CHAPTER FOUR

CURRENT OJD TESTING REGIMES

Introduction

4.1 The following chapter outlines the current testing, screening and surveillance methods and the development of new tests for OJD in accordance with the National OJD Program. It examines the costs associated with testing and discusses the limitations of the tests, particularly in relation to the specificity and sensitivity of current tests.

Current Testing Methods

4.2 A number of diagnostic and screening tests are used to detect OJD infection. As a diagnostic/screening tool, these tests are not one hundred percent accurate. However, they provide a strong indication of the prevalence of OJD infection in a sheep flock by detecting:

- the presence of bacteria in faeces and/or body tissues;
- the presence of antibody in the blood;
- an immune response by white blood cells, and
- changes in the structure and appearance of infected tissue (pathology).[1]

4.3 The current approved diagnostic tests are serology or blood tests. Screening tests include Pooled Faecal Culture, which has been recently endorsed for use in New South Wales and in abattoir surveillance nationally. [2]

4.4 Positive tests are referred for further definitive testing to either confirm or negate the initial diagnosis through histopathology or tissue culture, which detects changes in tissue structure.[3]

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1 Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control*, March 2000, p. 18.

2 NSW Agriculture, *NSW Agriculture Ovine Johne's Disease Policy Manual*, August 1999, Section 4, pp. 4-5.

3 NSW Agriculture, *NSW Agriculture Ovine Johne's Disease Policy Manual*, August 1999, Section 4, pp 4-5.

Serology/Blood Tests

4.5 There are two tests that detect antibodies to *M. Paratuberculosis* in blood:

- Agar Gel Immunodiffusion Test (AGID) and
- Absorbed Enzyme Lined Immunosorbent Assay (ELISA).[4]

4.6 Both tests are highly specific at 99% or greater, but with low sensitivity of 30%. The AGID test is the most commonly used and will detect 30-50% of infected sheep in an infected flock. As an OJD screening test for infected flocks, the best results are achieved when AGID is performed on individual blood samples from 450 sheep two years of age or older. The test should detect a 2% prevalence rate of infection with 95% confidence. [5]

4.7 The ELISA test uses a different type of technology and reagent to AGID. ELISA assays are not as commonly used as AGID and is only available through the Elizabeth MacArthur Institute at Camden in New South Wales or the Orange Agricultural Institute. The ELISA test is not suitable for individual sheep, but is used to screen flocks. The sensitivity of the ELISA test can be increased to 50% if the specificity cut off is lowered to 95%. This will detect more truly positive infected sheep, but with an increase in the number of false positive results.[6]

4.8 Costs for the AGID test are between $4.50 to $7.00 and the ELISA test between $3.85 to $8.50 per sheep.[7]

Histopathology

4.9 Histopathology seeks to confirm an initial diagnosis from serology tests by detecting the presence of bacteria or changes in the appearance of tissues in post mortem sheep. It is considered to be the definitive test for the presence or absence of OJD.[8]

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4 NSW Agriculture, *NSW Agriculture Ovine Johne's Disease Policy Manual,* August 1999, Section 4, p.4.

5 NSW Agriculture, *NSW Agriculture Ovine Johne's Disease Policy Manual,* August 1999, Section 4, p.4. See also, Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control,* March 2000, p. 23.

6 NSW Agriculture, *NSW Agriculture Ovine Johne's Disease Policy Manual,* August 1999, Section 4, p.4. See also, Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control,* March 2000, pp. 20-23.

7 Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control,* March 2000, p. 20.

8 NSW Agriculture, *NSW Agriculture Ovine Johne's Disease Policy Manual,* August 1999, Section 4, p.5. See also, Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control,* March 2000, p. 24.

