MEAT & LIVESTOCK
AUSTRALIA

On farm

Evaluation and Comparison of Two Methods of Abattoir Surveillance for Detection of Ovine Johne's Disease

Project number OJD.007

Final Report prepared for MLA by:

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1. Summary

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

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

Trained inspectors were stationed in abattoirs to examine not less than 50% and up to 95% of the abdominal viscera from all lines of adult sheep slaughtered during each kill shift (about 10 to 15 lines per shift at the two major export abattoirs). Where visible lesions suggestive of OJD were observed, fixed tissue samples were taken for confirmatory histopathology from up to three suspect sheep in each suspect line. Inspectors were not told the identity of the trial lines, but were aware that there was a trial line to be killed during the particular shift.

The inspectors detected gross lesions suggestive of OJD in 34 (97%) of the 35 eligible trial lines. The only trial line in which lesions were not detected by inspectors was a line of cross-bred ewes introduced as adults to a property on which OJD had only been reported at low prevalence in merinos. Microscopic lesions diagnostic (31 lines or 91%) or suggestive (3 lines or 9%) of OJD were identified in all 34 lines detected by inspectors. The average proportion of sheep in these 34 lines with gross lesions suggestive of OJD as reported by inspectors was 21% (range <1% to >90%).

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

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

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

Five lines of sheep obtained from infected properties were retrospectively excluded from the trial on the basis of later epidemiological information that indicated they did not meet the selection criteria. All of these lines were negative on both histopathology and culture.

For 7 of the 9 negative control lines (from properties not known to be infected with OJD), no visible lesions of OJD were detected. In 2 of the 9 control lines, however, inspectors reported 2% and 4% of sheep with suspect lesions, but all fixed tissue samples submitted from these lines were negative on histopathology.

Positive PIC results however were obtained from 2 of the 7 control lines in which no visible lesions were detected, one line from an area of Western NSW where OJD has not been reported to date, the

other from an area where OJD had not been reported at the time. Whether these positive PIC results result from true flock infection or transient environmental contamination of cull sheep during transportation or holding in abattoir lairage is uncertain.

The results of this trial indicate that visual and tactile monitoring of the viscera of cull sheep at slaughter for lesions suggestive of ovine Johne's disease is a highly sensitive and reliable strategy for the detection of OJD infected flocks in which deaths from OJD are occurring.

The estimated 95% confidence intervals of 91.5% and 100% for the sensitivity estimate of 97% obtained from these data suggest a practical working estimate of the sensitivity of visual/tactile examination of viscera for lesions suggestive of OJD in lines of >300 adult sheep as a screening test for the selection of samples for a definitive histopathology test would be 90% for any sheep population in which OJD has been recognised for many years.
Whether this abattoir screening technique will be sufficiently sensitive for the routine detection of flocks that have been recently infected with OJD or flocks with persistent low prevalence infection is however less certain.

2. Main Report

2.1 Background and industry context

Cost-efficient disease surveillance strategies are a prerequisite for effective disease control.
For control of ovine Johne's disease in Australia, surveillance methods are necessary to:

a. Define the current geographic extent of the epidemic of this disease
b. Identify infected properties and flocks from which the disease is spreading
c. Monitor the progress of control programs to limit further spread

Until late 1999, OJD surveillance in Australia was largely restricted to forward and backward tracing of infection between properties. Whilst such tracing strategies can effectively identify infected properties within known trading blocs or particular localities, tracing cannot identify infected properties that are unconnected to another property that is already known to be infected by any identifiable trading history or close proximity. To identify such unconnected properties, a cost-effective method of random disease surveillance is required. Alternative methods for random OJD surveillance such as on-property flock testing could effectively achieve this objective but would not be cost-effective.

Abattoir monitoring is well recognised as an efficient and cost-effective random surveillance strategy for many animal diseases and has been widely used in the past in animal disease control programs in Australia. Not surprisingly, an investigation of the effectiveness and practicality of abattoir monitoring for OJD was given a high priority in the business plan of the National Ovine Johne's Disease Control and Evaluation Program (NOJDP). This project directly addresses that NOJDP research priority.

