OJD Research Update & Control Workshop

University of Sydney
Veterinary Science Conference Centre
Monday 24th & Tuesday 25th September 2001

MEAT & LIVESTOCK
AUSTRALIA
### FORUM PROGRAM

#### Day 1

<table>
<thead>
<tr>
<th>Start time</th>
<th>Session time</th>
<th>Subject</th>
<th>Presenter</th>
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<tbody>
<tr>
<td>10.00</td>
<td>20</td>
<td>Registration and coffee</td>
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<tr>
<td>10.20</td>
<td>10</td>
<td>Welcome and introduction</td>
<td>Peter Rolfe</td>
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<tr>
<td>10.30</td>
<td>15</td>
<td>Workshop Objectives, process and roles</td>
<td>Ian Crook</td>
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<tr>
<td>10.45</td>
<td>10</td>
<td>Investigating mortalities due to OJD</td>
<td>Helen McGregor</td>
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<tr>
<td>10.55</td>
<td>15</td>
<td>Significance of OJD and progress with the disease - a farmer perspective</td>
<td>Terry Hayes</td>
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<tr>
<td>11.10</td>
<td>15</td>
<td>Significant of OJD and progress with the disease - a farmer perspective.</td>
<td>Stuart Reid</td>
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<tr>
<td>11.25</td>
<td>10</td>
<td>Cross species transfer of Johne’s disease</td>
<td>Richard Whittington</td>
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<tr>
<td>11.35</td>
<td>15</td>
<td>Epidemiology - sources and importance of infection in lambs.</td>
<td>Kym Abbott</td>
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<tr>
<td>11.50</td>
<td>20</td>
<td>Survival of the organism in the environment</td>
<td>Richard Whittington</td>
</tr>
<tr>
<td>12.10</td>
<td>75</td>
<td>WORKSHOP – Development of Findings and Implications for each subject so far.</td>
<td>Small groups</td>
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<tr>
<td>1.10</td>
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<td>Review morning session, domestic arrangements for lunch and accommodation overnight.</td>
<td>Ian Crook/Peter Rolfe</td>
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<tr>
<td>1:15</td>
<td>45</td>
<td>Lunch</td>
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<tr>
<td>2.00</td>
<td>60</td>
<td>PLENARY SESSION – presentation of butchers paper small group outputs –</td>
<td>Six Groups – 10 minutes per group includes time for questions of clarification and arrival at consensus</td>
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<tr>
<td></td>
<td></td>
<td>A. Key Finding</td>
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<td>B. Implications for the designated stakeholder categories</td>
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<tr>
<td>3.00</td>
<td>15</td>
<td>Break</td>
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<tr>
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<td>10</td>
<td>Vaccination studies</td>
<td>Peter Windsor</td>
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<tr>
<td>3.30</td>
<td>15</td>
<td>Eradication of OJD by destocking</td>
<td>Pat Taylor</td>
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<td>3.40</td>
<td>10</td>
<td>Detection of OJD in abattoirs</td>
<td>Laurie Denholm</td>
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<tr>
<td>3.50</td>
<td>10</td>
<td>Gamma Interferon test - potential for early diagnosis</td>
<td>David Stewart</td>
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<tr>
<td>4.00</td>
<td>10</td>
<td>Tracer weaner as a tool for detection of infection</td>
<td>Leslie Reddacliff</td>
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<td>4.10</td>
<td>15</td>
<td>Development of other diagnostics</td>
<td>Ian Marsh</td>
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<td>WORKSHOP – Development of Findings and Implications for second batch of 6 subjects.</td>
<td>Groups</td>
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<tr>
<td>5.25</td>
<td>5</td>
<td>Review and domestic arrangements</td>
<td>Ian Crook</td>
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<tr>
<td>5.30</td>
<td>15</td>
<td>Questions and comments for the day – outline for the next day activities</td>
<td>All, Ian Crook plus MLA</td>
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<td>7:00 for</td>
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<td>Drinks and Dinner</td>
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<td>7.30</td>
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<tr>
<td>Time</td>
<td>Duration</td>
<td>Activity</td>
<td>Organizer</td>
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<tr>
<td>8:30</td>
<td>15</td>
<td>Review and scene setting, travel arrangements for later in the day.</td>
<td>Ian Crook</td>
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<tr>
<td>8:45</td>
<td>60</td>
<td><strong>PLENARY SESSION</strong> – presentation of butchers paper small group outputs –  &lt;br&gt; A. Key Finding  &lt;br&gt; B. Implications for the designated stakeholder categories</td>
<td>Six Groups – 10 minutes per group includes time for questions of clarification and arrival at consensus</td>
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<tr>
<td>9:45</td>
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<td>Morning break – tea and coffee</td>
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<tr>
<td>10:00</td>
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<td>Workshop session – draft the Tips and Tools for the first 6 subjects.</td>
<td>Groups</td>
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<td>11:00</td>
<td>60</td>
<td><strong>PLENARY SESSION</strong> – presentation of draft Tips and Tools for each of first six subjects.</td>
<td>Six Groups – 10 minutes per group includes time for questions of clarification and arrival at consensus</td>
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<tr>
<td>12:00</td>
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<td>Lunch</td>
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<td>12:45</td>
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<td>Groups</td>
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<td>60</td>
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<td>Six Groups – 10 minutes per group includes time for questions of clarification and arrival at consensus</td>
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<tr>
<td>2:45</td>
<td>15</td>
<td>Review, process from here, thanks and close</td>
<td>Ian Crook, Peter Rolfe to close</td>
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<td>3:00</td>
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Foreword

Ovine Johne's disease continues to create challenges not the least are the methods that can be used to reduce the impact of the disease on individual farms. The aims of this forum are twofold. Firstly it will provide the most recent results of the research that has been conducted under the national program. Secondly, it will be an opportunity for key personnel involved in the management of the disease to develop generic control strategies that may be applicable for particular production systems. This is an opportunity to revisit the first principles of disease control.

This forum is not about disease control policy and it's implementation. There are many other forums for that debate to occur. This group will concentrate very clearly and precisely on technical issues on how to control the disease on a farm and by extension over a number of farms in districts and regions.

There are a number of producers at this forum and we particularly welcome their presence and contribution. As veterinarians, advisers and researchers with experience in disease control, it is very necessary to have the hard edge of experience to weigh the options that may be presented. There are participants from all sections of the advisory network across Australia, but with particular contributions from NSW as the state with the largest number of affected farms. This is a unique opportunity to take advantage of the knowledge and experience of all those assembled to develop the most up to date recommendations and to foresee future strategies that may be applicable when more information is known.

The outcomes of this forum will be a better informed community of advisers armed with information that has been critically reviewed that can be used to reduce the impact of the disease for individual farmers. I encourage you to participate actively in the discussion and the workshop sessions.

I would like to thank Gilly Simos and Jaime Lawton for the large effort behind the scenes to make this forum happen.

Dr Peter Rolfe
Program manager, Animal Health and Welfare
Meat and Livestock Australia.
Investigating Mortalities due to OJD
Helen McGregor, Kym Abbott, Peter Harper, Andrea Britton, University of Sydney

The causes of mortality and the effects of whole flock vaccination against OJD are being studied on a Johne's infected property in the Southern Tablelands of NSW. Early estimates based on stock inventories indicated losses of up to 25% between September 1999 and September 2000. Post mortem studies take place four times a year. Initial results from the two post mortem studies prior to vaccination (April and July 2000), supported the first estimates of mortality rate.

Approximately 10.7% of adult sheep tested were seropositive in prevaccination testing. All sheep were vaccinated in September 2000 in order to reduce OJD contamination of pastures, incidence of clinical OJD and the death rate due to OJD. The faecal excretion rate of Mycobacterium avium subsp. paratuberculosis in 2-year-old sheep has been chosen as an index of the prevalence of OJD in the flock, supporting other measures including mortality rates and serology. Monitoring of the changing cause of mortalities, rate of mortality, seroprevalence and faecal excretion rates will continue until December 2003 when the first lambs vaccinated will reach three years of age.
Investigating Mortalities due to OJD

Study Property
- Location
- Enterprise
- History Flock
- History OJD
- Management systems
- Flock management
- Southern Tablelands NSW
- Fine wool Merinos
- Established 1856
- Diagnosed 1996
- Following voluntary testing
- Cell grazing
- Set-stocked lambing in September-October
- Shearing August-September

Study Aims
- Assess sampling method
- Investigate mortalities
- Estimate true OJD mortality rate
- Measure OJD mortality rate over several years following whole flock vaccination
Determining a True Mortality Rate

- Can we find the sheep?
- Are dead sheep submitted for postmortem?
- Can we collect and store appropriate tissues?
- Is there adequate record keeping?
- Are we confident of an unbiased result?

Time of Observations

- Commenced April 2000
  - Month 0-April 2000
  - Month 3-July 2000
  - Month 6-October 2000
  - Month 9-January 2001
  - Month 12-April 2001
  - Month 15-July 2001

Necropsies

- Systematic examination of all mobs
- Identification of dead/dying sheep
- Standard post mortem inspection protocol
- Collection of appropriate tissues
  - OJD diagnosis- histology and bacteriology
  - Worm counts- intestinal and abomasal
  - Non OJD diagnosis
Histopathology

- Tissues sampled
  - Ileo-caecal junction
  - Terminal ileum (3 sites 1 meter apart)
  - Colon
  - Caudal jejunal node
  - Ileo-caecal node
  - Liver

Histopathological Diagnosis

- OJD lesions were categorised objectively;
  - Focal granulomatous enteritis: small aggregations of >3 macrophages within lymphoid tissue and or mucosa +/- scarce/moderate numbers AFB
  - Zonal granulomatous enteritis: zonal or locally extensive accumulations of macrophages with scarce/moderate/abundant AFB
  - Diffuse/Severe granulomatous enteritis: diffuse infiltration of macrophages within lymphoid tissue and mucosa. Moderate to abundant AFB

Determination of Cause of Death

- Sheep are determined to have died from OJD alone if there are;
  - Clinical signs of OJD
  - Histopathological evidence of diffuse, severe OJD
  - Absence of other causes of death
  - Clinically insignificant worm counts
Determination of Cause of Death

Sheep with histopathological evidence of focal or zonal granulomatous enteritis, or the diffuse severe form complicated by other disease states were classified as "dying with OJD"

If no evidence of OJD was observed, other causes of death were recorded.

