OVINE JOHNE’S DISEASE
An update of Australian
and International Research

JULY 2002

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# OVINE JOHNE’S DISEASE RESEARCH and DEVELOPMENT UPDATE JULY 2002

## Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Executive summary</strong></td>
<td>3</td>
</tr>
<tr>
<td><strong>Researcher summaries of the 7th International Colloquium on Paratuberculosis, Bilbao, Spain, June 11th – 14th, 2002</strong></td>
<td>10</td>
</tr>
<tr>
<td>Overview of the research results presented at the 7ICP – Bruce Allworth</td>
<td>11</td>
</tr>
<tr>
<td>Summary report on diagnostic methods for Johne’s disease – Debbie Cousins</td>
<td>14</td>
</tr>
<tr>
<td>Conference Overview – Jack Gwozdz</td>
<td>18</td>
</tr>
<tr>
<td>Summary report on the molecular biology of Johne’s disease – Ian Marsh</td>
<td>22</td>
</tr>
<tr>
<td>Aetiology of paratuberculosis: genome issues and genomics – Mark Tizard</td>
<td>27</td>
</tr>
<tr>
<td>Pathogenesis of Johne’s disease – Chris Lambeth</td>
<td>31</td>
</tr>
<tr>
<td>Vaccination against Johne’s disease – Peter Windsor</td>
<td>35</td>
</tr>
<tr>
<td>Wild hosts of Mycobacterium avium subsp. paratuberculosis – Paul Cleland</td>
<td>38</td>
</tr>
<tr>
<td>Epidemiology of Johne’s disease – Richard Whittington</td>
<td>42</td>
</tr>
<tr>
<td>Public health implications of Johne’s disease – David Kennedy and Bruce Allworth</td>
<td>46</td>
</tr>
<tr>
<td>Impact of control programs on sheep producers – Frank Tobin</td>
<td>50</td>
</tr>
<tr>
<td><strong>Summary of OJD-related papers presented at the UK Sheep Veterinary Society Meeting, May 2002 - Helen McGregor</strong></td>
<td>52</td>
</tr>
<tr>
<td><strong>Reports on the OJD Research &amp; Development projects currently in progress under the National Ovine Johne’s Disease Control and Evaluation Program</strong></td>
<td>56</td>
</tr>
<tr>
<td>Cross species transmission of OJD: Phase 2 – Cattle – Barbara Maloney</td>
<td>57</td>
</tr>
<tr>
<td>Exposure factors leading to establishment of OJD infection and clinical disease in lambs – Helen McGregor</td>
<td>59</td>
</tr>
<tr>
<td>Ewe/lamb transmission and comparison of diagnostic tests – Chris Lambeth</td>
<td>61</td>
</tr>
<tr>
<td>i) A longitudinal study of OJD and the effects of whole flock vaccination with Gudair™ – Helen McGregor</td>
<td>63</td>
</tr>
<tr>
<td>ii) Biological and economic impacts of OJD in NSW flocks – Russell Bush</td>
<td>63</td>
</tr>
<tr>
<td>Validation of the gamma interferon test for diagnosis of OJD – David Stewart</td>
<td>65</td>
</tr>
<tr>
<td>An Australian evaluation of Gudair™ OJD vaccine – Jeff Eppleston</td>
<td>68</td>
</tr>
<tr>
<td>Evaluation of eradication strategies for OJD – Pat Taylor</td>
<td>71</td>
</tr>
<tr>
<td>Economic evaluation of control options – Stewart Webster</td>
<td>72</td>
</tr>
<tr>
<td>Development of computer models to describe the epidemiology of Johne’s disease in sheep – Evan Sergeant</td>
<td>74</td>
</tr>
</tbody>
</table>
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Evaluation and comparison of PFC and AGID as flock screening tests for OJD (OJD.022)

Brainstorming session – suggested areas for future research
Executive Summary

This report provides a summary of the national and international research that is being conducted into Ovine Johne’s Disease (OJD). A one-day research update forum was held at the University of Sydney on the 25th July 2002 and was attended by researchers and key producers from throughout Australia.

The forum had three main components:

1. To present an overview of the international research that was presented at the 7th International Colloquium on Paratuberculosis (7ICP) held in Bilbao, Spain on the 11-14th of June 2002.
2. To provide updates on the OJD research and development projects currently underway as part of the National Ovine Johne’s Disease Control and Evaluation Program (NOJD). New projects and those currently under consideration for research funding by Meat and Livestock Australia were also presented.
3. To generate discussion on ideas and priorities for future research that will provide tools and technology for the management of the disease.

This report contains reviews of the 7ICP prepared by researchers who attended the colloquium; progress reports on Australian research projects; executive summaries from key OJD research projects that have been completed; details on new research projects currently under consideration and outcomes from the brainstorming session on future research directions. The report also contains a summary of the OJD related papers presented at the recent UK Sheep Veterinary Society meeting held in May 2002.

Status of Australian & International OJD Research & Development

Australia is at the forefront of many aspects of OJD research, particularly in terms of assessing disease prevalence, assessing vaccine efficacy and applying diagnostic techniques. Throughout the world there is an increasing amount of epidemiological information regarding Johne’s disease, and emerging technologies in the area of molecular biology are starting to produce a greater understanding of the causative organism, Mycobacterium avium subsp. paratuberculosis (Mptb) at the DNA and protein level. It is hoped that this information will lead to future improvements in the area of diagnosis, vaccination and disease control.

An increasing body of evidence suggests that eradication of OJD may not be currently feasible or technically possible. However the increasing evidence of a link between Mptb and human Crohn’s disease and the resulting public health concerns have reinforced the need for Johne’s disease (JD) control in farmed livestock.

In Australia there is an increasing acceptance of the importance of vaccination in OJD control. Although current vaccines do not prevent infection, they do significantly modify the response of vaccinates to infection and appear to provide a valuable tool for use in OJD control programs.

The status of our current knowledge of OJD and future research directions for this disease are summarised below. More specific information is presented in the research reports later in this publication.

Pathogenesis

Major new areas of pathogenesis research include studies into gene expression in both Mptb and host cells following infection, the identification of virulence factors of the organism and the immune response to infection.

Phagocytosis of Mptb by macrophages is a crucial stage in the pathogenesis of JD and both host and pathogen factors play a role in deciding the outcome of this interaction. Research results presented at the 7ICP highlighted the ability of Mptb to alter macrophage gene expression during phagocytosis allowing it to evade the host’s defensive mechanisms. Differences in gene expression have also been found between macrophages from animals with subclinical and clinical disease and phenotypic differences reported between macrophages within lesions and those in adjacent unaffected areas. Further work to characterise the genes involved, and the mechanisms employed by the organism may potentially lead to the development of strategies for intervention.
Two potential virulence factors for Mtb have been identified. One, a major membrane protein, has been shown to play an important role in epithelial cell invasion while the second protein is thought to provide protection against bactericidal mechanisms of the host cell. Further work in this area is likely to identify additional virulence factors and may lead to the development of strategies to prevent or control infection.

Research into various aspects of the immune response to infection with Mtb has produced conflicting and confusing results. However, it is clear that the immune response varies between individual animals, which contributes to the poor performance of diagnostic tests that target the immune response. Genetic factors are also hypothesized to influence the susceptibility of exposed animals to paratuberculosis and nutritional factors have been suggested as a predisposing factor in the development of clinical signs.

In Australia a PhD student supported by the NODJP is studying the mechanism by which the OJD organism invades intestinal cells and causes disease using cell cultures made from foetal intestinal tissue. Mtb is being compared to non-pathogenic strains of mycobacteria.

Diagnosis

The major culture system for Mtb used in Australia is the BACTEC system, which works well for both cattle and sheep strains of the bacteria. However it is only semi-automated and involves the use of a radiometric label. For these reasons this system has been phased out of human pathology labs and is not viewed favourably in the USA and Europe. Automated culture systems including the TREK ESP II and MGIT 960 are currently under evaluation and although it is unlikely that they will provide additional sensitivity for culture under existing conditions, they may provide improved throughput, cost and safety.

Various direct PCR (polymerase chain reaction) methods detecting Mtb in faeces and milk are under development and evaluation in numerous countries including Australia. At this stage none are standout successes and in most cases PCR is proving less efficient than culture. The use of real time PCR may offer advantages in terms of turn around time, specificity and sensitivity. The costs of PCR are still high and the issue of acceptance of DNA results alone needs to be considered and resolved. The specificity of the IS900 target used in PCR has still not been completely resolved. However, changes to ANZSDP have already been made in Australia to address this situation. The current requirements in Australia demand further testing using additional PCR targets, or sequencing, if a positive diagnosis will result in a change of status of a property.

No major improvements in the serological diagnosis of paratuberculosis were reported at the 7ICP. Humoral antibody tests are unlikely to provide significant improvements in diagnosis due to the biology of the disease. Detection of cell mediated immunity or other specific host/pathogen responses may provide alternative technologies.

In Australia the gamma interferon (IFN-γ) test is being evaluated in field trials to determine a possible application in the national OJD control program. This test has the potential to identify infected sheep earlier than bacterial culture and antibody tests, prior to exposure of other livestock, since the cell-mediated response occurs before fecal shedding and conversion. Specificity and sensitivity trials are currently in progress. Results to date suggest that the estimated sensitivity of the IFN-γ test, in comparison to culture and histopathology interpreted in parallel, was about 67% and 52%. The estimated specificity is > 98% in uninfected flocks. However in infected flocks a high number of false positives have been detected. It is considered probable that the interferon-γ memory response may persist in previously exposed sheep that have completely eliminated the infection. Further trials are continuing.

Abattoir surveillance has proved to be an efficient and low cost method for diagnosing OJD. In the last 3 years over 14.5 million sheep have been examined by this method in 20 abattoirs around Australia. Previous studies concluded that visual/tactile examination of viscera for lesions suggestive of OJD in lines of >300 adult sheep could achieve a sensitivity of 90% in sheep populations in which OJD had been recognised for many years. A new project is currently underway to compare the ability of experienced meat inspectors to detect visible signs of OJD in sheep from both high and low prevalence regions. Results of this trial are expected later this year.
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# OVINE JOHNE'S DISEASE
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## Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Executive summary</td>
<td>3</td>
</tr>
<tr>
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<td>10</td>
</tr>
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<td>11</td>
</tr>
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<td>14</td>
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<td>18</td>
</tr>
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<td>22</td>
</tr>
<tr>
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<td>27</td>
</tr>
<tr>
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<td>31</td>
</tr>
<tr>
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<td>35</td>
</tr>
<tr>
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<td>38</td>
</tr>
<tr>
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<td>42</td>
</tr>
<tr>
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<td>46</td>
</tr>
<tr>
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<td>50</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Project</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross species transmission of OJD: Phase 2 – Cattle – Barbara Maloney</td>
<td>56</td>
</tr>
<tr>
<td>Exposure factors leading to establishment of OJD infection and clinical disease in lambs – Helen McGregor</td>
<td>56</td>
</tr>
<tr>
<td>Ewe/lamb transmission and comparison of diagnostic tests – Chris Lambeth</td>
<td>57</td>
</tr>
<tr>
<td>i) A longitudinal study of OJD and the effects of whole flock vaccination with Gudair™ – Helen McGregor</td>
<td>59</td>
</tr>
<tr>
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<td>63</td>
</tr>
<tr>
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<td>63</td>
</tr>
<tr>
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<td>63</td>
</tr>
<tr>
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<td>65</td>
</tr>
<tr>
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<td>68</td>
</tr>
<tr>
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<td>71</td>
</tr>
<tr>
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<td>72</td>
</tr>
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<td>74</td>
</tr>
</tbody>
</table>
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Several trials are evaluating the specificity and sensitivity of a range of tests in detecting \textit{Mptb} infection in individual animals. This includes the IFN-\gamma test and direct PCR. A longitudinal study is evaluating the value of gut tissue biopsy as a tool for early diagnosis of infection. Biopsy studies are also helping to define the progression of infection and possible recovery in individual animals. Two tissue culture methods (sedimented versus centrifuged) are also being compared to define the most sensitive technique.

\textbf{Molecular Biology}

Much of the research presented at the 7ICP was heavily influenced by current and emerging techniques in the field of molecular biology, which is providing a better understanding of \textit{Mptb} at the DNA and protein level.

Mapping of the \textit{Mptb} genome is nearing completion and it is hoped that the complete gene sequence will be published before the end of 2002. This will provide a critical resource for basic and applied research that will be central to future studies of the nature of \textit{Mptb} and the mechanism by which it causes disease.

\textit{Mptb} specific microarrays are being developed to study the genes involved in the pathogenesis of \textit{Johne's} disease and to determine how and when these genes are regulated during the infection process.

PCR based diagnosis is an extremely important area of continued research in JD. As previously mentioned the specificity of IS900, the DNA marker currently used as the specific indicator for \textit{Mptb}, is under question as several studies have shown cross reactivity with environmental mycobacterial species. Research on the use of new DNA markers for use in PCR in both faeces and milk was presented.

Although there are no direct indications for new control measures in the immediate future as a result of these basic research efforts, the scene is now set for significant steps forward. Understanding the functional genomics of \textit{Mptb} should help to identify useful targets for diagnostic, vaccine and other disease control applications.

\textbf{Epidemiology and Control}

Although a wealth of epidemiological information was presented at the 7ICP most related to areas that had been well covered in previous meetings. This reinforced confidence that concepts implicit in current control programs in Australia were consistent with international best practice.

\textit{Epidemiology}

Much of the current epidemiological research related to on-farm control of OJD is being conducted in Australia, as the development of strategies to assist in the control of OJD infection within flocks is considered vital for producers who elect not to undertake an eradication program.

The major aim of OJD control programs is to limit the exposure of susceptible sheep to heavily contaminated pastures. Lambs and young sheep are considered to be most susceptible to contracting the infection. A major Australian research study is currently investigating at what age, and from what source, lambs become infected. At this stage the researchers have concluded that lambs exposed to the organism prior to weaning have a higher risk of dying before 32 months of age compared to those exposed after weaning. Sheep born into infected ewe flocks have a higher rate of faecal excretion at 18 months of age and a higher risk of dying before 20 months of age compared to sheep born in uninfected ewe flocks on contaminated pasture.

A second study is investigating whether lambs can be infected prior to birth, and if so to correlate this with the stage of the disease in the ewe. The study is also investigating whether \textit{Mptb} is present in milk and colostrum. Results to date indicate that intrauterine transmission does occur, however confirmed results are yet to be analysed.

Research is also underway to investigate whether the level of pasture contamination with \textit{Mptb} influences the incidence of OJD, the incubation period of the disease and the timing of diagnosis in sheep of different ages.

Computer simulation models have been developed to predict the likely rate of spread of OJD on infected farms and throughout infected districts and the potential influence of control strategies on disease spread.
A study on the prevalence of paratuberculosis in cattle exposed to sheep with OJD revealed that cattle could occasionally become infected with the S strain of the organism. The researchers suggested that cattle being reared in OJD endemic areas should have minimal contact with infected sheep or manure, particularly during their first 12 months of life. Serological screening of cattle may be necessary as part of OJD surveillance and control programs.

New Australian projects under evaluation or soon to commence include an assessment of risk factors that lead to OJD prevalence and mortalities in infected flocks; an evaluation of the effects of subclinical and clinical disease on productivity; and the relative susceptibility of adult sheep of different ages exposed to \textit{Mptb}.

\section*{Eradication}

An evaluation of the efficacy of a destocking-restocking strategy to eradicate the S strain of \textit{Mptb} from individual properties in Australia is currently underway. To date seven properties (from 12 assessed at this time) have failed the eradication process. The inability to identify OJD free replacement stock has been identified as the cause of at least some of these failures. The study is continuing.

Reports from Iceland where vaccination of sheep has been compulsory in OJD endemic areas since 1966 suggest that eradication by vaccination may not be possible. In 2001 the infection reappeared on two properties which had been declared paratuberculosis free, in an area where vaccination had ceased 3 years earlier. The reason for the outbreak is still unclear however it has raised the question of whether eradication by vaccination is possible or not.

\section*{Vaccination}

The use of vaccines against \textit{Mptb} has historically generated variable results and in general vaccine trials have not been particularly rigorous. However, overseas field experience and limited experimental data has indicated the potential for vaccination against \textit{Mptb} to reduce the on-farm impact of OJD in sheep flocks.

A major Australian field trial evaluating the efficacy of the killed vaccine Gudair\textsuperscript{TM} is providing excellent long term information on the vaccine effects on cellular and humoral immunity, faecal shedding of the organism, mortality rates, condition score, bodyweight, wool productivity and vaccine site lesions.

The vaccine has been shown to significantly stimulate both the cellular & humoral immune systems in the majority of vaccines, however, the persistence of these responses has been variable. Vaccination of lambs has significantly delayed and reduced shedding of \textit{Mptb} compared to controls. To date there have been only two OJD mortalities in vaccines compared to 32 in control sheep. The incidence of vaccination injection site lesions has decreased from initial levels of almost 50\% to around 20\% of vaccines retaining lesions 18–24 months after vaccination. No difference between control and vaccinated sheep has been detected in the productivity traits of growth, body condition, greasy fleece weight or fibre diameter. This data has assisted the registration of Gudair in Australia and supports a major role for Gudair vaccine for managing the on-farm impact of OJD in Australia.

A plenary presentation at the 7ICP on control of paratuberculosis by vaccination suggested that there has been a “conspiracy of silence” regarding the benefits of vaccination. This has been attributed to concerns regarding the complication of vaccination on Tuberculosis diagnosis, trading of vaccines and the risk of self-administration of the vaccine. A review of the literature provided conclusive evidence that vaccination results in significant decreases in clinical signs, bacterial shedding and necropsy lesions.

There is emerging consensus that eradication of endemic \textit{Mptb} may not be “possible, necessary or profitable” and that vaccination is likely to be a long-term control strategy.

Two new Australian research projects which are currently in the planning or early stages will investigate the change in faecal shedding following vaccination of adult sheep and changes in the prevalence of shedding of \textit{Mptb} following the commencement of a vaccination programme in both high and low prevalence flocks.
Control programs

Control programs for JD, either voluntary or involuntary, have been initiated in many countries including Australia, Iceland, the Netherlands, Sweden, Argentina, Japan, Spain and Norway. These programs have met with variable success.

The Australian NOJDP is a $40 million, six year program which aims to deliver the necessary information on the distribution of the disease and the technological tools and information needed for an informed decision on the future management of JD. At the same time the program aims to control the disease during this evaluation phase. The program is currently in its fourth year and has made significant progress, including extensive surveillance that has enabled better definition of the disease distribution, the implementation of 21 research projects and the implementation of a communications program. Following a mid-term review a revised three-year plan has been adopted which in addition to ongoing commitment to research and surveillance also incorporates a specific Control subprogram and a new national Financial Assistance subprogram. Despite its success, the Program continues to face a number of challenges. The identification of infected flocks through increased surveillance, together with trading restrictions placed on infected and suspect flocks and flocks in high risk areas has brought opposition from those affected. The issue of financial assistance is currently being addressed through both national industry and State initiatives. Delivery of an effective assistance package is essential to ensure the successful completion of the Program.

Japan has a compulsory surveillance program for all adult cattle which must be tested every five years. Reactors are culled and compensation paid. Reactor cattle cannot be slaughtered in an abattoir and cannot be used for human consumption. This has potential implications for trade in livestock between Australia and Japan.

In Sweden JD is a notifiable disease in cattle and reactors must be culled. There is an assurance program based on testing all animals over 24 months annually by pooled faecal culture.

Iceland implemented a program of compulsory vaccination of all sheep in endemic areas in 1966. Despite this program Mptb has recently reoccurred in sheep in an area that had been declared free of paratuberculosis and where vaccination had ceased three years earlier. Vaccination has now been re-instituted.

A feature of many of the reports of control programs for JD in other countries is the need for a protracted campaign.

Wild Hosts

There is mounting evidence from around the world that the host range for Mptb is much wider than originally described although there is significant host preference. The cattle strain particularly appears to be able to infect a range of non-ruminant hosts. JD infection has been demonstrated in several wildlife species including rabbits, hares, rats, mice and various species of birds, carnivores and macropods. However the capacity of wildlife to deliver an infectious dose of Mptb to domestic livestock is unknown. It is also uncertain whether Mptb can cycle independently in wildlife populations in the absence of infected ruminants.

Three key papers were presented at the 7ICP: -

The first from Scotland isolated Mptb from rabbits harvested from both JD infected and non-infected farms (cattle). Mptb was also isolated from a range of non-grazing wild species including foxes, stoats, weasels, crows, rooks, jackdaws, rats, woodmice and hares. This suggests that animals and birds that predate on rabbits are very likely to become infected with Mptb. Bird and rodent faeces could potentially contaminate pasture and stored feedstuffs. Rabbit isolates of Mptb were orally dosed into neonatal calves resulting infection and disease. Rabbits are currently considered to have the greatest potential to be involved in the epidemiology of JD in domestic ruminants in Scotland.

Mptb was also isolated from wild ferrets on both the north and south islands of New Zealand. The source of infection for ferrets could be cattle, sheep, farmed or wild deer or rabbits. To date rabbits in NZ have not been examined for the presence of Mptb.
The third study examined the level of contact between cattle and rabbit faeces in the grazing environment and rodent faeces via stored concentrate feed. This study demonstrated a high level of contact by cattle with pasture contaminated with rabbit faeces. When concentrate feed was studied cattle did show an aversion to contaminated feed, however significant quantities of rodent faeces were ingested.

*Mptb* has not been found to infect rabbits and macropods on mainland Australia. Studies on western grey kangaroos and tammar wallabies on Kangaroo Island have demonstrated a significant prevalence of histological lesions and the presence of *Mptb* from the tissues of the macropods surveyed. How commonly JD occurs in macropods and under what conditions is currently unknown, although the population density of both sheep and macropods is important.

The prevalence of *Mptb* in such a wide range of wildlife species around the world raises serious questions regarding the feasibility of eradication programs for paratuberculosis in some circumstances. In Australia this risk appears low in most situations based on research results so far.

**Economic Impact**

The economic impact of OJD infection within flocks and the returns associated with different on-farm management options are an important consideration in the long-term management of the disease. A longitudinal study on the biological and economic impact of OJD has commenced in NSW. This study will obtain an accurate estimate of both crude mortality rates in infected flocks and the proportion of the mortality directly attributable to OJD.

Deterministic, spreadsheet based, representative farm models have been constructed to quantify the financial consequences of three OJD management options – status quo, control with vaccination and decontamination through destocking. Results to date demonstrate that positive returns to an investment in a vaccination program are earned almost immediately, and vaccination was the most profitable short-term management option investigated. The associated costs and high risk of failure of decontamination programs do not make this option attractive at this time for most producers.

**Public Health**

The link between Crohn’s disease in humans and JD in animals received a high profile at the 71CP. There is growing evidence that *Mptb* may play a role in Crohn’s disease in some people and with improvements in culture techniques *Mptb* is increasingly isolated from gut biopsies from Crohn’s patients. In one laboratory over 80% of cases are now testing positive. Additionally open treatment studies with antibiotics specific for mycobacteria are reporting high rates of remission and recovery in Crohn’s patients. There is still the unresolved issue of whether these isolations reflect a primary pathogen or a comensal organism.

*Mptb* is found in milk from infected cattle and although pasteurisation *per se* is highly effective in killing the organism some reputable laboratories still report survivors after commercial pasteurisation of raw milk. Milk and other dairy products have therefore been suggested as a route of *Mptb* infection for humans as has been contamination of food and water sources with animal faeces.

It is interesting to note however that *Mptb* has been found in Swedish and Finnish Crohn’s patients at a similar incidence (around 50%) as patients from South Wales. This finding was unexpected as the Scandinavian countries have a much lower incidence of JD. In addition, a regional case control study conducted in the USA failed to identify exposure to animals or dairy products as a risk factor for the disease in humans. The origins and host specificity of the *Mptb* found in Crohn’s patients in now being queried, after genotyping of human isolates provided evidence for “humanised” strains.

Australia is in a unique position of having two similar areas of the country with distinctly different JD status, namely south-eastern and south-western Australia. This provides an opportunity to investigate the incidence of Crohn’s disease and the *Mctb* status in human populations that have been potentially exposed, and not exposed, to animal strains of *Mptb*. Knowledge of the occurrence of *Mptb* in raw and pasteurised milk in Australia may also assist the dairy industry to manage JD and the growing perceptions about milk as a source of the organism.

Due to public health concerns Mike Collins, the President of the International Association for Paratuberculosis, identified the need to erect multiple hurdles from the farm gate to the consumer. These
Include keeping herds and flocks JD free, reducing disease prevalence in infected herds and flocks, reducing the number of infected animals going to slaughter for human consumption and maintaining effective pasteurisation and appropriate cooking methods.

It is likely that there will be increasing pressure to maintain food safety. There are also potential trade implications for failing to control JD in our livestock. Japan currently does not allow *M. tuberculosis* positive cattle to be slaughtered in abattoirs or to be utilised in food products for human consumption, and this may serve as a warning for the future direction of food safety.
RESEARCHER SUMMARIES
OF THE
7TH INTERNATIONAL COLLOQUIUM ON PARATUBERCULOSIS

BILBAO, SPAIN
11-14TH JUNE 2002
OVERVIEW OF THE RESEARCH RESULTS PRESENTED AT THE 7ICP

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The Colloquium comprised 100 oral presentations over the four days, delivered by over 80 speakers. There were four invited one-hour papers covering the major topics of the conference, and the remaining papers were 15-minute presentations. In addition there were poster presentations, with a further 113 abstracts being represented.

Key topics of the Colloquium included molecular techniques, diagnostics, public health and vaccination/control. Approximately 20 Australians attended out of the 257 delegates present, with participants coming from most livestock-orientated countries. MLA assisted 8 attendees and they will provide detailed reports on their areas of expertise. David Kennedy and I presented two papers each, relating to the NOJDP, BJD program and the MAPs.

Below are some notes on the key areas.

Molecular Techniques

- *Mycobacterium avium* subsp. *paratuberculosis* (*Mptb*) genome sequencing is almost completed
- Many new techniques are being used and aim to identify better diagnostic or expressive antigens.
- None of the new techniques are applicable to the field as yet; all require extra work.
- There was some disagreement on the usefulness of various techniques; in general micro-array technology was considered the most useful tool.
- There was disagreement on how *Mptb* is defined – the role of IS900 in diagnosis is questioned given doubt over specificity.

*To be useful, we require agreed definition on what is *Mptb* and an appropriate bank of well-characterised *Mptb* isolates to test new techniques.*

Diagnosis

- Results between labs is variable (and in discussions afterwards it was apparent that this variation was not necessarily related to culture media or type of tests).
- There are still difficulties in many countries with culture.
- In some cases, ELISA and culture appear to identify different animals; in other cases, no real advantage in seen in using both tests. It is difficult to come away with a definite outcome on the value of culture versus ELISA or combinations.
Culture is used in Holland for assurance (cattle) after initial ELISA.

A number of papers discussed the interpretation of tests in heavily infected herds to minimise misclassification but retain control.

