact is provided in Table 1. This analysis covered the 46 projects we reviewed in Sections 4.2 and 4.3, with the large and currently ongoing P.PSH.0576 (Diagnostic, predictive and preventative tools for JD in sheep and cattle) rated separately for each of its four key

[11] http://www.safeworkaustralia.gov.au/sites/swa/about/publications/pages/work-related-injuries-fatalities-australian-farms, accessed 18 May 2015

themes: genomics, vaccines, diagnostics, and animal models and field trials. The result was a total of 49 rated items.

Table 1 shows that the 25 projects (or 51 per cent) which received a rating on the scientific significance scale of Moderate or Low accounted for $9,926,086 (or 46 per cent) of the total commitment to JD research managed by MLA. Similarly, the 18 projects (or 37 per cent) which received a rating on the adoption and impact scale of Moderate or Low accounted for $10,274,259 (or 48 per cent) of the total funding. The nine not rated projects (or 18 per cent), which included all current projects, accounted for $7,808,961 (or 36 per cent). These funding preponderances rested to a large extent on the University of Sydney's diagnostics and basic research program, which has received approximately $14m in total funds for a wide range of objectives, but has proportionately fewer concrete deliverables. The result was not unexpected, however, as basic research is by nature high-cost and exploratory and is usually associated with a lower certainty of absolute success and of delivery of tangible industry outcomes/tools.

This observation can be contrasted with many of the generally lower-cost observational studies that helped to clarify important aspects of the epidemiology, management or control of JD (in particular, OJD) in Australia. Observational studies are in general less expensive than laboratory-based basic research and may provide definitive results in a relatively shorter period of time.

Table 1. Distribution of rankings for scientific significance and for adoption and impact (No. Projects, $'000's)

	Very High		High		Moderate		Low		Not Rated**		Total	
Scientific Significance												
Epidemiology	2	$186	4	$105	7	$1,803	3	$377	1	$478	17	$2,949
Diagnostics and Basic Research	0	$0	2	$289	6	$3,407	5	$3,920	7	$7,096	20	$14,712
Impact	0	$0	0	$0	0	$0	3	$335	0	$0	3	$335
Control	0	$0	4	$2,137	1	$84	0	$0	2	$1,234	7	$3,454
Management	0	$0	0	$0	0	$0	0	$0	2	$28	2	$28
*Totals**	*2*	*$186*	*10*	*$2,530*	*14*	*$5,294*	*11*	*$4,632*	*12*	*$8,834*	*49*	*$21,477*
Adoption and Impact												
Epidemiology	3	$177	5	$369	5	$1,594	3	$331	1	$478	17	$2,949
Diagnostics and Basic Research	2	$262	5	$81	1	$1,312	7	$6,014	5	$7,043	20	$14,712
Impact	0	$0	2	$285	0	$0	1	$50	0	$0	3	$335
Control	2	$1,602	3	$619	1	$973	0	$0	1	$261	7	$3,454
Management	0	$0	0	$0	0	$0	0	$0	2	$28	2	$28
Totals	*7*	*$2,040*	*15*	*$1,353*	*7*	*$3,878*	*11*	*$6,396*	*9*	*$7,809*	*49*	*$21,477*

**totals eliminate rounding in individual projects*

***all current projects were ranked as not rated*

4.5 Significant remaining Johne's disease knowledge gaps

Based on review of the JD research funded by MLA to date; consultation with JD researchers, Animal Health Australia and the cattle and sheep industry; and taking into account the expertise of the authors, the following significant remaining knowledge gaps have been identified:

Epidemiology of JD:

- The epidemiology of the sheep strain in beef cattle in Australia, i.e. whether the sheep strain can adapt to and persist in a beef breeding herd after contact with infected sheep has stopped, and whether cattle can then transmit disease to disease-free sheep.
- The epidemiology of the sheep and/or cattle strain in deer in Australia, in particular disease prevalence in farmed and feral deer and whether the sheep strain of Mptb can infect and persist in farmed and feral deer, with deer becoming a potential source of infection for sheep.
- The epidemiology of BJD in beef cattle, i.e. regional herd prevalence and information to clarify the determinants for the successful establishment and persistence of JD within northern and southern beef cattle herds of differing commercial characteristics and in different geo-climatic zones.