Results

Mortality Rate by Inventory Year 0

- Shearing Sept '99-Sept 2000
- Losses
  - Adults: 1947
- Total at risk: 9071
  21.5 % of adult sheep

The average daily death rate consistent with 1947 deaths per year is 5.3 sheep per day, or 26.7 sheep in a five day period.
Mortality Rate by Inventory Year 1

- Post shearing September 2000 – June 2001
- Total at risk: 9276 (>12 months)
- Total losses in adult population: 1011
- 10.9% of adult sheep

Abomasal and Small Intestinal Worm Counts

<table>
<thead>
<tr>
<th>Month</th>
<th>Total Worm Count</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0 – 3600</td>
</tr>
<tr>
<td>3</td>
<td>2400 – 24400</td>
</tr>
<tr>
<td>6</td>
<td>0 – 16500</td>
</tr>
<tr>
<td>9</td>
<td>0 – 3400</td>
</tr>
<tr>
<td>15</td>
<td>0 – 40,000</td>
</tr>
</tbody>
</table>

Four animals in month 3 and five animals in month 15 were considered to have significant worm counts. These animals were considered to have died with ovine Johne’s disease.
### Mortality Rate Estimated by Sampling

<table>
<thead>
<tr>
<th>Period (month)</th>
<th>No. of Mort</th>
<th>% with JD</th>
<th>% other causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0+</td>
<td>20</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>9</td>
<td>5</td>
<td>50</td>
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</tr>
<tr>
<td>12+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15+</td>
<td>1</td>
<td>50</td>
<td>0</td>
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</table>

* sheep were not available for necropsy

### Mortality Rate/Cause of Death

#### YEAR 0

<table>
<thead>
<tr>
<th>Age/yr</th>
<th>No. Dead</th>
<th>% from JD</th>
<th>% with JD</th>
<th>No JD</th>
</tr>
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<tbody>
<tr>
<td>0-1</td>
<td>3</td>
<td>0</td>
<td>55</td>
<td>6</td>
</tr>
<tr>
<td>1-2</td>
<td>18</td>
<td>22</td>
<td>38</td>
<td>11</td>
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<td>2-3</td>
<td>7</td>
<td>28</td>
<td>57</td>
<td>14</td>
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<td>3-4</td>
<td>9</td>
<td>44</td>
<td>33</td>
<td>0</td>
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<tr>
<td>4-5</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5-6</td>
<td>11</td>
<td>31</td>
<td>18</td>
<td>0</td>
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<tr>
<td>Total</td>
<td>49</td>
<td>41</td>
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#### YEAR 1

<table>
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<tr>
<th>Age/yr</th>
<th>No. Dead</th>
<th>% from JD</th>
<th>% with JD</th>
<th>No JD</th>
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</thead>
<tbody>
<tr>
<td>0-1</td>
<td>9</td>
<td>0</td>
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<td>78</td>
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<td>0</td>
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<td>2-3</td>
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<td>69</td>
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<td>0</td>
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</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>48</td>
<td>30</td>
<td>22</td>
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</tbody>
</table>
• For Year 0
  • Based on gross post mortem results and histopathology
  • We can attribute 68% of all adult deaths to OJD and estimate the annual OJD mortality rate of adults to be 14.6% in 1999/2000

• For Year 1
  • We can attribute 60% of all adult deaths to OJD and estimate the predicted annual OJD mortality rate of adults to be 8.8% in 2000-2001

Acknowledgements
• NSW Stud Merino Breeders Association
• CSL
• Yass Rural Lands Protection Board
• Proprietors of the study property
• National Ovine Johne's Disease Control and Evaluation Program
• Meat and Livestock Australia
Managing OJD – A Personal Experience
Terry Hayes, Middle Arm

Appreciating reality……something was wrong. From 1991 we saw wasting sheep, production losses. Then we lost half our breeding ewes in 1995.

Diagnosis
January 1996

Regulatory environment at the time
• Quarantine
• Eradication

Property environment
• Despair
• Aimlessness
• Frustration
• Anger

OJD management
Nothing until early 1998, then a process of evaluation.

What was the problem?
• Deaths
• Reproduction losses
• Poor lambing
• Difficulty marketing stock

Was it OJD?
University investigation on 60 sheep found 100% positive.

What were the possibilities?
• Eradicate and start again
• Change enterprises
• Carry on

Our decision
• Carry on
• Reduce sheep
• Increase cattle
• Reduce stress
• Better parasite management
• Better nutrition for weaners
• Spell country with cattle for weaners

Deaths normally began at 15 months. The first group under this strategy was no different. We needed a circuit breaker. Vaccine was introduced in June 2000.

Conclusion
• Every property must want to know its stock disease status
• Every property must want to know the status of its trading partners
• Every property must know the status of its neighbours

Properties should adopt wholistic stock management that will:
• Provide adequate nutrition
• Control parasites
• Control introductions
• Include vaccine when appropriate
• Minimise stress
  - Regulations must allow properties to remain viable
  - Managers must see light at the end of the tunnel
  - Programmes must not be long winded
  - Extension services must be respected.
Managing OJD – A Personal Experience
Terry Hayes, Middle Arm

We operate a fine wool enterprise on the Southern Tablelands of New South Wales. The flock is self-replacing and apart from rams purchased, has been closed for 60 years.

Some of our rams “did” badly around 1991. By around 1994 we were barely buying enough rams to keep sufficient on the property for breeding.

Our lambing percentage, by this time, had dropped to 40 – 45%. A “tail end” was noticeable in the ewes.

Was it the effect of footrot? Was this damn drench not working? The seasons have been pretty ordinary! Income is down, Vets are bloody expensive!

A ram died three days after it arrived on the property from our stud source. Why?

1995 was a tough winter. I joined 1290 ewes. At weaning I had 640 ewes left and weaned 230 lambs. At the end of that spring I still had a “tail end” in the ewes.

I rang the district vet – he was free.

The flock was diagnosed with OJD in January 1996.

I believed that the severe losses were due to OJD, however this was to be disputed by a large number of people who, at that stage, believed that OJD was of no significance – “it’s been here for fifty years”.

University investigation of 60 of our sheep in 1997 using histopathology confirmed that all had OJD.

At the time the flock was diagnosed, the regulatory environment was directed towards quarantine and eventually eradication by total destocking.

My property environment until early 1998 was despair, aimlessness, frustration and anger. On property management after OJD diagnosis had come to a standstill.

No one was sure what direction policy would take.

Standstill was common to almost all infected properties on the Southern Tablelands of New South Wales at that time.

A realisation of the need to be positive and have direction dawned in early 1998. We made a conscious decision to go forward based on “bugger them.”

Evaluating Our Position

We set about evaluating our position. The problems crystallized down to:

- Disease Transfer
- Poor lambing
- Production losses
- Deaths
- Difficulty marketing stock
After the disastrous winter of 1995 our deaths seemed to plateau out to 13 – 15% in
the 18 month to 3 year old sheep down to around 5% at 5-6 year old. Before 18
months of age deaths didn’t occur.

Production losses centered around devaluation of wool due to factors such as
increased quantities of tender, short and yellow wool. I don’t really know if this was a
result of disease or as a result of lower quality sheep being retained due to an
inability to cull effectively because of the overall deaths occurring in the flock.

Lambing percentage was low at around 40 – 45% requiring a larger than desirable
number of ewes to be joined.

Because of the quarantine we were forced to dispose of stock only through abattoir.
This did not disadvantage me but some producers did report savage discounting
because of lack of competition in the market place. Off lines were most difficult to
move.

We were satisfied, particularly after the university investigation on our flock, that OJD
was our primary problem.

IT WAS SIGNIFICANT – MANAGEMENT NEEDED TO TAKE IT TO ACCOUNT

What were the possibilities?

• We could eradicate and start again.
• We could change enterprises.
• We could carry on.
• We could sell out and retire.

Eradication did not seem feasible scientifically. Disinfection of land and water had
not been investigated. Status of neighboring properties was unknown to us as was
the status of most flocks from which we would source replacement stock. It was
going to be very expensive and there was not an offer of compensation. The only
attraction was that it would have removed the regulatory monkey from our back but at
the risk of still having the disease.

Changing enterprise lent toward cattle because we had the expertise and the
infrastructure. Some of the property did not lend itself well to cattle because we had
an amount of land that was unimproved. Fat lambs were considered about equal to
cattle except that cattle were attractive because of the destocking value for OJD from
a regulatory point of view.

Selling out to retire looked attractive except that we were too young and the value
of the property and livestock would have been severely effected because of the
quarantine. We further considered it would have been an admission of defeat from a
problem that we felt, left to our resources, we could rise above. We are the third
generation of our family to occupy this property and we did not want to surrender to
what we perceived as an incompetently managed program based on whim rather
than science and sound economic analysis. My grandparents founded the property
and my parents and Cecily and I continued building the enterprise completing nearly
100 years of work and investment. We were not about to stand by and watch it
disappear without some struggle.
Should we carry on? We considered the finewool enterprise more suitable and more profitable. The livestock were more valuable to us than to cash them out at slaughter prices. We decided to pursue finewool and attune the enterprise to take into account the need to overcome the problems we had identified at the time.