Pooled Faecal Culture (PFC)

4.10 Pooled Faecal Culture (PFC) was endorsed by Veterinary Committee as an approved test in November 1999. PFC involves the collection of single pellet samples of sheep faeces, which are pooled, with up to fifty sheep in each pool. Faecal samples are tested using BACTEC (radiometric) culture and are incubated for twelve weeks. Positive samples are then tested using Polymerse Chain Reaction (PCR) to confirm that bacteria growth is *M. Paratuberculosis.* Positive samples are also subcultured onto a solid medium for a further incubation period of ten weeks to determine whether or not slow growing *M. Paratuberculosis* bacteria can be isolated. Therefore, for a pool to be considered positive to OJD, there must be positive growth determined by BACTEC culture, a positive PCR reaction and positive growth on a solid medium.[9]

4.11 It is considered that testing seven pools of faecal pellets from fifty sheep provides 95% confidence in detecting a 2% level of infection. This is a favourable outcome when compared to serology tests, which require the testing of 450 sheep to provide similar sensitivity confidence levels.[10] Prowse states that PFC has a specificity of 100% and sensitivity of between 30-50%.[11]

4.12 An advantage of PFC is that it can determine whether a sheep is infectious and shedding bacteria as opposed to sheep that are infected and displaying signs of infection by antibodies or lesions, but not shedding bacteria.[12] However, a recent study suggests that it may be difficult to diagnose infection by using PFC in flocks with a low prevalence of sheep with OJD.[13]

4.13 Pooled Faecal Culture can detect infection earlier than other tests. Dr Richard Whittington told the Committee:

> The evidence, I think, is overwhelming that the organism can be detected in the faeces of animals long before those animals have produced a detectable immune response that is the basis for blood testing … pooled faecal culture has a role in many circumstances where it is desired to find the infection at the earliest possible stage. Based on our understanding of the way the disease develops, pooled faecal culture is most probably going to give a

9 *Submission 65A*, NSW Agriculture, pp 8-9. See also, Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control*, March 2000, p. 21.

10 *Submission 65A*, NSW Agriculture, p. 9.

11 Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control*, March 2000, p. 20.

12 *Submission 65A*, NSW Agriculture, p. 9.

13 Whittington et al, NSW Agriculture, Elizabeth Macarthur Agricultural Institute, Use of Pooled Fecal Culture for Sensitive and Economic Detection of *Mycobacterium avium* subsp. *paratuberculosis* Infection in Flocks of Sheep, *Journal of Clinical Microbiology*, 38, 7, July 2000, p. 2554.

> positive result before any of the other commonly available tests, including abattoir surveillance.[14]

4.14 The Pooled Faecal Culture test has been well received by industry and producers. In evidence to the Committee, NSW Agriculture stated:

> We believe the reliability of the pooled faecal culture test is extremely high. When we were getting approval nationally with the Veterinary Committee for use of the pooled faecal culture test in New South Wales as an approved test, we had ongoing discussions with the New South Wales OJD Advisory Committee to factor in the concerns of industry about introducing a new test which employs PCR genetic technology …. we put in place acceptable risk strategies to ensure that the benefit of the doubt was given to any producer whose sheep yielded a positive pooled faecal culture test.[15]

4.15 Mr David Moen, representing the New England Ovine Johne's Disease Coordinating Committee stated in evidence that:

> ...all sheep two years of age are tested, particularly ones in poor condition – then you have got 95 per cent confidence of picking up OJD in that flock. It is a very good test. It is a far superior test to the serological test. There are very few false positives.[16]

4.16 Another advantage of PFC is that it enables the screening of a large number of samples at a lower cost than serology testing. Prowse states that PFC is approximately 40-50% the cost of serology tests. The cost of PFC is currently about $100 per pool.[17]

4.17 Researchers at the Elizabeth Macarthur Agricultural Institute have calculated that the costs of a flock diagnosis using PFC are approximately 30% of serology tests. This conclusion is based on the assumption that equal numbers of sheep are tested. Sample collection costs also vary, with PFC costing approximately $0.50 per sheep for faeces and serology costing $1.00 per sheep for blood. This excludes the costs of containers, needles and syringes. Actual PFC testing costs about $2.00 per sheep, compared to serology at approximately $6.00 per sheep. These figures are based on a pooling rate of 50.[18]

4.18 Prowse's report, *Ovine Johne's Disease – A scientific review of the current knowledge of ovine johne's disease in Victoria and elsewhere, its impact in Victoria,*

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14 *Evidence,* Dr Richard Whittington, pp. 441-442.