New diagnostic tests, including basic research:

- The potential for abattoir surveillance to be used as a surveillance tool for JD and other endemic diseases of beef cattle in Australia, including appropriate methodology and how surveillance data can be used to facilitate overseas trade and inform policy to mitigate risk(s) to the beef industry nationally.
- Validation of the HT-J PCR as an individual animal test for cattle.
- Increased understanding of ways to better utilise the HT-J PCR test in both cattle and sheep, including as individual or flock/herd tests.
- The development of an individual animal test or panel of tests for use in cattle that will identify animals in the early stage of infection, prior to faecal shedding of Mptb, that will either develop clinical disease, develop sub-clinical disease and shed Mptb in their faeces or clear the infection.
- Genetic markers and breeding values to allow selection of cattle and sheep that are unlikely to develop clinical disease.

Control options for JD in cattle and sheep:

- The long-term efficacy of vaccination in sheep, in particular the potential ability of the Gudair® vaccine to reduce shedding and infection in the medium to long term as suggested by projects OJD.033 and P.PSH. 0309, and the implications of this for industry.
- Increased understanding of the efficacy and potential role of the Silirum® vaccine in controlling BJD in Australian cattle, including the ability of the vaccine to reduce the number of clinical cases within a herd, ameliorate the clinical signs of JD, reduce sub-clinical productivity losses and reduce faecal shedding of Mptb. In addition, the ability of the vaccine to eliminate shedding and infection in the long-term, as

research is suggesting may be possible for sheep flocks vaccinated with the Gudair® vaccine, could be investigated as an option to de-stocking infected beef herds.

5 Discussion

5.1 Research that addressed knowledge gaps identified in 1998

Early research commissioned by MLA and industry as part of the National OJD Control and Evaluation Program 1998-2004 addressed the knowledge gaps identified by Hussey and Morris (Hussey and Morris, 1998) and has made a valuable contribution to the management of both OJD and BJD in Australia. When questioned in the past, industry and government representatives have freely volunteered this research programme as the most valuable aspect of the OJD control programmes undertaken in Australia over the past 15 years (Lloyd, 2011).

Key deliverables from the research programme for the sheep industry have been abattoir surveillance and confirmation of the efficacy of the Gudair® vaccine in Australian sheep. In recent years, abattoir surveillance has been expanded to become a valuable endemic disease surveillance tool for the industry. The Gudair vaccine has been widely adopted as a control tool for OJD in Australian sheep flocks.

The research programme has also delivered a cost-effective test to detect infection within a flock, the pooled faecal culture test. Subsequent to the adoption and acceptance of this test for use in sheep, MLA-managed research has resulted in the test being approved for use in cattle and goats.

Other valuable findings from the early research programme were that wildlife (kangaroos and rabbits) are not important reservoirs for JD in Australia, something that was not known previously, and that the disease was not having a significant negative impact on the meat processing sector. These findings, along with research projects which revealed that de-stocking was not a viable means of eradication in endemic areas, and that cattle and sheep in Australia are most commonly infected with different strains of Mptb, were fundamental in shaping Australia's JD control and management programmes over the past 15 years.

The epidemiology of the disease was closely studied in a small number of infected sheep flocks over a number of years. This revealed information on the age-related susceptibility of infection and the potential for lateral spread between paddocks, and between infected farms and neighbours. This information, combined with the results of studies on the survivability of Mptb in the environment, has provided producers with information on risk management and on grazing management options for the within flock control of OJD.

5.2 Research that addressed knowledge gaps identified in 2010

The majority of the knowledge gaps identified to MLA by WoolProducers Australia and Sheepmeat Council of Australia in 2010 have been filled.

The long-term performance of the Gudair® vaccine was investigated in two projects, P.PSH0309 and OJD.033/P.PSH.0565. The results from both projects continued to

demonstrate declining prevalence of disease with continued use of the vaccine in most vaccinated flocks. Although Mptb shedding was still detectable in many of the vaccinated flocks in these studies, in a small number of flocks detectable shedding had ceased.