*Tests are far from perfect and need to work with JD with this knowledge.*

**Public Health**

- The link between Crohn's Disease (CD) and JD in animals had a high profile at the 71CP. A one-hour address by J. Hormon-Taylor continually asserted that *M. tub* caused JD in animals and was shown to be entering the food chain and CD patients had a much higher rate of *M. tub* infection, therefore *M. tub* caused CD. There was no chance to publicly discuss his assertions, which was disappointing. His address graphically demonstrated the severe outcomes in human patients with CD. It was also noted that his abstract had not been accepted for presentation at the recent (May 2002) International Gastroenterology Meeting.

- Treatment with antibiotic combinations specific against *M. tub* gave 70% success, and cures were quite spectacular (noted the need to see the outcome from the double-blind trial in Australia, which people seemed to think was concluding in June 2002).

- There was some doubt as to the form of *M. tub* in humans – cell-wall deficient? – requires 10 months to culture.

- General feeling that the prevalence of JD was increasing worldwide, as was the prevalence of CD. No connection shown, but guilt by association.

- Milk is a source of *M. tub* – pasteurisation will not eliminate all organisms. The risk associated with a low-level of *M. tub* post-pasteurisation was not quantified.

- Water was also suggested as a potential source for humans with animal effluent contaminating water sources, especially given the hardness of *M. tub* in the environment. Other sources (tomatoes, radishes) also suggested.

- Ground beef was questioned and cooking of commercial hamburgers may not provide sufficient temperature or time to kill *M. tub*.

In summing up the Colloquium, Mike Collins (President, IAP) noted concerns over public health issues and identified the need to put up multiple hurdles from the farm gate to the consumer:

- keep herds/flocks free,
- reduce prevalence in infected herds/flocks,
- reduce the number of infected animals going to slaughter for human consumption,
- maintain pasteurisation and appropriate cooking methods.

(NB: Difficult to gauge how much the influence of attracting more $ for research influences discussions on public health).

**Vaccination/Control**

- Vaccination results have historically been variable and in general trials have not been particularly rigorous.
- General consensus that vaccination will decrease clinical signs and this role is currently being underplayed.
- Main problem in Europe is cross-reaction with TB (not an issue in Australia).
- The role in minimising milk excretion was queried.
Most countries were considering control of JD.

- Some still at the stage of characterising the extent of the disease.
- Some have too much disease and are concentrating on decreasing within-herd prevalence.
- Holland has a similar assurance scheme to Australia based on testing, time, and management.
- Norway is looking at cattle having found the problem in goats.
- US is using testing programs to reduce within-herd prevalence and to identify low-risk herds.
- Iceland has used zoning (via fencing country) and vaccination.

**Take-home messages from Control papers:**

- **increased awareness for on-farm control**
- **use of testing (with appropriate interpretation), vaccination and management (largely dairy cattle) to achieve control**
- **there is likely to be increasing pressure to decrease likely contamination of food products**
- **use of low-risk approach for trading**
- **eradication very difficult**

**Australia's position in relation to other countries**

The presentations highlighted the amount and quality of the work currently underway in Australia, particularly in relation to OJD. It was also easy to understand the various approaches by certain countries given their current prevalence of JD. For example, most European countries, the UK and parts of the US have very high levels of JD and subsequently high levels of environmental contamination. The transfer to non-farmed animals under such circumstances is inevitable and has been well documented; but despite this the relevance of infected "wild" animals in the transmission of JD between farm animals is still not clear.

It is also clear that under such levels of contamination, researchers and policy makers are constantly being confronted by "unexpected" outcomes which result in anecdotal explanations. In most cases the level of investigation behind such explanations and subsequent validation is inadequate. However, it should act as a warning in our higher prevalence areas.

Australia's current "control" approach would appear well justified in the climate of food safety, which had a high profile at the Colloquium. Much of the information directly applicable to our programs came from research within Australia, and with the exception of the molecular work, Australia is leading the way in terms of assessing prevalence, assessing vaccine efficacy and applying diagnostic techniques. While this does not suggest we "have it right", it does suggest that our moves forward should be largely determined by information from within Australia.
SUMMARY REPORT ON DIAGNOSTIC METHODS FOR JOHNE’S DISEASE

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Summary

Eight oral and seven poster presentations were made in the culture section and 11 oral and 14 poster presentations were made in the immunology section. One presentation in the aetiology section was relevant to diagnosis and several in the section on molecular biology included aspects that had some relevance to diagnosis. Because of the latter some crossover with this report and the report on molecular biological aspects of the Colloquium may occur.

Methods of diagnosis for Johne's disease (JD) are currently based on 2 core principals:

1. The detection of the infectious agent M. paratuberculosis (Mptb) or a specific component of that agent

2. Or the detection of the host's response to Mptb.

This report will be divided into these two components.

Key Papers/Posters

An excellent overview of disease diagnosis, especially as it relates to Australian conditions was presented by Prof Richard Whittington.

Detection of agent

Culture

The TREK™ ESP II system is an automated (like the BD MGIT 960) culture system that detects changes in pressure in the incubation tube being used for culture. TREK™ Diagnostic systems have placed their ESP II into a number of USA laboratories for evaluation (Gardner et al S4.05) and are interested in doing an evaluation in Australia. The marketing manager has made some preliminary inquiries regarding moving the ESP II system into Australia. No reports comparing the sensitivity of TREK™ with BACTEC 460 were presented at the Colloquium and most of the laboratories are primarily testing for bovine JD.

A comparison of the MGIT and BACTEC systems reported by Grant et al (S4.06) proved equivalent for culture of Mptb from milk. The MGIT system uses a non-radiometric system that can be automated (960 tubes – MGIT 980) and is the technology that has replaced BACTEC 460 in most human laboratories for culture of Mycobacterium spp. Improvements in the formulation of the MGIT media (which utilises a fluorescent indicator) for culture of Mptb was reported by Beaty (S4.P2). Addition of bovine serum albumin in particular had a favourable effect on growth. However the study did not appear to test ‘S’ strains of Mptb and further work would need to be carried out to determine whether this modified medium provided equivalent or additional sensitivity for culture under Australian conditions.
Machackova et al (S4.01) reported the existence of what were called 'uncultivable' strains of Mptb from the Czech Republic. Basically their culture technique (using conventional Herold's [HEYM] medium) was less successful with sheep, goats and mouflan (big horned sheep) than cattle. Only one isolate of 'S' strain was reported. The reason for lack of culture success is likely to be that they were not using appropriate media for growing S strains (i.e. they were only uncultivable on HEYM) rather than the existence of uncultivable strains.

Several groups reported the use of pooled faecal culture (PFC) in cattle. In the USA, PFC is being considered as an alternative to ELISA testing for determination of herd status and identification of individual animals warranting individual faecal culture. Van Schaik et al (S4.04) used a modeling approach to conclude that the optimum pool size varied, depending on the objective of testing, the herd size and the prevalence. Pooling was not considered to be useful in smaller (<250 cows), low prevalence (<5%) herds. Up to 5 animals per pool was considered to be useful in high prevalence herds. If the objective was to identify infected animals, then the costs increase in high prevalence herds because of the costs of repeat testing on individual animals. Gardner et al (S4.05) reported an experimental study including 29 herds ranging in size from 300 to 1500. Sixty cows ≥ 2nd lactation were tested by ELISA and PFC (n=10 pools) and individual culture using the TREK™ ESP II system. The relative sensitivity of PFC was 50% when compared to individual culture under these conditions and consequently the practicality of using PFC in low prevalence herds is questionable.

Contamination rates in cultures are reportedly high in some batches of cultures in Europe. Some studies aimed at solving this problem were reported (Homston et al S4.P4, van Weering et al S4P5 and Weber et al S4P6). Few firm conclusions were presented other than that made by Weber et al that re-testing cattle following contamination of a faecal culture was not cost efficient if the contamination occurred after more than 8 weeks of incubation. Not re-testing animals that produced contamination after 8 weeks would have meant a decrease of 25% in re-testing while the estimated relative sensitivity of individual faecal culture would still have been 98.5%.

A preliminary study examining volatile organic compounds (VOC) was reported (Phillips et al S4.08). The detection of VOCs as a breath test for tuberculosis is currently being evaluated in a clinical trial by groups in the USA. VOCs were captured in the headspace of cultures of Mptb and analysed by automated thermal desorption/gas chromatography/mass spectrometry. One hundred and seventy five different VOCs were detected from Mptb. Whether these VOCs prove to be specific for Mptb and whether this could develop into a practical test for diagnosis remains to be seen.

PCR

Collins (S1.03) reported the preliminary findings of a novel DNA sequence to distinguish between sheep and cattle strains of Mptb. However, this technique has only been used on a few organisms and the robustness of the technique (and its ability to work from other sources such as BACTEC vials) has yet to be validated. Bolske et al (S5.01) presented the recently published finding of a false positive IS900 PCR result for a Mycobacterium spp. isolate designated 2333, including the inability of this strain to be differentiated from Mptb by PCR-REA. This report confirmed previous findings (Cousins et al 1999) of the existence of rarely encountered mycobacteria that contain IS900-like elements. The use of alternative PCR targets for identification of Mptb, namely p34 and i57, were reported as negative with isolate 2333. Primer pairs targeting these genes need to be evaluated on the collection of similar isolates from Australia to determine the value of alternative PCR targets.

Several papers reported in-house variations of PCR methods for direct detection in faeces or milk including the use of immunomagnetic PCR and PCR-ELISA (S5.03, S5.06, S5.07, S5.08, S5.09, S5.10, S5.11, S5P2, S5P3, S5P4, S5.P5, S5.P6, S5.P8, S5.P10, S5.P11, S5.P12, S5.P13, S5.P14). The majority of these reports were research based and had been tried on experimental or limited numbers of 'real' samples. In particular, Hemon et al (S5.03) reported the use of PCR and nucleic acid based sequence based amplification (NASBA) assays for the ssrA gene and its tRNA transcript to detect viable Mptb in milk. This technique currently cannot differentiate between Mptb and M. avium, but further work may make it more specific. Kojima et al (S5P3) reported preliminary results of direct PCR technique that was more sensitive than culture and less subject to the presence of inhibitors, but the technique was evaluated on faecal samples from only 4 experimentally infected calves. McKee et al (S5.011) reported the use of IM-PCR and
culture for detection of \textit{M.\textit{p}}tb from ‘in-line’ milk filters as a means of screening farms for Johne’s disease. The use of a ribolyser was reported to increase the sensitivity by 100-1000 times.

Two groups (Willemsen \textit{et al} S5P6, O’Mahony & Hill S5.P8) reported the use of real time (RT) PCR for detection of \textit{M.\textit{p}}tb in artificially seeded and naturally infected faeces and DNA and from purified cultures. Although both of these studies were preliminary reports, real time PCR may offer advantages in speed, throughput, sensitivity and specificity and future research in this area may provide some advantages to diagnostic systems.

\textbf{Identification of \textit{M. paratuberculosis}}

There was some obvious contention about the specificity of IS\textit{900}. In reality, the number of isolates that contain an IS\textit{900} like sequence are likely to be rare but if IS\textit{900} PCR alone is used for identification, a false diagnosis could be made. The isolates found so far have all been mycobactin independent and consequently, if the dependency test is performed on a suspect culture routinely, a false diagnosis will not occur. However, a mycobactin dependency confirmation is not an option when PCR is performed directly on faeces, tissue or milk. Other PCR targets reported that might be useful for the identification of \textit{M.\textit{p}}tb included p34, f57, IS\textit{Mav2} and the ‘Collins’ target.

\textbf{Immunology}

No standout improvement in diagnostic immunology was reported. Several papers (Storset S.01, Antognoli \textit{et al} S3.02) examined the immune response of artificially or naturally infected young cattle over time, while others compared different ELISAs across a set range of sera (Van Maanen \textit{et al} S3.05, Dargatz \textit{et al} S3.07, Couquet S3.P7, Jackson S3.P8, Garrido \textit{et al} S3.P9, Ferreira \textit{et al} S3.P11) with very little significant difference. The most significant factor affecting variation in the IDEXX ELISA performed in different laboratories across the USA was kit batch (35%) (Dargatz \textit{et al}). Two papers reported the use of IFN-\gamma for detection of \textit{M.\textit{p}}tb; one (Stewart \textit{et al} S3.09) of which reported preliminary results of a field evaluation of the test under Australian conditions.

Huda \textit{et al} (S3.P1) evaluated the use of ELISA on milk and serum samples from a small number of infected (n=6) and non-infected (n=2) herds. Sensitivity of the milk ELISA could be adjusted to that of the serum ELISA by altering the cut-off values but it should be remembered that altering the cut-off to enhance sensitivity, almost invariably results in a reduction in specificity. Although milk is an easier sample to collect, it is unlikely the test would prove to be a significant improvement to other diagnostic methods because of its low specificity.

Kramsky \textit{et al} (S3.P14) reported a preliminary study on the use of Protein G conjugate as a sero-diagnostic aid for non-domestic hoofstock. Protein G has the potential to be used for diagnosis of paratuberculosis of different animal species and especially wildlife.

\textbf{Implications and Relevance to Australia}

\textbf{Culture systems}

The BACTEC system used throughout Australia works well for C and S strains of \textit{M.\textit{p}}tb although as it is only semi-automated there are logistical issues for laboratories handling large volumes of work. It also uses a radiometric label and this is the reason why the system has been phased out in human pathology laboratories and is not viewed favourably in European and American laboratories. There are limited comparisons of the more automated systems with BACTEC 460. TREK ESP II systems and MGI T 960 companies are interested in evaluations. It is unlikely that these systems will provide additional sensitivity for culture under existing conditions, although throughput requirements, cost and safety need to be considered.

\textbf{DNA detection}

Various direct PCR methods for faeces and milk are being developed and evaluated in numerous countries (including Australia). At this stage, none of them are standout successes. In most cases, PCR is less efficient than culture, although the assays appear to perform better on milk samples (presumably because there are less inhibitors) than on faeces. The use of real time (RT) PCR may offer advantages in terms of
turn-around time, specificity and sensitivity. The costs of PCR are still high and the issue of acceptance of DNA results alone needs to be considered and resolved.

**PCR identification**

The specificity of IS900 was not resolved completely. Changes to the ANZSDP have already been made to meet this situation. The current requirements require further testing with additional PCR targets, or sequencing, if the diagnosis will result in a change in status of a property. Several requests have been made to Australian researchers for the ‘false positive’ isolates, as they will play a key role in the evaluation of alternative PCR targets. One such isolate has been reported in Europe and the rest have originated in Australia. Discussion with ‘holders’ of these isolates suggests that an Australian study examining the performance of all of these isolates with all currently known PCR targets would be a worthwhile exercise before supplying DNA or isolates to overseas groups. Such a study could be performed in a short time frame. Consideration should be given to any commercial benefit of the isolates.

**Immunology**

Despite the fact that many groups have spent many years altering and optimising various components of the ELISA test (and they continue to do so) no major improvements on the serological diagnosis of paratuberculosis has been reported. Humoral antibody tests are unlikely to provide significant improvements in diagnosis because of the biology of the disease. Detection of cell mediated immunity or other specific host/pathogen specific responses may provide alternative technologies.
Report from the International Colloquium on Paratuberculosis
Bilbao, Spain 11-14th June 2002

CONFERENCE OVERVIEW

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Summary

Approximately 200 papers were submitted to the Seventh International Colloquium on Paratuberculosis (7ICP). The programme was divided into a number of sessions that dealt with various aspects of paratuberculosis, ranging from etiology, pathogenesis, diagnosis, epidemiology, control and public health.

The Victorian Institute of Animal Science presented results of four studies. There were several papers on the pathogenesis of paratuberculosis. A number of investigators have used DNA micro-arrays and differential expression analysis methods to study the interactions between the host/macrophages and M. paratuberculosis (Mptb) at the gene level. Although small numbers of animals and limited number of genes have been examined, the results of these studies were concordant. As one would expect, differences in gene expression have been noted between infected and non-infected macrophages, as well as between animals at various stages of infection. The significance of these findings is difficult to determine, as the differentially expressed genes have not been identified. Similarly difficult to interpret were findings of a study in which gene expression have been analysed in Mptb grown in artificial media and the bacteria cultured in amoebas. In addition, the relevance of observed differences at the RNA remains controversial as this experiment was performed at room temperature. Nevertheless, the ability to maintain viable Mptb within amoebas under experimental conditions raises the possibility that protozoa could serve as a vector in which multiplication of the bacterium may occur outside the mammalian host. This would have serious implications to the epidemiology of paratuberculosis.

An elegant study showed that Mptb inhibits presentation of antigens and interferes with phagosome-lysosome functions in macrophages at the site of infection in natural hosts. These mechanisms could play a crucial role in the survival of the organism. It is also likely that they contribute to slow and erratic development of immune responses in paratuberculosis, as reported by several investigators. The host and environmental factors also influence the immune responses. Stabel et al have reported that parturition produces immunosuppression in cows. It remains unclear if this profound effect of parturition is due to stress or increased concentration of foetal steroids.

Numerous investigators presented data that further supported the generally accepted domna that antibody and cell-mediated immune responses are inversely related in paratuberculosis. Strong gamma-interferon responses to Johnin PPD have been noted in calves that were able to control Mptb infection but not in animals shedding the organism in faeces. Potential virulence factors of Mptb have been identified using in vitro systems. Further in vivo studies would be required to confirm the role of these factors in the pathogenesis of paratuberculosis. Several DNA sequences unique to Mptb have been identified in comparative studies of M. avium and M. paratuberculosis genomes. It is likely that some of these sequences code for virulence factors or species specific antigens. The identification of these molecules could facilitate the improvement of diagnostic tests that measure the cell-mediated immune response and assist the development of new generation vaccines. Although the results of studies evaluating the diagnostic potential of various recombinant antigens are promising, it seems unlikely that any of them could be used alone in the currently available CMI tests. It is worth noting that these antigens were derived from Mptb grown in artificial media. The possibility of different antigens being expressed in infected animals has been
addressed by studies conducted by CSIRO. Differences between in vivo and in vitro antigen expression have been noted but the significance of these findings remains to be determined.22,23

Stratmann and Gerlach have reported an isolation of high affinity peptides that bind specifically to Mptb using a phage library.24 This novel method for the detection of the organism raises prospects for the improvement of tests that provide direct evidence of infection with Mptb. Stewart et al have presented results of a field trial evaluating a gamma-interferon assay for the diagnosis of ovine paratuberculosis.25 The reported sensitivity and specificity of the assay were 66% and 98%, respectively. In my opinion, this well designed and conducted trial, along with an excellent evaluation of antibody ELISA presented by Jubb and Galvin26 and the study by Stratmann and Gerlach,24 should be considered as the most significant papers presented during the colloquium.

The use of anamnestic ELISA after intradermal inoculation of Johnin PPD may improve serological detection of animals infected with Mptb.27 Alternatively, the likelihood ratios could be used to optimise the ELISA's discriminatory power.28 Although this is not a new concept and there are several pitfalls, this method of interpretation of results has the advantage of being independent of the prevalence of infection, which is frequently unknown. The results of a Danish study evaluating a milk-ELISA for the detection of infected herds seem promising.29 Interestingly, others have detected antibodies against Mptb in milk of individual cows only during the first 2 days after calving.13

A comparative study of two culture systems for Mptb, BACTEC and MGIT, found that both systems have similar analytical detection limits.30 The MGIT system does not involve radioactive materials and is less laborious than the BACTEC. Further studies on clinical samples are required to fully evaluate the potential of the MGIT system. Several investigators presented studies on PCR-based methods for the detection of Mptb in clinical samples such as faeces and milk.31-35 In the past, PCR systems lacked sensitivity due to inhibition of the amplification process. The immunomagnetic separation of Mptb before PCR,31-32 the use of novel methods of DNA extraction33-34 and adaptation of ELISA technology35 appear to minimise the problems associated with PCR inhibition.

Of importance is the question of whether any serological test or tests that directly detect animals shedding Mptb in faeces will have a major impact on the control of the disease. Available data suggest that significant reduction in the prevalence of infection may be difficult to achieve using serological tests and/or faecal culture in test-and-cull control programs.26,36 The growing number of wildlife vectors further complicates this already complex and politically sensitive issue.37-38 Although there is more and more evidence that vaccination may play an important role in control of paratuberculosis in sheep, cattle and goats,39-43 it remains unclear if eradication of the disease by vaccination is possible.44

A group led by Hermon-Taylor has presented a study in which Mptb was detected in 17 of 18 intestinal samples from Crohn's disease cases.45 These results are intriguing. The high detection rate has been attributed to the improvements in culture and PCR methods used in this study and testing full-thickness surgically resected tissues instead of traditional mucosal biopsies.45 According to Bull et al., the genetic make up of human isolates significantly varies from animal isolates of Mptb.46 This is a fascinating finding as it suggests that the isolates evolved and spread within the human population.

Key Papers/Posters

- Stratmann J and Gerlach GF. Isolation of high affinity peptides for the detection of Mycobacterium avium subspecies paratuberculosis in milk. (24)


- Jubb TF, Galvin JW. Testing to control Johne's disease in dairy herds in Victoria. (26)

Implications and Relevance to Australia

Data presented at the 7ICP suggest that significant reduction in the prevalence of infection may be difficult to achieve using serological tests and/or faecal culture in test-and-cull control programs. This is in agreement with results of an ongoing 12-year study in cattle at VIAS. The use of gamma interferon assay in such programs should be considered. Considering the cost of the assay and logistic problems, it may be warranted to investigate the use of a skin test as an alternative/frontline test. The isolation of high affinity peptides that bind specifically to \textit{Mptb} offers some hope for the improvement of tests that provide direct evidence of infection. There is a growing body of evidence that vaccination may play an important role in control of paratuberculosis. However, because of the possible link between to \textit{Mptb} and Crohn’s disease it is unknown if both producers and consumers will accept wide use of vaccine.

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SUMMARY REPORT ON THE MOLECULAR BIOLOGY OF JOHNE’S DISEASE

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Summary

Research presented at the 7th International Colloquium on Paratuberculosis (7ICP) was heavily influenced by current and emerging techniques in the field of molecular biology. Many of the presented reports detailed the use of the tools of molecular biology in an attempt to develop new and improved diagnostic tests for paratuberculosis (Johne’s disease). Other reports focused on the application of the latest developments in genomics and proteomics to further improve our understanding of the aetiology of Johne’s disease (JD) and the mechanisms involved in the pathogenesis of Mycobacterium avium subsp. paratuberculosis (Mptb), the causative agent of JD.

In contrast to the 6th International Colloquium on Paratuberculosis (6ICP) the 7ICP covered many new and exciting areas in JD molecular biology research. Molecular biology at the 6ICP was primarily focused on the development of polymerase chain reaction (PCR) diagnostic tests and restriction fragment length polymorphism (RFLP) epidemiological studies. However, from the 7ICP it is evident that more attention is now being applied to the molecular biology of Mptb, both sheep and cattle strains and their respective hosts, using the emerging techniques in molecular biology. These studies should help overcome the current shortfall in our understanding of the disease and the organism.

Key Papers/Posters

This review is focused on the oral and poster presentations of the 7ICP from the molecular biology section of the program. While this is not a comprehensive review of this topic at the 7ICP, the remaining molecular biology reports will be reviewed elsewhere (Molecular Biology Review of the 7th International Colloquium on Paratuberculosis by Mark Tizard). In this review, oral and poster presentations have been grouped under the following headings: genomics, proteomics and polymerase chain reaction.

Genomics

At present our understanding of Mptb at the DNA level is extremely limited. To gain a better understanding on how this organism causes disease we need to identify the genes involved in pathogenesis and determine how and when these genes are regulated during the infection process. The development of microarray technology will certainly provide the opportunity to achieve this. However, as with any new technology there are pros and cons.

In one of the first reports on the use of microarray technology to identify differences between Mycobacterium avium subsp. avium (MAV) and Mptb, Zhai et al. (2002) developed a microarray with 3924 open reading frames (ORF) from Mycobacterium tuberculosis (MTB) plus 647 ORF’s from either Mptb or MAV in an attempt to exploit the gene homology between Mptb and other closely related mycobacteria. While still in the early stages of validating this approach the results to date seem to indicate that the homology between Mptb and MAV might be suitable for studying gene expression between the subspecies. However, little if any
results were obtained with the MTB ORF's suggesting there is insufficient homology between Mptb and MTB at the DNA level to use MTB microarrays to study gene expression in Mptb.

Microarray technology was also presented with respect to the organism and the host. Bull et al. (2002) demonstrated the usefulness of applying this technology to study the effects of gene expression in the organism. A microarray of Mptb specific PCR products representing housekeeping genes and genes known to be associated with pathogenesis was constructed. This was used to compare Mptb that had been internalised by protozoa, to simulate Mptb in an intracellular environment, and cultured Mptb. The results demonstrated up-regulation of several genes in the intracellular Mptb and an active mycobacterial promoter associated Mptb gene induced in the intracellular environment. Romano et al. (2002) used microarrays to measure gene expression in human macrophages after infection with Mptb. Strong changes in the expression of 24 genes were observed indicating a strong pro-inflammatory response. Many of these genes were associated with the production of molecules involved in recruiting regulatory and effector cells to the infection site. Watkins et al. (2002) reported the development of a ruminant immuno-inflammatory gene microarray to be used to investigate the three different types of intestinal pathology observed in sheep: infected but asymptomatic, paucibacillary or multibacillary. Each of these studies demonstrated how microarray technology can be applied to studying the association between Mptb and the host and to identifying the mechanisms of Mptb pathogenesis under conditions previously un-examinable.

Other key studies that were presented with regard to gene expression included those from Doran et al. (2002) and Granger et al. (2002). The first of these reported a modified method of in vitro induced antigen technology (IVIAT) to determine Mptb genes that were up or down regulated in the in vivo environment. The results from this study indicated the presence of yet uncharacterised potential protein antigen associated with the in vivo state of the disease. The second study used real time PCR, targeting specific RNA transcripts, to evaluate gene expression of Mptb isolated from macrophages (in vivo) compared to cultured Mptb (in vitro). For this study real time-PCR was used as insufficient RNA was obtained for microarray analysis. Differential expression of two genes was observed. Interestingly, both studies identified differences in the regulation of the katG (catalase peroxidase) gene from Mptb with respect to in vivo and in vitro environments.

Proteomics

Proteomics is the study of the proteome, the complete set of proteins of an organism, and is an emerging technology in microbiology. A single study was presented, as an oral presentation and in a poster, on the use of proteomics in JD research. Beddome et al. (2002) and Shiell et al. (2002) presented their work on the identification of secreted proteins from MAV and Mptb using a liquid phase proteomics approach. To date fifteen proteins that respond in an IFN-γ release assay have been identified and are being evaluated with respect to their potential in diagnostic testing. To date this is the first report of the use of proteomics in JD research.

Polymerase chain reaction

A considerable number of oral and poster presentations were devoted to the development and/or optimisation of new or existing PCR for the detection of Mptb. Prior to reviewing these it should be noted that one of the key issues raised at the 7ICP was the specificity of IS900 PCR. This issue was first addressed at the 6ICP where Cousins et al. (1999) identified environmental mycobacteria with IS900-like DNA sequences that cross reacted in IS900 PCR. As a result it was suggested that confirmation of PCR results with respect to Mptb required the addition of a restriction endonuclease analysis (REA) step.