2.2 Project Staff

Planning and design of this project and original drafting of the MLA application were undertaken by Dr Ian Lugton in mid 1999. Dr Laurence Denholm implemented Dr Lugton's design, commencing in late 1999 when Dr Lugton was transferred to other duties in NSW Agriculture. Dr Denholm managed the project for the next eight months until he went on long-service leave in June 2000. Thereafter the project was managed by Mr Maurie Ryan. Four National Abattoir Surveillance Program inspectors worked on the project – Messrs Gary Murphy, James Smith, Tony Ware, and Tony Hogben.
BACTEC cultures and IS900 PFC were undertaken at the Elizabeth Macarthur Agricultural Institute by Dr Richard Whittington on pooled mucosal scraping samples prepared by Ms Sonya Lane and Ms Jody Sargeant at the Orange Agricultural Institute. Data was collated by Mr Ryan and Ms Lane. The final report was written by Dr Denholm.

2.3 Project objectives

The objectives of the project were, by 30 June 2000, to have:

1. Determined the practicality of implementing post-mortem surveillance for OJD in lines of slaughter sheep at NSW meatworks that slaughter significant numbers of caste-for-age sheep

2. Compared two alternative techniques for OJD surveillance in abattoirs for practicality, relative sensitivity and cost. These techniques are (1) screening by visual inspection of intestinal tracts followed by histological examination of suspect lesions and (2) BACTEC culture of pooled ileal mucosal scrapings from 10 to 20 randomly selected sheep in each line of slaughter sheep.

3. Obtained crude estimates of the sensitivity of these two alternative detection techniques in sheep populations with a high prevalence of flocks with longstanding infection, utilising culled sheep from known infected properties

4. Obtained and stored for future culture and analysis, matched samples of caudal jejunal lymph node, rectal faeces and terminal ileum, to facilitate a future study to determine whether the sensitivity of lymph node culture is comparable to that of intestinal culture, given that collection of lymph nodes has significant practical and cost advantages over the collection of intestinal tissue in abattoirs, and to also determine whether faecal culture is as sensitive as intestinal tissue culture, given that there are significant advantages of ante-mortem collection of faeces in abattoir lairage over the collection of intestinal tissues on the dressing chain.

2.4 Introduction

The best method of abattoir monitoring for any chronic disease – that is, what exactly it is that can be most successfully monitored - is determined by the nature of any pathology of the particular disease that can be detected in infected but apparently normal animals presented for slaughter.

For some diseases, visible or palpable lesions are present in apparently normal animals and can be readily detected by trained meat inspectors during carcase dressing or routine examination of viscera.

For these diseases, of which tuberculosis is perhaps the best known example, visual and/or tactile examination backed by histopathological confirmation is usually the optimal surveillance strategy on economic grounds.

Large numbers of animals can be inspected at abattoirs for these diseases for little cost, with expensive laboratory tests restricted to confirmatory testing on those animals in which suspect lesions are detected at the abattoir. However, the specificity of visual and tactile examinations is often low. Accordingly, examination on the kill chain is used only as a screening test with a formal diagnosis made only after a more specific laboratory test.

For other chronic diseases in which visible or palpable lesions are not readily detected in slaughter stock that have been exposed to infection, the detection of infection by abattoir monitoring depends exclusively on samples collected at random for laboratory testing. Specimens for laboratory examination may be taken from all the animals in the line of animals submitted from the property for slaughter, or alternatively, specimens may be taken only from a random sample of the animals.

Where the prevalence of infection in a line of animals is low, sampling will reduce the likelihood of detection. By chance, none of the few infected animals in the line may be included in the sample. In principle at least, in such situations a high sensitivity screening test applied to the majority of animals

in the line followed by a highly specific laboratory confirmatory test is likely to be, overall, more effective than a highly specific highly sensitive test applied only to a small random sample of animals in the line - even if the screening test has a relatively low specificity. However, to determine which is the best strategy – screening or random sampling – it is necessary to have some estimates of the sensitivity, specificity and costs for both screening and definitive tests.

When this project began, abattoir surveillance had been recognised as a potential key surveillance strategy of the NOJDP. There were however two major sources of uncertainty. First, it was not known whether or not visible lesions of Johne's disease could be readily detected in lines of apparently normal aged cull sheep at slaughter. Although OJD is widespread throughout the world there are few reports of effective abattoir surveillance for OJD in small ruminants overseas. Secondly, it was not known whether the identification of lines of cull sheep at abattoirs would be sufficiently reliable to allow effective tracing of any OJD suspect sheep to their properties of origin. Reliable sheep identification is recognised as a key determinant of the success of any abattoir surveillance system.