Breed susceptibility has been investigated (P.PSH.0576). All of the breeds examined (Merino, Merino cross White Suffolk, Border Leicester, Poll Dorset) were susceptible to Mptb infection and developed clinical OJD, but, within the short 14-month timeframe of the study, Merino and Suffolk cross Merino breeds developed the most disease. If the assessment timeframe had been longer, more of the Poll Dorset and Border Leicester sheep might have developed clinical disease because infection was detected in many of the animals.

Adoption drivers and blockers affecting the Sheep Health Statement were investigated in MLA Project B.AHE.0057 (Taylor et al. 2011). The findings of this study have previously been summarised for industry as part of a review of the National OJD Management Plan 2007-12 (Lloyd, 2011). This review also investigated reasons for prevalence areas changes and the application of the Assurance Based Credit scheme in managing risk.

Sheep movement volumes and patterns have recently been reviewed and reported (East and Foreman, 2011).

Specific research to investigate adoption drivers and blockers affecting the uptake of the Gudair® vaccine has not been funded by MLA. During the review of the National OJD Management Plan 2007-12 in 2011, industry representatives and others interviewed indicated that they thought occupational health and safety issues might be limiting the uptake of the vaccine (Lloyd, 2011). The limited consultation undertaken as part of the current review suggested that this may no longer be the case, with the shrouded vaccinator now more widely accepted and livestock contractors being engaged to vaccinate sheep. No human adverse reactions associated with Gudair® were reported by the Australian Pesticides and Veterinary Medicines Authority between 2009 and 2013[12]. (Australian Pesticides and Veterinary Medicines Authority reports since 2013 were unavailable at the time of this review). In contrast, there were 14,425 Agriculture workers compensation claims for other injuries during the same time period[13].

Diagnostic test development has been an area with differential outcomes in the JD research program, especially over the past few years. The early research on culture methods and improvements was significant and necessary for current assessment of JD status. DNA typing methodologies allowed strains to be characterised, which has strong epidemiological value, if the need arises. Recent sheep strain infection in cattle may be an example of such a need.

More recently, some success has been achieved in PCR technology, with the development of the HT-J PCR and the culture assay methods. While demonstrated as an accurate test and comparable to international methods, the HT-J PCR remains labour-intensive and expensive to perform and there is considerable room for improvement in its practicalities if

[12] http://apvma.gov.au/node/10946, accessed 15 May 2015
[13] http://www.safeworkaustralia.gov.au/sites/swa/about/publications/pages/work-related-injuries-fatalities-australian-farms, accessed 18 May 2015

widespread use is to be achieved. However, the relatively low level of use makes it an unattractive area for serious commercial involvement. State and private veterinary diagnostic laboratories continue to offer JD diagnostic testing services around Australia. The data provided to us by the JD Technical Working Group for the 2014 calendar year indicates an overall low adoption of tests by industry, with various export tests (especially the JD ELISA) the only large volume service.

The need for Mptb antigens or epitopes that provide a level of specificity was well established (Refer section 4.2.2.10; OJD.025) and research is yet to yield a solution despite considerable effort in Australia and overseas. The use of such specific antigens in whole blood IFN testing has been an Australian innovation that has largely transformed human tuberculosis infection diagnosis worldwide since 2004, allowing diagnosis in the presence of vaccination. In contrast, methodological investigations in the JD research program funded by MLA have not delivered the main goal of improved test specificity and practicality, suggesting that the specific antigens needed may not exist.

Considering vaccine research, our review has found a lack of clarity in focus and consensus in expected outcome between producer groups, funding bodies, and research groups. No fixed program goals have been set for vaccine research, and the general understanding has been an expressed desire for a 'better, safer and cheaper' vaccine than Gudair® and presumably Silirum®, for which less information is currently available. Working to produce a vaccine without quantified, written and agreed objectives of vaccine efficacy will inevitably lead to wasted effort and resource.

Expectations of vaccine research may be overly optimistic. Producers accustomed to the efficacious and economic virtues of other routinely used vaccines may underestimate the difficulties of vaccine development against mycobacterial disease, which has yet to be effectively achieved in humans, where aggressive reactive adjuvants are not acceptable. Thus it should be widely accepted by all parties involved that an equally effective, yet less reactive alternative to Gudair®, is an extraordinarily difficult project, on a par with developing an effective tuberculosis vaccine, where extensive resources and intellectual effort have been devoted over more than a century without success beyond the largely ineffective BCG. In this context, the solution to Mptb vaccination issues will likely reside within the mycobacterial research area in total, rather than through short-term applied research projects in Mptb. The review team has no expectation of a deliverable vaccine from current efforts, and the researchers confirm this expectation.