A NEW STRATEGY

We needed to reduce the infection on the land and within the livestock.
The strategy:
- Reduce Stress.
- Improve parasite control.
- Destock country of sheep.

**Stress Reduction:** Stress seemed to us to be a significant factor, where there was infection, in bringing on the clinical disease. Lambing would be put back from mid July until the last week in August in an attempt to increase lambing percentage. The dual effect of joining after the autumn break and lambing in the better weather would achieve this. We anticipated the number of ewes required to be joined would be less. Shearing would move to February. Taking away the double stress near lambing would help the ewes and shearing after weaning would reduce the premature weaning associated with putting ewes and lambs in a shearing shed together.

**Parasite Control:** We adopted broadly the recommendations in Drenchplan and decided to carry out worm monitoring before the second recommended drenching time, after the Autumn break and prior to lambing. We would regularly do testing against drench resistance. Sheep would always be weighed before drenching and manufacturers instructions would always be adhered to.

**Destocking:** We would increase cattle numbers against sheep so that we would have sufficient cattle to destock a large enough part of the property on which to run our weaner sheep. Whilst this strategy would not necessarily eradicate all bacteria on the land, we were confident it would lead to a significant reduction. This would slow the onset of the disease and be beneficial in reducing other internal parasites.

**Three Years On**

Deaths from OJD had begun in the flock when sheep were around 16 to 18 months of age. The first death in the first mob to be managed under the new strategy was right on cue at 17 months of age. There seemed sufficient infection to carry on with these sheep as weaners onto spelled country to lead to deaths at the normal starting age. The following months demonstrated that the number of deaths would be as normal.

We needed a circuit breaker.

Vaccination was commenced in June 2000. Current plans are to carry that on for two generations or ten years.

Today our losses are running at about 10% in untreated sheep.

Sheep that were vaccinated at 2 years of age are still suffering losses of around 3%. Peculiarly most of the losses are in the wether portion of that mob.
Sheep vaccinated at the same time but that were 1 year old, have had no deaths due to OJD. I would without the vaccine, have expected them to begin to die in January of this year.

Sheep vaccinated as lambs in November 2000 have had no deaths due to OJD. I would not however, have expected any deaths at this stage in any event. Trial work being conducted on some unvaccinated sheep from this mob will provide a good control group since they are under intense scrutiny. These unvaccinated sheep are running separately.

Properties that have discovered the infection when it has been at low levels have been able to sustain low losses for long periods using the sorts of strategies that we attempted. Care with introductions is most important in these circumstances.

**Management and The Policy Environment**

Developing management strategies will be a waste of time unless the policy environment is conducive to universal industry adoption.

Every property must know its disease status of its trading partners and neighbours.

Good OJD management strategies will include all facets of animal husbandry. Properties need to remain viable if the labour force and resources are to be available for good husbandry.

Problems need to be faced positively and managers must have confidence in their capacity to emerge from the disease situation.

Quarantine must not be used beyond obtaining ongoing commitment to reducing the problem. Regulatory requirements must be soundly based and achievable practically and economically. Regulations are best left for recalcitrants.

Extension services will never sell any message unless the message is soundly based and the extension service is credible. Such services have no hope whatsoever if they can't get onto the farms.

Unless policy can be reasonably stable, confusion will reign supreme in the minds of producers.

**REMEMBER:** Policy on the run one day, have extension officers looking down the barrel of a gun.
Issues I will raise in the discussion

Presenter:

Topic:

The key message for me from this presentation was:

I would like clarification on:

I would like to add the following points to the discussion:
Managing OJD – A Personal Experience
Stuart Reid, Berridale

Murlingbun, a property of 1500 Ha carrying a self replacing merino flock of 5000 and a breeding herd of 160 cows, is situated 40 kms west of Cooma in the foothills of the Snowy Mountains at 1100-1200 metres.

Joined exclusive club June 2000. Stud ewes bought Woodstock area Jan 15 1996. Joined map programme May '96 with 50 sheep blood tested including some of above gained MN1. Post mortem 2 sheep each year and fell over in June 2000 with a commercial wether showing positive. Immediately had pooled samples taken from the commercial sheep and individual blood tests and pooled culture of the Studs. Commercials came up positive and the studs negative. A clean block with separate woolshed was leased for the studs but a further pooled culture test 6 mths later showed the studs to be infected also. This was devastating news. Here I was only the 2nd property on Monaro in a Control area with the “dreaded disease”. I felt like a leper. It surprised me how few phone calls I got with support of what I was facing. I had a self replacing merino flock breed on Merryville blood lines for 70 years with a micron 18 to 20 and my only market was the abattoirs.

My wife Jan gave me great encouragement not to let it beat you. I looked at destocking but the economics didn’t stack up and after getting a phone call from a client who had been through that process and ended up with another case of OJD and 2 lots of foot rot, the chances of staying clean weren’t good.

The above sheep were the only ones I had bought in 6 years as I was using an AI programme and could really see progress being made. The capital asset loss associated with getting OJD is quite staggering $30.00 a head over 5000 would easily be possible and more like $50.00 or more. The loss of trading has been significant in the first year alone, in the order of $25000.00.

If there is a positive to come out of this whole mess, its this; it is only 15 months since my flock was quarantined and I now have access to Gudair vaccine. This I believe is the only way I will lower the risk to my neighbours, and lower the infection build up on my place.

When I was first quarantined I immediately split my property in 2 isolating my 3-5 year old sheep to the side I felt carried the most infection and the weaners and hoggets to the second area. Because weaners and hoggets are less likely to be shedding, this would give me cleaner country. As time went on I realized that to be able to keep the side with the young stock relatively clean I needed to vaccinate the weaners before they came to the cleaner side or give up having a self replacing flock.

If the long term aim is still for eradication of OJD I would consider converting to cattle over time if adequate financial help was available. This may be the only way that you could remove the loss of land value. It really depends how quickly OJD spreads from my place and how many neighbours become involved. My feeling is it probably has already spread but will take time to show up. So vaccination may have the same effect as destocking without the financial cost.

There needs to be a lot more evidence to prove destocking is worthwhile and what time frame is required especially in our cool climate. I would be very surprised if 2 summers would be adequate. I also believe that it would be very cost effective if the vaccine was subsidised for that first initial vaccination where half or more of your flock is being done. Also I don’t think you should need 5% deaths to get the vaccine but simply a commercial decision on your part. Looking back it has surprised me how quickly losses in the 3-4 year old sheep have built up to where it is over 50 for a 6 months period or about 6%. 
Issues I will raise in the discussion

Presenter:

Topic:

The key message for me from this presentation was:

I would like clarification on:

I would like to add the following points to the discussion:
Cross species transfer of Johne's Disease
Richard Whittington, NSW Agriculture

Johne’s disease is caused by a bacterium with the technical name *Mycobacterium paratuberculosis*. There are two main types, one that commonly infects sheep (S strain), and another that commonly infects cattle (C strain). Tests developed in Australia enable the two strains to be identified rapidly.

In Australia, JD in sheep is almost always caused by S strain and JD in cattle by C strain. Isolates of the organism from 168 sheep from 33 farms were typed as S strain and isolates from cattle from 63 farms were typed as C strain in a study that concluded in December 1998, results that agreed with an earlier and smaller survey. However, cattle occasionally become infected with S strain, but probably only when they are raised as calves with infected sheep. Goats also get S strain but tend not to be badly affected, which makes it harder to detect. Overseas, C strain occurs in many species and this indicates that it is quite promiscuous, but S strain is mostly confined to sheep. Over time in Australia we can expect to find C strain in most farmed species, including sheep.

Based on observations over about a century it is clear that the organism prefers to live in the gut of grazing animals, especially ruminants. For this reason Johne’s disease is very unusual in animals other than cattle, sheep, goats, deer and a few other herbivores. However, if artificially large doses of the organism are given by mouth or injected, many species can become infected: mice, guinea pigs, rabbits, horses, pigs and even chickens. In 1997 rabbits in Scotland were found with severe infection and it was associated geographically with farms with Johne’s disease in cattle. Further research led to the discovery of infection in carnivores in the same area – foxes, weasels and stoats - but these animals had very mild lesions. The bacteria were isolated also from a range of animals that had no signs of disease – several birds, rats, mice and hare. More recently the infection has been detected in wild ferrets in New Zealand. *M. paratuberculosis* C strain has also been isolated from people and a link has been proposed with Crohn’s disease.

Two surveys have been completed of rabbits and kangaroos on OJD affected farms in Victoria and NSW. No evidence of infection was found. However, infected wallabies have been found on Kangaroo Island, SA and the lesions in some were quite severe. A survey is planned to see how common this is.

Under most circumstances Johne’s disease is passed from one farm animal to another and wild animals are probably not an important management consideration. The exception is where the disease is to be eradicated, but even then it is unclear whether wildlife pose a risk – it depends on how common the infection is and the degree of contact with farm animals. This is being studied in Scotland.

Semi-wild farm and exotic species such as deer and goats are also a potential source of infection. White-tailed deer and bison in the USA and alpine ibex in Europe harbour the infection, but it might not be a simple matter of these species exchanging infection with farm animals. For example we were recently able to prove that bison in the USA are infected with a unique strain and have not acquired the infection from cattle.

More extensive typing is required whenever wildlife are found with the infection as it is possible that they might have their own infection cycle. In Australia we need to be vigilant and monitor wildlife and feral animal populations for signs of infection. We also need to continue to monitor the strain of infection occurring in farmed animals.
Cross species transfer of Johne’s disease

Richard Whittington
NSW Agriculture

Why bother about cross species infection?

- Eradication of OJD
  - do we need to get rid of cattle too??
  - what about rabbits and kangaroos??