Over the last two years, abattoir surveillance for OJD has been rapidly developed as the recognised surveillance strategy for OJD in Australia, largely on the basis of interim results from this project. There was however no scientific basis for such development until this project commenced.

2.5 Materials and methods

2.5.1 Study design

The primary objective of this study was to determine whether the cost-effective method of visual/tactile examination of viscera from cull animals at abattoirs could be used to successfully identify sheep flocks with OJD. When this trial began this was unknown.

Although the trial involved a direct comparison of two potential surveillance techniques – visual/tactile examination and random sample pooled intestinal culture (PIC), the original purpose of including the PIC treatment in the design was to provide a positive control to demonstrate that the trial lines of sheep were in fact OJD infected in the event that no visible/tactile lesions were detected. The PIC was intended as a "gold standard" for comparison with the physical examination. In the event however, PIC turned out to be a very poor reference standard for this comparison. However, when the trial commenced there was a possibility that visible lesions of OJD would not be detectable in clinically normal lines of cull sheep from known infected properties. If this had been the case, the PIC results would have been critical to demonstrate that the trial lines, or at least some of them, were in fact infected with OJD.
Culture samples were however taken at random rather than from sheep with identified gross lesions. Although culturing from sheep with lesions would have increased the rate of culture positives, this would not have allowed an independent comparison of the two monitoring techniques. In any case, it would not have been possible within the limitations of the abattoir environment to collect culture specimens from the sheep selected for histopathology without potentially compromising the primary objective of the trial by alerting the inspectors to the identity of trial lines. The decision to culture a random sample of sheep was a deliberate design element, given it was not known in advance whether any lesions could be detected.

The design of this project involved the submission of lines of known OJD infected sheep for routine slaughtering at abattoirs where inspectors were stationed to examine all lines of sheep for signs of OJD. The inspectors were not told the identity of the trial lines in advance, but were usually aware that there was a trial line to be killed in the particular shift. The trial was designed as a "blind" trial, but may have been somewhat less blind than expected. Inspectors were always aware of the locality of origin of the sheep as this is recorded on the abattoir daily kill schedule. In two case, however, the inspectors were certainly aware of the identity of the line. In one of these cases, the sheep were followed after sale to a Victorian abattoir and monitored at slaughter by staff from the Victorian Department of Natural Resources and Energy rather than a regular inspector. In the other case an inspector was given the name of the owner of the line by an abattoir worker and recognised that

name as the owner of a previous trial line. The prevalence of sheep with suspect lesions in these two lines as reported by the inspector was 2% and 25% respectively, suggesting that the second line at least would not have been difficult to detect.

2.5.2 Selection of Lines of Sheep

2.5.2.1 Selection Criteria

The key selection criteria for trial lines were designed to ensure to a high degree of reliability that all of the trial lines were infected with OJD. In other words, the criteria were designed and applied in such a way as to eliminate lines of sheep from the trial for which there was any doubt about their infected status. As a result, most but certainly not all of the lines used in the trial were likely to have been heavily infected.

Lines were only accepted as "trial lines" if they met one of the following criteria.
Deaths from OJD had been confirmed in the mob from which the trial line was derived; or
If no deaths from OJD had been observed in the mob from which the trial line was derived, the mob had been born on an infected property at a time when OJD deaths were occurring in other mobs on the property and the trial line had not been isolated from the infected mobs (ie had grazed contaminated pastures); or.
The mob from which the trial line was derived had been introduced to the infected property not less than three years before slaughter and at a time when OJD deaths were occurring in other mobs on the property and the trial line mob had not been isolated from infected mobs (ie had grazed contaminated pastures).

2.5.2.2 Lines from NOJDP Trial 1.1 Property Destocking

When this trial was originally planned it was estimated that between 60 and 80 lines of OJD infected sheep could be obtained from the OJD infected properties that were destocking as part of NOJDP Trial 1.1. This however was not the case. There were two reasons for the marked reduction in the number of lines available from Trial 1.1 flocks.
First, the original design of Trial 1.1 called for destocking of 150 infected properties in NSW but ultimately less than 20% of this agreed number of NSW properties were actually destocked under Trial 1.1. Secondly, by the time the funding of this abattoir surveillance project (OJD-007) was approved, destocking had been completed on most of the NSW properties that did participate in Trial 1.1. NSW Agriculture attempted to contractually bind participants in Trial 1.1 to provide infected lines of sheep for this abattoir project, but by the time these contracts were prepared destocking had already been completed on most of the properties. Only 14 of the 60 to 80 lines of infected sheep from Trial 1.1 properties expected to be available were actually available. These came from 5 different properties.