15 *Evidence,* Dr Ian Links, NSW Agriculture, p. 257.

16 *Evidence,* Mr David Moen, p. 179.

17 Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control,* March 2000, p. 21.

18 Whittington et al, NSW Agriculture, Elizabeth Macarthur Agricultural Institute, Use of Pooled Fecal Culture for Sensitive and Economic Detection of *Mycobacterium avium* subsp. *paratuberculosis* Infection in Flocks of Sheep, *Journal of Clinical Microbiology,* 38, 7, July 2000, pp. 2550-2556.

and an assessment of potential strategies for its eradication and control, summarises a number of diagnostic tests for OJD. The tests discussed in this report are repeated in table 4.1.

Table 4.1 Summary of Diagnostic Tests for OJD Discussed in Report

Test	Sample	Sensitivity	Specificity	Cost	Use
AGID/AGIT	Blood	30%	99.9%	$4.50-$7.00	Testing suspect flocks
ELISA	Blood	30%	99%	$3.85-$8.50	Screening flocks
Histology	Tissue	<50%	~90%	$60.00	Confirmation of infection
Pooled Faecal Culture	Faeces	30-50%	100%	$100.00	Demonstrates freedom from disease

Abattoir Surveillance

4.19 Abattoir surveillance is a cost effective method of detecting infected sheep and monitoring the incidence of OJD infection in flocks. It complements current testing methods by identifying suspect flocks for further diagnostic testing and assists in other surveillance based on trace-backs from infected flocks.[19]

4.20 Abattoir surveillance involves the inspection of the intestinal tract of sheep for changes to the intestine representing signs of infection. A sheep with OJD will demonstrate thickening to the wall of the intestine, including the bowel and enlargement of lymph nodes and ducts.[20] (See photographs provided by Dr Frecklington at pages 17-19).

4.21 The primary limitation of abattoir surveillance is the difficulty in tracing infection sources. Abattoir surveillance is also not as effective in detecting OJD during its early stages, or if disease prevalence in a flock is low. Despite this, it is considered to be successful in detecting infection that is well established or

19 *Submission 65A,* NSW Agriculture, p.6.

20 Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control*, March 2000, p. 25. See also, *Evidence*, Dr Bruce Kefford, p. 131.

widespread in flocks. It has been credited with detecting a number of infected flocks not previously identified.[21]

4.22 The cost of conducting abattoir surveillance varies, depending on the number of sheep lines going to slaughter. A line is any group of sheep consigned to an abattoir and might vary from 50 to 500 animals. It usually averages between 200 and 300 sheep with producers generally submitting one or two lines of sheep per property a year. The approximate cost is between $30 and $60 per line. This compares favourably with the average on farm investigation cost of $1,800. [22]

Specificity and Sensitivity of Current Tests

4.23 Current tests for OJD have a high specificity but a low level of sensitivity.[23] The level of specificity indicates the ability of a test to correctly ascertain the infected status of a sheep at flock level. For example, a positive result from a highly specific test will most likely indicate that an animal is truly infected.[24]

4.24 The difficulty with current tests is their low level of sensitivity. This means that at flock level, the test might only detect fifty percent or less of infected sheep in that infected flock.[25] In general, tests have maximum sensitivity in detecting infection in sheep that are demonstrating the clinical symptoms of infection, ie. weight loss. However, there are exceptions to this and it is recommended that two or more different types of tests are used to confirm infection. Further, low sensitivity tests will provide valid results when enough sheep in a flock are tested.[26]

4.25 Along with the difficulties experienced due to the low sensitivity of current tests is that of inaccurate results. These are termed either false positives or false negatives. A false positive result is one where a sheep that is not truly infected returns a positive test for OJD. The number of false positive reactions is related to the level of test specificity. Specificity is affected by the potential exposure of sheep to

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21 *Submission 65A,* NSW Agriculture, p.8. See also, Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control,* March 2000, p. 25.