- Control of OJD
  - are my cattle at risk if I run them on OJD infected pasture?
  - can I use cattle to prepare pasture for sheep?

Strains of Mycobacterium paratuberculosis
**Strains of M. paratuberculosis**

- In Australia
  We are in the early stages of the epidemic
  - S strain - mostly infects sheep
    - 33 farms, 168 isolates from sheep all S strain
  - C strain - infects cattle, goats, deer, alpaca, men
    - 63 farms, 160 isolates from cattle, goats, alpaca, all
      C strain
    - but S strain sometimes infects calves, goats

**Strains of M. paratuberculosis**

- Overseas
  The epidemic is more mature
  - S strain still mostly infects sheep, - deer in NZ
  - C strain infects most species

- Conclusion
  - S strain is relatively choosy
  - we can regulate BJD separately from OJD

**Usual transmission of Johne's disease in Australia**
Transmission of Johne’s disease globally

Host range of *M. paratuberculosis*

- Natural disease
  - cattle, sheep
  - goats, deer
  - camelids
  - rabbit, fox, stoat
  - weasel (Scotland)
  - ferrets (New Zealand)
  - wallaby (SA)
  - wildlife e.g. deer,
    alpine ibex, bison
  - zoo animals

- Experimental disease
  - mice
  - guinea pigs
  - rabbits
  - horses
  - pigs
  - chickens

Possible transmission of Johne’s disease in bison

USA?
Strain typing DNA test

Bison  Cattle  Sheep

Actual transmission of Johne's disease in bison

Bison JD  B strain

Possible transmission of Johne's disease

Scotland?  Australia?
Conclusions for Australia

- OJD spreads from sheep to sheep (mainly)
- BJD spreads cow to cow and to other hosts
- Wildlife might be a risk for livestock but wildlife can have their own independent infection cycle
- Wildlife/livestock contact is an issue
- We need to be vigilant (there is an active project on risk of OJD spread to cattle)
- We should expect to see BJD in sheep
Understanding host factors in the transmission of OJD within flocks
KA Abbott, The University Of Sydney

An understanding of the factors which influence the prevalence of OJD within flocks is essential to the development of effective management strategies for flock owners. The factors which are potentially important include

- A variation with age in the susceptibility of sheep to OJD
- A difference in susceptibility to OJD between ewes, rams and wethers
- A difference in susceptibility to OJD between sheep of different breeds
- A genetic variation in susceptibility within flocks, which can be exploited through breeding programs
- A variation in susceptibility within flocks which can be exploited through management practices, particularly those which reduce stress.

To date, the first of these has received the most attention and, if real effects exist, this factor offers the most readily applied strategies for reducing disease prevalence.

Current research project – Epidemiology I
This project is a collaboration between Kym Abbott (The University of Sydney) and Richard Whittington (NSW Agriculture). Helen McGregor (The University of Sydney) is the project manager. The experimental site is at Arthursleigh Farm, a large grazing farm belonging to the University and situated near Goulburn, in the southern Tablelands of NSW. The site covers 200 ha and the experiment includes 500 Merino sheep born in spring, 1999.

The project aims to identify the major risk factors for young sheep associated with the development of OJD by 3 years of age and to quantify some of the factors discussed in broad terms in the introduction above.

Proposed research project – Epidemiology II
A further examination of age susceptibility is planned to commence in December 2001. This project will, however, determine the relative susceptibility of mature adult, young adult and weaner sheep to infection at a range of exposure levels. This project is a collaboration between The University of Sydney, NSW Agriculture and CSIRO.
Understanding host factors in the transmission of OJD within flocks

KA Abbott
The University of Sydney, Camden, NSW

An understanding of the factors which influence the prevalence of OJD within flocks is essential to the development of effective management strategies for flock owners.

The factors which are potentially important include:
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- A difference in susceptibility to OJD between sheep of different breeds
- A genetic variation in susceptibility within flocks, which can be exploited through breeding programs
- A variation in susceptibility within flocks which can be exploited through management practices, particularly those which reduce stress.
To be useful, age resistance must be teased out further, to determine

- The precise relationship between age and susceptibility including a determination of the age at which resistance becomes effective (if it exists at all)
- The relationship between age of first exposure and age of disease onset
- The relationship between age resistance and size of challenge in determining the outcome of exposure
- How well these relationships match the variation in infectivity which can be achieved with affordable practices on farms. For example, there may be such a low threshold of infection that an infective dose is exceeded in virtually all infected flocks.

A high prevalence of infection is likely to be associated with

- A high mortality due to OJD infection, we know that up to 15% of adult sheep (> 12 months) may die of OJD per year.
- Reduced reproductive rate, as a consequence of clinical disease of ewes during pregnancy or lactation and, probably, the reduction in fertility consequent on reduced liveweight in sub-clinically affected animals.
- A reduced productivity of wool
- An increased proportion of poor quality wool
- An increased risk of OJD transmission within the flock
- An increased risk of transmission to other flocks.

Current research project – Epidemiology 1

- This project is a collaboration between Kym Abbott (The University of Sydney) and Richard Whittington (NSW Agriculture). Helen McGregor (The University of Sydney) is the project manager.
- The experimental site is at Arthursleigh Farm, a large grazing farm belonging to the University and situated near Goolburn, in the southern Tablelands of NSW.
- The site covers 200 ha and the experiment includes 500 Merino sheep born in spring, 1999.
Aims

- The project aims to identify the major risk factors for young sheep associated with the development of OID by 3 years of age and to quantify some of the factors discussed in broad terms in the introduction above.
- Specifically, lambs have been exposed to OID infection either before weaning, after weaning or both and those exposed before weaning were exposed to either infected ewes, infected pastures or both.
- A primary outcome of the experiment will be the development of a set of recommendations for owners of OID-affected breeding flocks to enable them to reduce the impact of OID in their flocks using grazing management strategies.
- The experiment will also provide substantial information on the transmission of OID within flocks and the time-course of disease in relation to the age of first exposure.

Methodology

- Two flocks of related ewes, one from an OID-free source and one from an OID-infected source. Both lambed on the experimental site.
- Ewes from the uninfected source lambed on either OID-free pastures or OID-contaminated pastures.
- Ewes from the infected flock lambed on either contaminated pastures or pastures which were kept at low levels of contamination by frequent movement of the flocks during lactation.
- Lambs from all groups were weaned on to clean or infected pastures.

Preliminary results

- Nine out of 70 faecal pools (7 sheep per pool) were culture positive at 12 months of age. Five of the positive pools arose from sheep which had infected dams, four from sheep with uninfected dams lambed on 'contaminated' pastures. No positive pools arose from sheep which remained unexposed until weaning.
- Five sheep have died of OID by 23 months of age. The first deaths occurred at 17 months of age. Four deaths have occurred amongst the 210 animals born from the infected flock (2.0%). One death has occurred amongst 149 animals (0.7%) born to uninfected ewes but on contaminated pastures. None of the 70 animals exposed only since weaning has died.
Ongoing work

- Further fecal culture is underway to determine the fecal excretion rate of *M. paratuberculosis* in the experimental sheep at 18 months of age.

- Collection of fecal and blood specimens and production measures are currently continuing at 6 weekly intervals.

Proposed research project – Epidemiology II

- A further examination of age susceptibility is planned to commence in December 2001.
- This project will, however, determine the relative susceptibility of mature adult, young adult and weaner sheep to infection at a range of exposure levels.
- A collaboration between The University of Sydney, NSW Agriculture and CSIRO.

Methodology

- Sheep of the three different age groups will be exposed to four different levels of pasture contamination (zero, low, medium and high).
- The development of OJD in the sheep will be monitored for 2.5 years using a range of microbiological and serological tests, including the novel sheep gamma interferon test.
- A total of 840 sheep are required for the experiment.
Survival of Johne’s Disease in the Environment
Richard Whittington, NSW Agriculture

Johne's disease is caused by *Mycobacterium paratuberculosis*. Information about the length of survival of this bacterium in the environment can assist in minimising the impact of the disease on affected farms and to plan eradication programs based on destocking. A study of this issue was completed in June 2001 and the main findings are summarised here.

The sheep strain of *M. paratuberculosis* 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.

In the first part of the study pasture contamination on OJD affected farms was investigated. It 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. Contamination was not detectable after one complete summer in most sites previously found to be infected. Of course we do not know when or to what degree each site was contaminated so these findings need to be used with great caution.

In the next part of the study we controlled some of the variables by working in pasture plots and pasture boxes. The duration of survival exceeded 12 months in faecal pellets in the shade but was much less in unshaded places. Pasture and weed growth provided shade which assisted survival. Sunlight is a very significant factor influencing survival of the organism. Moisture levels and application of lime to plots did not appear to influence survival.

The organism moved out from faecal pellets into the surface litter as pellets broke down and entered the soil profile. Pasture emerging through contaminated faeces became contaminated. The organism moved away in run-off water and survived for prolonged periods in drinking-trough water. The duration of survival in water was longer than in soil in the same environment.

Decay rates for the organism were estimated to enable modelling of survival. There was a rapid decay phase lasting about 6 weeks during which the vast majority of viable bacteria declined. Then there was a slow decline phase lasting many months. A means of estimating decontamination intervals for pasture was proposed based on reasonable assumptions of prevalence of OJD, faecal shedding levels, bacterial decay rates and rates of soil consumption by grazing sheep.

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. Speculation that short destocking periods (particularly 4 months) might be adequate for eradication must end.

Decontamination intervals following transient contamination of land (eg recent trace forward in rams) can be shorter than those following long term contamination by an endemically infected flock.

Short pasture spelling periods, such as those used for internal parasite control, are ideal to increase the safety of pasture for young sheep on infected properties by significantly reducing contamination levels.
Survival of Johne's Disease in the Environment

Why study the survival of *M. paratuberculosis*?