6 Conclusions/recommendations

Examining the large body of work on the epidemiology and impact of JD, on-farm control options, diagnostic options and other basic research, it is clear that a great deal of the research performed has been adopted into JD policy and has guided JD (particularly OJD) management and control in Australia. Diagnostic work has not always been fruitful, but it has provided tools for the maintenance of this system, albeit not at the price point or with the ease of use that would be desirable for the producer or within a commercial veterinary diagnostic laboratory. Nevertheless, along with the efficacy of the Gudair® vaccine, the research has in total contributed to an environment where, in the general sense (and noting

there are particular exceptions) OJD has been maintained as a persistent irritation, rather than a severe economic constraint for industry.

Less is understood about the epidemiology of JD in beef cattle or the most effective and efficient methods for its control. There is no evidence that any strain of JD (cattle, sheep or bison) is widespread in beef cattle, but surveillance data are lacking and absence of evidence does not prove absence of disease.

Many of the research projects reviewed were assessed to have immediate deliverables, even though these projects often lacked physically marketable outcomes. Nevertheless, their knowledge outcomes were directly relevant and significant in guiding regulatory and on-farm practice to the financial benefit of producers. The bulk of projects, if not the bulk of expenditure, fit within this category. Nevertheless, extension work to ensure such knowledge is in the hands of industry may still be needed.

Projects within the areas of diagnosis and vaccine improvement necessarily involve the development of products or methods, either fully commercial as physical products, or transferred as methods only to the end users. As noted in the individual project commentary, in recent years it is projects in the area of diagnosis and vaccine improvement that are less evidently delivering commercially viable, marketable outcomes. Public and private laboratory testing services are adopting and offering the improved diagnostics; however, there appears to be relatively little demand for them.

Another group of projects, or sub-projects, must be considered pure research, such as investigations into pathogenesis or genomics. Such work may serendipitously result in marketable outcomes but, in general, is unlikely to produce such outcomes in a timeframe less than decades. It is, however, a matter for others to assess the significance of ensuring the value of long-term basic investigations, and the requirement for maintaining a deep scientific knowledge base of JD within Australia.

Because of the high cost and inherent risk of basic research, we propose that it should follow a strategic plan that extends beyond the outcomes of primary experiments to the raft of practical considerations that are likely to determine how easily or cost-effectively these can be implemented to industries' advantage. This will often include considerations of the manufacture and/or widespread application of products and, as relevant, their outlook for successful commercialisation.

MLA has used independent Australian and international experts to guide research priority setting the past as well as the JD industry advisory/steering committees. However further, wider consultation by MLA to obtain specialist advice in the scientific field prior to funding, with a specific view of seeking the best collaborative teams, would likely enhance project outcomes. We recommend that any future studies in diagnostics, genomics or proteomics, or in any field requiring specialised skills and intensive capital, be structured to include specialist external advice and collaboration, not only from academic sources. Within the laboratory based research there has been a heavy dependence on the resources and in-depth JD knowledge at the University of Sydney, where field experience and clinical expertise lies. Leveraging this knowledge resource by ensuring future projects have effective collaboration with specialist expertise, including that residing in commercial areas, will likely yield more practical outcomes in less time and with less wasted effort. Basic research should

be carefully prioritised, with the perceived needs of industry subject to a rigorous analysis of their technical and practical underpinning. We repeatedly heard, for example, a call for a 'better, safer and cheaper' vaccine for OJD. However, it is our assessment that the currently available alternative (Gudair®) is: (a) overall, far more efficacious than any other purported mycobacterial vaccine; (b) associated with virtually no reported self-injection injuries, following the advent of the shrouded vaccinator; (c) acceptable to the sheepmeat processing sector; and (d) very unlikely to be made cheaper by any basic research, given the relatively small population of potential users. We are not aware of an assessment that systematically addressed these criteria and that differs from our conclusion.

We also recommend to MLA the value of critical, independent review before, during and after any larger, higher-risk research effort. In addition, the value of recruiting specialists with appropriate expertise to project manage these studies on behalf of industry should not be underestimated.