2.5.2.3 Lines solicited by advertisement and bounty

As a result of the problem described in 1.5.2.2, an attempt was made to obtain the remaining required 86 lines of infected sheep from other infected properties. A letter was sent to the owners of all known infected properties in NSW offering to pay a bonus of $200 for prior notification of the slaughter any line of 200 or more sheep from an infected flock at either of the two major export works in NSW. However, unfortunately the mailing of this notice to all OJD affected producers in NSW coincided with a significant rise in political opposition amongst these producers to the introduction of abattoir surveillance for OJD.

As a result of this opposition, in response to the notice of a $200 bounty, the number of producers who telephoned to express their opposition to the project and say they were NOT prepared to submit lines of infected sheep for this purpose was more than double the number of producers who telephoned to offer infected sheep for the project.

Nonetheless, over the next six months, twenty-six lines of sheep were obtained from 15 known infected properties whose owners supported the project. Subsequent epidemiological investigations however indicated that 5 of these 26 lines originating from 3 properties did not meet the strict criteria for inclusion in the trial (see 4.5.2.5 below). Hence the 35 lines of infected sheep used in this project comprised 14 lines from Trial 1.1 properties and 21 lines from 12 other infected properties. Nine of these 21 additional lines were obtained from one property that was totally destocked over the first half of 2000, although not under Trial 1.1

2.5.2.4 Other Lines included in Trial

Although not a part of the original project proposal, fresh intestinal specimens were obtained from 9 lines of sheep that were killed in 9 different shifts at which samples from 9 of the 35 “trial lines” were collected. These additional lines originated from areas of NSW where OJD was not known to exist and were intended to act as negative controls for the trial lines.

Specifically, these negative controls were collected to identify any problems with cross-contamination of samples in the mucosal scraping culture process. There were several potential sources of culture cross-contamination, including:
Transient infection of sheep during transport or holding in abattoir lairage. Uninfected sheep could become positive on intestinal culture as a result of ingesting *M. ptb* in the contaminated environment during the period immediately prior to slaughter.
Cross-contamination of intestinal tracts during carcase dressing process and collection of tied-off gut loops
Cross-contamination of mucosal samples during laboratory scraping process
Cross-contamination during the laboratory culture and PCR process.

Samples from control lines were processed by the same techniques used for the trial lines.

2.5.2.5 Lines Excluded from Trial on Epidemiological Grounds

As stated above, five lines were retrospectively excluded from the trial as a result of epidemiological investigations. The reasons for exclusion were as follows:

One line obtained from a prospective participant in Trial 1.1 was excluded when extensive serological testing on the property failed to disclose any evidence of OJD infection in any sheep on the property. The property was probably not infected.
One line from a recently diagnosed infected property was excluded. Infection had been detected at low prevalence in a mob set-stocked in a paddock immediately adjacent to a known infected neighbour. OJD had not been detected in any other mob on the property and there had been no contact or common grazing between the infected mob and the mob from which the potential trial line was derived.
Three lines were excluded because they all came from one apparently uninfected mob of sheep on a property that had carried two mobs of sheep, both purchased. OJD had been detected at low prevalence in the other mob but not in the mob from which the three excluded potential trial lines were derived. The mobs were run separately, although some paddocks had been grazed by both mobs in the last year. The first mob was probably infected when purchased. There was no evidence of OJD transmission occurring on the property. The second mob from which the potential trial line was derived may not have been infected.

2.5.3 Abattoir Procedures

2.5.3.1 NASP Inspectors

All of the inspectors used in this project were tertiary qualified Meat Inspectors previously employed by the Australian Quarantine Inspection Service at major sheep export works. These inspectors were further trained according to the approved NOJDP National Abattoir Surveillance Program (NASP)

training manual and all had passed the NASP competency examination. The inspectors were all experienced in working on the sheep viscera inspection chain of major export mutton works.

Inspectors were able to determine the beginning and end of each line of sheep on the chain by a tag placed on the gambrel of the first sheep in each line by abattoir staff. Inspectors were instructed to avoid taking samples from the first 10 or last 10 sheep in any line wherever possible because there was a theoretical potential for occasional mixing of sheep in the holding pen immediately prior to the stunning point, at the time of changeover between successive lines.