- For eradication based on destocking
  - when will my pastures be completely safe?
- For managing the disease by grazing
  - how long do I need to spell pasture?
  - can I control OJD and parasites?

What is already known?

- Northern hemisphere
  - 11 months survival in bovine faeces
  - 18 months survival in water
  - survival factors unknown, but urine toxic
- Australia
  - the environment is harsh
  - 15 month destocking was recommended ~1996
  - 4 month destocking???
Research plan

- Field-based studies in NSW with OJD
  - on-farm survey
  - pasture plot and box trials
  - water trough pilot study

On-farm study

- 5 farms in endemic area; ~25 sites per farm

<table>
<thead>
<tr>
<th>Site</th>
<th>No</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil/pasture</td>
<td>106</td>
<td>22*</td>
</tr>
<tr>
<td>Water</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Sediments</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>Yards, sheds</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

*Vamps, dry exposed hill sides, lightly shaded areas, gullies; these sites were fenced off

On-farm study

- Culture positive sites were resampled
  5 and 12 months later - all negative

- Conclusion - survival less than 1 summer is possible but..............
  - no info. about level of contamination at start
  - low-lying areas had greater bacterial counts
Pasture plots

Pasture boxes

Maximum duration of survival on soil

<table>
<thead>
<tr>
<th>Month</th>
<th>Site</th>
<th>Source</th>
<th>Shade</th>
<th>Max survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan 98</td>
<td>Boremore, Plots</td>
<td>part</td>
<td>28 wks</td>
<td></td>
</tr>
<tr>
<td>Nov 98</td>
<td>ditto</td>
<td>Plots</td>
<td>nil</td>
<td>3-5 wks</td>
</tr>
<tr>
<td>Nov 99</td>
<td>Boremore</td>
<td>Plots</td>
<td>nil</td>
<td>2 wks, 70% 12 wks</td>
</tr>
<tr>
<td>Jan 00</td>
<td>Camden</td>
<td>Boxes</td>
<td>nil</td>
<td>12 wks, 70% 16 wks, 100% &gt;55 wks</td>
</tr>
</tbody>
</table>
Soil temperatures - Camden

- Weekly max, average, min

Survival in water

- Soil in pasture boxes, full shade - 28 weeks
- Dam water sediment in troughs, shaded - 48 weeks
- The study is continuing

What happens to the organism?

- It moves away in water
- It gets carried up onto pasture
- It enters the surface layers of soil and litter
We counted numbers of bacteria in pellets and soil

Counts

Assessing risk - Scenario 1 - eradication endemic infection
- 2000 head flock grazing 100 ha; 1600 > 1 year old
- Illimiting; light timber; vegetation confined; disease free
- Flock profile PFC tests indicate 10% adult sheep shedding
- Estimate 10% advanced cases shedding in average of 10^7 fg
- Fecal output 150 g/sheep/day
- Total contamination over 100 days of winter =
  100 x (100 x 10^7 x 750 x 100 - 1 x 10^7 fg) - 1 x 10^9 g/ton soil
- Sheep consume about 400 g/ton/day
- At end of winter viable organisms consumed = 1.2 x 10^6/day
- Commence dosing; dose rate assumed to be 1 kg per 3 months
- After 12 months viable organisms consumed = 1.2 x 10^6/day
- Contaminant - need more than 12 months dosing because 10^6 organisms might be enough to set up infection
Assessing risk - Scenario 2
- manage pasture to reduce losses
  - 3000 head flock grazing 600 ha, 1000 = 1 year old
  - At end of winter viable organisms consumed = 1.2 x 10^9/day
  - Commence pasture stocking. Decay rate assumed to be 3 logs per month in first 2 months
  - After 2 months only small numbers of viable organisms are consumed each day
  - Conclusion - pasture stocking is likely to be beneficial because it reduces the level of challenge
  - It would be beneficial to maintain an open pasture structure to maximise exposure to sunlight
  - Focus off low-lying and heavily shaded areas

Assessing risk - Scenario 3
- transient contamination
  - 100000 introduced, confined to 100 ha, OJD detected 3 months later
  - One cow has early histopathological lesions, assumed to be shedding 10^7 pg
  - Fecal output 0.5 kg/day
  - Total contamination over 100 days of winter = 10^7 x 0.5 x 100 = 10^9 / 10^6 = 10^3/gram soil
  - Sheep minimum about 40 pg soil/day
  - At end of winter viable organisms consumed = 3 x 10^9/day - danger
  - Commence decontaminating. Decay rate assumed to be 1 log per 3 months.
  - After 6 months viable organisms consumed = 3 x 10^9/day
  - Conclusion - 6 months decontaminating may be sufficient

Conclusions
- Eradication requires 15 months; this will be sufficient in most but not all cases
- Forget 1 summer decontaminating
- We need property specific programs to account for variable rates of contamination - use flock profiling by PFD
- Pasture management should be effective in reducing the severity of OJD
Vaccination Studies
Peter Windsor, NSW Agriculture & Jeff Appleston, Central Tablelands RLPB.

Background
Vaccination against OJD has been used in several overseas countries reportedly with encouraging results, however there are few scientifically controlled trials demonstrating its effectiveness. This field trial, funded by the NOJDPC, aims to evaluate the efficacy of Gudair for the control of OJD in Australian genotypes in pastoral environments. This paper presents some preliminary data on the impact of vaccination on cellular and humoral immunity, faecal shedding of *Mycobacterium paratuberculosis* (*M. ptb*), mortality and lesion development at the site of vaccination. The results of this trial will be used to support registration of the product.

Methods
On each of 3 heavily infected properties around Bathurst, 200 2-3 month old Merino lambs were treated with Gudair while 200 were left unvaccinated. Property visits occurred at 2 and 6 months post vaccination (pv) and approximately every 6 months thereafter. Liveweight, condition score and injection site lesion diameters were recorded at every visit while blood samples for gamma interferon (IFN-γ) and Elisa antibody determination, and faeces for PFC testing, were taken every 6 months pv.

Results
- Vaccination has stimulated both the cellular and humoral immune systems. A high proportion of vaccines had positive titres for IFN-γ and Elisa antibodies 2 months pv, compared to controls.
- Shedding of *M Ptbb* commenced in control animals on all 3 properties beginning at around 9 months of age, while in vaccines no shedding has been detected as yet (12 months pv).
- Mortalities due to OJD have been diagnosed in controls in 2 of the 3 properties, but no OJD has yet been diagnosed in vaccines.
- Injection site lesion up to 45 mm in diameter were present in up to 50% of vaccines 2 months pv, but less than 20% of vaccines had palpable lesions 15 months pv. No control animals developed injection site lesions.

Discussion
These preliminary results indicate that vaccination with Gudair can stimulate immunity against OJD and at least delay the onset of bacterial shedding and deaths due to the disease. It is critical that further data is collected to determine the duration of this response. If this response is shown to last for the majority of an animals life then vaccination with Gudair will play a major role in controlling the effects of this disease on-farm. A prolonged reduction in shedding in infected animals and a resultant reduction in exposure in subsequent generations of lambs should result in a major reduction in expression of this disease.

However it is important that the injection site lesions are properly monitored and any increased cost of processing be accurately determined.
The use and effectiveness of Gudair vaccine for the control of OJD in Australian sheep flocks

P Windsor, J Epplestone, R Waittngton, K Thorneberry, A Dillen and S Jones
NSW Agriculture, Camden Vaccines R & P
CSL MLA

MLA PROJECT OJD.009 OBJECTIVES

- To assess impact of Gudair treatment of lambs on
  - sheep-to-sheep transmission pre-shearing
  - post-shearing
  - scrotal swelling
  - rectal temperature
  - production index
  - bovine ephemeral fever
  - bovine respiratory disease
  - MRSA
  - cross-mediated immune response between genotypes
  - hybrid environment on Australian merino genotypes

- NPA replication

MLA PROJECT OJD.009 Trial design

- 3 sites: property of Balchard
  - 1 property: 1960s
  - 2 properties: 2000s
  - 3 properties: 2000s
  - 200 vaccinated vs. 200 control per site (200 retained)
  - Properties were 2 and 6 months, and every 3 months
  - 5 year trial
**Sampling schedule**

- Body weight, condition score, and feces measured at every visit.
- Feces - anal 5 pools of 40 pigs, rumen 10 pools, 26 sheep pools.
- Days 1, 12, 18 months post.
- Days 2, 3.
- Blood for IFN-γ and Elisa antibody levels.
- Days 1, 9, 17, 18, 24.
- Days 2, 3, 6, 12 months.
- Gross and histopathology for diagnosis throughout trial.
- Abattoir surveillance at completion.

**Disease prevalence on trial properties**

- Property 1: 10 of 100 Elisa positive.
  - 1% with abnormalities.
- Property 2: 12 of 150 Elisa positive at 3 months.
  - 1% with abnormalities.
- Property 3: 22 of 50 Elisa positive.
  - 4% with abnormalities.
- Clinical sheep run with trial flock.
Faecal shedding - Proportion positive pools:

<table>
<thead>
<tr>
<th>Property</th>
<th>Group</th>
<th>2nd wk pv</th>
<th>6th wk pv</th>
<th>60th wk pv</th>
<th>12th wk pv</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.5</td>
<td>0.5</td>
<td>210</td>
<td>210</td>
</tr>
<tr>
<td>2</td>
<td>Vaccine</td>
<td>0.5</td>
<td>0.5</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>0.5</td>
<td>0.5</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Vaccine</td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Prevalence of shedding increased from 0% to a maximum of 3.9% (1 out of 3) on Prep 3.
Significant difference in shedding rate between C and V on Prep's 1 and 2.
OVA mortality commenced in C's on Prep's 1 (July) and 2 in mid 2001.