In a key oral presentation on PCR diagnosis with IS900, Böske et al. (2002) reported the identification of an environmental mycobacteria (strain 2333) with an IS900-like element that not only cross reacted in IS900 PCR but could not be differentiated from Mptb by REA. Strain 2333, which type more closely to M.\textit{coccii} and the \textit{Mycobacterium sp. strain IMVS B7657}, based on 16S rRNA sequencing, gave identical PCR/REA results to Mptb. This result was achieved when examined with 5 common PCR tests for IS900 of which three included REA. This is the first report of non-Mptb mycobacteria giving this result in PCR plus REA. Even though the IS900-like element from strain 2333 has only 94.4% homology with IS900 from Mptb, as defined in the literature, this result raises serious questions about the specificity of IS900 PCR/REA for PCR based tests for JD.
Two PCR based studies were reported that might provide new opportunities to overcome this specificity issue. Enosawa et al. (2002) reported a new DNA amplification procedure called loop-mediated isothermal amplification (LAMP) in which four *Mtb* DNA targets (IS900, HspX, DnaJ and F57) are amplified simultaneously in a multiplex reaction. While only two (IS900 and HspX) of the four target sequences have been amplified to date, using DNA derived from cultured bacterial suspensions, continued research on this technique may be of benefit to *Mtb* PCR diagnosis. Similarly, Englund et al. (2002) described a PCR targeting the p34 and f57 genes in *Mtb*. The advantage of the p34/f57 PCR is that it will differentiate *Mtb* from MAV, MTB and *Mycobacterium bovis*. Englund et al. (2002) also reported a randomly amplified polymorphic DNA (RAPD) system for the differentiation of *Mtb* from other mycobacteria. Both the p34/f57 and RAPD PCR could differentiate strain 2333 from all *Mtb* tested.

Opportunities to overcome the specificity issues of IS900 also exist in the development of real time-PCR. Real time-PCR is a progressive technique in the field of DNA amplification and offers several advantages over existing PCR techniques. Real time-PCR offers quantitative results and eliminates the need for electrophoresis, thus making the process quicker and avoiding the potential contamination issues associated with conventional PCR. Two posters were presented on the development of real time-PCR for JD. Two detection chemistries were offered, the first study by Willemsen et al. (2002) used the popular TaqMan (Roche) chemistry and the second study by O'Mahony & Hill (2002) used the DNA stain SYBR green. While both papers claimed high sensitivity neither demonstrated specificity with regard to IS900-like environmental mycobacteria. However, these are among the first of the real time-PCR studies to be presented in JD research and therefore ongoing research will be required to fine tune this technique. With numerous alternate real time-PCR strategies exploiting different combinations of *Mtb* DNA targets and detection chemistries, this is surely an area of JD research that is certain to escalate in popularity with the view of developing new sensitive, specific and cost effect diagnostic tests for JD.

Several studies were presented on the development of improved IS900 PCR for the detection of *Mtb* in faeces. These included one tube nested PCR, internal PCR controls and improved DNA extraction procedures. While providing an interesting insight into alternate methods, most studies failed to demonstrate the application of these tests to clinical samples or failed to demonstrate the cost of the proposed test. It would be helpful for future colloquiums if publications on diagnostic PCR tests could provide clinical sensitivity (real samples) not just analytical sensitivity (spiked samples) and costing. The cost of the test is an important issue as a test must be affordable especially when the value of the animal is low. An expensive test is more likely to be a research tool rather than a diagnostic test.

With the ever increasing concern regarding the association between *Mtb* and Crohn's disease a considerable number of oral and poster presentations were devoted to the detection of *Mtb* in milk. Herron et al. (2002) presented a report on the use of PCR and nucleic acid sequence based amplification (NASBA) in an attempt to identify viable *Mtb* in milk. The PCR/NASBA assay was used to target the RNA transcript from the SsrA gene. While the technique proved to be successful the results could not differentiate between *Mtb* and other members of the MAC. However, further refinement of this technique would provide a useful tool in JD research. Stratmann and Gerlach (2002) also presented a new technique to identify *Mtb* in milk. Unique and specific peptides derived from filamentous phages that bind to *Mtb* were coupled to immunomagnetic beads. The peptide-bead complex was then used to isolate *Mtb* from milk samples followed by confirmation of *Mtb* by ISMav2 PCR. ISMav2 PCR at this stage does not appear to suffer from the same specificity issues as IS900 but may suffer reduced sensitivity as a result of there being only a few copies of ISMav2 in the *Mtb* genome compared to fifteen to eighteen copies of IS900. Unlike the Herron technique, which identifies viable *Mtb*, the Stratmann technique requires culture to demonstrate viability.

A number of studies including those from Dijonne et al. (2002), Ocepek et al. (2002), Nilson et al. (2002) and McKee et al. (2002) described immunomagnetic separation-PCR (IMS-PCR) techniques for the identification of *Mtb* in milk. While each was successful in demonstrating the presence of *Mtb* in milk it was the McKee study that was of particular interest. In this study pre-vat in-line filters in the milking system were targeted for the isolation of *Mtb*, not the milk directly. This is an interesting and logical choice as these filters may concentrate the bacteria still bound to faeces or other particulate material. The authors proposed that this technique may be suitable for identifying JD infected dairy herds. However, validation of the technique is still in process.
Implications and Relevance to Australia

It is clear from the content of the research presented at the 7ICP that the JD research community has endorsed the need to apply the new and emerging molecular biology techniques to obtain a better understanding of Mptb at the DNA and protein level and how this organism causes disease.

With the Mptb genome nearly completed the development of Mptb specific microarrays will make it possible to analyse gene expression and regulation in the in vivo environment and improve our understanding of the mechanisms by which Mptb causes disease. Similarly, proteomics will provide a new and valuable tool in addressing JD research. However, to date there are certain issues that limit the application of proteomics to JD research but these will certainly be overcome.

Finally, PCR based diagnosis is an extremely important area of continued research in JD. We need to overcome the IS900 specificity issue and develop new sensitive and specific PCR to aid in the rapid, definitive and cost effective diagnosis of the disease. To do this real time-PCR appears to be the natural progression as this offers many potential benefits over conventional PCR such as sensitivity, specificity, timeliness and minimisation of handling amplified product. The research presented at the 7ICP in these areas clearly indicates that we are progressing in the right direction to find answers to the many questions that still remain on this disease. Consequently, the outcomes from these second-generation molecular biology studies will benefit control programs for the disease.

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AETIOLOGY OF PARATUBERCULOSIS: GENOME ISSUES AND GENOMICS

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Summary

Etiology is defined as “The study of the causes and modes of operation of diseases”. The principal cause of Johne’s disease, the infectious agent Mycobacterium paratuberculosis (Mptb), was identified more than a century ago. The papers in this session did not reveal any great leaps forward in our understanding of other causative factors or the modes of operation of this disease. However this session represented a landmark in Johne’s disease research as it unveiled the near-to-complete genome sequence of the organism, arguably the most significant step forward in this field of study since the first identification of the bacterium by Professor Heinrich Johne. This represents a critical resource for basic and applied research that will be central to future studies of the nature of Mptb and the mechanism by which it causes disease.

Questions were raised over the specificity of current DNA (IS900) based tests. New methodologies were presented for identification, typing and epidemiology of the M.avium family of organisms particularly Mptb. But already the biggest impact is likely to come from the genome studies and the previously unknown genes now identified as unique and specific for Mptb.

Key Papers/Posters

The significance of determination of the genome sequence for Mptb was reflected in its position as opening paper and keynote address of the Colloquium (Kapur et al 2002). This paper, presented by Dr Vivek Kapur, addressed the current status of the genome-sequencing project. On completion this project will reveal the gene content and organisation for a laboratory strain of a bovine isolate known as K10. The genome is currently represented in 18 separate segments (contigs) which comprise 4.92 million base pairs of coding sequence - capable of producing 5178 potential proteins and gene products. These 18 segments still need to be joined to give one continuous chromosome and current estimates indicate that there are only 2500 base pairs missing from the final sequence. The integrity of the vast quantity of data generated is assured by repetitive overlaying of sequences determined to give, in this case, 6-fold coverage i.e. most sequences have been read at least six times in different clones or reactions. Completion of the sequence, annotation of the genes encoded and its public release is expected before the end of 2002.

The genome sequence is also being determined elsewhere for Mycobacterium avium subsp. avium strain 104 (The Institute for Genome Research) and this data has been used for comparison with the Mptb genome. This has shown that 500,000 base pairs (approximately 500 genes) are missing from the Mptb genome. However it was found that the complete suite of genes required for the synthesis of mycobactin are present in Mptb - an interesting observation in light of the fact that mycobactin dependence for growth, is universally accepted as a definition for this organism on primary culture. This is a clear demonstration that it is as important to understand how, when and why particular genes are used, as much as which genes are present in a bacterial agent. This is the discipline of functional genomics (i.e. the study of which genes are transcribed into gene products or proteins).
Another important observation emerged from this and related studies (reported in part by Kiehnbaum et al. 2002) on the relationship between members of the *M. avium* family of organisms. A method known as AFLP (amplified fragment length polymorphism) was used to type and group various isolates of *M. avium* and *Mtb* in combination with IS900 testing. The data showed that the DNA marker currently used as a specific indicator for *Mtb* is found in isolates from a number of groups of the *M. avium* family. Whilst there may be minor variation between these markers they are unlikely to be distinguished by current tests. This data is discussed elsewhere (Summary Report On The Molecular Biology Of Johne’s Disease by Ian Marsh) and was supported by presentations at this Colloquium by Bolske *et al.* (2002) and Whittington (2002) which were the cause of considerable debate.

The presentation by Dr. John Bannantine addressed in more detail the comparison between the genomes of *Mtb* K10 and *M. avium* 104 (Bannantine *et al.* 2002). This comparison was performed on just over half of the genome sequence and showed that the sequences are 98-99% identical. Currently 27 genes of *Mtb* K10 are missing from *M. avium* 104 and the predicted total on completion of the project is for around 100 genes to be specific for *Mtb*. In contrast more than 500 genes of *M. avium* 104 are missing from *Mtb* K10. Further analysis of the 27 genes, apparently specific for *Mtb*, has shown that 21 of these are in fact unique and absent from a range of *M. avium* and other mycobacterial species. These 21 genes, and those soon to be revealed, have great potential value in diagnosis both as DNA targets and possible protein antigens for use as immunodiagnostic reagents. Many elaborate technical procedures have been used over the past decade to identify genes unique to *Mtb* and yet these studies have yielded only a handful of useful targets of which only one (IS900) is in regular use. Another such approach was reported at this Colloquium (Kiltgaard 2002) which seems to have been more successful than preceding studies with the identification of nine novel gene fragments, which are currently only partially characterized. However within 6 months the genomic studies should be able to provide a much more extensive and definitive list of unique genes and DNA elements specific for bovine *Mtb*.

A number of interesting papers and posters provided new opportunities for epidemiological studies of Johne’s disease outbreaks. Characterisation of mycobacterial interstitial repetitive units (MIRUs) and variable number tandem repeats (VNTRs) were both shown to have value in rapidly distinguishing *Mtb* strains using PCR based methodologies (Sidi-Boumedine *et al.* 2002; Nishimori *et al.* 2002). However it appeared that greater strain variation could be identified using amplified fragment length polymorphism (AFLP) another PCR based technique (Kiehnbaum *et al.* 2002). These methods have the advantage of requiring only small amounts of sample and of giving a rapid read-out time. Bartos *et al.* (2002) used the older, more material and labour intensive method of restriction fragment length polymorphism (RFLP) and presented an extension of their earlier epidemiological studies – the most significant outcome being the apparent association of a human isolate of *Mtb* with a local cattle isolate.

In the area of functional genomics a number of papers were presented in the Pathogenesis and Molecular Biology sessions showing data on *Mtb* using microarray analysis essentially based on *M. tuberculosis* genome information and small numbers of selected genes from *Mtb* (Bull *et al.* 2002; Zhai *et al.* 2002). These reports gave an indication of the potential for application of this technology. Bull *et al.* used the unorthodox approach of bacteria engulfed by protozoa for their “in vivo” model of *Mtb*. Functional genomics of the host was addressed by a number of papers including that of Coussens *et al.* (2002) which used microarray analysis of white blood cells from JD affected cattle to show significant down-regulation of more than 30 immune cell genes on stimulation with *Mtb*. These and related studies are reviewed elsewhere (Summary Report On The Molecular Biology Of Johne’s Disease by Ian Marsh).

In terms of other factors related to the cause of disease the only two presentations were on survival of the organism in soil (Schroen *et al.* 2002) and in forage (Katayama *et al.* 2002). Survival of *Mtb* in soil was found to be lower in elevated temperatures and low moisture conditions. In alfalfa hay processed for forage, survival of *Mtb* was found to be adversely affected by increased moisture, processing temperature, ammonia concentration and processing time respectively.

**Implications and Relevance to Australia**

The needs of the Australian sheep and cattle industries are for improved control measures including improved diagnostics and farm/stock management practices.

The 7th International Colloquium on Paratuberculosis has provided:
New “specific” genes that will be investigated for their potential to improve diagnosis of Johne’s disease – and more to be uncovered in the near future.

The basic knowledge to undertake functional genomics (on the bacteria and host) and the first indications that there is valuable data to be gathered.

New tools to conduct epidemiological studies.

Although there are no direct indications for new control measures in the immediate future, as a result of these basic research efforts the scene is now set for significant steps forward in the foreseeable future. Uncovering genes known to be absolutely specific for M. tuberculosi allows development of potential diagnostic reagents that can be assessed in infection models and field situations. These and other genes could also be applied to the development of sub-unit vaccines for combined use with new diagnostics.

Other control measures could arise from a better understanding of the bacterium and its relationship and interactions with the infected host – through the application of functional genomics. This may be used to modify farm or stock management practices in ways it is not yet possible to predict but that may involve animal stress or nutritional factors, resistance and susceptibility, soil or water treatments and so on. Many other areas of research will lead eventually to the provision of full sheep and cattle genome data (to supplement the incomplete libraries that are currently used). However only JD research will provide the required outputs for functional genomics of M. tuberculosi. This will help to determine which of the 5000 plus genes of the bacterium are useful targets for diagnostic, vaccine and other applications for disease control.

Issues over the specificity of IS900 as a marker need to be addressed for future veterinary diagnostic tests. The question mark that this throws over older studies on the putative link between JD and Crohn’s disease may be outweighed by new culture data (McMinn et al. 2002). This data seems to indicate association of M. tuberculosi with Crohn’s tissue (94% and 25% in controls) and the ability to culture live organisms from up to 52% of surgical tissue samples. Should this data be verified concerns over food safety issues are likely to arise.

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PATHOGENESIS OF JOHNE’S DISEASE

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Summary

Presentations were given in the pathogenesis section of the colloquium in diverse areas. Some presented new information from completed studies, while others described experiments using new techniques that are ongoing. The major areas covered were phagocytosis of *M. paratuberculosis* (*Mptb*) (with particular emphasis on macrophage gene expression), gene expression of *Mptb*, virulence factors of *Mptb*, some aspects of nutrition, new experimental models for infection, pathology, host genetic resistance/susceptibility and many presentations on the various aspects of the immune response to infection. There were also presentations in the pathogenesis section on new vaccines and evaluation of diagnostic tests. These areas are covered by others.

Phagocytosis of *Mptb* by macrophages is a crucial stage in the pathogenesis of Johne's disease (JD), both from the point of view of the host and the pathogen. Both host and pathogen factors play a part in deciding the outcome of this interaction. So to understand how *Mptb* evades and survives the normal defensive mechanisms of the macrophage, the interaction must be looked at from both sides. Several presentations were made examining this relationship.

A particular area of interest is macrophage gene expression during phagocytosis. This was examined by several groups, with most using a functional genomics approach (microarray). Three hundred and eighty amplicons have been identified representing genes whose expression is altered during phagocytosis. The gene expression profile for phagocytosis of *Mptb* is distinctly different from that for *E.coli* (which is readily degradable) or for latex beads (a positive control for phagocytosis experiments). The comparison reveals that some genes are activated while others are suppressed. The altered genes discovered thus far are involved in energy metabolism, calcium binding, cell signaling and macrophage migration. A study of 6 genes that are activated by exposure to *Mptb* demonstrated that their activation is severely delayed in comparison to *E.coli*, suggesting that *Mptb* enters macrophages in a manner that prevents immediate activation, thus giving them time to avoid degradation. Increased cytosolic calcium is an early event following phagocytosis and is involved in activation of kinases, cytoskeletal changes and phagolysosome development. A flow cytometric study of cytosolic calcium revealed no significant change following uptake of *Mptb*. These reports provide evidence that *Mptb* is able to alter gene expression of macrophages in order to evade their defensive mechanisms.

Differences in gene expression have also been found between macrophages from animals with subclinical and clinical disease. The most significant difference is the down-regulation of 30 genes in clinical animals compared to subclinical animals. Phenotypic differences between macrophages within lesions and those in adjacent unaffected areas have also been reported. Macrophages within lesions showed lower expression of MHC II (important for antigen presentation to CD4 T cells), CD88 (a marker for lysosomal membranes) and CD11b. This demonstrates that the alterations in gene expression are manifested by differences in phenotype.

There were also a number of presentations on bacterial factors. Again using microarray technology gene expression was compared between intracellular and extracellular *Mptb*. The intracellular *Mptb* showed several differences in gene expression, but the most significant was found to be a marked increase in the
expression of a gene called Kcat, which is a Mptb specific catalase. In addition two virulence factors have been suggested. A 35kDa protein, called major membrane protein (MMP) has been shown to be surface localized and to play an important role in the invasion of bovine epithelial cells. A 34kDa protein, which is similar to a trypsin-like serine protease, has been shown to increase the survival within macrophages when cloned into M.smegmatis. It is suggested that the protein may afford protection against the bactericidal mechanisms of the host cell, possibly by degrading damaged proteins associated with the mycobacterial cell wall. Using in-situ hybridization, cell wall deficient forms of Mptb have been identified within paratuberculosis lesions from some cattle. The presence of these cell wall deficient forms may explain why the organism is not always readily cultured and possibly also why JD pathology may be seen, but no acid-fast bacilli seen.

Many presentations on the various aspects of the immune response to paratuberculosis were given, with an emphasis on the role(s) of various cytokines (IL-1β, IL-2, IL-4, IL-8, IL-10, IL-18, IFN-γ, TNF and TGF-β). Several techniques have been used including the use of gene knockout mice, in-situ hybridization to detect cytokine mRNA levels, quantitative RT-PCR to detect cytokine mRNA levels as well as the standard diagnostic tests for cell mediated immunity (CMI) and humoral immunity.

In particular the switch from a Th1 immune response to a Th2 immune response was examined. In subclinical cattle a parallel shift from a Th1 to Th2 response prior to faecal shedding occurred in about half of the culture positive cattle, indicating that the shift from Th1 to Th2 is a gradual and variable process. In a comparison between cytokine levels in tuberculoid and lepromatous lesions in cattle however, it was found that Th2 cytokines were expressed more in the lepromatous group than in the tuberculoid group and Th1 cytokines were expressed more in the tuberculoid group than in the lepromatous group. This suggests that formation of lepromatous lesions or tuberculoid lesions may be influenced by Th1/Th2 cytokine production. A role for IL-18 has been suggested in the switch from Th1 to Th2, with IL-18 being expressed less in the lepromatous lesions than in tuberculoid lesions. Another study using IL-18 knockout mice concluded that it has little or no role in granuloma formation, but suggested that a lack of IL-18 may relate a bactericidal effect.

The decline of IFN-γ levels is thought to be an important step in the progression from subclinical to clinical disease. IL-10 and TGF-β, which down-regulate production of IFN-γ, are expressed more in animals with clinical disease than in healthy or subclinical animals. This correlates with IFN-γ levels that in the same study were shown to be higher in subclinical animals than clinical animals. Another study, however, found no clear relation between individual animals IL-10 and IFN-γ responses over time.

Several immunological and microbiological parameters from a longitudinal study of experimentally infected calves were also reported. The earliest changes detected were a minor response in the peripheral blood mononuclear cell (PBMC) population within 3 months, which then rises exponentially over the first year of infection before reaching a plateau. Within this population there is a particular increase in γδ T-cells, possibly indicating a role for these cells in infection. Also a dose-response relationship between lymphocyte stimulation and bacterial load has been noted. This work is ongoing.

Other factors that affect the development of paratuberculosis have been identified. In beef cattle the microelements selenium (Se) and copper (Cu) have been identified as being of importance. Both Se deficiency and Cu deficiency (primary or secondary) may predispose to development of clinical signs of paratuberculosis. In dairy cattle the periparturient period is associated with immunosuppression that can result in the presentation of clinical signs. Dietary energy supplementation during this period was investigated, but while it affected some parameters, overall it did not preclude immunosuppression.

Genetic factors are hypothesized to influence the susceptibility of exposed animals to paratuberculosis. A transmembrane glycoprotein, bovine leukocyte antigen DRB3 (BoLA-DRB3), was investigated to determine its possible role in resistance/susceptibility. Preliminary allele frequencies in infected animals revealed 4 alleles that had a frequency above 15%. Further work is needed to investigate the role of this gene and others.

The spectrum of pathological lesions in paratuberculosis affected small ruminants has been examined and reported previously. Similar studies in cattle have not been performed until now. In cattle the lesions are divided into 3 major types: focal, multifocal and diffuse, with a further division of diffuse lesions into multibacillary, lepromatous and intermediate. The major differences between the lesions in cattle and those in small ruminants is that there were large numbers with intermediate diffuse lesions, as opposed to well
defined multibacillary or lepromatous lesions. Additionally focal lesions in cattle were seen primarily in the ileal lymph nodes rather than the intestinal lymphoid tissue, as seen in small ruminants.

Finally, reports on the development and implication of several models for experimental infection with Mptb were presented. In the development of an experimental model for OJD to test vaccine efficiency and immune reactivity, several key points were elucidated. Lambs aged 4-14 weeks are equally susceptible to infection. The optimal challenge dose is between 10^5 and 10^6 cfu. Both oral inoculation and direct instillation into the tonsillar crypt are suitable to infect lambs. A mouse model was used to compare virulence of strains of Mptb isolated from different host species. C57/BL6 mice were inoculated with either bovine, pigmented ovine, non-pigmented ovine or human strains. The bovine strain was shown to be most virulent, while the pigmented ovine and human strains were least virulent, as determined by histopathology and liver culture. This however, may just be a reflection of the growth rate of different strains as the mice were killed 8 weeks post-infection.

Key Papers/Posters


Mptb enters intestinal cells via several mechanisms that are yet to be fully elucidated. In this study the role of major membrane protein (MMP) in this process was evaluated. Immunoelectron microscopy of Mptb bacilli labeled with MMP-specific antibodies demonstrated the protein is localized to the surface of the bacteria. Cattle with JD were shown to produce antibodies against MMP, but did not produce IFN-γ, suggesting the protein elicits a humoral but not cell-mediated immune response. Anti-MMP antibodies inhibited the invasion of cultured MDBK cells by 30%, as did the fusion protein MBP/MMP (MMP fused with maltose binding protein). Similar experiments in low oxygen tension, simulating conditions in the intestine, showed decreased invasion by 60%. From these results it is clear that MMP plays a role in the invasion of epithelial cells, but does not appear to be the sole mechanism by which invasion occurs. The authors suggest that MMP is a virulence factor for Mptb, which may be important in the initiation of infection in vivo. This is important as most previous work in this area has looked at M.tuberculosis rather than Mptb.


Gene expression profiles of PBMCs from control-uninfected, clinical JD positive and subclinical JD positive Holstein cows were compared using a bovine-specific cDNA microarray system. PBMCs from a control cow and an early clinical JD positive cow responded similarly to stimulation with the general mitogen ConA, with activation of 119 genes. Stimulation of PBMCs from the control-uninfected animal with Mptb resulted in activation of 6 genes involved in phagocytosis. Stimulation of PBMCs from subclinical cows with Mptb resulted in significant activation of numerous genes (pattern of activation similar to that seen from stimulation with ConA). Major differences were seen, however, when the PBMCs from clinical cows were stimulated with Mptb. In this group there was significant down-regulation in expression of >30 genes (no down-regulation seen in the other groups) and also activation of >100 genes. So within this small sample set there was a dramatic difference in the overall response of PBMCs from cows with clinical disease compared to those with subclinical disease. The cause of this difference between subclinical and clinical disease may be important in the control of disease.


Mptb is an intracellular pathogen, and so the expression of genes by intracellular organisms, as opposed to extracellular or cultured organisms, may highlight the molecular mechanisms behind the pathogenesis of paratuberculosis. A microarray was constructed of specific PCR products from a bank of Mptb specific genes, mycobacterial housekeeping genes and genes previously associated with mycobacterial pathogenesis. Intracellular organisms were obtained by infecting cultures of Acanthamoebae polyphaga,
while extracellular organisms were grown in amoebic culture medium only. After 4-8 weeks the mRNA was extracted from the Mptb. cDNA was generated, labeled and hybridized to microarrays. These were then read and ratios of intracellular/extracellular signals calculated. Results showed that the intracellular organisms had decreased expression of DNA synthesis genes, increased expression of GS cassette genes, modestly increased expression of IS900 associated genes and most notably dramatically increased expression of a Mptb specific catalase (Kcat). These genes may play an important role in the pathogenesis of paratuberculosis.

Implications and Relevance to Australia

Studies on macrophage gene expression highlight the fact that Mptb is capable of altering host gene expression (at different stages of infection) and this is manifested in phenotypic changes. Further work is needed in this area to characterize the genes whose expression is altered and to elucidate the mechanisms by which Mptb is able to do this. With this knowledge, strategies for intervention may be possible. Two potential virulence factors have been identified, but there are likely to be many more and further work in this area may lead to the development of strategies to prevent or control infection with Mptb. There is still much to learn of the immune response to Mptb. Some studies are contradictory, but this is not surprising as a variety of systems and methods are used in this area. It is clear, however, that the immune response is variable between individual animals and this is a factor in the poor performance of diagnostic tests that target the immune response.
VACCINATION AGAINST JOHNE’S DISEASE

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Summary

There were 7 papers and 2 posters presented which were relevant to vaccination:

1. Our progress report on MLAOJD.009 evaluating Gudair™ on 3 high prevalence Mycobacterium avium subsp. paratuberculosis (Mptb) infected properties in NSW. Of interest to Spanish delegates (RA Juste of NEIKER, JF Garcia-Marín of University of Leon and staff of CZ Veterinaria) as despite numerous papers on the effects of vaccination, definitive long-term studies that encompass all aspects of the vaccine are needed; the Australian work is appreciated.

2. Was followed by a presentation by V Fridriksdottir of the University of Iceland entitled: “Paratuberculosis in sheep in Iceland – is eradication by vaccination possible?” The history of Mptb infection in Iceland is that a sheep strain infecting sheep and cattle was introduced from Germany in 1933 and since 1966 vaccination of sheep in endemic areas has been compulsory, considerably reducing losses. In 2001 the disease re-appeared on a farm in an area that had been declared free from paratuberculosis and where vaccination had ceased 3 years earlier (50/400 animals were seropositive on one farm and one seropositive on an adjacent farm). The seropositives were culled and the rest vaccinated, however the cause of the re-infection is unknown and doubts were expressed that eradication by vaccination is possible.