2.5.3.2 Visual/Tactile Examination of Viscera and Sampling

Inspectors were employed under contract to examine not less than 50% of all viscera from lines of adult sheep slaughtered during any kill shift on which they worked. For most lines the inspectors actually examined 70% to 90% of the viscera.

This inspection involved locating and palpating the terminal ileum for mural thickening, visual examination of the ileum and jejunum for any sign of segmental mural thickening, visual examination of the mesenteric lymph nodes, particularly the caudal jejunal lymph node, for any sign of lymphadenopathy and examination of the ileal and mesenteric serosa for signs of lymphangitis, particularly lymphatic cording.
Where any such lesions were detected, entire intestinal tracts from up to three suspect sheep in each suspect line were removed from the viscera chain and placed in a plastic bag for subsequent collection of intestinal and lymph node samples fixed in 10% neutral buffered formol saline. Inspectors were instructed to collect the samples from any abnormal (thickened) area of small intestine regardless of the location of the lesion along the alimentary tract if there was no such lesion in the ileum.

2.5.3.3 Collection of Fresh Tissues for Culture

Staff were also engaged to collected fresh intestinal tissues. This work was undertaken in the so-called “runners room” where sausage casings are prepared. This is a room below the kill floor proper where intestinal tracts arrive via a chute from the viscera chain above. Using tags applied by the inspector on the viscera chain above, the beginning and end of each line of sheep was identified for the staff collecting the fresh intestinal samples. Avoiding the first few sheep at the beginning or the last few sheep at the end of the line (for the reason explained above), the staff selected 10 small intestinal tracts at random from the line. A loop of intestine approximately 10 cm in length and close to the ileo-caecal valve was isolated and the ends tied off firmly with string prior to transection, leaving the loop sealed by string at both ends. The isolated intestinal loops were on ice within 45 minutes of death. These tissues were despatched by courier on the same day as collection and arrived at the Orange Regional Veterinary Laboratory on the following morning.

On the day of receipt at the laboratory, mucosal scrapings were collected from each intestinal loop. All scraping work was undertaken in a pre-sterilised laminar flow biohazard cabinet, with instruments and cutting boards changed between batches.

Each intestinal loop was pinned out on a cutting board using ordinary sewing pins passed through the intestinal wall distal to the string. The uppers surface of the wall was then sterilised with a heated spatula. Using scissors and forceps a cut was made through the seared wall, at the same time reflecting the edges away from the opening. This was done in such a way that neither the scissors nor the cut edges were allowed to come in contact with the mucosa on the wall of the intestine opposite to the cut. The cut edges were then pinned down using more sewing pins.

Finally the mucosa was scraped from an area of approximately 25 sq cm of the intestinal wall in the centre of the preparation, using the rounded end of a normal laboratory spatula, taking care to avoid including or contacting any mucosa adjacent to the cut edges. The scraped mucosa was then placed in a sterile plastic conical tube.

This process was continued until scrapings from all ten intestinal loops from the same line were in the same conical tube. This process took approximately 1.5 hours for the ten loops. Tubes were stored at –70^{o}C until dispatch to the Central Veterinary Laboratory at Camden.

These pooled intestinal mucosal scrapings were collected from 34 of the 35 trial lines, 9 control lines and the 5 lines eliminated from the trial on epidemiological grounds. Scrapings were not collected from one trial line as a result of a last-minute decision by the abattoir to postpone killing the sheep until the night shift rather than the day shift as had been arranged. When advised of this, the NASP inspector agreed to work a double shift but it was not possible to have NASP staff in place to collect fresh intestinal samples.

Results of pooled intestinal culture were obtained from the Central Veterinary Laboratory for 33 of the 34 pooled samples submitted.

2.5.3.4 Data Recording and Verification

To ensure there had been no error in the identification of the trial line or control line of sheep at the abattoir, a visit was made to the abattoir some weeks after each line was killed and the abattoir records were checked. Proper identification and maintenance of the integrity of the line during the slaughter process was confirmed for each of the 35 trial lines.

2.5.4 Laboratory methods

2.5.4.1 Histopathology

Formalin fixed intestinal tissue and lymph node were embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin and Ziehl Neelsen stain[1].