ELISA antibodies - % positive:

Gamma IFN - % positive:

(10m C: 2%, 12.8% yr lesion challenge; Prep 1, C: 33.2%)
Issues I will raise in the discussion

Presenter:

Topic:

The key message for me from this presentation was:

I would like clarification on:

I would like to add the following points to the discussion:
Eradication of OJD by Destocking
Pat Taylor¹, Kevin Thornberry¹ & David Hall², NSW Agriculture, ¹Orange Agricultural Institute

Background
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. In 1999 contracts were signed between AHA, MLA and NSW Agriculture to respectively fund, administer, and execute a project (Trial 1.1) to evaluate the efficacy of Property Disease Eradication Plans to eradicate OJD from up to 50 properties in south-eastern Australia. Details of the current status of that project follow.

Method
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.

Table 1 Selected evaluation sites by state and year of restock.

<table>
<thead>
<tr>
<th>State</th>
<th>1998/9</th>
<th>2000</th>
<th>2001</th>
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<tbody>
<tr>
<td>NSW</td>
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<td>Victoria</td>
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<td>0</td>
<td>5</td>
<td>7</td>
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<td>South Aust</td>
<td>0</td>
<td>3</td>
<td>1</td>
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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 post restock.

Results
Of the 41 sites that have restocked to date, 17 have purchased from MAP flocks (10 MN1; 4 MN1and MN2; 3 MN2) with no pre-purchase testing and 24 have purchased from non-assessed flocks. Of the latter, 20 were successful in securing flocks that passed MAP equivalent assurance tests and four are awaiting PFC’s. Of the purchased flocks that were tested, 12 were via PFC only, 7 were via serology only and 5 were via PFC and serology.

Of the 7 sites that were restocked in 1998/99, two have been confirmed (re)infected via histopathology, two have been confirmed (re)infected via PFC and two are OJD negative at around 24 months post restock. Faecal samples from the remaining site will be collected in October. Investigations to attempt to determine whether Mptb was reintroduced or persisted on these sites are in progress.
Detection of Ovine Johne's Disease in Abattoirs as a Surveillance Tool
Laurence J Denholm, NSW Agriculture

Cost-efficient and effective disease surveillance strategies are a prerequisite for effective disease control, *inter alia* (1) to define regional prevalence and geographic extent of infection, (2) to identify properties from which infection is spreading and (3) to monitor progress of control. When a national program to control ovine Johne's disease (OJD) in Australia began in 1998, the only method of surveillance in use to determine regional disease prevalence was the tracing of infected sheep followed by on-farm flock testing. That method was validly criticised for bias based on lack of randomness. Monitoring sheep at abattoirs was thought likely to be more cost-effective and random, but whether it was possible to detect OJD lesions in normal sheep at slaughter and whether a routine monitoring system could be practically implemented in Australian meatworks was unknown. Maintaining the integrity of sheep line identity to the point of viscera inspection during processing was critical. Although detection of infected flocks by abattoir monitoring had been used in small ruminant JD control programs overseas, an estimate of the sensitivity with which OJD lesions could be detected in normal sheep at slaughter in Australia was not available. A sensitivity estimate was deemed to be essential for the design of any future national OJD abattoir surveillance program.

A trial was established to (1) develop and determine the practicality of a surveillance system in which specially trained meat inspectors screen the viscera of adult sheep for macroscopic lesions suggestive of OJD, with definitive diagnosis by histopathology on suspect lesions, (2) compare this system with a pooled intestinal culture (PIC) system involving PCR-BACTEC on ileal mucosal scrapings from 10 sheep selected randomly from each slaughter line, and (3) obtain estimates of sensitivity for these two methods.

This trial demonstrated both the practicality and utility of surveillance for OJD by visual/tactile screening of viscera at abattoirs followed by laboratory histopathology. The estimated sensitivity of the method in a sheep population with a high prevalence of flocks with longstanding OJD infection was 97% (95% confidence intervals of 91.5% and 100%). As implemented, PIC was impractical and had a sensitivity of only 55%.

Caution must be exercised in any use of the sensitivity estimate for visual/tactile detection of OJD from this or any other trial. Any sensitivity estimate will be affected significantly by factors such as (1) the training and experience of inspectors, (2) the number of sheep in lines submitted for slaughter, (3) the prevalence of sheep in these lines with detectable lesions of OJD, and (4) the proportion of sheep in each line from which viscera are inspected. Further research to refine the estimate of sensitivity for abattoir surveillance obtained from this trial is not warranted unless that research is also designed to assess the effect of such factors on the sensitivity of the technique.

The potential additive effect of these factors will make any sensitivity estimate from a trial in which these factors are uncontrolled and unknown quite unreliable. In particular, sensitivity estimates from high OJD prevalence sheep populations must not be applied to surveillance in low or unknown prevalence populations. The 97% estimate from this trial in NSW is probably an overestimate of the sensitivity for any low OJD prevalence sheep population. Conversely, the current NOJDP "working" estimate of sensitivity of 30% for low prevalence populations may be a significant underestimate where abattoir inspectors are skilled and examine at least 250 sheep in each slaughter line.

Despite this expected failure to obtain a sensitivity estimate with wide applicability, the trial did show that abattoir surveillance will be an effective and cost-efficient tool for control of OJD if accompanied by a sheep identification scheme that ensures reliable traceback to property of origin for any sheep with OJD that is detected at an abattoir.
Detection of ovine Johne’s disease in abattoirs as a surveillance tool

Dr Laurence Denholm
NSW Agriculture

The MLA Project: OJD-007

"Evaluation and Comparison of Two Methods of Abattoir Surveillance for the Detection of Ovine Johne’s Disease"

Dr Ian Lugton (Project Design & MLA Application)
Dr Laurence Denholm (Nov 99 to March 2000)
Mr Maurie Ryan (June 2000 to completion)
Dr Laurence Denholm (Final Report, June 2001)

Objectives

- Determine the practicality of implementing post-mortem surveillance for OJD in lines of adult sheep slaughtered at NSW meatworks.
- Compare two potential monitoring techniques
  - standard "meat inspection" screening of viscera
  - pooled BACTEC-PCR on a random sample of sheep
- Obtain crude sensitivity estimates for the two techniques in a sheep population with a high prevalence of infected flocks.
Major Problem

- Design called for 100 lines of infected sheep
- Only 35 of the 100 lines were obtained

Reasons
1. Changes and delay in Trial 1.1
   - Could only obtain 14 lines of extended 60 lines
2. Lack of cooperation from other producers
   - Could only obtain 24 lines from 12 properties
   - Despite offers of £200 bounty for information of date and location of slaughter

Results - Practicality of Methods

- Visual/tactile screening method was a practical, cost-efficient and effective method for routine OJD surveillance.
- Pooled intestinal culture was a costly, impractical and less effective method for routine OJD surveillance.

Results - V/TS-HC

- V/TS-HC detected macroscopic lesions of OJD in 34 (97%) of the 35 eligible lines
- Inspectors reported 21% (range 1% to 90%) of sheep in these 34 lines had gross lesions of OJD
- Microscopic lesions diagnostic (91%) or suggestive (9%) of OJD were detected all 34 lines
- Microscopic lesions diagnostic (74%) or suggestive (9%) of OJD were detected in 80 (81%) of the 99 individual sheep from which samples were submitted by inspectors
Results - PIC

• PIC detected 19 (58%) of the 35 eligible lines.

• PIC detected 18 (55%) of the 34 lines detected by V/TS-HC.

Results - Negative Controls

• V/TS-HC was negative on all of nine (9) negative control lines obtained from areas of NSW where OJD is not known to occur.

• PIC was positive on 2 (22%) of the 9 negative control lines obtained from areas of NSW where OJD is not known to occur.

• Whether these positive PICs were false positives due to post-formigate cross-contamination between lines or true positives reflecting higher sensitivity of BACTEC-PCR could not be determined.

Sensitivity Estimates

• Visual/tactile screening of viscera with histopathology confirmation: 97%

• Pooled intestinal BACTEC-PCR (random sample of 10 sheep/line): 58%
Conclusions (1)

- Subject to some preconditions, visual/tactile screening with histopathology confirmation is an effective and cost-efficient abattoir surveillance method for OID
- Pooled BACTEC-PCR methods of abattoir surveillance are not likely to be as sensitive as V/TS-HC for detection of OID in slaughter sheep unless high numbers of animals are sampled for BACTEC-PCR.

Conclusions (2)

- The optimum diagnostic method for abattoir surveillance may vary between sheep populations with different disease prevalence
- V/TS-HC is likely to be more cost-efficient than BACTEC-PCR in high disease prevalence sheep populations and is not subject to potential errors from post-farmgate cross-contamination between slaughter lines

Discussion

- In a long-standing low prevalence situation, pooled faecal BACTEC-PCR might be more sensitive than V/TS-HC if:
  - persistent sub-clinical shedding occurs, but progression to clinical disease is rare;
  - cross-contamination is not a problem, and
  - BACTEC-PCR sample size is high (similar to V/TS-HC).
Discussion (2)

- In temporary low prevalence situations (eg following recent OJD incursion), VTS-HC might well be more sensitive than BACTEC-PCR if:
  - within-flock sheep infection rates are low, but progression of these few cases to clinical disease does occur;
  - VTS-HC sample size if greater than BACTEC-PCR sample size.