3. The invited plenary presentation by Ramon Juste (detailed below) on “Control of paratuberculosis by vaccination” included a review of the vaccine, when it can be expected to be useful, and a meta-analysis of available published literature on Mptb vaccination. Ramon expressed appreciation we presented MLAOJD.009.

4. The presentation by Juan Frederico Garcia-Marín on “An ovine histopathological model for the evaluation of the efficacy of vaccines against paratuberculosis” showed that after experimental oral infection of 2-4 month old vaccinated lambs, all vaccines had limitation of lesions to intestinal lymphoid tissue and regression of the lesions with time. This is evidence that the vaccine decreases the level of infection and modifies the pathological response to infection.

5. A presentation by JFJ Huntley (Merkal Award Lecture) of Iowa State University on “Expression library immunisation of mice identifies 5 clone pools that offer protection against challenge from Mptb” gave the impression that designer DNA vaccines are getting closer. Five of 26 clone pools (each pool containing an average of 1500 clones of novel Mptb genes) demonstrated a 100-fold reduction in Mptb colonisation of liver, spleen, mesenteric lymph node and ileum. This study used C57BL/6J mice (6 weeks old) immunised with 2 Ig of DNA (plus 0.5ug gold beads, using beta-galactosidase expression as evidence of success of vaccination) in the abdominal area via gene gun delivery. The mice were boosted 3 weeks later and challenged 2 weeks later with Mptb (108 bacteria per mouse) via tail vein.
6. The presentation by MV Geijo of NEIKER on "Paratuberculosis vaccination responses in cattle, sheep and goats" examined Elisa, IFN-γ and blood PCR responses in 3 age cohorts (15 days, 6 months and 1.5 to 2 years) in the 3 species. Five to nine animals were vaccinated and there were 5-9 control animals. Different patterns of immune response were noted according to species (goats had small changes in IFN-γ compared with cattle), age and clinical history (by PCR Mptb was as frequently detected in PTB-free as in PTB-affected herds, both before and after vaccination).

7. The presentation by J Uzonna of the University of Pennsylvania on "Effect of IL-12 on immune response to paratuberculosis vaccination in calves" involved 3 experiments examining 2 vaccine formulations (commercial versus field strain), time of vaccination and adjuvant effect of IL-12. Calves were vaccinated with Strain 18 +/- IL-12 at 2-7 days of age then orally challenged with Mptb 3 weeks later, versus oral challenge at 2-3 days and vaccination with Strain 18 +/- IL-12 3 weeks later, versus vaccination with Mycopar +/- IL-12 at 2-7 days then challenged at 3 weeks ((killed at 49 days and cultured). The study demonstrated that the severity of infection was reduced when the vaccine was given before challenge. The field strain vaccine showed better protection; all vaccines had high concentrations of IFN-γ from prescapular and ileocecal lymph node cultures, and whilst IL-12 had no effect on IFN-γ production, some calves vaccinated with IL-12 prior to challenge were culture negative. None of calves vaccinated after challenge were protected.

8. The poster by Reyes et al of the University of Leon on "Evaluation of different adjuvants in the vaccination against paratuberculosis in sheep" compared vaccination of sheep (2 to 4 per group) with a killed vaccine containing MONTANIDE ISA 266 adjuvant, compared to Gudair containing Complete Freund's Adjuvant (CFA). The vaccine containing ISA 266 adjuvant induced enhanced cellular responses (IFN-γ and skin test) and better immunohistochemical distribution of antigen in the lymph nodes which were less severe (no ulceration). The vaccine had a similar level of efficacy in controlling infection as Gudair.

9. The poster by Garcia-Pariente et al of the University of Leon on "Paratuberculosis vaccination of adult animals in two flocks of dairy sheep" documented results at 12 months post-vaccination in 2 flocks of 250 Assaf sheep (22.2% and 11.7% ELISA positive respectively). Seventy percent of sheep were vaccinated with Gudair regardless of their age while 30% were left unvaccinated. Milk production, immune responses and losses were recorded (shedding results unavailable yet). At 12 months after vaccination no differences were detected in milk production. IFN-γ responses in vaccinated declined from over 90% +ve at vaccination to about 50%. ELISA responses decreased from 90% to about 80% and AGF from above 70% to about 20%. Clinical cases were reduced, with flock A culling 56 sheep during the 12 months with 28 examined by histology. Of 17 culls (11 vaccinated and 6 controls) in the first 6 months, 9 had diffuse lesions, 2 had focal lesions and 6 had no lesions, compared to 11 culls (5 vaccinated and 6 controls) in the last 6 months, 3 having diffuse lesions, 4 with focal lesions and 4 with no lesions. The authors claim evidence that vaccination of adult sheep reduces the number of clinical cases; those in which vaccination has no effect having to be culled in the first 6 months followed by a decrease in sheep with severe diffuse lesions and an increase in sheep with focal disease i.e. vaccination limiting disease to focal lesions.

Key Paper

The plenary presentation by R Juste on "Control of paratuberculosis by vaccination" described the current vaccines (killed or attenuated) mostly containing 3167F Weybridge strain (whole cell appears better than cell-fractions) with an oil/paraffin/pumice powder adjuvant (eg CFA) to cause locally persistent, subcutaneous inflammation; not available from oral vaccines (considered "unconventional" route of administration).

He considers there is evidence that whilst "vaccination does not completely protect from infection, and therefore by itself cannot lead to Mptb eradication", there are only 3 reports of vaccine failure (involving oral administration, use of sonicated cells and a comparison of vaccine effects on shedding versus other controls respectively). Further, the literature contained conclusive evidence that vaccine caused significant decreases in clinical signs (96%, 45.2%, 46%), bacterial load (79.2%, 53.4%, 82.9%) and necropsy lesions (…, 92.9%, 96%) in vaccinated cattle, sheep and goats respectively. He inferred a "conspiracy of silence against vaccination" which has led to limited documentation on the benefits of vaccination. He attributes this
to concerns regarding the complication of vaccination on TB diagnosis, trading of vaccinates, risk of self-administration (reported as 1 in 4000 doses in the USA compared to Spain where self-inoculation is rare ie 1 in 500,000 doses?) and uncertainty as to whether vaccination of cattle reduces excretion of Mptb in milk. Potential improvements to vaccines include sub-unit vaccines, naked DNA, deletions & marker antigens, iscoms, microspheres and emulsions, mucosal immune stimulation and re-vaccination.

Implications and Relevance to Australia

Australia has an increasing acceptance of the importance of vaccination in OJD control, possibly because we have evidence of recent introduction and zonal distribution of the disease and have eradicated (or nearly eradicated) TB (plus sheep are resistant to TB).

Vaccination of cattle with Gudair or other vaccines (which are currently being evaluated in cattle in Spain and some research in USA) for control of Mptb is an option for future consideration, particularly when the Crohn's disease story achieves agro-political impact.

New technologies suggest possibilities for improved vaccines i.e. better adjuvants and possibly DNA vaccine formulations. However, with the important limitation that current (& future?) vaccines do not prevent infection, but do significantly modify the response of vaccinates to infection, we currently have a powerful tool for OJD control.

With minimal documentation of vaccine control strategies, our challenge is working out the best ways to use it (eg vaccinating animals prior to challenge), compiling the cost-benefits of vaccination, and determining the need for sustained use of vaccine where within-flock prevalence of mortality is reduced to satisfactory levels.

The view was advanced in the summation that embracing "vaccination may be considered an election to live with Mptb" (M Collins) & authorities need to consider implications of this in decisions on eradication versus control. Australian experience (at least in NSW), suggests that to not vaccinate is to live with increasing amounts and effects of Mptb. There is an emerging consensus that eradication of endemic Mptb is "not possible, necessary or profitable" (R Juste) and that vaccination is likely to be a long-term Mptb control strategy. The Iceland experience suggests that re-emergence of Mptb can be expected on cessation of vaccination.
WILD HOSTS OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

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Summary

There is mounting evidence from throughout the world that the host range for Mycobacterium avium subsp. paratuberculosis (Mptb) is much wider than originally described. Johne's disease (JD) infection has been demonstrated in several wildlife species including rabbits, hares, rats, mice and various species of birds, carnivores and macropods. However, further research is required to investigate the capacity of wildlife to deliver an infectious dose of Mptb to domestic livestock, and whether Mptb can cycle independently in wildlife populations in the absence of infected domestic ruminants.

There are important implications for the control of JD in cattle and sheep in Australia if wildlife species can become reservoirs of Mptb.

Key Papers/Posters:

Three key papers relevant to current research into JD wildlife epidemiology were presented:

1. The potential role of wildlife in the epidemiology of paratuberculosis in domestic animals.
   Grelg A, Moreeun Research Institute, Edinburgh UK.

   Paratuberculosis was first described in wild rabbits in Scotland in 1990. A small follow-up survey of 33 rabbits was conducted in 1997. Mptb was isolated from 20/27 rabbits harvested from JD infected farms and 3/6 rabbits harvested from non-infected farms. This finding exposed a major gap in the understanding of JD epidemiology by indicating that non-ruminant wildlife could potentially transmit Mptb to domestic livestock.

   A further study was undertaken to determine the potential of other wildlife species to harbour Mptb and to identify natural wildlife-ruminant transmission routes. Four farms greater than 30 km apart with a history of JD in cattle were selected as study sites. Wildlife species were collected opportunistically as part of vermin control programs on these farms. Duodenum, ileum, caecum, colon and mesenteric lymph nodes were collected for culture and histopathology and faeces for culture. Positive results are given in Table 1:
Table 1. Mptb culture and histopathology results of wildlife species harvested from four JD infected cattle farms in Scotland.

<table>
<thead>
<tr>
<th>Species</th>
<th>Culture Positive</th>
<th></th>
<th>Histopath Positive</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tissue</td>
<td>Faeces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fox</td>
<td>23/27</td>
<td>3/27</td>
<td>12/26</td>
<td></td>
</tr>
<tr>
<td>Stoat</td>
<td>17/37</td>
<td>1/6</td>
<td>1/13</td>
<td></td>
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<tr>
<td>Weasel</td>
<td>2/4</td>
<td>N/A</td>
<td>2/4</td>
<td></td>
</tr>
<tr>
<td>Crow</td>
<td>36/60</td>
<td>4/12</td>
<td>1/60</td>
<td></td>
</tr>
<tr>
<td>Rook</td>
<td>3/53</td>
<td>1/1</td>
<td>0/53</td>
<td></td>
</tr>
<tr>
<td>Jackdaw</td>
<td>1/38</td>
<td>N/A</td>
<td>0/38</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>3/35</td>
<td>0/7</td>
<td>0/23</td>
<td></td>
</tr>
<tr>
<td>Wood mouse</td>
<td>3/88</td>
<td>2/2</td>
<td>1/68</td>
<td></td>
</tr>
<tr>
<td>Hare</td>
<td>1/6</td>
<td>0/3</td>
<td>0/4</td>
<td></td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>90/348</strong></td>
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</tbody>
</table>

Negative results were obtained for the house mouse (0/89), pigeon (0/74), sparrow (0/47), vole (0/26), pheasant (0/4), badger (0/1) and buzzard (0/1).

To determine rabbit-ruminant transmission by experimental infection, neonatal calves were orally dosed with rabbit isolates of Mptb and were euthanased after 6 months. Seven of eight calves were culture positive and three of eight were positive on histopathology.

The results of this work indicate that animals and birds that predate on rabbits or scavenge rabbit carcasses are very likely to become infected with Mptb. Carnivores probably do not pose a serious threat to farmed livestock since they are unlikely to ingest carnivore faeces. Bird and rodent faeces on the other hand could contaminate pasture and stored feedstuffs.

Based on the results to date, rabbits are considered to have the greatest potential for being involved in the epidemiology of JD in domestic ruminants in Scotland.


This paper reported the serendipitous finding of eight feral ferrets infected with Mptb as part of investigations into the presence of bovine TB in wildlife in New Zealand.

Fresh and fixed tissue samples were collected from ferrets that had macroscopic lesions suggestive of TB. The only gross lesions present were enlarged mesenteric lymph nodes. Fresh tissues were cultured for mycobacteria using BACTEC and Mptb was confirmed by demonstrating the presence of IS900 and mycobactin dependency.

Six of the eight ferret isolates were S strain and the remaining two were C strain. Infected ferrets were found on both the north and south islands.
A notable feature of the ferrets infected with *Mptb* was the presence of large numbers of acid-fast bacilli in their livers. This finding suggests that ferrets are highly susceptible to systemic infection.

The possible sources of infection for ferrets in New Zealand include cattle, sheep, farmed and/or wild deer and rabbits. Rabbits have not been examined for the presence of *Mptb* in New Zealand.


This paper described two experiments to determine the level of contact between cattle and rabbit faeces in the grazing environment and between housed cattle and rodent faeces via stored concentrate feed.

Faeces from infected rabbits have been demonstrated to contain high numbers of viable *Mptb*, suggesting that they input millions of bacteria per hectare per day onto pasture. In addition, during the winter months livestock are housed and fed stored concentrates that may become contaminated with rodent faeces potentially containing rodent-derived *Mptb*.

In the grazing experiment, remote monitoring in conjunction with a stratified survey of sward height was used to quantify the grazing behaviour of cattle on pasture contaminated by varying levels of rabbit faeces. Four pasture treatments were created by contaminating 40 plots 0.5 x 0.5 m² in area with 0, 10, 50 or 250 fresh rabbit faecal pellets. When compared to the control pasture treatments, the cattle did not avoid plots heavily contaminated with rabbit faeces, suggesting that they were likely to contact fresh rabbit faeces when grazing. The demonstrated high contact by cattle with pasture contaminated with rabbit faeces indicates that the potential for transmission of *Mptb* from rabbit faeces is high.

In the stored feed concentrate experiment, ten cattle were individually presented with three repeats of five feed treatments. The five treatments were: no contamination; 20 mouse faecal pellets; 80 mouse faecal pellets; 20 rat faecal pellets and 80 rat faecal pellets per 400 grams of feed. Each animal was penned individually and presented with a feed treatment in a bucket to which they had been previously acclimatised. The time taken for each animal to consume the feed treatment presented was recorded, either until the feed was finished or until five minutes had elapsed. Immediately after each treatment had been completed, all rejected feed and faeces were recovered. The remaining feed was weighed and faeces counted.

The level of rodent faeces contamination had a significant effect on the time taken for cattle to consume feed and the proportion of feed rejected, with most feed rejected from high contamination treatments. Treatments with rat as opposed to mouse faeces took longer to be consumed and had higher amounts of feed rejected. However, despite rejection of contaminated feed and faeces, significant quantities of rodent faeces were ingested by cattle.

Thousands of rodent faeces per farm per day enter livestock feed in Scotland over the winter months. Despite selective rejection by cattle, feeding behaviour alone was not sufficient to avoid ingestion of significant amounts of rodent faeces. It was concluded that cattle were at risk of transmission of *Mptb* via stored feed contaminated by infected rodents.

**Poster Presentations**

Poster presentations relevant to JD in wildlife included evidence of JD in wild red deer in Belgium (Linden and Godfroid, Institute of Tropical Medicine, Antwerp). These posters described the presence of *Mptb* in wild red deer clinically affected with emaciation and bloody diarrhoea and a survey of hunter-killed adult red deer where 7.8% were found to be ELISA positive to *Mptb*. A poster titled Paratuberculosis in Wild Ruminants in the Czech Republic (Machackova, Veterinary Research Institute, Brno) described the prevalence of *Mptb* in 3147 wild and domestic red, roe and fallow deer and mouflons. *Mptb* has been isolated from 150 animals so far. The prevalence of JD in wild ruminants is low (0.7%) but is higher in game parks (2.8%) and higher still in farmed deer (14%).
Implications and Relevance to Australia:

- *M. ptb* has a broad host range and JD in several wildlife species in many countries has been demonstrated. The cycling of *M. ptb* in wildlife species independent of the presence of infected domestic ruminants has not yet been demonstrated anywhere in the world. However, it remains a distinct possibility with important implications for JD control programs.

- If wildlife are consistently capable of delivering an infectious dose of *M. ptb* back to domestic ruminants, then eradication of JD is currently not feasible, since it would require elimination of all susceptible species to achieve decontamination.

- If farmed species alone are destocked followed by restocking of uninfected animals during a PDEP, it may be several years before cross infection from wildlife back to domestic livestock is detected.

- A study of western grey kangaroos and tammar wallabies on Kangaroo Island has demonstrated a significant prevalence of histological lesions and the presence of *M. ptb* from the tissues of the macropods surveyed. How commonly JD occurs in macropods, and under what conditions, is currently unknown.

- The significance for the JD control program in sheep and cattle in Australia that JD can occur in macropods (and possibly other wildlife) is unclear, but it suggests that macropods are potential reservoirs for *M. ptb*. The level of risk to domestic ruminants will depend on the prevalence of JD in macropods, the strain type involved, the excretion rate of infected individuals, the size of the infectious dose required for macropods, sheep and cattle and the probability of exposure to this infectious dose.

- Further work is required in Australia to clarify whether or not macropods and rabbits are reservoirs for *M. ptb* under our grazing and climatic conditions. The change in prevalence of JD in macropods could be measured by continued field surveillance at sites already identified on Kangaroo Island. Quantitative faecal culture results from fresh macropod scats collected on both endemically infected and destocked properties should provide evidence about the capacity of infected macropods to contaminate pasture. Pen trials may also be necessary to determine what constitutes an infectious dose of *M. ptb* for macropods, rabbits and other potential wild hosts, to determine how readily the organism transmits within these species.
Report from the International Colloquium on Paratuberculosis
Bilbao, Spain 11-14th June 2002

EPIDEMIOLOGY OF JOHNE'S DISEASE

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Summary

There were over 70 papers or posters related to epidemiology in its broadest sense in the areas of strain variation, test validation/interpretation/procedures, country prevalence/incidence/occurrence data, animal health economics, cross species transmission/wildlife/plant/invertebrate reservoirs, risk factors, environmental survival, transmission and market assurance. There were several papers using computer models to understand/predict the epidemiology of JD or tests for JD. Most papers reinforced the knowledge already implicit in control programs in Australia and will be outlined briefly in this report.

Key Papers/Posters

Note: papers related to methods in molecular epidemiology, for example strain typing, and papers on epidemiological evaluation of diagnostic tests will not be covered here to avoid duplication of other reviews, but implications of such work will be summarised if considered relevant. (Letters/numbers in brackets refer to abstract numbers from the conference handbook)

Strain variation

Machackova et al (S4.01) (Czech Republic) reported paratuberculosis in ruminants caused by uncultivable strains. The data was confusing and it was unclear whether strains were uncultivable, grew slowly or were victims of methodology. There was certainly some evidence of variation in growth rates of various isolates, but whether these constitute different strains was very unclear. It is usual to see variation in growth rate from animal to animal within an infected flock/herd in Australia and this may relate to numbers of organisms present and methods as much as it might to strain variation.

Test validation, interpretation and procedures

Collins (S3.06) (USA) advocated use of likelihood ratios to facilitate more meaningful interpretation of diagnostic ELISA test results. The concept is analogous to accepting results as OD or ELISA ratio and taking action with individual cows based on the magnitude of the response. The assumption is that animals with higher responses are more likely to be infected and at higher risk for shedding.

Gardner et al (7.018) used Bayesian approaches to model ELISA test interpretation.

Van Schaik et al (S7.020) (Cornell USA) examined ways to use continuous (kinetic) ELISA output to interpret results for herd-level detection.

Van Schaik (S7.021, S7.022) modelled variation in ELISA and faecal test results over time for individual cows and found associations with parity and proximity to calving, with random cow effects. There was a delay between culture positive and ELISA positive status. Nielsen (S7.023) (Denmark) had similar ideas and concluded that inclusion of cow and population information would aid test interpretation.
Wells et al (S7.024) argued that ELISA interpretation should be based on the likely prevalence of infection in dairy herds: 33% of ELISA +ve cows were shedders where prevalence of shedding was <5% whereas 88% of reactors were shedders where the prevalence of shedding was >15%. This has implications for test and cull. Overall, only 40% of faecal shedders were ELISA positive.

Using ELISA as the basis for a test and cull program, Jubb et al (S7.02S) (Australia) noted a high rate of confirmation of paratuberculosis in culis (70-89%).

Dargatz et al (S3.07) (USA) compared ELISA performance across 5 labs in the US. Variation was of the order of 20%. This is comparable to the variation seen in Australia. There was a call for greater standardisation internationally using a standard panel of sera. Similar recommendations were made by Couquet (S3.P7) (France).

Ocepek et al (3.P6) (Croatia) reported that Corynebacterium pseudotuberculosis infection (CLA) can cause cross reactions in ELISA for JD in sheep.

Whitlock et al (S4.03) noted considerable variation in the ability of media sourced from various laboratories to support growth of the organism. This has serious implications for assessment of tests such as ELISA when culture is used as the gold standard. It may explain the wide discrepancies seen in the literature when culture and serology are compared in different laboratories.

Holmstrom et al (S4.P4) (Sweden) reported herd clustering of cases of mould contamination of faecal cultures. Contamination of culture is sometimes associated with particular herds/flocks in Australia.

Weber et al (S4.P6) (Netherlands) studied the cost effectiveness of re-culture of freshly collected faeces from cattle that had previously yielded a contaminated faecal culture result. They concluded that it was not cost effective to do so if contamination occurred >8 weeks after starting the culture; this was explained by the fact that by 8 weeks most culture positive samples have shown growth of Mtb.

Jubb et al (S7.02S) (Australia) analysed results from the Victorian test and cull program, which is based on ELISA, and estimated test sensitivity of 10-15%.

**Country reports: Occurrence/Incidence/Prevalence**

Several authors used milk tests to screen herds for paratuberculosis. Djonne et al (S5.010) (Norway) reported more widespread occurrence in goats in Norway than was thought to be the case (IM3-PCR).

Sevilla et al (S7.01) (Spain) conducted PCR on pellets from 200 bovine bulk milk samples and found 8-10% to be positive. Corti and Stephan (S7.P6) screened bulk milk in Switzerland using PCR and found 20% positive.

Several authors noted rising prevalence of paratuberculosis in their countries since 1990 (Yayo et al, S7.03, Czech Republic; Ocepek et al S7.02, Slovenia). There were many other reports of zero or culture prevalence within ranges already known or expected globally.

Sternberg et al (S7.034; S7.P5) (Sweden) presented data on bovine JD indicating very low prevalence associated with imported animals. The disease is notifiable and stamped out upon detection. There is an assurance program based on testing all animals > 24 months old by faecal culture, using pooling, repeated annually. JD is also uncommon in cattle in Norway (S7.P35).

There were also reports on occurrence, incidence or prevalence in various species from Argentina (S7.P1), Czech Republic (S7.P2), Uruguay (S7.P3), Italy (S7.P4), Brazil (S7.P6), Portugal (S7.P10), Spain (S7.P11) and Mexico (S7.P12).

There were papers on control approaches from Australia (sheep and cattle) (S7.031, S7.032, S7.035, S7.036, S7.037, S7.038, S7.P9), South Africa (S7.P29 – sheep), Spain (S7.P30 - goats), USA (S7.P34 - cattle) and Norway (S7.P35). A feature of many reports was the need for a protracted campaign.

In Japan there is a compulsory surveillance program of all adult cattle, tested every 5 years, with culling of reactors and compensation. Reactor cattle cannot be slaughtered in an abattoir (Yokomizo S7.P33).

**Animal Health Economics**

Abbott et al (S7.04) (Australia) reported objective mortality rates in sheep.

Kudahl et al (S7.06) (Denmark) modelled milk losses in relation to milk ELISA values and estimated losses of up to 1318 Kg energy corrected milk in second parity cows.
Chaffer et al (S7.07) (Israel) found little effect on milk production in seropositive cows (prevalence around 7%).

Cvetnic et al (S7.P23) reported annual losses of 2.7 to 7.4% due to clinical JD on a dairy farm in Croatia.

**Cross species transmission and wildlife, plant and invertebrate reservoirs**

Holstad et al (S7.014) (Norway) noted that transmission between goats and cattle in Norway was suspected, probably C strain.

Grieg et al (S7.010) (Scotland) reviewed the rabbit-carnivore-cattle interaction which is well known and has been published already.

Hutchins et al (S.013) (Scotland) studied the behaviour of cattle exposed to rabbit faeces on pasture and rodent faeces in feed and noted little avoidance. They concluded that rabbits posed a threat based on the potential for contact with cattle and the concentration of the organism in infected rabbit faeces.

De Lisle et al (S7.01) (New Zealand) reported isolation of S strain from wild ferrets in New Zealand and noted possible sources as sheep, farmed deer or possibly rabbits. Rabbits have not yet been sampled. Great concern was implied by the speaker’s tone.

In another fascinating account from the Czech Republic Pavlik et al (S7.012) recovered the organism from a wide range of environmental sites as well as invertebrates (worms, flies), and the leaf & stem tissues of plants grown in contaminated soil (lettuce, tomato, radish). Earthworms shed the organism in their faeces for up to 48 hours after exposure (passive shedding).


**Risk factor studies**

Roussel et al (S7.05) (Texas USA) used serology and faecal culture in cattle to identify infected cattle herds and reported significant risk factors as being breed (Brahman-non-Brahman), calf suckling on a dairy nurse cow, running water sources, with spatial clustering in certain parts of Texas.

**Survival of the organism**

Schroen et al (S1.05) (Vic torian Institute of Animal Science) presented work already well-known in Australia, concluding from trials in plastic trays in a lab that dry soil and high temperature reduced survival of the organism.

Katayama et al (S1.06) (National Institute of Animal Health, Shizuoka, Japan) presented an informative paper, the significance of which did not seem to register with participants. They studied the effect of ammonia concentration, moisture and temperature on survival of the organism in silage. Ammonia concentrations >1% were detrimental to the organism. These findings explain observations made decades ago that there is poor survival in slurry (a mixture of faeces, straw and urine) compared to faeces. These findings may have practical application and deserve greater attention. The same group (S7.P21) studies the effect of UV light at lab level and concluded that organisms could survive inside dung or behind grass (i.e. shaded) for months even under exposure to sunlight.

**Transmission**

Van Roermund & de Jong (S7.015) (The Netherlands) modelled transmission parameters (susceptible to latent to infectious) using existing data from 21 infected dairy herds and found that transmission was best explained if calves were also able to transmit infection. The implications did not make much biological sense.

Van Roermund et al (S7.016) modelled between herd spread to estimate the effectiveness of current surveillance in herds believed to be uninfected. Yearly pooled faecal culture of all cows > 2 years old resulted in transmission rates of <1 per infected herd, which was considered satisfactory. Testing all cows >1 year old every 2 years gave a similar outcome.
Valente et al (S7.P24) described a within-herd model for transmission in dairy cows.

**Market assurance/replacement stock**

Weber et al (S7.019) (The Netherlands) modelled variations to the current Dutch cattle assurance testing regime in an attempt to find something cheaper. Of 9 approaches examined, 4 herd examinations at 2-year intervals using pooled faecal culture with individual faecal culture of positive pools led to the lowest risk of infection being undetected and the lowest cost.