2.5.4.2 Pooled Intestinal Culture

Pooled intestinal mucosal scrapings from suspect lines were cultured in a radiometric system (BACTEC)[2]. This method ensures isolation of ovine strains of *M. avium* subsp. *paratuberculosis*. Identification of any *M. avium* subsp. *paratuberculosis* in the BACTEC cultures was undertaken by an IS*900* PCR-REA[3] using P90/P91 and M56/M119 primers.

2.6 Results

2.6.1.1 Visual Examination

Inspectors detected gross lesions consistent with OJD in 34 (97%) of the 35 known infected trial lines. The only trial line in which visible lesions were not detected by the inspector was a line of cross-bred ewes introduced as young adults to a property on which OJD had only been reported in merino sheep at low prevalence.

Microscopic lesions diagnostic (31 lines or 91%) or suggestive (3 lines or 9%) of OJD were identified in all 34 lines in which suspect lesions were detected by abattoir inspectors. There were no trial lines in which the inspectors failed to detect OJD as a result of the presence of some other cause of intestinal lesions similar to those of OJD.

From the 35 trial lines, inspectors submitted suspect lesions from a total of 99 sheep. Histopathology, positive (73 or 74%) or suggestive (7 or 7%) of OJD, was detected in 80 (81%) of those sheep.

The average proportion of sheep in the 34 lines with lesions suggestive of OJD as reported by the inspectors was 21% (range <1% to >90%) indicating a high prevalence of OJD infection amongst

many of these lines of sheep. However, of the 35 trial lines inspected, 10 lines had a reported within-line prevalence of sheep with OJD lesions of <2%. Inspectors detected lesions in 9 of these 10 "low prevalence" lines. In three of these 9 lines, inspectors detected only two suspect sheep in each of the lines - 300, 383 and 398 sheep respectively, but of these sheep, 1 or 2 from each line were positive on histopathology.

Amongst the 9 "negative control" lines, lesions were detected in two lines originating from areas of Far Western NSW where OJD has not been reported. In one case the inspector reported 4% of the sheep had typical OJD lesions, in the other case 2%. However, none of the samples submitted for histopathology from these lines revealed any signs of OJD.

Of the 5 lines that were retrospectively excluded from the trial on epidemiological grounds, no lesions were detected at inspection in the abattoirs for 4 of these lines. In the fifth line (which came from the same property as two of the other rejected lines) lesions were detected in one sheep, but these were negative on histopathology.

2.6.1.2 Pooled Intestinal Culture (PIC)

Of the 33 PIC results available for trial lines, 19 (58%) were positive for *M. ptb* and 14 (42%) were negative. The only trial line in which no visible lesions of OJD were detected by inspectors, a line of introduced crossbred sheep, was positive on PIC.

PIC detected 18 (55%) of the 33 lines detected by visual examination and histopathology for which PIC results were available. (PIC results were not available for one of the 34 samples.)

All of the five lines of sheep that were retrospectively excluded from the trial on the basis of epidemiological information that indicated they had not met the original selection criteria were negative on PIC.

No lesions of OJD were detected by abattoir inspectors in 7 of the 9 "negative control" lines. Suspect lesions were however detected in 2 of these 9 control lines, but lesions in the sheep from which samples were taken were negative on histopathology.

Positive PIC results however were obtained from 2 of the 7 negative control lines in which no visible lesions were detected. One was a line from an area of Western NSW where OJD has not been reported to date, the other from an area of NSW where OJD had not been reported at the time.

2.7 Discussion

This trial provides a crude estimate of 97% for the sensitivity of abattoir surveillance for OJD by visual/tactile examination of the viscera from cull sheep. However, it must be noted that the trial lines used in this project were selected in such a way that the average prevalence of sheep with lesions of OJD in these lines was probably significantly higher than could be expected in any sample of OJD infected lines taken randomly from the population of OJD infected properties in NSW. From the inspectors' estimates, the mean prevalence of sheep with lesions of OJD in these trial lines was 21%.

Notwithstanding this deliberate bias in the design of the trial, it should be noted that the skilled inspectors used in this project were able to detect 9 of 10 (90%) lines in which the reported prevalence of sheep with suspect OJD lesions was <2%.