6.1 Research to address remaining knowledge gaps

Based on our review of the JD research funded by MLA to date and its apparent successes and failures, and taking into account the expertise and experience of the authors, the following researchable knowledge gaps are suggested to MLA for consideration. However, before embarking on any future JD research effort, we strongly recommend that MLA negotiate a clear vision with and for industry as to what future JD research will seek to deliver. Bodies such as the JD Research Advisory Group and the previous National OJD Programme Steering Committee should be reviewed, interrogated and possibly re-constituted as a way of strengthening the outcomes of future JD research efforts. Combined or separate complimentary processes for cattle and sheep may be required, taking into account all relevant stakeholders and the current review of the National BJD Strategy.

<u>Epidemiology of JD:</u>

- The epidemiology of the sheep strain in beef cattle in Australia, i.e. whether the sheep strain can adapt to and persist in a beef breeding herd after contact with infected sheep has stopped, and whether cattle can then transmit disease to disease-free sheep.

  This was an issue raised during the MLA-directed consultation and which we were aware of previously. There are several research options that could be used to address this issue, such as cross-sectional and longitudinal studies in cattle naturally infected with the sheep strain, or experimental infection models. The latter are likely to require significant research funding. To date, MLA has funded several projects that have investigated cross species infection with Mptb (TR.022, OJD.005, OJD.016, P.PSH.0206 and P.PSH.0301). Before embarking on additional research, it is suggested that MLA consider a critical, desktop review of what is currently known about cross species infection between cattle and sheep, drawing on the previously funded research; epidemiological, laboratory and other records of cross species infection; and the international literature. Possible experimental or field designs and approaches could be considered as part of this review, including any limitations or advantages. This may provide guidance as to what, if any, additional research is

required. The role of deer in the epidemiology of JD in Australia could be included in this review.

- Further information on the epidemiology of JD in beef cattle, i.e. regional herd prevalence and information to clarify the determinants for the successful establishment and persistence of JD within northern and southern beef cattle herds of differing commercial characteristics and in different geo-climatic zones.

  Very little is currently understood about the epidemiology (and control) of JD in Australian beef cattle herds. The situation is made difficult by the current regulatory environment in which herds identified with JD can be significantly penalised. The National BJD Strategy is currently under review and the outcome of this process may result in a greater ability to carry out observational epidemiologic studies to investigate the distribution and prevalence of herds infected with cattle, sheep or bison strains. Further observational studies could then be used to investigate the farm-level and within-farm risk factors that influence the likelihood of infection, the seriousness of the disease within a herd and its long-term persistence. Options for the on-farm management of JD in beef herds are likely to differ significantly from those of dairy herds (with a generally higher prevalence of disease and with more direct control over individual animals).

<u>New diagnostic tests, including basic research:</u>

- The potential for abattoir surveillance to be used as a surveillance tool for JD and other endemic diseases of beef cattle in Australia, including appropriate methodology and how surveillance data can be used to facilitate overseas trade and inform policy to mitigate risk(s) to the beef industry nationally.

  During the MLA-directed consultation for this review we learned that Cattle Council of Australia had asked Animal Health Australia to investigate whether abattoir surveillance is feasible as a surveillance tool for JD in Australian beef cattle. While these investigations may be largely ones of implementation, it is possible that strategic, targeted research could be beneficial. It is suggested that MLA liaise with Animal Health Australia to determine the rationale and requirements for research.

- Diagnostic test development, including validation of the HT-J PCR as an individual animal test for cattle; increased understanding of ways to better utilise the HT-J PCR test in both cattle and sheep, including as individual or flock/herd tests; and the development of an individual animal test or panel of tests for use in cattle that will identify animals in the early stage of infection, prior to faecal shedding of Mptb, that will either develop clinical disease, develop sub-clinical disease and shed Mptb in their faeces or clear the infection.

  Within a flock or herd infected with JD there are likely to be animals recently infected, with sub-clinical infection and with clinical infection. Some will be shedding Mptb in their faeces and some will not. Some will have readily detectable immune responses to Mptb (either humoral or cell mediated) and others may not. For these reasons, we

believe it is unrealistic to maintain that a single test can be developed that will detect animals in all stages of infection.