Gardner et al (S7.026) modelled the risk of introduction of paratuberculosis with replacement heifers. There was low benefit in testing using ELISA and faecal culture if animals were sourced from known low-prevalence herds, but there was increasing benefit as prevalence increased. Purchase from test negative source herds without further testing had lower risk than purchasing tested individuals from random sources.

**Implications and Relevance to Australia**

There was a wealth of epidemiological information presented at Bilbao, but mostly in areas already well covered in previous meetings. This reinforced confidence that concepts implicit in current control programs in Australia were consistent with international best practice. There was some very new material and of note and with relevance for Australia were:

- many calls for use of all the data (i.e. continuous data) (rather than just dichotomous positive or negative status) from bovine ELISA tests to be considered in test and cull and herd surveillance programs
- ongoing questions about the sensitivity of ELISA, particularly in low prevalence situations, with estimates as low as 10%
- variation in test performance between labs (both ELISA and culture)
- the low prevalence of bovine JD in Sweden and Norway, and strong efforts to maintain this situation, as we wish to do in Australia
- the high prevalence of JD in many species in many countries
- increased use of surveillance and market assurance testing in developed countries
- in Japan, a major trading partner for Australia, the compulsory surveillance program with test and cull (with financial compensation) for bovine JD, and removal of reactors from the human food chain
- objective estimates of economic losses in sheep and cattle
- increasing evidence of cross species transmission, infection of wildlife and the first evidence of environmental contamination within plant tissues, all of which have relevance for disease transmission and the control programs in Australia
- the very small number of informative risk factor studies
- an explanation from Japan for prior observations of poor survival of the organism in bovine slurry (straw/urine/faeces mixture), attributable to ammonia content, with possible application in supplementary feed preparation.
- several computer models of disease transmission and market assurance testing to complement those models developed in Australia
PUBLIC HEALTH IMPLICATIONS OF JOHNE’S DISEASE

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Summary

Although the public health session was a relatively short one, its significance to attitudes to, and investment in work on, M. paratuberculosis (Mptb) makes it deserving of a fairly full coverage. Following an overview paper there were another four papers on varying subjects related to isolation and typing of Mptb from people, pasteurisation efficacy and survival in cheese. Abstracts were published for 8 posters and a further poster on an epidemiological case-control study from Colorado was displayed.

Although no contrary material was presented or discussed, the overview presentation and those about isolation of the organism and treatment success added weight to the hypothesis that Mptb plays a role in Crohn’s disease in some patients. At the same time, it was proposed that some Mptb found in humans may not be of recent animal origin, but “humanised” strains and that sewerage may be a source of contamination of human water supplies. The opportunity should be taken in Australia to investigate the Crohn’s disease incidence and Mptb status of human populations potentially exposed, and not exposed, to animal Mptb, such as in south-eastern and south-western Australia.

Pasteurisation per se is highly effective in killing Mptb in milk but some reputable laboratories still report survivors after commercial pasteurisation of raw milk. The effectiveness of pasteurisation in the field will be affected by the initial contamination by Mptb and by the efficacy of the heat treatment, but also by engineering or plumbing factors. Knowledge of the occurrence of Mptb in Australian milk would assist the dairy industry manage JD and perceptions about milk as a source of Mptb.

Key Papers/Posters

Mycobacterium avium subspecies paratuberculosis (Mptb) and its relation to Crohn’s disease.
Hermon Taylor J. Dept of Surgery, St George’s Hospital Medical School London, UK. (Overview)

The overview by Prof John Hermon-Taylor was a biased presentation that supported the hypothesis that Mptb causes Crohn’s disease. Despite this weakness, the presentation highlighted recent findings that:

- as techniques improve in his laboratory, the rate of identification of organisms consistent with Mptb in Crohn’s patients has increased; it was claimed that 80% of cases are now positive.

- half of the 63 biopsy samples from Crohn’s patients in South Wales and of the 36 from Sweden (almost free of JD) and Finland (thought low JD prevalence) have tested positive in culture.

- Italian work identified Mptb in granulomas in Crohn’s patients.

- open treatment studies with rifabutin and clarylthromycin are reporting high rates of remission and recovery in Crohn’s patients.
- Genotyping of isolates from humans has found differences to animal types of *M. tuberculosis* suggesting that a "humanised" strain may be present in people.

- Treated sewage discharges into one UK water supply appear to contain DNA consistent with *M. tuberculosis*.

The presentation avoided discussing other findings that did not support the hypothesis.

**Human health**

**Reliable detection of MAP in human intestine by optimised PCR, MGIT culture and protozoan culture.** McMinn EJ et al. St Georges Hospital, London and Centre for Ecology and Hydrology, Lake Windermere, UK. (Paper)

This lab has now cultured 120 biopsy specimens for 6 months in the MGIT system, including 17/18 Crohn’s biopsies and 4/16 uninflamed gut sections in an open study. In a current double-blind study, *M. tuberculosis* has been isolated from 23/44 surgical resections in MGIT and has survived (and apparently replicated) in an amoeba, *Acanthamoeba polyphaga*.

**Genotyping of human isolates of MAP provides evidence of “humanised strains”**. Bull TJ et al. St Georges Hospital, London, UK and University of Central Florida, USA. (Paper)

Culture in MGIT of 7 US isolates found 6/7 to be MPIL type 3 (RFLP type B/C5) but lacking the IS900 sequence at locus 5 resulting in the *desA1* gene remaining intact, suggesting that there may be a type of *M. tuberculosis* adapted to humans. It was noted that culture in MGIT should be run for at least 10 mths.

**Fistula healing in MAP positive Crohn’s disease patients following rifabutin and macrolide antibiotic treatment.** Shafran I et al. University of Central Florida, Orlando, FL, USA (Two abstracts and poster)

Two scheduled oral presentations by Shafran were not given but there was a similar poster. The abstracts report that treating Crohn’s patients with clarithromycin and rifabutin found a 60% response in 16 weeks and 80% in two years. Most patients were reported to have healed and gone off anti-inflammatory drugs or immunotherapy. Treatment of patients over an average of 12 months (range 3-17 mths) with these drugs and a lactobacillus probiotic reported 26/42 maintaining clinical remission without other medication. Fistulae healed in 5 patients.

**Regional case-control study of Crohn’s disease.** Hirst HI et al. Colorado State University and Centres for Epidemiology and Disease Control, Fort Collins, CO, USA. (Poster, no abstract)

To identify potential risk factors for Crohn’s disease across three age groups (child, teenage, adult) a questionnaire was sent to 2,000 members of the Crohn’s and Colitis Association (response 33%) and 1,000 of the Multiple Sclerosis Society in Colorado (19%). Using a case-control approach with 320 Crohn’s cases and 453 controls (latter from both groups), a logistic regression model found only one significant risk factor: ie working in finance, insurance or real estate as an adult. Factors such as exposure to animals and eating dairy products did not increase risk. A model to analyse cumulative exposure is now being developed.

**Routes of exposure**

**Impact of commercial HTST pasteurisation on Mycobacterium avium subsp. paratuberculosis in naturally infected cows’ milk.** Grant IR et al. Queen’s University of Belfast and Dept of Agriculture and Rural Development, Belfast, N. Ireland, UK. (Paper)

As well as summarising the 2000 UK milk survey, this paper presented the results of pasteurising work on Irish milk, in which 10 of 144 (6.7%) commercially pasteurised milk samples from two heavily infected herds were culture positive. It was noted that larger milk samples were cultured (50ml) and milk was allowed to rest at 4 degrees for 24-72 hours after pasteurisation before culture, both of which appeared to increase recovery rates.

In hard and semi-hard raw milk cheeses, D values (time for one log reduction in numbers of live organisms) of 28 days and 45 days respectively were reported. These are consistent with earlier US work that reported 60 days for soft raw milk cheeses. Factors affecting the viability of *Mptb* are the initial cooling of the cheese (eg 50 degrees for 40 mins), increasing pH with ageing and salt concentration.

Survival of *Mycobacterium avium* ssp. *paratuberculosis* at high temperature short time pasteurisation in a pilot plant pasteuriser at elevated temperatures and extended holding time. Hammer P et al. Federal Dairy Research Centre, Institute for Hygiene and Food Safety and Institute for Dairy Chemistry and Technology, Kiel, Germany. (Poster)

Heat treatment of inoculated raw milk in a spiral holder tube at 72-90 degrees for 40-60 secs reduced concentrations by 5 to 6 logs but survivors were cultured in 6-8 weeks in 45/48 experiments. It was proposed that there may be some heat activation of some *Mptb* cells. (Note: level of turbulence generated in system uncertain)

The recovery of *Mptb* following heat treatment of inoculated milk in a turbulent-flow pasteuriser is not adversely affected by decontamination and antibiotic selection. Pearce LE et al. NZ Dairy Research Institute, Palmerston North and AgResearch, Wallaceville, NZ. (Poster)

Inoculated milk samples treated at 62 to 72 degrees for 15 secs were evaluated to see if recovery was affected by decontamination and antibiotics in culture in Bactec and on Herrold's egg yolk. Bactec was superior to HEY and decontamination had no effect. Recovery was improved by antibiotic selection (eg PANTA PLUS in Bactec). As previously found in this turbulent system, there were no survivors at 72/15, indicating a 7-10 log kill of *Mptb*.

Implications and Relevance to Australia:

**Human health**

The rate of culture of *Mptb* and of detection of DNA consistent with *Mptb* in Europe has increased in recent years with improved techniques. Similarly, open treatment studies indicate that rifabutin/clarithromycin treatment is improving the health of patients. On the other hand, the Colorado study found that exposure to animals and dairy products did not increase risk for Crohn's disease. The findings of the organism in Swedish and Finnish patients at the same rate (50%) as those from South Wales were unexpected, given that the exposure to *Mptb* from animals should be much lower in the two Scandinavian countries. Though still not conclusive, the session overall added weight to the hypothesis that *Mptb* is playing a role in the pathogenesis of Crohn's disease. The origins and host specificity of the *Mptb* in Crohn's patients is now being queried with "humanised" strains proposed.

These findings are likely to increase concern that *Mptb* is involved in Crohn's but also increase attention to a possible human strain cycling in people. Arguments to control JD as a potential human health issue are unlikely to abate.

It could be argued that, while most of the work on *Mptb* in people is conducted in Europe and North America, *Mptb* is likely to be detected because the infection is endemic in cattle and, to some extent, other animal species. Australia is in the unique position of having two similar areas of the country with distinctively different JD status: south-west and south-east Australia. There are large urban populations with similar demographics, for instance in Perth and Melbourne, which should have very different levels of exposure to *Mptb* of animal origin, if in fact there is any exposure of urban people occurring.

Major contributions to the understanding of the possible role of *Mptb* in Crohn's disease could be made by:

- measuring and comparing the incidence of Crohn's disease in these populations (taking into account travel, exotic dairy food consumption), and
- investigating biopsy specimens for the presence of \textit{Mptb}.

Differences in adjusted incidence rates could be an indication of attributable risk if no \textit{Mptb} was identified in WA. On the other hand, detection of \textit{Mptb} in WA cases would present a challenge to our current understanding of \textit{Mptb} which may lead to a better understanding of \textit{Mptb}. Such research will be discussed with NHMRC and others as part of the proposed NBJDP. The results of the Australian double-blind treatment trial are not expected until early 2005.

\textbf{Routes of Exposure}

Results on the efficacy of pasteurisation under commercial type conditions continue to differ with NZ work showing complete killing and Irish work detecting survivors. The German survival results require further scrutiny. In summary, pasteurisation is highly effective but there is evidence in the UK of survival following the factory pasteurisation process. It is recommended that Australia investigate the rate of occurrence of \textit{Mptb} in raw and pasteurised milk. Observations of the apparent recovery of heat-damaged \textit{Mptb} allowed to rest for 48 hours after pasteurisation should be taken into account in any further work here.

Another notable item in this session was the reported survival and perhaps replication of \textit{Mptb} in amoeba. If this is occurring in nature, it would increase the profile of water as a route of exposure to animals and people.
Report from the International Colloquium on Paratuberculosis
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IMPACT OF CONTROL PROGRAMS ON SHEEP PRODUCERS

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Implications and Relevance to Australia

The 7th International Colloquium on Paratuberculosis was significant in that there was little progress in the area of control programs nor information that could be added to the knowledge we already have in Australia with regard to control and eradication of OJD.

There were no breakthroughs in the key areas required (improved tests, decontamination of the environment, relevance of different strains, length of time required to decontaminate soil etc) to give us improved techniques to seriously consider that eradication of OJD is possible now, or in the near future. In fact one could argue that there were a number of areas where concern was expressed over current knowledge and practices that apply to Johne’s disease (JD) such as cross infection, other hosts, wild life, current tests, decontamination etc.

It is quite apparent that decisions on how we manage OJD from here on will be made with the limitations that we currently have. The current programs are not providing acceptable solutions to the problems that are being created. It is more likely that continuation of the current approach to the management of OJD will contribute more to the failure of Australia to successfully control OJD than if we did nothing at all.

There are no magic cures or major breakthroughs on the horizon to make the task of managing OJD any easier. Essentially successful control of OJD will depend on the level of cooperation that we get from the Australian sheep industry. The real question is what will it take to deliver that level of cooperation?

There is no doubt that Australia is leading the world in the area of research into JD, particularly in the area of applications and understanding of disease minimisation strategies on farm. This is one area that we will expand on our knowledge in the short term. Australian research programs specifically looking at improving OJD minimisation on farm and at what steps can be taken to lessen the impact of OJD in flocks, have the potential to greatly enhance affected sheep producers ability to control the disease. However there needs to be more work done on how these results can be implemented into a farm management plan.

There is a huge imbalance between the resources involved in the research and development side of OJD compared to the implementation of programs on farm. There needs to be additional resources channelled into solving some of the current problems created by the existing program.

There is a critical need to change how OJD is managed in Australia, so as to regain the confidence of the sheep industry, and furthermore attend to the desperate needs of those already caught up in the current program.

There is so much confusion surrounding the current management of OJD in Australia that the sheep industry has lost confidence in the program, and furthermore, that to participate in the program is too great a risk to their business.
The message that is being communicated to sheep producers is that there is a significant risk associated with testing and being part of the Market Assurance Program, the stakes are too high and far outweigh any apparent benefit. The sheep industry cannot afford to fund an acceptable financial assistance package that will allow the current program to continue.

The practice of pitting sheep producer against sheep producer as is happening must stop, as it is only serving to split an industry that needs to work together if progress is to be made. All states need to agree on a uniform approach to managing OJD - they all need to accept that OJD is a sheep industry problem and not just one state's problem alone. Putting up artificial barriers is not the answer nor should it be used as a means to justify a state's lack of involvement in finding solutions.

So what do we do from here on?

- The first issue to tackle is to communicate to the sheep industry that eradication of OJD is not currently possible, nor is it likely to be in the near future.
- The next step is to clearly articulate to all what our objectives are.
- Currently there is no incentive for infected sheep producers (not suffering high mortality rates) to lower their level of infection.
- There needs to be a system of disease status progression for infected sheep producers so that they can justify implementing a disease management plan. This will ultimately lead to a lower level of infection in the environment and help protect against further spread from infected properties.
- The current program does not justify why an infected flock is a risk to the sheep industry and is quarantined. Sheep producers must have some input into determining the status of their flocks and what options they have.

If eradication of OJD is not an option, then control of the disease is the only alternative available. The question is how do we control OJD and what procedure do we use?

There needs to be considerable discussion on the following issues:

- What constitutes an infected flock?
- What is the relevance of a low level of infection in a responsibly managed flock?
- Should all infected flocks be classified the same?
- How can trading options be created for infected flocks?

It would achieve more if all OJD infected flocks were set a goal to reduce their level of infection by 1 or 2% rather than concentrating on trying to eliminate infection on a few properties.

The bar has been set too high, we need to lower the bar, take smaller steps, move away from destroying businesses and look at ways of getting cooperation.

There is an alternative, and that is, if it is too difficult, and we can not get agreement on how to move forward, then remove OJD from the Notifiable Diseases Register. Continue on with research programs to help in the management of OJD on-farm which is still supported by the majority of sheep producers, provide technical support and let market forces dictate what needs to happen as with most other diseases.
UPDATE FROM THE UNITED KINGDOM

SUMMARY OF OJD-RELATED PAPERS PRESENTED AT THE UK SHEEP VETERINARY SOCIETY MEETING

MAY 2002
Report from the UK Sheep Veterinary Society Meeting
May 2002

UPDATE FROM THE UNITED KINGDOM

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Conference attended: Sheep Veterinary Society meeting, UK. May 2002

Title of paper presented

“Estimation of mortality rates from paratuberculosis in a high prevalence flock in NSW Australia.”

A report of the preliminary results from a study to estimate the crude mortality and the OJD-attributiable death rate in a suspected high prevalence flock using a novel method requiring four sampling periods per-annum in which the flock was visited every day for five consecutive days and all dead sheep necropsied. The crude and OJD-attributable mortality rates will be estimated over several years following whole flock vaccination to monitor changes in the death rate in the flock following vaccination and to examine the relationship between flock seroprevalence, faecal excretion rate and mortality rate.

Key Papers in Johne’s disease section

2. “Johne’s Disease, general introduction and zoonotic potential.” Irene Grant. Queen’s University, Belfast, Northern Ireland.

Overview of papers presented

“The future of the UK sheep industry.” John Thorley, National Sheep Association, UK.

The impact of FMD has left many areas of the UK struggling to rebuild a devastated rural economy and industry. Non-farming organisations are adopting certain aspects of the rehabilitation process, as it is widely viewed that the UK must no longer be regarded to be in the grip of the crisis. Intervention on the part of the tourist industry and conservation groups will ensure that the British countryside is restored to its former glory. The tourist industry is actively working to re-establish the picture postcard image of the British rural landscape supported by the view that many of the hill and upland areas of the UK will only be adequately maintained in the future if livestock are returned to them. Active importation and redistribution of livestock within Britain carries the risk of spread of transmissible disease through the UK’s open internal rural market.

The outlook from the industry point of view is positive but there is open recognition of the need for increased surveillance and vigilance to prevent the spread or importation of further notifiable disease.

The difficulty of sourcing satisfactory genetic stock often overshadows disease importation concerns. Restocking with an economically viable livestock population is costly and time consuming, with often only
basic risk management procedures followed. Government and veterinary surgeons are providing guidance for producers in risk management through restocking, including a specific plan for JD.

The unresolved issue of the zoonotic potential of paratuberculosis for transmission in pasteurised dairy products continues to be of concern both in industry and government. The relationship between Crohn's disease and paratuberculosis continues to attract funding and attention. It is recognised that there are likely multifactorial causes for the development of the disease, possibly including genetic susceptibility, a currently undefined environmental trigger and immune hypersensitivity. Collection of data from histopathology and epidemiological studies is continuing.

The epidemiology of paratuberculosis is poorly explored or understood in the UK. Environmental spread issues and the role of wildlife and domestic species in the transmission of the disease is creating concern amongst producers following the identification of cattle strains in the faeces of rabbits. The same strain types have been identified in cattle and rabbits grazing the same areas. Anecdotally, it has been observed that in some Scottish herds investigated there seemed to be a correlation between the size and proximity of the rabbit populations to the degree of paratuberculosis related disease in the herd. It has been established that cattle do not necessarily selectively graze around rabbit faeces under common UK farming conditions but may ingest relatively large quantities of potentially infective faeces. The interaction between domestic species and wildlife in the transmission of Mtb is the subject of current collaborative research within Europe. The role of the national flock in the epidemiology of the disease is regarded more as that of a carrier along with goats and the camelid species, rather than a primary host or a concern within their own industry.

An overview of the disease worldwide

Mortality figures and discussion of the economic contribution paratuberculosis can make to on-farm losses both in the form of deaths from OJD and animals dying where management related disease may have been accelerated by the presence of moderate to severe OJD. The investigation of mortality rates explored, with the introduction of the concept of crude and OJD attributable mortality rates. Focus on the disease in Australia, with discussion of the investigation of mortality rates holding political value in enabling affected producers to implement control with vaccination, and the immediate need to assess the true contribution of the disease to production losses for a more valuable economic assessment and implementation of alternative control options.

Points of discussion/further interest

Epidemiology and pathogenesis

Large gaps are recognised in knowledge of this area of the disease in the national flock. This is exacerbated by producer perception of the disease, the value of individual animals and the structure of the sheep industry in the UK. It is recognised that there is wide variation in climate and industry structure between the UK and other affected countries and there is a need to generate UK specific data, particularly for the implementation of successful surveillance and control programs. Many producers still regard OJD as a disease that affects other countries and is not likely to be a significant problem in the UK due to the differences in industry structure and priority.

Diagnostic tests/surveillance

The sensitivity of current tests is a cause for concern with producers. Implementation of testing and control programs without satisfactory evidence of improved monitoring and identification techniques is unlikely to be well received by producers in the wake of FMD control regimes.

Vaccine use

The use of an imported live vaccine is limited in the UK. Vaccine use is focused mainly in the dairy industry where both the economic and control benefits are more easily demonstrated. Vaccination in the sheep population has been demonstrated to be useful in controlling the disease but the economics of widespread use are not attractive.
Implications and Relevance to Australia:

1. The long-term impact on rural industry and the government following the importation of exotic disease into a naïve population.

2. Techniques for the development and implementation of successful and sustainable disease surveillance programs including the value of producer confidence in testing regimes and test reliability.

3. The implications for the dairy industry worldwide of the zoonotic potential of paratuberculosis and its role in Crohn’s disease.

4. Epidemiological investigations, particularly with reference to domestic-wildlife interaction and interaction between the different domestic species. Establishing the relationship between a primary host and its environment. Strain typing within these populations.
PROGRESS REPORTS
ON
AUSTRALIAN
OJD R&D PROJECTS

MANAGED BY
MEAT AND LIVESTOCK, AUSTRALIA

FOR THE
NATIONAL OVINE JOHNE'S DISEASE CONTROL AND EVALUATION PROGRAM
NOJDP RESEARCH PROGRESS REPORT

CROSS SPECIES TRANSMISSION OF OVINE JOHNE'S DISEASE: PHASE 2 - CATTLE

PROJECT NUMBER: OJD.016

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Background and Objectives

Australian Johne’s disease (JD) control and assurance programs assume that sheep and cattle strains of Mycobacterium paratuberculosis (Mptb) are epidemiologically distinct infections. Four cases of JD in cattle from Central Tablelands properties, which occurred from 1989 to 1995, were retrospectively typed as S strain in 1998. Follow-up investigations in 1999 detected another two subclinical animals from one of these properties.

This project aims to assess the prevalence of disease in cattle exposed to sheep infected with OJD, and to determine likely risk factors associated with cattle developing infection (or passive transfer) of OJD and hence modifications that may need to be applied to OJD control/eradication plans.

Methods and Results

1808 animals from 12 properties were sampled by ELISA to detect antibodies against JD and by faecal culture to detect shedding of organisms. One animal was found to be culture positive (S strain) and all were serology negative. Follow-up investigations of that animal were all negative.

Additionally, two properties undergoing CattleMAP testing outside the project had reactors, which were confirmed as S strain and histologically positive. Animals from both properties had exposure to OJD infected sheep.

An estimate of prevalence in susceptible cattle on OJD infected farms was made using a BJD risk assessment model (Cannon & Garner). Some risk factors were descriptively reviewed.

Figure 1: Estimated Herd Prevalence of OJD in Susceptible Exposed Cattle
(Estimates calculated using Cannon & Garner's BJD Risk Assessment Model, based on estimated 200 exposed cattle herds within Central Tableland's RLPB)

<table>
<thead>
<tr>
<th>Within-herd Prevalence</th>
<th>Equiv. no. 100% sensitive herd tests</th>
<th>Estimated OJD exposed herd prevalence</th>
<th>Estimated number of infected herds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>median</td>
<td>upper 95%</td>
</tr>
<tr>
<td>1.00%</td>
<td>4.2</td>
<td>15%</td>
<td>51%</td>
</tr>
<tr>
<td>2.00%</td>
<td>5.7</td>
<td>11%</td>
<td>41%</td>
</tr>
<tr>
<td>2.50%</td>
<td>6.1</td>
<td>11%</td>
<td>38%</td>
</tr>
<tr>
<td>5.00%</td>
<td>7.2</td>
<td>9%</td>
<td>33%</td>
</tr>
</tbody>
</table>
Conclusions to Date

The prevalence of OJD in exposed susceptible cattle is very low, but it does occur. S strain infection of cattle may be an emerging problem in OJD endemic areas.

Where cattle are being reared in OJD endemic areas, care should be taken to minimise contact with infected sheep or manure, particularly during the first 12 months of life.

Cattle MAP guidelines should include the possibility that OJD will transmit to cattle, particularly in OJD endemic areas.

Where cattle have had significant contact with infected sheep, serological screening of cattle may be necessary as part of an OJD surveillance and control program.
EXPOSURE FACTORS LEADING TO ESTABLISHMENT OF OJD INFECTION AND CLINICAL DISEASE

PROJECT NUMBER: OJD.002A

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Background and Objectives

The principle objective of this study is to relate the origin and timing of exposure to Mptb to the development of infection in a cohort of young sheep. The experiment will demonstrate if, and how, pastures of low infectivity can be prepared and effectively used to reduce the level of OJD infection, potential production losses and mortalities from the disease. With this knowledge steps may be able to be taken by producers to protect lambs from infection at the most critical stages and thereby reduce the prevalence and economic cost of the disease in a flock.

Methods and Results

The project commenced in Sept 1999, with progeny born onto the site to identified infected or clean dams in Oct/Nov 1999. The field component of the trial will terminate in September 2002 with the slaughter and postmortem examination of all remaining animals on site. Tissues will be collected for culture and histopathology.

Birth and weaning paddocks are of known infection status. Lambs will remain in weaning paddocks until the experiment is terminated in September 2002. Animals are blood and faecal sampled at 3 monthly intervals. Body weights are recorded at 3 monthly intervals from weaning until euthanasia. Wool weights will be recorded on 3 occasions at shearing each year.

To June 2002, 26 sheep have died and been examined at post-mortem. Gross pathology is suggestive of the presence of OJD infection in most cases.

Culture of faecal specimens taken from all animals at 12 and 16-17 months old are near completion. Early results suggest that there is a higher rate of faecal culture positive animals (12% to 15%) in those exposed to infected ewes compared to those exposed only to infected pastures pre-weaning (5%) or post-weaning (3%).
Conclusions to Date

Interim results suggest that:

1. Sheep exposed to OJD before weaning have a higher risk of dying before 32 months of age than sheep exposed only after weaning.

2. Sheep born in infected ewe flocks have a higher risk of dying before 20 months of age than sheep born in uninfected ewe flocks on contaminated pasture.

3. Sheep born in infected ewe flocks have a higher rate of faecal excretion at 18 months of age than sheep born in uninfected ewe flocks on contaminated pasture.
EWE/LAMB TRANSMISSION AND COMPARISON OF DIAGNOSTIC TESTS

PROJECT NUMBER: 0JD.017

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Background and Objectives

To control OJD, accurate information is needed on the mode(s) of transmission of infection between sheep. Most of the information on transmission of OJD has been extrapolated from the disease in cattle.