 Preconditions for effective abattoir surveillance for OJD

- Producer and processor support
- Reliable individual sheep identification with effective ID tracking to viscera inspection point
- Qualified and accredited inspectors
- Periodic testing and reaccreditation of inspectors
- QA approach with regular audits

Further Work

- Compare V/T Screening Histo Confirmation with Pooled Faecal BACTEC-PCR in a low prevalence sheep population
- Determine whether post-farmgate cross-contamination between slaughter lines precludes use of culture methods for OJD surveillance in high prevalence populations.
Gamma interferon for diagnosis of OJD
David Stewart and Mark Tizard, CSIRO Livestock Industries, Geelong

Background
For an effective OJD control program, it is important that diagnostic tests can identify infected sheep prior to commencement of bacterial shedding, pasture contamination and exposure of other livestock to infection. The γ-interferon test has the potential to fill this role since the assay detects a cell-mediated immune response, the first and most important immunological reaction of animals to infection with mycobacteria. The results of research in Australia and New Zealand provide evidence that the γ-interferon test in its current format may be a useful laboratory tool for diagnosis of OJD.

Methods
Field trials are being undertaken, funded by MLA, to validate the γ-interferon test for diagnosis of OJD in terms of establishing the diagnostic accuracy (specificity and sensitivity) of the assay in unexposed and infected sheep flocks before it can be considered for endorsement and adoption as an official standard diagnostic test. CSL Limited will provide the test kits for these trials.
- The specificity trial would involve flocks on 6 uninfected properties in the SE of Australia and WA.
- A sensitivity trial is underway in NSW in collaboration with Richard Whittington, EMAI, NSW Agriculture. The infected commercial flock will be tested with the γ-interferon assay with subsequent follow-up diagnostic bacterial culture and histopathology evaluation at slaughter.
- The interferon test will be incorporated in a longitudinal study on a naturally infected experimental flock of sheep in collaboration with Richard Whittington, EMAI and Kym Abbott, University of Sydney. This trial will provide information on the relationship between interferon responses during the 3-year period of the trial and the final infection status of individual animals following their autopsy.

Discussion
The potential applications of the γ-interferon test include:
- Demonstrating flock freedom from disease. High specificity (≥ 98%) required.
- Identification of weaners, not exposed to OJD, for movement to clean properties and establishment of infection-free flocks. Monitoring for at least 3 years required. High specificity (≥ 98%) required.
- A vendor decision-making tool as part of a package of diagnostic tests for high value sheep in infected stud flocks. High sensitivity (≥ 70%) for the γ-interferon test required as well as repeat testing.
- A test and cull program for reactor sheep in conjunction with a property disease management plan and flock profiling with other tests. High sensitivity (≥ 70%) and repeat testing required.

The limitations of the current γ-interferon test include:
- A time limit for initiation of the culture phase of the assay and implications for the logistics of sample collection and transport.
- The possibility of false positives in different environments due to exposure to closely related bacteria. A separate MLA funded project is in progress to identify and clone specific M. paratuberculosis antigens that do not provoke these cross-reactive responses.
Gamma interferon for diagnosis of OJD

Background

- Gamma interferon assay developed by CSIRO in collaboration with CSL Limited (Wheeler et al, 1993)
- One technology licensed to CSL Ltd.
- Kit marketed for diagnosis of Mycobacterium haemophilum infection (Bovigen®®): Applicable also to diagnosis of M. avium infection - the cause of bovine Johne's disease (G.J. Rothman et al, 1990)
- Research groups in Australia and New Zealand demonstrate potential uses for OJD diagnosis

Gamma interferon for diagnosis of OJD

- Bovigen®® is a standardized culture and enzyme linked immunosorbent assay for vaccination immunity (VCA)
- CWA reactive occurs in the early phase of infection with bacterial shedding and antibody occurring in the later stage of disease
- Bovigen®® detects interferon-γ induced by PPD-stimulated lymphocytes synthesized by exposure to mycobacteria
- Production and release of IL-10 by T-cells is an objective measure of a type 1 CD1 response
- A potential diagnostic tool to identify infected sheep before post-mortem examination and exposure of other farm stock
Gamma interferon for diagnosis of OJD

Time Course of JD in an infected animal and the key diagnostic variables

Goat 523 (OJD strain)
Gamma interferon for diagnosis of OJD

Methods

Validation of the γ-Interferon test

- For each strain of sheep, determine the diagnostic sensitivity and specificity.
- Evaluate the test's ability to detect differences in γ-Interferon levels between infected and uninfected sheep.
- Perform experiments using sheep sera collected from infected and uninfected animals.
- Analyze the data using statistical methods to determine the test's accuracy.

Results provided by Dr. Sarah Johnson

Discussion

- The potential benefits include:
  - Improved diagnosis of OJD, reducing the need for invasive procedures.
  - Enhanced monitoring of the disease, enabling more effective herd management.
  - Potential for early intervention, improving livestock health and productivity.

Further research and clinical trials are needed to validate these findings.
Gamma interferon for diagnosis of OJD

Discussion

- The potential limitations include:
  
  - A new method of diagnosis: adaptation and methodological
    improvements are required for practical and efficient
    implementation.
  
  - Differences in disease and environmental factors affecting infection.

- The drawbacks of the present examination method due to
  current limitations and its modification.

- A summary of the presented method and a discussion on its practical application.

References (if applicable)
Use of Tracer Weaner Sheep as a Tool for Detection of Infection on Farm
Leslie Reddacliff, NSW Agriculture

Eradication of ovine Johne's disease requires destocking sheep and spelling land until *M. a. paratuberculosis* has died out. After spelling there is no simple way to assess the risk that any residual contamination poses to livestock. Ovine strains of *M. a. paratuberculosis* have been shown to survive for up to 13 months on shaded pasture, but such studies will not determine whether infectious doses of the organism remain. Ultimately the risk can only be assessed using live sheep. Such an approach is used in other disease control programs, particularly for viral diseases, and the animals involved are known as tracers or sentinel livestock. A major disadvantage for use of tracer animals in Johne's disease is that the incubation period is measured in years, so there is a need to detect infection soon after it has occurred. The tracer weaner study described here was undertaken for this reason. Work in the UK forty years ago demonstrated that low oral doses of *M. a. paratuberculosis* could produce infection detectable by organ culture in most sheep as early as 3 months after first infection. Significantly, this is many months to years before the likely development of clinical disease. We sought to investigate whether such early detection of *M. a. paratuberculosis* could be employed to detect infection in tracer weaner lambs used as "vacuum cleaners" to pick up organisms from pasture.

In pilot laboratory study we demonstrated that Australian ovine *M. a. paratuberculosis* isolates were capable of colonising the intestines of recently weaned merino lambs after oral dosing at levels likely to be encountered in the field, and that the organism could be detected in these lambs 7 and 14 weeks after first infection. The organism was cultured from tissues of each sheep that received 10^7 or more organisms. Weaners given lower doses were not infected. Skill tests were positive in 66% of infected weaners and negative in uninfected sheep. Gel and ELISA tests and faecal culture were not useful, but results for gamma interferon showed some correlation with infection status. These results suggested that the skin test might be useful to select individual sentinel weaners for culture.

We then attempted to validate these findings in pastured sheep. Groups of merino weaners were placed onto pasture of high, moderate, low or zero infectivity. Weaners were necropsied 2, 4, 6 and 12 months after first exposure, and examined by culture and histopathology. All cultures were negative at 2 and 4 months. At 6 months 20 % of weaners on heavily or moderately infected pasture were cultured positive. By 12 months 75% from the highly exposed group (25% had microscopic lesions), and 11% from the moderate and low exposure groups (no lesions) were culture positive. The skin test was not predictive of culture status in these pastured animals.

These findings indicate that organ culture from weaners may detect infection 6 to 12 months after exposure to infected pasture. This could give early indication that infection is still active, but negative results will not guarantee that pasture is clear.
Tracer weaner as a tool for detection of infection on farm
L Reddick
(WSW Agriculture)

The tracer weaner concept
Several animals will be challenged with other diseases.
For practical use in CJD, require a method for early detection.
100% in UK implicated the organism in first month by culture of intestines and nodes.

Experimental infections: Laboratory pilot trial
- High security animal house under controlled conditions.
- Australian natural weaner groups.
- Australian sheep CJD isolates.
- CJD infections with relevant bovine.
Dosage of viable \textit{M. avium subsp. paratub}

- Continuous: 0
- Low: 10
- Medium: 100
- High: 1000 organisms per dose

- Mouse intestine
- Mouse spleen
- Mouse thymus

Dosing animals

Necropsy sampling for culture and histopathology

- Proximal jejunum
- Terminal ileum
- Esophageal wall
- Lymph nodes
- Liver
- Bone marrow
- Thymus gland
- Mediastinal lymph nodes
- Splenomegaly
- Nodule formation
Culture results five weeks after last dose

- Short regime (7 wk after first infection):
- Long regime (14 wk after first infection):
- No positive cultures from control, low or medium-dosed groups.
- All six lambs given highest dose were culture positive in at least one tissue.
- No gross or microscopic lesions

Other tests

- Foetal liver culture
- Faecal culture
- Serology (ELISA)
- γ-interferon
  - Skin test for active infection by giving positive skin test on pregnancy
  - Only faecal culture selected positive

Skin test appearance at 72 hours
Results: skin tests

- Using cutoff of 0.16 mm (mean + 3sd)
  of control sheep values for thickness increase
- At 7 wks after first infection: 33% sensitive
  (detected one of three infected sheep)
- At 14 wks after first infection: 100% sensitive
  (detected three of three infected sheep)
- No uninfected sheep positive: 100% specific

Conclusions: progression to field trials warranted

- Experimental infection with doses that
  are plausible under field conditions
- Infection detected in tissue culture 7 to
  14 weeks after first exposure
- Skin test shows promise for targeted,
  necropsy
- Defined range of samples for collection at
  necropsy

Field trials vs lab trials

- Timing of infection is unknown
- Does not overwhelm
- Exposure to a single parameter
- Exposure may help with seasonal
  controls
- Depends on sheep raising behavior and
  that needs to be understood
Field trials

Clean lambs weaned from pastures of different infection, up to 12 months post weaning. Each 100 lambs:

1. Highly infected pasture: 100 lambs infected and treated with an anthelmintic at day 0.

2. Moderately infected pasture: 100 lambs infected and treated with an anthelmintic at day 0.

3. Lightly infected pasture: 100 lambs infected and treated with an anthelmintic at day 0.

4. Clean pasture: 100 lambs infected and treated with an anthelmintic at day 0.

Field trials

- **Sampling times:** 2, 4, 6 and 12 months after weaning
- **Skin testing:** All surviving lambs at each time point
- **Necropsy:** 10 lambs from each infection level at each time point. Selected on basis of skin test (biggest and smallest)

Field trials results: culture and pathology

- **Two and four months post weaning:** No infection detected in any group.
- **Six months post weaning:**
  - 20-25% of moderate infection group infected. No growth in untreated, 60-70% growth in treated.
  - 20% of heavily infected group infected. No growth or microscopic lesions.
Field trials, results:
culture and pathology

- 12 months post weaning:
  75% (6/8) of heavily exposed group infected, no gross lesions, 2 with microscopic lesions.
  11% (1/9) of moderately exposed group infected, no lesions.
  11% (1/9) of lightly exposed group infected, no gross or microscopic lesions.