Instead, we recommend that the research emphasis shift to investigating how the currently available diagnostic tools (pooled faecal culture, HT-J PCR test, ELISA, abattoir surveillance with histopathology, the PCR culture assay method including strain typing), along with the current available control options (Gudair® and Silirum® vaccines and grazing management) can be better used as a whole to manage JD in Australian beef cattle and sheep.

- Genetic markers and breeding values to allow selection of cattle and sheep that are unlikely to develop clinical disease.

  If the current University of Sydney project (P.PSH.0576) indicates that genomic selection for increased resistance to JD is feasible, it is recommended that MLA commission a review of how this might be practically applied within the Australian farming context. It is strongly recommended that this review be conducted by those with specialist expertise in animal breeding or genomic selection, and that it consider the potential for genomic selection for increased resistance to JD to impact on selection for productivity traits or more economically important diseases, such as internal parasites and flystrike in sheep, or cattle tick and buffalo fly in cattle.

<u>Control options for JD in cattle and sheep:</u>

- The long-term efficacy of vaccination in sheep, in particular the potential ability of the Gudair® vaccine to eliminate shedding and infection in the medium to long term as suggested by projects OJD.033 and P.PSH. 0309, and the implications of this for industry.

  Most Australian sheep flocks operate as self-replacing enterprises and purchase little stock, restricted largely to stud rams (East and Foreman, 2011). In some flocks that introduce little or no new stock the potential for vaccination to eradicate OJD in the long-term may be an economically attractive, i.e. the annual overhead cost of vaccination might eventually be phased out. However, the risk of re-introducing the disease would need to be carefully managed, for example by maintaining good farm biosecurity and purchasing rams only from long-standing JD Market Assurance Programme seedstock ram breeders. Not all producers would be prepared to manage this risk.

  To further investigate this potential use of the vaccine, we suggest that MLA investigate the potential for a second cross-sectional survey, preferably in the same flocks sampled in project P.PH.0309, using a combination of the HT-J PCR test and pooled faecal culture as assessment methods. If the prevalence of farms with detectable shedding has continued to decrease, options that could be considered for future funding might include: a) the development of a vaccinate and test protocol (i.e. time after the implementation of vaccination to begin testing, and how may negative tests are required and by which test method); b) an economic assessment

of the benefit to cost ratio of stopping vaccination; and c) a risk-based decision tool to guide producer decision making.

As part of option (a) it may be possible to use flocks known to be free of apparent shedding to apply and evaluate a range of high-sensitivity diagnostics at the flock level as a way of defining microbiological status, i.e. freedom from infection in the shorter (10 years after the start of vaccination) compared to the longer (15 years after the start of vaccination) term.

We do not recommend an additional round of testing in project P.PSH.0565 because only eight of the original twelve flocks were available at the most recent, sixth sampling. In addition, the researchers have stated in Milestone Report 5 that they believe only five or six properties would be available for another round of testing. However, these properties could be incorporated into the second cross-sectional study described above.

- Increased understanding of the efficacy and potential role of the Silirum® vaccine in controlling BJD in Australian cattle, including the ability of the vaccine to reduce the number of clinical cases within a herd, ameliorate the clinical signs of JD, reduce sub-clinical productivity losses and reduce faecal shedding of Mptb. In addition, the ability of the vaccine to eliminate shedding and infection in the long-term, as research is suggesting may be possible for sheep flocks vaccinated with the Gudair® vaccine, could be investigated as an alternative to de-stocking infected beef properties.

  It is suggested that MLA liaise with Zoetis, Animal Health Australia, Dairy Australia and State Departments to develop a programme of research to investigate the potential for the Silirum® vaccine in managing JD in Australian beef and dairy cattle. As part of this, it may be possible for MLA to help the dairy industry commence calf-hood vaccination with the Silirum® vaccine through the Producer Demonstration Site programme. As part of monitoring the effectiveness of vaccination in these cooperating herds, preferred diagnostic test technologies could be investigated.

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8 Appendices

8.1 Research projects included in this review

8.2 Terms of reference for the review

8.3 Hussey Morris report

8.4 Documents provided for review

8.5 Stakeholder consultation

8.6 Ovine Johne’s disease research needs position paper, 27 May 2010