22 *Evidence,* Dr Bruce Allworth, Animal Health Australia, pp. 412-413.

23 *Evidence,* Dr Stephen Prowse, p. 78.

24 Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control,* March 2000, pp. 18-19. See also *Evidence,* Dr Prowse, p. 78.

25 *Evidence,* Dr Stephen Prowse, p. 78.

26 Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control,* March 2000, p. 19.

environmental organisms closely related to *M. Paratuberculosis.* Test results might also return a false negative diagnosis for infected sheep.[27]

4.26 Prowse states that the high specificity of current diagnostic tests means that there are very few false positives.[28] However, the Committee notes that the quarantine restrictions and de-stocking option of the National OJD Program has a devastating impact on affected producers. This is of particular importance if quarantine restrictions and de-stocking is initiated, based on a false positive test result. The fact that there are false positive results highlights the need for an accurate diagnostic test with both high specificity and sensitivity. The Committee also notes the need for a diagnostic test that can detect early OJD infection.

Research on a More Reliable Test

4.27 Testing for OJD has developed significantly since the Committee's first report. The first report discussed the experimental use of PFC using Polymerase Chain Reaction (PCR). As discussed above, PFC has since become an approved method of screening for OJD in sheep flocks.

4.28 The Committee's first report also noted CSIRO research being conducted into testing for tuberculosis in cattle and the potential for adaption of this technique in sheep (Gamma Interferon). CSIRO's submission to this Inquiry indicated that a test had been developed to detect tuberculosis in cattle and that following further research it may be possible for the test to be applied to Ovine Johne's Disease. Trials in cattle indicate that specificity is in the range of 85-95% and a high sensitivity. However, further research is needed before specificity and sensitivity in sheep can be determined. [29]

4.29 Another difficulty with the potential use of the test is its cost. It is estimated that the test might cost between $10 - $15 per test, which may limit its use for the testing of sheep.[30]

4.30 The aim of research being undertaken at CSIRO is to produce a test with high sensitivity that can detect infection early, prior to the commencement of shedding and pasture contamination.[31]

4.31 Researchers at the Elizabeth Macarthur Agricultural Institute have commented on other tests by stating that:

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27 Dr Stephen Prowse, CSIRO, *Ovine Johne's Disease. A Scientific review of the current knowledge of Ovine Johne's Disease in Victoria and elsewhere, its impact in Victoria, and an assessment of potential strategies for its eradication and control,* March 2000, pp 18-19.

28 *Submission 62,* CSIRO Division of Livestock Industries, p.1.

29 *Submission,* CSIRO, pp1-2. See also, *Evidence,* Dr Stephen Prowse, p. 78.

30 *Submission 62,* CSIRO, p. 2.

31 *Evidence,* Dr Stephen Prowse, p. 78.

> … tests for cell-mediated immunity are not yet sufficiently developed for routine use, and tests for anti-*M. avium* subsp. *paratuberculosis* antibody are insensitive.[32]

Conclusions

4.32 Current OJD testing methods have some disadvantages, principally their low sensitivity and inability to detect early rates of infection. However, as a method of detecting OJD in flocks, current tests are highly effective.

4.33 The recent introduction and use of both the Pooled Faecal Culture test and abattoir surveillance enhances the effectiveness of detecting and screening for OJD. Clearly, Pooled Faecal Culture testing is superior to current serology tests both in sensitivity and cost.

4.34 CSIRO research into a more reliable test with higher sensitivity remains ongoing. However, the Committee notes that the development of a diagnostic test that is cost effective, both highly specific and sensitive and accurate in detecting early OJD infection should be given priority. The Committee is also of the view that adequate funding for further research and development needs to be made readily available.

32 Whittington et al, NSW Agriculture, Elizabeth Macarthur Agricultural Institute, Use of Pooled Fecal Culture for Sensitive and Economic Detection of *Mycobacterium avium* subsp. *paratuberculosis* Infection in Flocks of Sheep, *Journal of Clinical Microbiology*, 38, 7, July 2000, p. 2550.