Intrauterine and transmammary transmission have been studied in cattle. Several studies have shown intrauterine transmission rates of 26-35% for clinically affected cattle, and 10% for subclinical cattle. Studies on transmammary transmission have shown that 22% of subclinically infected cattle excrete the organism in their colostrum, while 3% excrete in their milk, and up to 45% of clinically affected cattle excrete the organism in their milk. While there is a single report of intrauterine transmission in sheep, there have not been any studies to estimate the rates of these modes of transmission in sheep.

This study aimed to determine whether foetuses of ewes with OJD are infected prior to birth, and then to correlate this with the stage of disease in the ewe. The study also aimed to determine whether M. paratuberculosis is present in the milk/colostrum of OJD infected ewes. And finally a comparison of the diagnostic tests used was undertaken.

Methods and Results

Comparison of diagnostic tests

A variety of diagnostic tests, including gel test (AGID), delayed-type hypersensitivity (DTH skin test) using avian PPD and gamma-interferon test using avian PPD were carried out on 124-145 ewes over a period of 1 year, before the entire flock was post mortem ed. At PM faecal and tissue culture and histopathological examination were performed. Comparative results of the test are summarized below:
Figure 1: Specificity and Sensitivity of Diagnostic Tests

<table>
<thead>
<tr>
<th></th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGID – Aug 00 (3 yr old)</td>
<td>100% (1/1)</td>
<td>2.6% (1/38)</td>
</tr>
<tr>
<td>AGID – Aug 01 (4 yr old)</td>
<td>83.3% (5/6)</td>
<td>12.5% (5/40)</td>
</tr>
<tr>
<td>DTH skin test (avian PPD)</td>
<td>40.7% (11/27)</td>
<td>28.9% (11/38)</td>
</tr>
<tr>
<td>IFN-γ (avian PPD)</td>
<td></td>
<td>Awaiting analysis</td>
</tr>
<tr>
<td>Histopathology</td>
<td>77.8% (28/36)</td>
<td>58.7% (27/46)</td>
</tr>
<tr>
<td>Individual faecal culture</td>
<td>100%</td>
<td>19.6% (9/46)</td>
</tr>
<tr>
<td>Tissue culture</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Ewe/lamb transmission**

Tissues were collected at PM from ewes and foetuses. A total of 51 out of 150 (34%) ewes had one or more tissues/faeces culture positive. Tissues from the foetuses of infected ewes (culture positive and/or hist positive) were then cultured and examined histopathologically. To date some intrauterine infection has been found, but results are awaiting final confirmation. No histopathological lesions, similar to the granulomatous lesions seen in adults, were seen in the foetal samples examined.

Milk samples were also collected and cultured. None of the milk samples have been culture positive. This result may reflect the poor quality of samples, as it is difficult to obtain a milk sample from sheep at PM, prior to parturition.

**Conclusions to Date**

The performances of the various tests used are seen above, with the results for the IFN-γ test still to be analysed.

The results from the ewe/lamb transmission work indicate that intrauterine transmission does occur in sheep, but confirmed results are yet to be analysed. When results are complete further conclusions may be possible. The results from the culture of milk from sheep are difficult to draw conclusions from, given the poor quality of samples obtained.
NOJDP RESEARCH PROGRESS REPORT

(I) A LONGITUDINAL STUDY OF OJD AND THE EFFECTS OF WHOLE FLOCK VACCINATION WITH GUDAIR

(II) BIOLOGICAL AND ECONOMIC IMPACTS OF OJD IN NSW FLOCKS

PROJECT NUMBERS:

(I) OJD.015

(II) OJD.023

(I) H Mcgregor, P Windsor, K Abbott, A Britton

(II) R Bush, J.A Toribo, P Windsor

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University of Sydney
PMB 3, Camden NSW 2570

Background and Objectives

Obtaining an accurate estimate of both the crude mortality rate in a flock and the proportion of the mortality directly attributable to OJD is considered important. The proposition that OJD may increase susceptibility to mortality from other causes, or that the presence of other pathogenic processes may increase the susceptibility of the flock to OJD, remains unresolved.

These two studies aim to estimate the crude mortality and the OJD-attributable death rates in selected NSW flocks using a method requiring four sampling periods per annum in which the flock is visited every day for five consecutive days and all dead sheep necropsied. The relationship between flock seroprevalence, faecal excretion rate and the mortality rate will be examined. We report here the preliminary results of these studies.

Methods and Results

(I) OJD.015: each study year commences with the annual shearing in August. Year 0 commenced in September 1999.

Preliminary mortality rate estimations:

In the year preceding vaccination, 43% of adult sheep deaths were from OJD alone and the disease was present in a further 50% of cases. In the 1st year after vaccination, 56% of the adult sheep deaths were caused by OJD alone, with the disease present in a further 40% of cases. From these figures and crude mortality estimations we have derived an "OJD attributable death rate" of 14.5% (±3.0) for the year preceding vaccination and 13.2% (±3.2) for the year post vaccination.
Figure 1: Longitudinal Study Preliminary Results

<table>
<thead>
<tr>
<th></th>
<th>Pre-vaccination</th>
<th>Post-vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total mortality rate</td>
<td>21.5%</td>
<td>17.8%</td>
</tr>
<tr>
<td>Mortality rate attributable to OJD</td>
<td>14.5%</td>
<td>13.2%</td>
</tr>
</tbody>
</table>

(II) OJD.23: Twelve OJD-infected flocks in 4 areas of southern NSW (Bungendore, Taralga, Gunning & Harden) with reported mortality rates >5% are enrolled in this study. To minimise vaccination effects these properties have not vaccinated sheep older than 1-year of age. A questionnaire is used to collect property and flock information and producer records to collect flock inventory information. Seasonal pasture variation is measured using pasture samples and monthly rainfall distribution.

Preliminary results based on gross pathology for the first two collections (autumn and winter of 2002) indicate the majority of animals died with or from OJD.

Figure 2: Preliminary results for autumn and winter collection periods

<table>
<thead>
<tr>
<th>Collection period</th>
<th>No. of sheep examined</th>
<th>Presumptive cause of death (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OJD alone</td>
</tr>
<tr>
<td>Autumn</td>
<td>65</td>
<td>55.5</td>
</tr>
<tr>
<td>Winter</td>
<td>152</td>
<td>51</td>
</tr>
</tbody>
</table>

Conclusions to Date

(I) OJD.015: The results to date confirm a high level of infection in this flock. The effects of whole flock vaccination are expected to become more apparent as the trial continues over the next 18 months.

(II) OJD.023: Considerable losses due to OJD have been observed on all 12 farms.
VALIDATION OF THE GAMMA INTERFERON TEST FOR DIAGNOSIS OF OVINE JOHNE’S DISEASE

PROJECT NUMBER: OJD.025

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Background and Objectives

The gamma interferon (IFN-γ) test is being evaluated in field trials to determine a possible application in the national ovine Johne’s disease (OJD) control program. The IFN-γ test has the potential to identify infected sheep earlier than bacterial culture and antibody tests and prior to exposure of other livestock since a cell-mediated immune response occurs before shedding and seroconversion.

The objectives are to: standardise antigen potency; define the cut-point criteria for a positive reaction; determine the specificity and sensitivity of the IFN-γ test in sheep flocks; define the relationship between IFN-γ responses at different time points over a 3-year period and final infection status.

Methods and Results

Specificity trials have been completed on 3 uninfected Merino flocks (2 in NSW and one in WA), each containing 120 sheep (40 lambs, 40 yearlings and 40 3-year old ewes). Three flocks (one each in NSW, Vic and SA) remain to be tested.

**Figure 1: Estimated Specificity of IFN-γ Test in 4 Different Regions**

<table>
<thead>
<tr>
<th>Cut-point</th>
<th>Riverina flock</th>
<th>WA flock</th>
<th>Dubbo flock</th>
<th>Longitudinal trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-N &gt;= 0.05</td>
<td>100</td>
<td>95</td>
<td>99.2</td>
<td>98.8</td>
</tr>
<tr>
<td>J-N &gt;= 0.05</td>
<td>99.2</td>
<td>92.5</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>J-A &gt;= 0.05</td>
<td>100</td>
<td>99.2</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>J-N &gt;= 0.05</td>
<td>98.3</td>
<td>95.8</td>
<td>98.3</td>
<td>99.1</td>
</tr>
<tr>
<td>J-A &gt;= 0.05</td>
<td>98.3</td>
<td>98.3</td>
<td>99.2</td>
<td>100</td>
</tr>
<tr>
<td>Mitocen-N &gt;= 0.05</td>
<td>88</td>
<td>55</td>
<td>92.9</td>
<td>63</td>
</tr>
</tbody>
</table>

1 CSL A Johnin PPD
2 CSL B Johnin PPD
A sensitivity trial has been conducted in collaboration with NSW Agriculture (also part of another NSW Agriculture project - OJD.024). Histopathology and radiometric culture of intestinal tissues was performed by EMAI on 145 necropsied pregnant 4-year old ewes in a commercial flock, exposed since birth. IFN-γ test kits (Bovimag™) with Avian and Johnin PPD are being provided by CSL Limited. Estimated sensitivity of the IFN-γ test, in comparison to culture and histopathology interpreted in parallel, was about 67% and 52% for the cut-points Johnin-Nil ≥ 0.05 and Johnin-Avian PPD ≥ 0.05, respectively. The predictive value for a positive result was 55% and 70% for these cut-points. The number of apparent false positives was reduced from 33% to 13% when the avian PPD background was subtracted from the Johnin response (J-A ≥ 0.05). In 3 uninfected flocks and in the flock prior to exposure to OJD in the longitudinal trial, specificity was > 98%. The longitudinal trial is being conducted in collaboration with the University of Sydney (also part of another University of Sydney project - OJD.028).

**Figure 2: Estimated sensitivity of IFN-γ test**  
\[ J-N \geq 0.05 \]

<table>
<thead>
<tr>
<th>Reference standard</th>
<th>Number</th>
<th>J-N &gt;= 0.05</th>
<th>IFN +ve</th>
<th>Sensitivity %</th>
<th>CI 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture +ve</td>
<td>46</td>
<td>30</td>
<td>65</td>
<td>49.8 - 78.6</td>
<td></td>
</tr>
<tr>
<td>Histopathology +ve</td>
<td>36</td>
<td>29</td>
<td>81</td>
<td>64.0 - 91.8</td>
<td></td>
</tr>
<tr>
<td>Culture or histopathology +ve</td>
<td>54</td>
<td>36</td>
<td>67</td>
<td>52.5 - 78.9</td>
<td></td>
</tr>
<tr>
<td>Total IFN +ves</td>
<td></td>
<td>66</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1CI 95% binomial confidence interval

**Figure 3: Estimated sensitivity of IFN-γ test**  
\[ J-A \geq 0.05 \]

<table>
<thead>
<tr>
<th>Reference standard</th>
<th>Number</th>
<th>J-A &gt;= 0.05</th>
<th>IFN +ve</th>
<th>Sensitivity %</th>
<th>CI 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture +ve</td>
<td>46</td>
<td>25</td>
<td>54</td>
<td>39.0 - 69.1</td>
<td></td>
</tr>
<tr>
<td>Histopathology +ve</td>
<td>36</td>
<td>23</td>
<td>64</td>
<td>46.2 - 79.2</td>
<td></td>
</tr>
<tr>
<td>Culture or histopathology +ve</td>
<td>54</td>
<td>28</td>
<td>52</td>
<td>37.8 - 65.7</td>
<td></td>
</tr>
<tr>
<td>Total IFN +ves</td>
<td></td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1CI 95% binomial confidence interval
Conclusions to Date

In the sensitivity trial, the relatively high number of apparent false positives indicated that either the reference standards for detection of infection were relatively insensitive or more probably that the interferon-γ memory response may persist in sheep that have completely eliminated the infection. Since the IFN-γ test had high specificity in unexposed sheep, it is likely that the positive interferon responses, in the tissue culture negative and or histopathologically negative sheep, detected prior exposure in sheep that are no longer infected. Since the IFN-γ assay also has reasonable sensitivity, the test shows promise as a screening test for detection of OJD and for certifying flock freedom from disease. Further trials are in progress to provide additional information on diagnostic test accuracy and the most appropriate cut-point.
NOJDP RESEARCH PROGRESS REPORT

AN AUSTRALIAN EVALUATION OF GUDAIR™ OJD VACCINE

PROJECT NUMBER: OJD.009

JEFF EPPLESTON
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Background and Objectives

Overseas field experiences and limited experimental data have shown the potential for vaccination against \textit{M. ptb} to reduce the on-farm impact of OJD in sheep flocks. The objective of this study is to determine the impact of vaccination with Gudair™, a killed \textit{M. ptb} preparation, on cellular and humoral immunity, faecal shedding of \textit{M. ptb}, OJD mortality rate, condition score, bodyweight, wool productivity and vaccine site lesions, in Australian Merinos run under Australian pastoral conditions.

Methods

On each of 3 properties experiencing significant OJD losses, 200 Merino lambs were vaccinated with Gudair™ at 1-4 months of age, while 200 control lambs were sham vaccinated with saline. Experimental animals are run together and assessed every 3 months and sampled (blood & faeces) every 6 months.

Results and Conclusion to Date

Immunological data indicate significant stimulation of both the cellular & humoral immune systems in the majority of vaccinates, however, the persistence of these responses is variable. There has been an increase in the proportion of control sheep with positive immune reactions, presumably reflecting the increase in OJD prevalence in this group. Vaccination of lambs has significantly delayed and reduced shedding of \textit{M. ptb} compared to controls. Individual faecal samples from animals in positive pools are being cultured. To-date there have been 2 OJD mortalities in vaccinates compared to 32 in control sheep. The incidence of vaccination infection site lesions has decreased from initial levels of almost 50% to around 20% of vaccinates retaining lesions 18–24 months after vaccination. No difference between control and vaccinated sheep has been detected in the productivity traits of growth, body condition, greasy fleece weight or fibre diameter. This data has assisted the registration of Gudair™ in Australia and supports a major role for Gudair™ vaccine for managing the on-farm impact of OJD in Australia.
Figure 1: Gamma IFN – % Positive

Figure 2: Elisa Antibodies - % Positive

Figure 3: Faecal Shedding – Proportion Positive Pools

<table>
<thead>
<tr>
<th>Property</th>
<th>Group</th>
<th>2</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>18</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>4/5</td>
<td>4/5</td>
<td>4/10</td>
<td>5/10 (10%)*</td>
<td>5/20</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Vaccinate</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>1/10 (2.5%)</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>0/5</td>
<td>5/10</td>
<td>8/10</td>
<td>9/20 (14%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Vaccinate</td>
<td>0/5</td>
<td>0/10</td>
<td>1/10</td>
<td>2/10 (0.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>0/5</td>
<td>2/10</td>
<td>10/10</td>
<td>16/20 (9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Vaccinate</td>
<td>0/5</td>
<td>0/10</td>
<td>0/10</td>
<td>3/20 (1.5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Figures in brackets are the percentage of animals shedding Mptb in each group
Figure 4: Mortalities Due To OJD

<table>
<thead>
<tr>
<th>Property</th>
<th>Treatment</th>
<th>No. OJD deaths</th>
<th>Age at first death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Vaccinate</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Vaccinate</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Vaccinate</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Figure 5: Incidence of Lesions in Vaccinates

Figure 6: Distribution of Lesion Size
NOJDP RESEARCH PROGRESS REPORT

EVALUATION OF ERADICATION STRATEGIES FOR OVINE JOHNE'S DISEASE

PROJECT NUMBER: OJD.001

PAT TAYLOR

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Background and Objectives

One of the objectives of the National Ovine Johne's Disease Control and Evaluation Program is to investigate the potential of destock-restock strategies to eradicate the S strain of Mptb from individual properties. To be successful the eradication process requires that infective sheep (and goats) be removed from the site for sufficient time for residual Mptb to expire, that replacement sheep are free of OJD and that the site is protected from contamination with Mptb from infected or suspect neighbouring sites.

A national project (Trial 1.1) was initiated in 1999 with the primary objective of evaluating the efficacy of this strategy to eradicate OJD from up to 50 properties that were implementing formally prescribed Property Disease Eradication Plans (PDEPs). Secondary objectives are to determine where possible the reason(s) for any failures to eradicate the disease.

Methods and Results

Sites were selected on the basis of having an approved PDEP and demonstrating sufficient infection in the year of destocking for a worthwhile test of the eradication process. Participants are required to restock with at least 700 low risk sheep within six months of completing an approved 15-month PDEP. Sheep may be purchased from MAP flocks or flocks tested to MAP equivalence (ie ~95% assurance of detecting ≥2% prevalence). The efficacy of the eradication process will be determined by culturing 10 pools of 50 faecal samples (ie ~99% assurance of detecting ≥1% prevalence) at 24 and 36 months after restocking.

| Table 1: Selected evaluation sites by state and year of restock |
|-----------------------|--------|--------|--------|--------|
| State                | 1999/9 | 2000   | 2001   | Total  |
| NSW                  | 5      | 16     | 12     | 33     |
| Victoria             | 2      | 0      | 5      | 7      |
| South Aust           | 0      | 3      | 1      | 4      |

| Table 2: Eradication results at around 24 months after restocking |
|----------------------|--------|--------|--------|--------|
| Result               | 2001   | 2002   | 2003   | Total  |
| Failed               | 4      | 2      | 1      | 7      |
| Succeeded            | 2      | 3      | -      | 5      |
| Not assessed         | 1      | 14     | 17     | 32     |

Conclusions to Date

Seven sites have failed the eradication process. Of these, three inadvertently purchased infected sheep. A fourth probably purchased infected sheep. Causes of the remaining three failures are unknown at this point. Five sites are apparently free of OJD at 24 months after restocking. Two of these are in the residual zone while three are in the control zone. Four of the five sites adjoin infected neighbours. The status of another 32 sites is yet to be determined.
NOJDP RESEARCH PROGRESS REPORT

ECONOMIC EVALUATION OF CONTROL OPTIONS

PROJECT NUMBER: OJD.001

STEWARD WEBSTER
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Locked Bag 21, Orange NSW 2800
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E-mail: stewart.webster@agric.nsw.gov.au

Background and Objectives

The returns associated with different on-farm OJD management options are an important consideration in the long-term management of OJD. This research aims to quantify the financial consequences of three OJD management options — status quo, vaccination and decontamination through destocking — for individual producers located in grazing-dominant areas.

Methods and Results

When considering the private incentives that govern producer behaviour in response to a disease, it is not the difference in cost between having an infected and uninfected flock that is relevant, but the avoidable component of that cost given present infection. In the case of OJD, only comparison of the infected status quo with realistic alternative scenarios, such as vaccination or decontamination, will provide insight into the management options most likely to be favoured by producers.

A deterministic, spreadsheet based, representative farm model was constructed for each of three high OJD prevalence regions: the Central Tablelands of NSW; Kangaroo Island, SA; and South Gippsland, Victoria. A background flock average mortality of 2.5%, additional OJD-related mortality of 5% and a vaccinated OJD-related mortality of 0.5% were used across locations to facilitate comparison. Other parameters, such as property area, carrying capacities, wool cut and quality, surplus sheep prices and fixed costs were based on detailed case studies and external data sources. Each model was used to calculate monthly cash flows and business equity over a 20 year period for each of three OJD management options: the status quo, vaccination and decontamination.

The net present values (NPV) of the variation in monthly cash flow between the status quo and the vaccinated and decontamination options were calculated over twenty years to allow comparison of the three management options in each location. Decontamination requires a much larger initial investment than a vaccination program, but results in higher annual net farm income once the initial enterprise mix is reinstated. Consequently, the NPV of decontamination overtakes that of vaccination over longer investment horizons.

The results demonstrate that positive returns to an investment in a vaccination program are earned almost immediately. Vaccination is the most profitable short-term option in the Central Tablelands and South Gippsland, although decontamination overtakes vaccination as the most profitable management option on Kangaroo Island within two years. However, decontamination caused significant declines in producer business equity over the first 16 months, particularly on Kangaroo Island. This has implications for decontamination as a strategy because financial institutions lend on the basis of business equity expressed as a proportion of the property’s land value, with few willing to lend beyond 50 per cent equity. It is therefore possible that a switch to a finance-intensive interim enterprise, such as yearling steers, could not be fully funded through short-term debt.
Figure 1: Net Present Value (NPV) Of Income Variation From Status Quo

<table>
<thead>
<tr>
<th>Period</th>
<th>Vaccination</th>
<th>Decontamination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Central Tablelands</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 years</td>
<td>$24,336</td>
<td>$17,604</td>
</tr>
<tr>
<td>7 years</td>
<td>$44,348</td>
<td>$50,432</td>
</tr>
<tr>
<td>10 years</td>
<td>$81,033</td>
<td>$110,623</td>
</tr>
<tr>
<td>15 years</td>
<td>$172,404</td>
<td>$241,718</td>
</tr>
<tr>
<td>20 years</td>
<td>$304,791</td>
<td>$414,089</td>
</tr>
<tr>
<td><strong>Kangaroo Island</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 years</td>
<td>$2,813</td>
<td>$16,085</td>
</tr>
<tr>
<td>5 years</td>
<td>$666</td>
<td>$66,182</td>
</tr>
<tr>
<td>10 years</td>
<td>$18,413</td>
<td>$192,879</td>
</tr>
<tr>
<td>15 years</td>
<td>$65,547</td>
<td>$376,035</td>
</tr>
<tr>
<td>20 years</td>
<td>$165,926</td>
<td>$642,564</td>
</tr>
<tr>
<td><strong>South Gippsland</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 years</td>
<td>$155,229</td>
<td>$161,718</td>
</tr>
<tr>
<td>15 years</td>
<td>$201,027</td>
<td>$223,343</td>
</tr>
<tr>
<td>20 years</td>
<td>$353,682</td>
<td>$416,910</td>
</tr>
</tbody>
</table>

Figure 2: Effect of Decontamination on Equity

Conclusions to Date

This analysis ignores the significant production, price and regulatory risks facing producers who may be considering the decontamination option. The risks that decontamination is unsuccessful (or reinfection occurs), that the price of restocker sheep rises during the destocked period and that government “rules” might change considerably, reduce the relative attractiveness of decontamination beyond what the above deterministic modelling results would indicate. The likelihood of such risks being realised is difficult to estimate, but there is evidence that suggests that these risks are significant.

Given the demonstrated medium-term profitability and less risky nature of vaccination as an OJD management strategy, it seems likely that few producers will attempt decontamination in the future without considerable subsidies.
NOJDP RESEARCH PROGRESS REPORT

DEVELOPMENT OF COMPUTER MODELS TO DESCRIBE THE EPIDEMIOLOGY OF JOHNE’S DISEASE IN SHEEP

PROJECT NUMBER: OJD.027

EVAN SERGEANT
Ausvet Animal Health Services
Phone: 02 6362 1598; Fax: 02 6369 1473
E-mail: evan@ausvet.com.au

Background and Objectives

Mathematical models can be used to solve real-world problems by translating them into mathematical descriptions that can then be analysed and solved using standard mathematical techniques. Although models provide only a very simplified representation of the real world, they can be very useful for comparing alternative interventions, or for identifying specific areas requiring further research, and quantifying the potential benefits of research. Disease modelling is particularly useful because it allows the investigation of a variety of disease control options that would often not be possible or practical to evaluate experimentally or in field trials.

The objectives of this project were to develop simulation models for the spread and control of ovine Johne’s disease within infected flocks and between flocks in a region.

Methods and Results

The pathogenesis, epidemiology and options for control of Johne’s disease in sheep were reviewed and mathematical models developed to simulate the spread of Johne’s disease within infected flocks, and between flocks on a regional basis. The models also allow the evaluation and comparison of control options at both flock and regional levels.

Simulated control strategies include management changes, vaccination and test & cull options in infected flocks and vaccination, surveillance and quarantine and movement controls on sale/purchase of sheep in the regional model. Results for each simulation are summarised as the mean, standard deviation and percentiles of multiple iterations for each output variable of interest.

Figure 1: Flock Model
Figure 2: Regional Model

Figure 3: Mortality Rates over Time with Uncontrolled Spread

Figure 4: Effect of Vaccination on Mortality Rates
Conclusions to Date

There is still inadequate data available to accurately estimate the true values for many of the parameters involved in spread and progression of OJD infection at both within-flock and regional levels. However, these models provide an opportunity to investigate the effects of assumed realistic values on the rate of spread of infection. In addition, the models allow estimation of the likely costs of disease, and the effectiveness and cost-benefit of proposed control strategies, particularly at the farm level.
NEW OJD RESEARCH & DEVELOPMENT
PROJECTS RECENTLY COMMENCED OR UNDER
CONSIDERATION

THE NATIONAL OVINE JOHNE'S DISEASE CONTROL AND
EVALUATION PROGRAM
NEW NOJDP RESEARCH PROJECTS

STUDY OF RISK FACTORS FOR OJD

PROJECT NUMBER: NOT ASSIGNED

INVESTIGATORS:

JENNY-ANN TORIBO & RICHARD WHITTINGTON
UNIVERSITY OF SYDNEY

Background and Objectives

Greater understanding of factors that influence OJD prevalence and mortalities in infected flocks is required to assist farmers reduce OJD losses. It is highly likely that straightforward relationships exist but that observation of these is confused by the long incubation of this disease at individual animal and flock levels. The objective of the proposed study is to identify risk factors for infection and for clinical disease.

Methods

This study is currently in the design phase. Further development of a causal web will clarify assumptions and hypotheses and define potential risk factors, and the accuracy with which these can be assessed both retrospectively and prospectively.

Discussion will define appropriate measures of OJD prevalence and mortalities. It is envisaged that a substantial number of infected flocks in the residual zone will be included in a cross-sectional study possibly followed by a 2-year prospective study.
NEW NOJDP RESEARCH PROJECTS

OVINE JOHNE'S DISEASE – SUBCLINICAL AND CLINICAL EFFECTS ON PRODUCTION

PROJECT NUMBER: OJD.002A (variations)

INVESTIGATORS:

HELEN McGREGOR, OM DHUNGYEL, RICHARD WHITTINGTON
UNIVERSITY OF SYDNEY

Background and Objectives

OJD infection in Australian sheep flocks has been demonstrated to cause a significant increase in the death rate of adult sheep in some affected flocks. Whilst these increased death rates undoubtedly have a large impact on the profitability of sheep raising, other forms of lost production, particularly wool production may also have a significant negative impact on farm incomes. For Australian sheep no investigations of the biochemical changes occurring during an OJD infection have been made. Further, there are no studies reported from any country of any investigations into measurable changes in protein metabolism or other chemical parameters of sheep during the pre-clinical phase of JD infection. Knowledge of these changes may shed light on mechanisms of disease.

Objectives

To measure and describe the changes in chemical metabolism and productivity in animals affected with OJD during the pre-clinical phase of the disease, and in those where disease progresses, during the progression from the subclinical to the terminal phase.

Methods and Results

Currently there exists a large (~500 sheep) experiment in which groups of sheep have been exposed to infection with OJD at different ages and from different sources. This experiment offers the opportunity to measure the subclinical effects of OJD infection on live-weight and wool production and to relate the changes in productivity and onset of clinical disease to underlying changes in physiology and metabolism. These measurements commenced in June 2001 when the sheep were 20 months old. The presence or absence of OJD will be determined by histopathology at the conclusion of the experiment when the animals are three years of age.