Field trial results: skin testing

Using pepsin-hydrolysis extract of 1% NMBV

- 2 months:
  Five animals in heavily exposed group.

- 4 months:
  One animal in moderately exposed group.

- 6 months:
  All sheep negative on sensitive.

- 12 months:
  All sheep negative on sensitive.

Conclusions for on farm use

- Pasture contamination can be detected within 6 to 12 mth using tracer weaner sheep.
- In heavily exposed environments all sheep have been infected by 12 months.
- In lightly contaminated environments negative results for weaner sheep in the autumn gives freedom of the pasture from infection.
- Skin tests are of no practical use.
PCR diagnosis of OJD
Ian Marsh, NSW Agriculture

Clinical signs of Johne’s disease, unlike other infectious diseases where animals become sick in days or weeks, are not usually seen for years. This makes diagnosis of *Mycobacterium avium* subsp. *paratuberculosis* (*M. paratuberculosis*) from affected properties extremely difficult. The problem has been compounded by difficulties in developing reliable, sensitive and specific diagnostic tests, particularly for sheep, where the predominant strain of *M. paratuberculosis* has been extremely difficult to culture. The use of DNA based detection techniques such as polymerase chain reaction (PCR) are now common for diagnosis of numerous plant, animal and human diseases. For a routine DNA based diagnostic test, on faecal samples, the procedure needs to be simple, robust and inexpensive.

Unlike previous attempts to use PCR to detect *M. paratuberculosis* directly in faecal samples, that had limited success or limited application, we report a direct-PCR that is performed on the DNA purified from faeces using a simple protocol. In a blind trial on 502 pooled faecal samples, previously tested by pooled faecal culture, 60% of the pooled faecal culture positive pools were identified by direct-PCR which resulted in 79% of the infected flocks being detected.

Like the majority of non-invasive diagnostic tests for Johne’s disease the test will be of little use with early sub-clinical *M. paratuberculosis* infections. However, the advantages of the direct-PCR test is that results can be available within a few days of receipt of samples at a laboratory and unlike culture based tests, samples from infected sources containing non-culturable organisms can be detected.

While the costs of direct-PCR are no greater than other flock diagnostic strategies problems remain in that the results of DNA-based diagnostic strategies are not yet well accepted by animal health regulatory authorities.
PCR Diagnosis of OJD

Ian Marsh
Ellesworth Mycoplasma Laboratory, NSW Agriculture,
Mumogly, New South Wales, Australia.

Diagnostic tests for OJD

Blood tests (serology)

Blood [Blood sample] Antibodies to M. ph

Sheep has been exposed to M. ph

Diagnostic tests for OJD

Tests for bacteria

Feces [Feces sample] Culture [Culture] Bacterial DNA

Sheep has been exposed to M. ph
**DNA tests**

Require suitable target DNA sequence

For Mycobacterium avium subsp. paratuberculosis we use a DNA sequence named IS900

Start with very few copies of the target sequence, undetectable

Produce millions of identical copies of the target DNA in a process called Polymerase chain reaction (PCR), detectable

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**PCR - principle**

<table>
<thead>
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<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
<th>Cycle 4</th>
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</table>

**X 10^6**

Millions of exact copies

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**Detection of DNA**

DNA

---
Detection of DNA

DNA

Add a dye that sticks to the DNA

Detection of DNA

UV
Light

Detection of DNA

<table>
<thead>
<tr>
<th>Sample</th>
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<th>Negative control</th>
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Diagnosis of QJD using PCR

At present we combine culture and PCR - pooled faecal test

New test where PCR is applied directly to faecal samples - Direct-PCR

Culture + PCR

Culture + PCR
Direct-PCR Trial

502 pooled faecal samples, previously tested by pooled faecal culture test
Pooled faecal culture results were withheld until completion of direct-PCR testing
Direct-PCR found 60% of 33 PFC positives or 79% of the 14 infected farms
Direct-PCR outcomes

Rapid test, results within 48 - 72 hours
Not suitable for identification of early cases
Cost is no greater than PFC
Issues remain about the acceptance of results from DNA tests by regulatory authorities
Direct-PCR is being further evaluated in current field trials
EXECUTIVE SUMMARIES FROM
KEY OJD R&D PROJECTS
CONDUCTED BY MLA AND THE
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 New South Wales, 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 was tested using restriction fragment length polymorphism (RFLP) analysis and other techniques. Isolates were obtained mainly from sheep and cattle from about 100 farms in New South Wales and Victoria, but some samples were included from Tasmania, South Australia and France.

Fourteen RFLP types were found. Type S1 was the dominant type in sheep in New South Wales, accounting for 95% of isolates, and was the only type found in sheep from Victoria. Several sheep isolates obtained in New South Wales 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 New South Wales 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 is 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 New South Wales 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 New South Wales and Victoria.
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*, 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 *M. avium* subsp *paratuberculosis* 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-infected 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 *M. avium* subsp *paratuberculosis* 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 *M. avium* subsp *paratuberculosis* 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.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 with 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 10³ 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 loam 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.
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 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 M. paratuberculosis 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 in pooled faecal samples, based on the hybridisation-capture polymerase chain reaction (HC-PCR) technique. HC-PCR was developed in the United Kingdom for the detection of M. paratuberculosis in tissues from Crohn's disease patients and from faeces of animals with Johne's disease. 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 ovine Johne's disease 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 *M. paratuberculosis*. Recent studies have shown that some environmental mycobacteria posses IS900-like elements that react in PCR assays for *M. paratuberculosis*. As a result, restriction endonuclease analysis (REA) of the amplified product is required to confirm results as DNA consistent with *M. paratuberculosis*. However, non-specific products from sources other than *M. paratuberculosis* 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 *M. paratuberculosis* (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 *M. paratuberculosis* in 66.6% of the culture positive pooled faecal samples.

A prospective, blind trial was performed with the new DPCR reaction on 326 pooled faecal samples submitted to EMAI for routine culture. DNA consistent with *M. paratuberculosis* 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. paratuberculosis* 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 transmutable to detection of Johne's disease 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. paratuberculosis* infection.
TR.073 - Pilot Study: Tracer Weaner Trial for Ovine Johne's Disease

Abstract

Eradication of ovine Johne's disease requires destocking sheep and spelling land until Mycobacterium avium subsp. paratuberculosis 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 M. avium subsp. paratuberculosis. 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 M. avium subsp. paratuberculosis may be higher than previously thought.

Executive Summary

Eradication of ovine Johne's disease requires destocking sheep and spelling land until Mycobacterium avium subsp. paratuberculosis 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 United Kingdom suggested that this might be possible, but the trials had been done with cattle strains of M. avium subsp. paratuberculosis. Consequently in this project small groups of Merino weaners were orally dosed on repeated occasions with graded numbers of an Australian sheep strain of M. avium subsp. paratuberculosis. 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 to 6.9 x 10^7 organisms. Infection was not established in weaners given lower doses. The next lower dose was 8.2 x 10^3. Therefore the infectious dose of this strain of M. avium subsp. paratuberculosis is somewhere between about 10^3 and 10^7 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 M. avium subsp. paratuberculosis 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 ovine Johne's disease, the behaviour of new diagnostic tests and vaccine efficacy.
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* 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 *Mycobacterium paratuberculosis* (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 *M. paratuberculosis* 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. Pasteur 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 M. paratuberculosis and single-celled aquatic animals.

Faecal shedding rates of the organism were determined using sheep with multibacillary OJD and amounted to $10^8$ viable organism 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 pasture 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 M. paratuberculosis.
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. paratuberculosis 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.005 - Cross Species Transmission of Ovine Johne’s Disease - Phase I

Abstract

Johne’s disease was investigated in fibre goats on several farms. The disease was caused by sheep [S] strains of Mycobacterium avium subsp. paratuberculosis. 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 is justified. A communication program is recommended to advise producers that ovine Johne’s disease in goats may not be obvious and that testing should be undertaken to ensure disease is not present. The impact of ovine Johne’s disease 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 ovine Johne’s disease 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 ovine Johne’s disease 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 M. avium subsp. paratuberculosis S strain to sheep rather than goats.

The circumstances that resulted in ovine Johne’s disease 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 ovine Johne’s disease in goats. As Johne’s disease 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 ovine Johne’s disease 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.
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 \textit{M Ptib}, 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 \textit{Mycobacterium avium} \textit{subsp paratuberculosis} (\textit{M Ptib}), 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 \textit{M Ptib}. \textit{M Ptib} 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 \textit{M Ptib} 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 \textit{M Ptib} excretion in semen collected from rams at an earlier stage of infection with OJD.