Assessing the Risks of Transmitting OJD in the Semen of Rams by Artificial Insemination

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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 *M Pt* \textsubscript{tb}, the bacteria that causes OJD, and that it is likely that bacteria could be transferred to the reproductive tract of ewes at mating or AI. These results indicate that quality assurance guidelines for the safe use of semen from infected flocks need to be developed further.

EXECUTIVE SUMMARY

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

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

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

This study was conducted as a first step in determining the risk of transferring OJD by semen from rams from OJD infected flocks. Its objective was to determine whether *Mycobacterium avium* subsp *paratuberculosis* (*M Pt* \textsubscript{tb}), 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 *M Pt* \textsubscript{tb}. *M Pt* \textsubscript{tb} 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 *M Pt* \textsubscript{tb} 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 *M Pt* \textsubscript{tb} excretion in semen collected from rams at an earlier stage of infection with OJD.
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1. BACKGROUND TO PROJECT

Ram producers who have OJD diagnosed in their flocks are currently restricted in selling their genetics in the form of live animals. 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.

A literature review by Morgan (1999) highlights the lack of information on the risk of OJD being transmitted between sheep in fresh or frozen semen by AI. The evidence extrapolated from cattle indicates that this risk is low. However intermittent excretion of low numbers of Mycobacterium paratuberculosis (M Ptb.) has been detected in the semen of bulls clinically infected with BJD. There is little or no information in non-clinical bulls. No similar studies have occurred in rams.

The initial research proposal submitted, aimed to determine the incidence of M Ptb excretion in semen of rams with different levels of OJD infection, but was subsequently reduced to the current objective of determining the incidence of M Ptb excretion in semen of clinical rams only.

2. PROJECT OBJECTIVES

The simple objective of this trial was to determine whether rams showing clinical OJD were able to excrete M Ptb in their semen. It was proposed that the results of this trial could then be used to determine whether further research is necessary before developing guidelines for the low risk use of ram semen from infected flocks.

3. METHODOLOGY

Fourteen rams with signs of clinical OJD were collected from 7 known infected properties in the Central Tablelands RLPB. These were transported to the Orange Regional Veterinary Laboratory where they were sampled as follows (under ACEC Approval No. ORA 99/013) taking as much care as possible to avoid cross contamination;

- Semen was collected by electro ejaculation directly into a sterile glass container.
- Each ram was then euthanased by overdosing with a barbiturate and the following samples collected;
  - Blood for GDPT testing
  - Fresh and fixed samples of ileum for histology and tissue culture
  - Testis, tail of the epididymides, seminal vesicle, ampulla, bulbourethral glands, and iliac and internal inguinal lymph nodes.

The blood sample and preserved sample of ileum were tested immediately at the Orange laboratory. The blood sample was tested using the agar gel immunodiffusion test (AGID) while the ileum was examined histologically for the presence of acid fast bacilli and other histological evidence of OJD infection.

The fresh tissues were sent to the Elizabeth Macarthur Agricultural Institute laboratory for culture for M Ptb.
4. RESULTS AND DISCUSSION

Eleven of the 14 rams sampled were histologically positive and only samples from these rams were cultured. The results for individual rams are presented in Table 1.

Table 1. Agar gel immunodiffusion (GDPT), histology and culture test results.

<table>
<thead>
<tr>
<th>Ram</th>
<th>GDPT</th>
<th>Histology</th>
<th>Culture</th>
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<td>semen</td>
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<td>1</td>
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<td>2</td>
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<td>- ve</td>
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</tr>
<tr>
<td>14</td>
<td>- ve</td>
<td>+ ve</td>
<td>- ve</td>
</tr>
</tbody>
</table>

+ ve – positive test result; - ve – negative test result; na – not available.

In summary;

- *M. Ptb* was cultured from the ileum of all 11 rams.
- *M. Ptb* was cultured from the semen of 3 of the 11 rams.
- *M. Ptb* was cultured from only one other tissue and that was from the seminal vesicles of a ram whose semen and other reproductive tissues did not contain *M. Ptb*.
- *M. Ptb* was cultured from the lymph nodes that drain the inguinal region of 8 of the 11 rams.

These results have identified the presence of *M. Ptb* in the semen of around 30% of clinical rams sampled. However the source of these bacteria has not been determined given that none of the reproductive organs that contribute secretions to semen in these 3 rams contained *M. Ptb*. One possible explanation is that the semen was contaminated with bacteria from the prepuce of these rams during semen collection. All efforts were made to minimize the risk of this contamination by wiping the prepuce before exteriorising the penis and directing the penis directly into the sterile jar. However these rams were often in the terminal stages of the disease and several were suffering from diarrhoea.

However the fact that one ram contained *M. Ptb* in his seminal vesicles, and that the lymph nodes draining the inguinal region in which the reproductive organs are contained, suggests that *M. Ptb* can transfer to the reproductive organs and supports the observations of intermittent shedding previously made in cattle. The risks of cross contamination in these tissues were much lower than in the semen samples.

Irrespective of the source of the bacteria in the semen this work has indicated that *M. Ptb*, the cause of OJD could be transmitted to the vagina of ewes by natural mating and collected in semen during semen storage procedures.
As outlined in the original project submission, further investigations into whether semen from non-clinical but OJD infected rams can contain \textit{M \textit{Ptb}}. and into quantifying the level of contamination in infected semen may be warranted.

5. SUCCESS IN ACHIEVING OBJECTIVES

This trial has been successful in identifying qualitatively that clinical OJD infected rams can shed \textit{M \textit{Ptb}}. In their semen. However due to the small numbers of rams involved and the possibility that semen can be contaminated during ejaculation, no quantitative information could be collected. Hence the likelihood that \textit{M \textit{Ptb}}. Was at a level that may result in infection in inseminated or naturally mated ewes could not be assessed.

6. IMPACT ON THE MEAT AND LIVESTOCK INDUSTRY

This study confirms that the semen of clinically infected rams can contain \textit{M \textit{Ptb}}. However, as outlined in the initial research proposal, it is only the first step in determining what level of assurance testing is required prior to using semen from infected flocks.

7. CONCLUSIONS AND RECOMMENDATIONS

It is concluded that the semen of clinically infected rams can contain \textit{M \textit{Ptb}}, which may then be transmitted to the female reproductive tract during natural mating or artificial insemination. However the risk of this transfer resulting in infection in the recipient ewe or in any resulting foetus has not been addressed. Further, it has not been determined whether non-clinical OJD infected rams excrete bacteria in their semen.

It is therefore recommended that if increased confidence is required about the risk associated with OJD transmission via semen from rams from an OJD infected flock additional research be conducted to determine the incidence and level of \textit{M \textit{Ptb}}. excretion in semen collected from non-clinical OJD infected rams. Quantification of excretion levels and the consequence of insemination of ewes with contaminated semen should be investigated experimentally to determine the incidence of foetal infection.

8. BIBLIOGRAPHY