Conclusion to Date

Sheep in the trial are measured to be losing 8-12kg bodyweight in the weeks prior to death. Other production characteristics await analysis. Biochemical assays will be conducted when all samples have been collected.
NEW NOJDP RESEARCH PROJECTS

THE RELATIVE SUSCEPTIBILITY OF ADULT SHEEP TO INFECTION WITH OJD

PROJECT NUMBER: OJD.002A (variations)

INVESTIGATORS:
HELEN McGRGOR, KIM ABBOTT, RICHARD WHITTINGTON
UNIVERSITY OF SYDNEY

Background and Objectives

There is evidence from studies with cattle that neonates are more susceptible to infection with *M. tub* than adult cattle. There is less clear evidence in the case of sheep and although a declining susceptibility with age is suspected in sheep, the extent of the relative susceptibility is not understood. Knowledge of the relative susceptibility of sheep of different ages to OJD is a key to the development of OJD-control strategies on infected farms, based on grazing management.

**Objective**

To be able to quantify the difference in prevalence between sheep which were first exposed to OJD at birth, at weaning, or at 3 or 4 years of age.

Methods and Results

As part of an existing project (OJD 002A) 480 pregnant ewes were purchased in August 1999. Serological testing and subsequent faecal culture indicated that they were free of OJD infection. The ewes were aged 3, 4, 5 and 6 years at the time of purchase and lambed in September the year of purchase. Post weaning in January 2000, the oldest half of the previously uninfected ewes were removed from the site and sold. Approximately 220 ewes aged 3½ and 4½ years remained. These ewes existed in two replicates and included ewes that had been exposed to OJD during lambing, lactation and for 8 weeks later, and ewes that had not deliberately been exposed to infection. These ewes have been allocated to two similar treatments and replicates.

In essence each treatment within each replicate consists of ewes which were either exposed to OJD for the lambing-lactation period or not exposed and which were 3 years or 4 years at the time of exposure. The amount of exposure is known and comparable with that of their lambs, which remain in the main experiment. These ewes remained on the experimental site until November 2001. Faecal culture and histopathology of the samples collected from these animals is underway. The determination of the prevalence of infection in them will allow a direct comparison between the two age groups, which will provide insights into the relative susceptibility of adults and lambs to OJD infection.
NEW NOJDP RESEARCH PROJECTS

EXCRETION RATE FOLLOWING WHOLE FLOCK VACCINATION

PROJECT NUMBER: OJD.015 (extension)

INVESTIGATORS:
HELEN McGREGOR, PETER WINDSOR, OM DHUNGYEL, RICHARD WHITTINGTON
UNIVERSITY OF SYDNEY

Background and Objectives

For many owners of OJD infected properties vaccination is considered to be their best option for reducing losses and managing the disease. Current recommendations confine vaccination to young animals however in high mortality flocks, whole flock vaccination has been considered. Documentation of the effects of whole flock vaccination on faecal excretion rates and mortalities in a high prevalence infected flock is necessary to enhance knowledge of the benefits of a whole flock vaccination strategy.

Objective

To estimate the change in faecal shedding attributable to vaccination of adult sheep

Methods and Results

Investigation of a high prevalence OJD infected flock in the NSW southern tablelands by periodic mortality study is currently in its 2nd year. High mortality rates due to OJD have been confirmed in this flock in both years (Crude v OJD-attributable mortality rates of 21.5% v 14.5% and 17.8% v 13.2% in years 0 and 1 respectively). Whole flock vaccination commenced in April 2000 and was permitted in an attempt to more rapidly reduce the contamination of pastures and the OJD-attributable death rate, than by vaccination of lambs only.

As part of the methodology of this study, faeces is collected from cohorts of vaccinates of 100-150 animals in all age groups and from identified non-vaccinated animals in the three youngest age groups. Sampling has been repeated each year since the commencement of the study, and will continue until 2003. The original project protocol allowed for only the two-year-old animals’ samples to be cultured each year, which could provide an estimate of changes in Mptb challenge. An extension to the project enables investigation of the effect of adult vaccination on faecal excretion rates over time.

Conclusion to Date

With studies confirming that Gudair does not prevent infection, yet there is an expectation that the vaccine will significantly reduce pasture contamination, the need to proceed with this extension to measure changes in faecal excretion rate following vaccination as a measure of likely changes in pasture contamination, has become clearer.
NEW NOJDP RESEARCH PROJECTS

EPIDEMIOLOGY OF OJD 2 – PASTURE CONTAMINATION LEVEL, AGE SUSCEPTIBILITY AND DIAGNOSTIC TESTS

PROJECT NUMBER: OJD.028

INVESTIGATORS:
HELEN McGREGOR, OM DHUNGYEL, RICHARD WHITTINGTON
UNIVERSITY OF SYDNEY

Background and Objectives

The aim of this project is to determine whether pasture contamination rates and age of sheep when first exposed influence the incidence of Johne’s disease, its incubation period and the timing of diagnosis.

The experiment also allows for the determination of the efficacy of diagnostic tests and the comparison of faecal culture, cell mediated immunity and serology as diagnostic approaches in sheep recently exposed to infection.

The outcomes include recommendations regarding flock testing after introduction of clean sheep to infected pasture and for control of OJD, based on knowledge of an age-based clustering of infection.

These outcomes will provide improved understanding of the development of ovine Johne’s disease, in particular the implications of the exposure of sheep of different ages to infection.

This project complements an existing study to determine when transmission is most likely to take place and whether management can be altered to reduce transmission.

Methods and Results

Pasture has been created with three grades of contamination, low medium and high by grazing infected sheep at set rates over a given time period before the introduction of uninfected sheep. Uninfected control pasture is also being used. Treatments are replicated. The uninfected sheep are of three ages; female weaner sheep, hogget ewes and 3 year old ewes. The final infection rate in each age class at each level of pasture contamination will be determined using faecal culture and immune tests at 3 and 6 month intervals. Post mortem, histopathology and tissue culture will be performed on all animals after 2.5 years.

Conclusion to Date

The experiment has just commenced.
NEW NOJDP RESEARCH PROJECTS

OJD ABATTOIR SURVEILLANCE PROJECT

PROJECT NUMBER: OJD.029

INVESTIGATOR:

TRACEY BRADLEY, DNRE VIC

Background and Objectives

Abattoir surveillance is an extremely efficient and low cost method for diagnosing OJD. In the last 3 years over 14.5 million sheep have been examined by this method in 20 abattoirs around Australia. Obviously the vast majority of those examined are found by inspection to be free of disease. The data from these negative sheep however, is not captured and in combination with a sheep ID system it could be used to provide information for negative assurance purposes along with other tests such as serology and faecal culture. What has been missing until now is an accurate estimate of the sensitivity of abattoir inspection.

Objective

To determine the sensitivity of abattoir surveillance by OJD inspectors.

Methods

- 1200 sheep from known infected farms were sent to Southern Meats in Goulburn.

- All sheep were subjected to an AGID prior to slaughter to ensure there were sufficient numbers of infected sheep. For 10% confidence intervals around the point estimate of the sensitivity at least 100 infected animals are required.

- Over a 2-day period 3 inspectors from different parts of Australia with different exposure to OJD examined all 1200 individually numbered guts in the works and their diagnoses were recorded. The inspectors were visually and acoustically separated but all examined the same viscera.

- Viscera were processed and sent to a laboratory for histopathological diagnosis ("gold standard") on up to 6 sections per sheep.

- A line of known negative sheep was included as a "dummy run" to ensure that inspectors weren’t “overdiagnosing" the presence of disease.

Results for this trial should be available in 2 months.
NEW NOJDP RESEARCH PROJECTS

CHANGES IN WITHIN FLOCK PREVALENCE OF _M. PTB_ SHEDDING FOLLOWING VACCINATION WITH GUDAIR IN HIGH AND LOW PREVALENCE FLOCKS

PROJECT NUMBER: NOT ASSIGNED

INVESTIGATOR:
JEFF EPPLESTON
CENTRAL TABLELANDS RLPB

Background and Objectives

While the results of Trial OJD 009 have been encouraging to-date, this research is being conducted in high prevalence situations for a single generation only. The potential value in vaccination is likely to become evident only over an extended period following vaccination of a number of successive lamb crops. Recent computer modelling (Sergeant, 2002) suggests that the rate of disease reduction in vaccinated flocks will depend on the efficacy of the vaccine and the prevalence of disease at the commencement of vaccination.

Objective

The objective of the proposed study is to observe changes in the prevalence of shedding of _Mptb_ following the commencement of a vaccination programme in OJD-infected flocks varying in initial OJD prevalence.

Methods

Up to 4 flocks from each of 3 disease prevalence levels will be selected from commercial flocks using existing surveillance data. These are High (>5% mortalities/ >4/7 positive pools) vs Medium (<5% mortalities/ 2-4/7 positive pools) vs Low (0 losses/ <2/7 positive pools).

In each flock the age groups will be split into 3 (1&2, 3&4 and 5&6 year olds), with the 2 older groups being sampled (7 pools of faeces) every 2 years to estimate disease (shedding) prevalence. At each consecutive sampling another age group will have been vaccinated as lambs so that the following comparison can be made.

- Sampling 1 3/4 yo non-vaccinates vs 5/6 yo non-vaccinates
- Sampling 2 3/4 yo vaccinates vs 5/6 yo non-vaccinates
- Sampling 3 3/4 yo vaccinates vs 5/6 yo vaccinates

Comparisons between the same age groups at different samplings will provide an estimate of disease prevalence in each flock at the commencement of vaccination, and will estimate any change in prevalence that occurs in subsequent generations.
EXECUTIVE SUMMARIES
FROM
KEY OJD R&D PROJECTS
CONDUCTED BY MLA AND NOJDP
TR.022 - DNA TYPING OF JOHNE'S DISEASE ORGANISMS

Abstract

This study was undertaken to determine whether Johne's disease in sheep and cattle in Australia can be considered to be separate diseases subject to independent control programs. Isolates of the bacterium that causes Johne's disease, namely Mycobacterium avium subsp. paratuberculosis, were typed genetically in order to see whether those in sheep were different to those in cattle. About 350 separate isolates were evaluated from about 100 farms in New South Wales, Victoria, Tasmania and South Australia. A new test was developed to enable the bacterium to be typed quickly and accurately. Johne's disease in sheep was almost always due to sheep strains of M. avium subsp. paratuberculosis while cattle were almost always infected with cattle strains. However, it was concluded that Johne's disease has occasionally spread from sheep to cattle in NSW, probably under unusual circumstances. Johne's disease may also spread from sheep to goats. As a result of this project Johne's disease control programs will be assessed on an ongoing basis as more information is obtained about the way the disease may be spread between farm animals.

Executive summary

This project was undertaken to determine whether Johne's disease in sheep and cattle can continue to be considered to be different diseases, subject to separate control and market assurance programs.

The specific objectives were to genetically type the bacterium that causes Johne's disease, namely Mycobacterium avium subsp. paratuberculosis, to compare isolates of this bacterium from cattle, sheep, goats and other farm animals, to develop a rapid laboratory method for distinguishing sheep isolates from cattle isolates and to identify whether cross-species infection occurs between sheep and cattle. All of the objectives were achieved.

A total of 354 isolates of M. avium subsp. paratuberculosis were tested using restriction fragment length polymorphism (RFLP) analysis and other techniques. Isolates were obtained mainly from sheep and cattle from about 100 farms in NSW and Victoria, but some samples were included from Tasmania, SA and France.

Fourteen RFLP types were found. Type S1 was the dominant type in sheep in NSW, accounting for 95% of isolates, and was the only type found in sheep from Victoria. Several sheep isolates obtained in NSW during the 1980's together with several isolates from sheep from France were of cattle type. These bovine types of M. avium subsp. paratuberculosis are currently not involved in the epidemiology of ovine Johne's disease in Australia and would appear to represent unusual events in the past.

Seven RFLP types were present in cattle. Types C1 and C3 were the most common, but C1 was not found at all in NSW and C3 was absent from beef cattle in Victoria. Type C5 was the next most common type, accounting for 7% of isolates. Several RFLP types were present in some geographic regions in Victoria, for example types C1, C3, C5, CU4 and C12 were found amongst beef and dairy cattle in Gippsland. Several types may occur in cattle on the one farm; 2 RFLP types were found on 6 farms while 3 RFLP types were found on 2 farms. In general it appeared that individual cows were infected with only one type, but one cow was infected with both C5 and CU4. Given the existence of geographical and farm enterprise restriction in RFLP type, the technique may be applied to trace the future spread of Johne's disease in the cattle industries.

A rapid typing method was developed based on the IS1311 gene. Over 400 isolates of M. avium subsp. paratuberculosis were examined with the new test and complete agreement was obtained with the RFLP test and host species.

The IS1311 test was further developed and used to examine animal tissues stored in archives. Several cases of Johne's disease in cattle were found to have been due to sheep strains of M. avium subsp. paratuberculosis. In each case it appeared that young calves had been reared in association with infected sheep. The disease did not appear to have become established in cattle herds and one herd has now tested negative in the cattle market assurance program. Serological investigation of cattle was also undertaken on
3 farms with ovine Johne's disease during this study. The cattle had grazed as calves with infected sheep and were > 2 years old when sampled. All were seronegative. These findings suggest that cattle have not commonly become infected with sheep strains of *M. avium* subsp. *paratuberculosis*, despite exposure to the organism.

Ovine Johne's disease was shown to have spread from sheep to fibre goats on a farm in NSW during this study. There had been a high degree of contact between infected sheep and the goats on the property over a long period.

The main conclusion from this work is that sheep and cattle in Australia tend to be infected with mutually exclusive strains of *M. avium* subsp. *paratuberculosis* however, cross-species transmission has occurred occasionally. This is vital information for industry because it provides general validation of the current recommendations for control of ovine Johne's disease and market assurance program testing of cattle, i.e. that cattle can safely graze pasture after removal of infected sheep. However, it also indicates a need for industry to continue to monitor the situation and avoid the grazing of calves where exposure to high levels of contamination from sheep could occur. Similar comments could apply to goats, although their susceptibility as adults to ovine strains of *M. avium* subsp. *paratuberculosis* is uncertain.

As a result of the findings of this study, a working group was established under Veterinary Committee to make recommendations on cross-species transmission. Detailed recommendations from this group will follow further analysis of data obtained from NSW and Victoria.
TR.007 TYPING OF JOHNE’S DISEASE ISOLATES

Introduction

*Mycobacterium paratuberculosis* (Mptb) is the cause of Johne’s Disease (JD), an inflammatory bowel disease of ruminants, characterised by the irreversible thickening of the bowel wall and the enlargement of intestinal lymph nodes. Clinical features of the disease in cattle are weight loss and diarrhoea. It commonly presents in cattle between 4 and 6 years of age, often after calving or during periods of nutritional stress. Sheep, alpaca and goats are also known to be infected with *Mptb* in Australia.

DNA techniques have been used in the investigation of the epidemiology of JD. The use of restriction endonuclease analysis (REA) and restriction fragment length analysis (RFLP) using Bst EII indicated that there were 2 major groups of Mptb in isolates that originated mostly from New Zealand (NZ) and Australia (I). These were called cattle (C) and sheep (S) strains because of the predominance of the species from which they originated. RFLP identified more differences within the larger groups than REA. Five C types (C1-C5) were identified in 34 clinical isolates from NZ, Australia, Canada, and Norway (19 cattle, 6 sheep and 9 goats). The common cattle type found in NZ, C1, was found to be identical to the Weybridge vaccine strain 316v. Two S types, S1 and S2 were found in 9 clinical isolates: 7 sheep and 1 goat from NZ were infected with S1 and 1 sheep from the Faroe Islands was infected with S2. A single ovine isolate from Canada appeared to be intermediate between the S and C strains and was designated an intermediate (I) strain (I). In a subsequent study, 5 sheep isolates from South Africa were all identified as the I strain (2). Until recently, RFLP had only been applied to the 6 Australian cattle isolates of Mptb included in the study of Collins et al (I). These 6 isolates, from 4 geographical regions, were typed into 4 groups (C1, C3, C4 and C5).

Although epidemiological evidence supports the fact that sheep and cattle are infected with different strains of *Mptb* in Australia, this had not been confirmed. Consequently, this work was designed to examine:

i) whether sheep strains in Australia were the same as in NZ; and

ii) whether polymorphisms detected in Australian isolates of *Mptb* would be useful in epidemiological investigations of Johne’s Disease in Australia.

The *Mptb* isolates were selected in conjunction with Dr David Kennedy, National Johne’s Disease Coordinator. During the term of the project, and following the successful cultivation of ovine strains in BACTEC 12B medium in Australian laboratories, additional ovine strains were submitted for testing.

All isolates were tested using DNA fingerprinting techniques developed at Agriculture Western Australia using Southern hybridisation and the IS900 DNA probe. Before the MRC contract, Agriculture WA had developed the procedure and tested a number of isolates that had been obtained from WA, Victoria and NSW. For the sake of completeness, and in an attempt to draw meaningful conclusions, the results of all testing (ie. that funded by MRC and Agriculture WA) are reported here.

Conclusions

This study has confirmed that the JD situation in Australia is similar to that found in NZ. All cattle and some other species were infected with cattle (C) strains and sheep were predominantly infected with sheep (S) strains. Thus, while the C strain infected other animal species, such as alpaca, goats, a rhinoceros and sheep, there was no evidence to support the hypothesis that S strains could infect cattle.

Because of the small number of isolates that had unique IS900 RFLP patterns, it is unlikely that IS900-RFLP would be useful in epidemiological investigations of JD. In addition, since no polymorphisms were detected among ovine isolates, it is unlikely that this fingerprinting technique would be useful in the investigation of OJD epidemiology, other than to clarify whether infection on newly diagnosed properties was due to members of the C or S groupings. Confirmation of infection with C or S strains may be useful to consider when the source of infection and possible transmission are being investigated on properties known to graze different animal species on the same pastures. It is possible that other, more useful methods of genetically typing *Mptb* may be developed for epidemiological investigations of JD. However, it appears that 18900-RFLP methods are not sufficiently sensitive for this purpose.
TR.055 - MYCOBACTERIUM PARATUBERCULOSIS SOIL TRAY TRIAL

Executive Summary

Reducing the survival of *Mycobacterium paratuberculosis* (*Mptb*) outside the host animal has long been attributed to environmental factors such as elevated temperature, pH and ultraviolet light and dryness although without direct scientific evidence of the contribution of these factors. The experiment reported here evaluates these four factors; ultraviolet radiation, soil temperature, pH, moisture plus organic matter, for their effect on the survival of *Mptb* from soil as measured by proportional recovery using the Whitlock double incubation and BACTEC culture method.

In this study, soil moisture and soil temperature were the most significant environmental factors affecting the survival or death of *Mptb*. Ultraviolet radiation appeared to have no effect and different soil types and variable sensitivity of culture obscured the effect of pH.

Results for different environmental factors were evaluated on the low organic soils where the recovery of organisms in culture was not affected by soil type. Sand of acid pH and low organic matter had no loss in analytical sensitivity compared to the *Mptb* contaminated faeces to which it was inoculated. Fewer organisms were recovered from other soil types mixed with the same faeces. Clay soil of acid pH and high organic matter had a detection limit of 10⁶ colony-forming units per gram. Tray trial results from the two low organic soils showed distinct effects between treatments, however differences were inconsistent in high organic soils due to the lower analytical sensitivity and low total number of *Mptb* isolations.

The results were statistically analysed by logistic regression and a model developed which provides predicted recovery estimates for each combination of treatments. Results are presented in terms of the predicted mean recovery with standard errors of the predicted means. The conditions applied were within the range of environmental exposure and results of the treatments are directly applicable to field situations. The rate of death of organisms may have been accelerated in high temperature treatments because the temperature was maintained constantly rather than with diurnal variation.

Soil dryness and high temperature resulted in shorter survival times for *Mptb* in low organic soil. After 8 weeks, there was approximately 100% survival in wet, low temperature (10°C) acid sand; survival in the same soil with cyclic moisture was reduced to 74% and in dry soils to 32% of initially detectable organisms. At higher temperature (30°C) there was an 81%, 37% and 10% survival for wet, cyclic and dry treatments respectively. The alkali foam low organic soil demonstrated a lower culture sensitivity but the same survival trends as for the acid sand for moisture and temperature were observed.

Alkaline soil pH indicated a weak influence with shorter survival in wet and dry soil at both high and low temperature. There was no effect by UV light at the levels used, which may have been too low in intensity or exposure to ultra violet light may not be effective in killing *Mptb* in soil due to low penetration of the soil.

From these results it should be concluded that dry soil, high soil temperature and possibly alkaline pH are significant in reducing survival of *Mptb* in soil and should be used to best effect when implementing control procedures on properties.

In contrast wet soil at low temperature due to protection from sunlight and possibly with acidic pH are conditions where *Mptb* is likely to survive for longer periods.

This study provides only semi-quantitative estimates of the death of *Mptb* under different environmental conditions. Further work quantifying the log reduction death of *Mptb* influenced by soil temperature and soil moisture in acidic low organic soil is recommended to assess the level of risk associated with restocking properties undertaking Johne's disease control.
OJD.003 - SURVIVAL OF JOHNE'S DISEASE IN THE ENVIRONMENT

Abstract

This report covers three MLA projects (TR.055, TR.055A and OJD.003) undertaken by NSW Agriculture to determine how long the sheep strain of Mycobacterium paratuberculosis (Mptb) survives in the environment to validate destocking recommendations for eradication of OJD. Survival of the organism was prolonged but finite. In the shade it lasted for 13 months while in the open in ungrazed pasture it lasted for 7 months. It survived for a shorter period in fully exposed pastures where grazing was simulated but for much longer in water than on pasture. These times were probably underestimates. Liming pasture did not reduce survival and moisture did not increase it. Shade was the most significant factor favouring survival. Further research is necessary to determine the mechanisms of survival, which include dormancy. Decay rates for the organism were determined for short term and long term destocking. These can be used to estimate how much time must be allowed to render pastures safe for control and eradication of OJD, respectively. When estimates of soil ingestion rates by grazing sheep are combined with within-flock OJD prevalence estimates and bacterial shedding rates determined by PFC, it is possible to make property by property recommendations for the purpose of control or eradication of OJD.

Executive Summary

This report relates to components of three MLA projects to determine the duration of survival of the sheep strain of Mptb (TR.055, TR.055A and OJD.003) in order to provide greater confidence in recommendations for destocking and decontamination for eradication of OJD being evaluated under the National Ovine Johne's Disease Control and Evaluation Program. A separate report covers work undertaken by Agriculture Victoria under TR.055.

There is very little information on pasture decontamination. The sheep strain of Mptb that is responsible for almost all cases of OJD in Australia was extremely resistant in the environment. The results were consistent with those from several laboratory experiments conducted overseas using the cattle strain.

Pasture contamination on OJD affected farms can be widespread and its location is generally not predictable. Low-lying areas may become a focus for contamination due to movement of faecal material. Under the conditions studied, contamination was not detectable after one complete summer in most sites previously found to be infected.

In plot and box experiments the duration of survival exceeded 12 months in faecal pellets in a shaded location but was much less in unshaded treatments unless vegetation was not grazed. Sunlight, including factors such as UV, visible and infra-red radiation, is a very significant factor influencing survival of the organism. Temperature flux was proposed as the reason shade was so important because UV radiation probably cannot penetrate pellets. Moisture levels and lime application did not appear to influence survival.

Decay rates for the organism were estimated and were found to be inversely proportional to the period of observation. There was a rapid decay phase lasting about 6 weeks during which the vast majority of viable bacteria declined. 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, and sometimes a period of apparent replication during which its numbers increased. Finally there was a slow decline phase lasting many months.

The organism moves from faecal pellets into the surface litter as pellets break down and also enters the soil profile. Thus it can be cultured readily from the surface layers of the soil. The duration of survival in soil was underestimated because the culture method was imperfect and the data on duration of survival needs to be interpreted with caution. Pasture emerging through contaminated faeces becomes contaminated with relatively high concentrations of the organism. The organism was also found to be associated with infective third stage larvae of intestinal nematode parasites that developed in the faeces of sheep with OJD. These larvae may be found on pasture and may contribute to infectivity of pasture.
The organism moves away from infected sites in run-off water and survives for prolonged periods in water. The duration of survival in water is longer than that in soil in the same environment. Based on studies of other bacteria including the related organism M. avium, there is potential for interactions between Mptb and single-celled aquatic animals.

Faecal shedding rates of the organism were determined using sheep with multibacillary OJD and amounted to $10^9$ viable organisms per gram or more than $10^{10}$ viable organisms per sheep per day. Sheep in earlier stages of the disease process would shed fewer organisms. Faecal output ranged from about 500 grams per day to over 1000 g per day among sheep.

A means of estimating decontamination intervals for pastures was proposed and three examples were given. Each was based on reasonable assumptions of prevalence of OJD, faecal shedding levels, bacterial decay rates and rates of soil consumption by grazing sheep.

Specific recommendations were made:

1. Decontamination intervals for eradication of OJD need to take account of: (a) Decay rates. Conservative estimates of decay rate should be used. (b) The level of infection in the flock prior to destocking which can be measured objectively by PFC (prevalence of faecal shedders and level of shedding). (c) The presence of environments likely to be conducive to survival and which might be fenced off. (d) The amount of soil ingested by sheep, which can be estimated based on type of soil, stocking rate, pasture type, rainfall and other factors. A decontamination interval of 15 months is likely to be sufficient in many but not all cases, depending on the assumptions used.

2. Decontamination intervals following transient contamination of land can be shorter than those following long term contamination by an endemically infected flock.

3. Decontamination intervals to reduce the impact of OJD by pasture spelling or management on endemically infected farms can be quite short because of the rapid decline phase. In general, the practices recommended for control of internal parasites will be beneficial for OJD control provided that adult sheep used to prepare pasture for young sheep are not heavy shedders of Mptb.

4. Simple spreadsheet-based computer models need to be developed to facilitate estimation of decontamination intervals for individual situations using a stepwise approach based on that outlined in this report. Further information may need to be gathered about rates of soil ingestion by grazing sheep in Australia, but much of this information may already exist in the literature. Probabilistic models that can account for incomplete knowledge can be developed using commercial software (eg @risk).

5. Knowledge about age susceptibility of sheep is required because shorter decontamination intervals might be possible if age-resistant sheep were used as restockers.

6. It is important to measure decay rates for contaminated faeces and soil in the winter months because it is possible that season may be less significant than local or micro-environmental shade influences. This would reduce the component of economic hardship imposed by being required to commence destocking at the beginning of summer. This research should be conducted using the pasture box method developed in this study. Greater flexibility should be given as to when decontamination can start in the summer period.

7. In future experiments using pasture boxes it is important that contamination with faeces be undertaken after transport of boxes rather than before transport because of the potentially deleterious effects of pooling of water caused by vibrations during transport. It would also be desirable to protect boxes from heavy rain using removable covers.

8. Specific recommendations have already been made concerning research on the survival of the organism in water and its association with aquatic invertebrates.
9. In vitro studies of the survival of the organism within faecal pellets exposed to measured doses of UV radiation are required to confirm that incident UV radiation does not sterilise organisms within pellets. Similar in vitro studies of the effects of temperature flux also need to be undertaken.

10. Basic research on dormancy and environmental replication of M. ptb is needed to support the NOJDP.

11. The findings of this study need to be discussed widely because it is impossible to consider all relevant technical issues nor to foresee all relevant present and future policy and farm management factors in a single report.

The results of this project will be of immediate benefit to industry. Reliance can be placed on the current recommendations related to a 15-month destocking period for eradication of OJD under most circumstances. Situations where this might not be sufficient include properties with very high prior prevalence of infection and a high proportion of infected sheep in advanced stages of the disease, where there are extensive areas of shaded environment favourable for survival of the organism, or where pasture and geographic factors result in high levels of soil consumption. Contaminated water is also a risk. Industry can also benefit by an end to speculation that shorter destocking periods (particularly 4 months) might be adequate for eradication. Short pasture spelling periods, such as those used for internal parasite control, are, however, ideal to increase the safety of pasture for young sheep on infected properties by significantly reducing contamination levels.

The results of this project will be of benefit to producers, their veterinary advisers, policy makers, disease control regulators and research coordinators, all of whom are faced with difficult issues.
OJD.012 - PILOT STUDY - ASSESSING THE RISKS OF TRANSMITTING OJD IN RAM SEMEN

Abstract

The impact on stud breeders of the current national program to limit the spread of OJD within the Australian sheep industry could be reduced if they could safely use semen from within their studs. Trading semen would be a means of maintaining some cash flow, and artificial insemination could be used to re-establish the studs' genotype within any destocking program.

This research has shown for the first time that semen collected from rams clinically infected with OJD can contain Mptb, the bacteria that causes OJD, and that it is likely that bacteria could be transferred to the reproductive tract of ewes at mating or AI. These results indicate that quality assurance guidelines for the safe use of semen from infected flocks need to be developed further.

Executive Summary

Within the current National OJD Control and Evaluation Program, owners of flocks infected or suspected of being infected with OJD are restricted in selling sheep for restocker purposes. This restriction has the largest impact on seed stock breeders who rely heavily on income from ram sales.

However selling genetic material in the form of semen would allow continued industry access to these genotypes, continued income by the seed stock producer, and perhaps retention of the bloodline during any destocking program.

Before guidelines for the sale of semen can be developed, the risk associated with semen transmitting the disease needs to be determined.

This study was conducted as a first step in determining the risk of transferring OJD by semen from rams from OJD infected flocks. Its objective was to determine whether Mycobacterium avium subsp paratuberculosis (Mptb), the bacteria causing OJD, is shed in the semen of rams showing clinical signs of OJD.

Eleven clinical OJD infected rams had semen and other reproductive samples collected and subsequently cultured for the presence of Mptb. Mptb was cultured from the semen from 3 rams and from the seminal vesicles of a fourth ram, indicating that the bacteria could be transferred to the reproductive tract of ewes at mating or by artificial insemination.

The sheep industry now has evidence that Mptb can be contained in ram semen and that it will be necessary to develop quality assurance guidelines for the use of semen collected from rams in infected flocks. To determine the protocols needed in these guidelines it is recommended that additional research be conducted to determine the incidence and level of Mptb excretion in semen collected from rams at an earlier stage of infection with OJD.
OJD.005 - CROSS SPECIES TRANSMISSION OF OVINE JOHNE'S DISEASE - PHASE I

Abstract

Johne's disease (JD) was investigated in fibre goats on several farms. The disease was caused by sheep [S] strains of Mycobacterium avium subsp. paratuberculosis (Mptb). The infection appeared to be less severe than the same infection in sheep in that fewer goats than sheep became infected, and fewer goats than sheep developed obvious signs of the infection. However, infected goats shed the organism in their faeces and therefore were able to spread the infection to other goats and sheep. Therefore inclusion of goats in the control program for ovine Johne's disease (OJD) is justified. A communication program is recommended to advise producers that OJD in goats may not be obvious and that testing should be undertaken to ensure disease is not present. The impact of OJD on the fibre goat industry is projected not to be great due to the small number of herds likely to be infected.

Executive Summary

This project was undertaken to investigate OJD in goats on farms where the infection was acquired from sheep. Prior to this study there was little or no information about the behaviour of the disease in goats, its detection, its mode of spread or the risk it posed to uninfected goats and sheep.

By summarising the available information about OJD in goats, and conducting surveys on several farms, a picture of the disease in fibre goats was developed. Infected goats most often were detected using laboratory tests and not because they had obvious disease and it appeared that the tendency to develop severe disease was less in goats than sheep. In addition, the proportion of goats infected was less than the proportion of sheep infected on two farms where the disease was established. The reasons for the different disease pattern in sheep and goats are uncertain, but may 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 Mptb S strain 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.

Industry can benefit from this information immediately as it provides an objective view of OJD in goats. As JD may not be obvious in fibre goats, producers need to undertake laboratory testing to ensure that the disease is not present in their herds. Control programs for OJD in sheep can justifiably continue to consider goats - infected goats shed the organism and can transmit the infection to other goats and sheep. A communication program is needed to disseminate this information to producers.
TR.050 - PREVALENCE OF JOHNE'S DISEASE IN RABBITS AND KANGAROOS

Following reports from Scotland that rabbits on JD-infected farms in the Tayside region were infected with M. avium subsp paratuberculosis (Mptb), a study of rabbits and kangaroos on OJD-infected farms in NSW was commenced. Three hundred rabbits and 300 eastern grey kangaroos from 10 farms grazing OJD-affected sheep flocks were killed and examined for evidence of JD between late 1996 and late 2000.

Two hundred and fifty three rabbits were tested by radiometric culture of their faeces, while 47 were examined by smear and ZN stain of tissues combined with histopathology of the lower small intestine and regional lymph nodes. No evidence of JD or the causative organism was detected in any rabbit.

For 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 but histopathological examination revealed no evidence of active infection which might cause multiplication of the bacteria within the kangaroo. It was concluded that the bacteria identified in this animal were bacteria which had been ingested from pasture contaminated by OJD-infected sheep and which had survived passage through the gut.

Considering information available from studies of wildlife in Kangaroo Island and of rabbits in Scotland, we conclude that the prevalence of JD in rabbits and kangaroos on OJD-affected farms in Australia is very low (less than 1% of the adult population) or zero. Nevertheless, we recognise that there is a risk that adaptation of the organism to wildlife hosts could occur in future. There is evidence that the grazing pressures exerted by rabbits and kangaroos on sheep pastures in Australia is similar to that exerted by rabbits on beef cattle pastures in Scotland. We hypothesise that the S strains responsible for all or most of the OJD infection endemic in NSW sheep flocks are more host specific than C strains, particularly the C strains identified in cattle and rabbits in the Tayside region of Scotland.

The positive finding of Mptb in the faeces of a kangaroo implies that there is a risk of physical transfer of organisms from one farm to another which may lead to transfer of infection from an infected flock to a neighbouring uninfected flock by kangaroos.

These results, together with the overseas findings, suggest that further research activities should be conducted into the host specificity of strains of Mptb and the species studied should include the common domestic animals farmed in Australia and the rabbit and kangaroo. Further action on this recommendation should be postponed until the current study on KI is completed.
TR.054 THE ROLE OF RABBITS IN THE TRANSFER OF BOVINE JOHNE'S DISEASE

Summary

In any disease control program eradication of disease depends upon the protection of disease free animals from potential sources of infection. Sylvatic hosts have the potential to thwart eradication campaigns. Wild bovids and ungulates are a source of bovine tuberculosis for North American cattle herds. Two non-ruminant wildlife reservoirs, possums and badgers, in New Zealand and England respectively, have hampered eradication of bovine tuberculosis.

The presence of a common wildlife reservoir of Johne's disease (JD) could render the eradication of JD impossible or compromise current control measures. In Scotland, rabbits have been demonstrated to be infected with M. paratuberculosis (Mptb). In order to assess the risk posed to JD control due to wildlife, this survey examined rabbits and another herbivore commonly found on Australian pastoral land, eastern grey kangaroos.

European Rabbits

The aim of the first part of this project was to survey European rabbits (Oryctolagus cuniculus) for the presence of Mptb in areas with endemic JD in sheep and cattle.

In total 310 rabbits, 4 hares and 3 feral goats were examined by culture.

No Mptb was confirmed in any of the animals sampled. Eight of 314 samples demonstrated growth of organisms other than Mptb.

The lack of any evidence of JD in the rabbits in the Victorian survey would suggest that, under the conditions examined, rabbits are unlikely reservoir hosts for JD. The survey was designed to examine a sufficiently large sample of rabbits to detect infection if it occurred in relatively low levels of 3% with confidence limits of 95%. To "prove" that a disease does not exist in a population the entire population must be sampled with a test of 100% sensitivity and specificity. This survey does not rule out that rabbits may occasionally or rarely become infected with JD but that if it occurs it is likely to occur in low enough numbers as to not be a significant risk to resident populations of farmed livestock. Because of the limited range of farms and environments in the survey it is impossible to rule out the presence of a "focus" or "hotspot" of JD in rabbits as exists in Scotland. Large scale surveys for diseases that occur rarely are expensive and logistically difficult. A larger survey examining a wider range of environmental conditions over a larger number of farms with smaller numbers of rabbits per farm similar to the Scottish survey (Griag et al,1995) would be worthwhile.

Eastern Grey Kangaroos

Thirty-seven female and sixty-three male kangaroos were sampled. Five faecal samples had some growth which was shown to be due to contaminants, all other faecal and tissue samples were found to be negative.

There was no evidence of Mptb in the kangaroos examined. Although no animals were found to be infected with JD in this survey it does not rule out that eastern grey kangaroos could become infected with paratuberculosis. It does however demonstrate that infection if it occurs is unlikely to occur at a significant level and eastern greys are unlikely to act as significant reservoirs of infection of JD.
TR.060 - DEVELOPMENT OF A RAPID COST EFFECTIVE TEST FOR OVINE JOHNE'S DISEASE BASED ON TESTING OF POOLED FAECES

Abstract

This project was undertaken to develop and evaluate a rapid, cost-effective, flock test for *Mycobacterium paratuberculosis* (*Mtb*) in pooled faecal samples, based on hybridisation-capture polymerase chain reaction (HC-PCR). However, a simpler direct technique (DPCR) was found to be more sensitive than HC-PCR. About 67% of culture positive pooled faecal samples were positive when tested using DPCR. In a blind trial, 83% of 12 farms identified by culture of pooled faecal samples were detected using DPCR. The cost of DPCR is no greater than that of other flock detection strategies. The test is suitable for use in the National Ovine Johne’s Disease Control and Evaluation Program. A constraint exists in that Veterinary Committee does not recognise the results of DNA-based tests for *Mtb* as being definitive. The costs of follow-up testing to confirm infection are high. Recommendations are made to improve the test and reduce its cost.

Executive Summary

This project was undertaken to develop and evaluate a rapid cost effective flock test for the detection of *Mycobacterium paratuberculosis* (*Mtb*) in pooled faecal samples, based on the hybridisation-capture polymerase chain reaction (HC-PCR) technique. HC-PCR was developed in the UK for the detection of *Mtb* in tissues from Crohn’s disease patients and from faeces of animals with Johne’s disease (JD). The technique was modified in Australia by NSW Agriculture at the Elizabeth Macarthur Agricultural Institute (EMAI) to enable the test to be evaluated for routine use in diagnostic testing.

The specific objectives of the project were to evaluate the HC-PCR technique on 100 infected and 100 non-infected faecal samples from individual sheep and pooled faecal samples. Critical control points were to be identified and further improvements made. The test was then to be evaluated on faecal samples tested previously by the pooled faecal culture (PFC) technique. At the conclusion of this trial the appropriate documentation was to be prepared so that the technology could be transferred to other laboratories with a view to incorporating the test into the testing regime for OJD in Australia.

During the initial evaluation of the HC-PCR method we included a simpler PCR test which was used to identify *M. paratuberculosis* DNA directly from the faecal extract. Surprisingly, we found the simpler direct PCR (DPCR) technique to be more sensitive than HC-PCR. We identified several aspects of the HC-PCR technique that could be responsible for its apparent lack of sensitivity including the length and location of the capture probe, deterioration of the capture probe over time and deletion of Southern blotting. Each of these was addressed, but modifications to HC-PCR did not greatly improve the sensitivity of the test. Further experiments identified inefficiencies in the hybridisation and capture events that were responsible for the lack of sensitivity. The work on HC-PCR ceased as the technical improvements required were likely to have been too time consuming and costly to identify. The remainder of the project was focused on the simpler DPCR technique.

Initially, DPCR was performed with the same PCR reaction used in HC-PCR. This targets IS900, a gene thought to be unique to *Mtb*. Recent studies have shown that some environmental mycobacteria possess IS900-like elements that react in PCR assays for *Mtb*. As a result, restriction endonuclease analysis (REA) of the amplified product is required to confirm results as DNA consistent with *Mtb*. However, non-specific products from sources other than *Mtb* in the faecal sample were amplified simultaneously and this made confirmation by REA difficult. We evaluated several new PCR reactions for IS900 and IS1311, another gene used to identify *Mtb* (Collins et al., 1997, Whittington et al., 1998, Marsh et al., 1999), on 107 pooled faecal samples that had been evaluated by culture. A PCR reaction with superior sensitivity to the original PCR reaction was identified and was suitable for REA. The new reaction confirmed DNA consistent with *Mtb* in 66.6% of the culture positive pooled faecal samples.
A prospective, blind trial was performed with the new DPCR reaction on 328 pooled faecal samples submitted to EMAI for routine culture. DNA consistent with *M. ptb* was found in 66.6% of the culture positive pools. When the results were analysed by farm 83% of the properties positive by culture were positive by DPCR. An attempt was made to improve the sensitivity of DPCR by increasing the amount of faeces used from 0.1 g to 2 g. This was not successful but remains an area of future opportunity.

A rapid test for the detection *M. ptb* in pooled faecal samples could be offered to industry. Results would be available within a few days of receipt of samples at a laboratory. The costs of the new test are no greater than those of other flock tests. The technology would be transferable to detection of JD in other species of livestock. Significant constraints exist to the immediate application of this technology, not the least of which is the perception by Veterinary Committee that the results of DNA-based tests alone are not definitive for *M. ptb* infection.
TR.073 - PILOT STUDY: TRACER WEANER TRIAL FOR OVINE JOHNE’S DISEASE

Abstract

Eradication of ovine Johne’s disease (OJD) requires destocking sheep and spelling land until *Mycobacterium avium* subsp. *paratuberculosis* (*Mtb*) has died out. There is no simple way to assess the residual level of contamination or the risk this poses to livestock. Consequently small groups of Merino weaners were orally dosed with *Mtb*. Within 7-14 weeks the organism could be cultured from various locations in the gastrointestinal tract and associated lymph nodes. In addition, the results of a skin test were positive in 66% of weaners in which the organism had established an infection and were negative in uninfected sheep. The results of tests for gamma interferon were positive in some infected weaners but also in some of the controls. An ELISA test was not useful at this early stage of infection. Overall, the results suggest that weaner sheep could be used as sentinels in an infected environment, but this requires validation in a controlled field trial. Furthermore, the results suggest that the infectious dose of *Mtb* may be higher than previously thought.

Executive Summary

Eradication of OJD requires destocking sheep and spelling land until *Mycobacterium avium* subsp. *paratuberculosis* (*Mtb*) has died out. At the conclusion of a decontamination period, currently deemed to be 2 consecutive summers or 15 months, there is no simple way to assess either the residual level of contamination of the environment or the risk residual contamination poses to livestock.

This study was undertaken to determine whether it is possible to detect infection in young sheep exposed to low levels of contamination. Previous experiments in the UK suggested that this might be possible, but the trials had been done with cattle strains of *Mtb*. Consequently in this project small groups of Merino weaners were orally dosed on repeated occasions with graded numbers of an Australian sheep strain of *Mtb*. Samples were collected from each weaner at regular intervals and evaluated using several tests.

Within 7 to 14 weeks the organism could be cultured from various locations in the gastrointestinal tract and associated lymph nodes of each sheep that was given a total dose of 1.3 - 6.9 x 10^5 organisms. Infection was not established in weaners given lower doses. The next lower dose was 8.2 x 10^4. Therefore the infectious dose of this strain of *Mtb* is somewhere between about 10^3 and 10^5 organisms, a value higher than that found in the UK experiments. Reevaluation of the methods used in the UK experiments suggest that the doses given may have been underestimated by a wide margin. Thus the present results suggest that the infectious dose of *Mtb* may be higher than previously thought with the implication that it may be possible to reduce decontamination periods for land. In other words it might be easier to achieve decontamination of land than previously imagined.

Although infection was found by culture of tissues of these weaners, there would be a need to culture all sentinel sheep unless an indirect method could be found to identify sheep most likely to be culture positive. Fortunately the results of a skin test were positive in 66% of weaners in which the organism had established an infection and were negative in uninfected sheep, regardless of whether they had been dosed with the organism or were in a control group. The results of tests for gamma interferon were positive in some infected weaners but also in some of the controls so this test was not of value. An ELISA test also was not useful at this early stage of infection. Overall, the results suggest that weaner sheep could be used as sentinels in an infected environment, and that the skin test could be used to select individual sentinel sheep for culture. This approach requires validation in a controlled field trial.

The experimental infection method developed in this study can be used later for other purposes such as evaluating the pathogenesis of OJD, the behaviour of new diagnostic tests and vaccine efficacy.
OJD.007 EVALUATION AND COMPARISON OF TWO METHODS OF ABATTOIR SURVEILLANCE FOR DETECTION OF OVINE JOHNE'S DISEASE (OJD) INFECTED SHEEP FLOCKS IN REGIONS WHERE SHEEP POPULATIONS HAVE A HIGH PREVALENCE OF OJD INFECTED

This project was designed to determine whether properties infected with ovine Johne's disease (OJD) could be identified by routine monitoring of cull sheep at abattoirs for lesions of OJD or the presence of *Mycobacterium avium* subsp. *paratuberculosis* (Mptb).

Thirty-five lines of sheep that met predetermined criteria for inclusion in the trial were obtained from 17 known infected properties (average line 330, range 50 to 683). The criteria were designed to ensure that all the trial lines were infected with OJD. In most cases the trial lines were probably heavily infected. Over a period of six months, these trial lines were delivered to abattoirs throughout New South Wales for slaughter, most to the two major export works. One line was killed in Victoria.

Trained inspectors were stationed in abattoirs to examine not less than 50% and up to 95% 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 in each suspect 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%) of the 35 eligible trial lines. 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. Microscopic lesions diagnostic (31 lines 91%) or suggestive (3 lines or 9%) of OJD were identified in all 34 lines detected by inspectors. The average proportion of sheep in these 34 lines with gross lesions suggestive of OJD as reported by inspectors was 21% (range <1% to >90%).

Tied-off loops of terminal ileum were also taken from 10 randomly selected sheep in each line of sheep killed during the shift for pooled intestinal culture (PIC). Apart from 9 lines selected at the abattoirs as negative controls on the basis that these lines originated from areas not known to be OJD infected, all intestinal samples from non-trial lines were discarded on receipt at the laboratory. For trial and control lines to be tested, the mucosa was scraped from approx. 25 sq cm of each of the 10 tied-off loops of terminal ileum after opening by sterile technique. The pooled scrapings from each line were submitted for intestinal culture by the BACTEC method, with positive cultures subjected to PCR and REA for the IS900 sequence of M. ptb.

Of the 33 culture results available from the 35 trial lines, 19 (58%) were positive for M. ptb and 14 (42%) were negative. The crossbred line described above in which no visible lesions of OJD were detected by inspectors was positive on PIC. PIC detected as positive for OJD only 18 (53%) of the 34 lines detected by visual examination and histopathology.

The apparent lower sensitivity of PIC in this trial may simply reflect the small random sample (10) of sheep in the PIC method used in comparison to the larger sample examined visually (>200 sheep in most trial lines), rather than lower sensitivity of the culture method per se.

Five lines of sheep obtained from infected properties were retrospectively excluded from the trial on the basis of later epidemiological information that indicated they did not meet the selection criteria. All of these lines were negative on both histopathology and culture. For 7 of the 9 negative control lines (from properties not known to be infected with OJD), no visible lesions of OJD were detected. In 2 of the 9 control lines, however, inspectors reported 2% and 4% of sheep with suspect lesions, but all fixed tissue samples submitted from these lines were negative on histopathology.
Positive PIC results however were obtained from 2 of the 7 control lines in which no visible lesions were detected, one line from an area of Western NSW where OJD has not been reported to date, the other from an area where OJD had not been reported at the time. Whether these positive PIC results result from true flock infection or transient environmental contamination of cull sheep during transportation or holding in abattoir lairage is uncertain.

The results of this trial indicate that visual and tactile monitoring of the viscera of cull sheep at slaughter for lesions suggestive of OJD is a highly sensitive and reliable strategy for the detection of OJD infected flocks in which deaths from OJD are occurring. The estimated 95% confidence intervals of 91.5% and 100% for the sensitivity estimate of 97% obtained from these data suggest a practical working estimate of the sensitivity of visual/tactile examination of viscera for lesions suggestive of OJD in lines of >300 adult sheep as a screening test for the selection of samples for a definitive histopathology test would be 90% for any sheep population in which OJD has been recognised for many years. Whether this abattoir screening technique will be sufficiently sensitive for the routine detection of flocks that have been recently infected with OJD or flocks with persistent low prevalence infection is however less certain.
<table>
<thead>
<tr>
<th>Issue/Research Topic</th>
<th>Outcome</th>
<th>Relevance to control</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPIDEMIOLOGY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross species transfer from sheep to cattle</td>
<td>Identification of risk factors for transmission</td>
<td>Improved management</td>
<td>Risk</td>
</tr>
<tr>
<td>Cross species transfer to/from wild hosts (rabbits and</td>
<td></td>
<td></td>
<td>There is a greater need to investigate sheep to cattle transfer than vice versa.</td>
</tr>
<tr>
<td>marsupials)</td>
<td></td>
<td></td>
<td>There is an overlap of zones for BJD and OJD</td>
</tr>
<tr>
<td>Pathogenesis and progression of disease</td>
<td>Identification of factors that can be manipulated to control the level of disease on infected properties</td>
<td></td>
<td>May only be a Kangaroo Island specific problem – is this work relevant to mainland Australia?</td>
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<tr>
<td>→ impact of stressors and management practices in a</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>range of environments</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Further work on efficacy and economics of Gudair:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>→ Impact on low prevalence flocks</td>
<td>Identification of transmissible levels and time to reach with vaccination</td>
<td></td>
<td></td>
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<tr>
<td>→ vaccination at different ages and response to</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>infectious challenge</td>
<td></td>
<td></td>
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<tr>
<td>→ will vaccine prevent lateral spread?</td>
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<tr>
<td>DIAGNOSIS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFC – refine for prevalence evaluation and monitoring</td>
<td>Improve assessment of prevalence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Investigate relevance of a single positive test</td>
<td>Risk assessment</td>
<td>Trade/movement</td>
<td></td>
</tr>
<tr>
<td>Define reagents to use in gamma interferon and ELISA</td>
<td>Differentiate vaccinates from non-vaccinates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tests to improve sensitivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Validation of PFC in goats</td>
<td>Encourage goat producers to enter GoatMAP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR</td>
<td>Identify DNA target that is known to be specific</td>
<td>Identification and</td>
<td>Policy issue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>diagnosis</td>
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INTRODUCTION

A "brainstorming" session was held at the conclusion of the presentations of the international research reviews and the research updates on the projects currently underway within the NOJDP.

The major criterion for new OJD research directions was that the work must provide outcomes that are relevant to the control of the disease. The table below summarises the suggestions made during this session.

The inclusion of a research topic in this summary does not automatically endorse the project for future funding from the NOJDP.
BRAINSTORMING SESSION

IDEAS FOR FUTURE RESEARCH DIRECTIONS

OVINE JOHNE'S DISEASE
RESEARCH & DEVELOPMENT UPDATE

25TH JULY 2002
UNIVERSITY OF SYDNEY
OJD.022 EVALUATION AND COMPARISON OF PFC AND AGID AS FLOCK SCREENING TESTS FOR OJD

Executive Summary

A Monte Carlo simulation model was developed to estimate the sensitivity of pooled faecal culture (PFC) and the agar-gel immuno-diffusion test (AGID) as flock-screening tests for ovine Johne's disease under a range of scenarios. The flock-sensitivity of a test is the level of confidence of detecting a specified prevalence of infection. Outputs from the model are probability distributions for the flock-sensitivities of the two tests for a given scenario. The model allows direct comparison of the tests under a variety of conditions, and considers the effects of:

- Variations in animal-level sensitivity of the tests;
- Variations in flock size, sample size and prevalence of infection;
- Variations in animal-level sensitivity with type of lesion (paucibacillary or multibacillary);
- Variations in the proportions of animals with paucibacillary/multibacillary lesions; and
- Reduction in animal-level sensitivity due to pooling effects in pooled faecal culture;

Uncertainty as to the true values of input parameters such as animal-level test sensitivity and proportion of sheep with different lesion types.

Comparison of model outputs with results from a field trial of pooled faecal culture, and with calculated estimates, confirmed that the model provides reasonable estimates of flock-sensitivity of the tests.

The model was used to estimate:

- the flock-sensitivities of current testing strategies;
- sample sizes required for pooled faecal culture and serology to provide desired levels of flock-sensitivity for surveillance and market-assurance testing; and
- the sample size required for serology to provide equivalent flock-sensitivity to pooled faecal culture under a range of scenarios.

The mean flock-sensitivities for a Check Test (sample size = 100) were 67% and 42% for PFC and AGID respectively, and for a Sample Test (sample size = 350 for PFC, 500 for AGID) were 98% and 93% respectively. When large flocks were sampled, sample sizes of 300, 350 and 450 provided a flock-sensitivity for PFC of about 95%, 98% and 99% respectively, to detect infection if present at a prevalence of 2% in the sampled population. Sample sizes for the AGID to provide equivalent sensitivity to PFC were generally 2 - 3 times the PFC sample size, depending on the assumed animal-level sensitivities of the tests.

When whole-flock testing was simulated, AGID flock-sensitivity was generally poor for smaller flock-sizes and low prevalence or animal-level sensitivities, whereas PFC flock-sensitivity remained high. The flock-sensitivity for PFC was ≧98% for all combinations of flock size, prevalence, percentage of paucibacillary lesions and animal-level sensitivities tested compared to ≧68% for the AGID.

Although the AGID appears to perform reasonably well in higher prevalence flocks, its flock-sensitivity in low prevalence or recently infected flocks is likely to be very low, unless sample sizes 2 - 3 times those used for PFC are used. This is particularly important in Australia at present, as the majority of flocks being investigated outside the Residual Zone (endemic area) are likely to be relatively recently infected and still have only a low prevalence of infection. In these circumstances, PFC should be the preferred test, and larger sample sizes or whole-flock testing should be considered to maximise flock-sensitivity.

In higher prevalence flocks, such as many of those in the Residual Zone, the AGID will provide a satisfactory flock-sensitivity and a more-rapid result than PFC, particularly if biased sampling is used to maximise animal-level sensitivity and provided sample sizes are adequate.