Under routine surveillance in abattoirs, OJD inspectors check the viscera of a high proportion of the sheep in each line. The "quality" of inspection is such that any sheep with visible or palpable lesions of OJD that is actually inspected is very likely to be identified as suspect by the inspector. In NSW, the average cull line is about 300 sheep and normally at least 200 are examined by NASP inspectors. Accordingly, skilled inspectors are likely to detect at least one infected sheep in a line, even if the prevalence of sheep with lesions is very low (<1%). This type of "low prevalence line" would be the

typical pattern of infection expected in the first few years after OJD is introduced onto a property, before infection has spread widely through the flock.

Further research is required however to confirm this predicted high sensitivity of abattoir inspection for those “low prevalence lines” in which there are some, albeit only a few, sheep with detectable gross lesions. The proportion of sheep in each line that is inspected will influence this sensitivity.

There is however, at least theoretically, a second type of low prevalence flock in which infection could persist at a low level in such a way that infected sheep do not develop, or rarely develop, visible lesions of the disease. In this hypothetical scenario, a significant proportion of sheep in the flock could be persistently infected but only at a low level, a level theoretically insufficient to induce the development of visible lesions. Abattoir surveillance using a visual/tactile screening test will not detect this type of low prevalence infected flock. There is however no evidence that such flocks exist. Lesions of OJD can be detected in goats at slaughter despite the apparently higher resistance of goats to infection.

The current estimate of sensitivity for abattoir surveillance that is used in the national OJD program is 30%. Where experienced, trained inspectors are working in a sheep population with a known moderate to high prevalence of OJD, this is probably a significant underestimate. Where inexperienced inspectors are working in a sheep population in which OJD is rare or unknown, the estimate of 30% sensitivity may be an overestimate. Some investigation of the effects of variability in training and experience of inspectors on the effectiveness of surveillance for OJD is warranted.

The data presented here suggest that the specificity of visual/tactile examination for OJD in sheep is high, but this data was collected from a series of lines deliberately selected to give 100% prevalence of infected lines. Data collected from routine abattoir inspection of sheep for OJD in a population with an expected true line prevalence of <10% infected suggests that specificity of the technique is lower, perhaps about 60%. This suggests that other diseases may on rare occasions cause lesions similar to those of OJD and this could interfere with the detection of OJD in such flocks if the prevalence of OJD was very low. The observation that 2 of 9 negative control lines had suspect lesions in 2% and 4% of sheep that were apparently not caused by OJD suggests this situations is likely to occur although it may be rare. Whether the alternate cause of these lesions will persist to cause ongoing interference with the diagnosis of OJD in lines of sheep culled from the same property in future years is unknown.

The data from this project suggests, but does not prove, that visual/tactile examination may be a highly sensitive technique for detection of lines of sheep in which the within-line prevalence of infection is quite low. Obtaining a reliable estimate of the sensitivity of visual/tactile examination for OJD in “low prevalence lines” is clearly a high priority for the national OJD program if abattoir surveillance is going to be used as a means of making regional disease prevalence estimates for such purposes as zoning.

In this particular project, abattoir surveillance by visual/tactile examination was significantly more sensitive that the culture method adopted – pooled intestinal culture of 10 randomly selected sheep. However, even if this had not been the case, in practice the collection of intestinal samples and scraping of mucosa is not a practical or economic technique for routine surveillance. Further development of this method is not recommended.

Abattoir surveillance by alternative culture techniques such as the pooled faecal culture (PFC) test should however be further investigated. It should be possible to collect faecal pellets in abattoir lairage fairly simply and economically from a significant random sample of sheep in any line, certainly from 100 sheep rather than the sample of 10 used in this trial. Collection of faeces in lairage would have the added advantage of permitting a check on eartag identification to ensure that no stray sheep are included in the sample.

A designed study to compare PFC on faeces taken pre-slaughter from sheep in lairage with post-slaughter visual/tactile examination of their viscera would now seem to be appropriate.

However, it must be recognised that all culture techniques will suffer from the same potential problem of post-farmgate contamination of intestinal contents. The detection of 2 culture positive lines amongst the 9 negative control lines in this project supports the hypothesis that this will be a problem for any culture method of surveillance, particularly in areas where OJD is common. In areas believed to be free of OJD infection, this would not be a problem.

Given the observations in this trial and the problem of maintaining effective visual inspection in areas where OJD is rare or unknown, it is suggested that the optimal technique for abattoir surveillance may vary between different abattoirs. For abattoirs that draw sheep from regions of moderate to high OJD prevalence, visual/tactile examination will be sensitive and efficient for detection of infected flocks. In these abattoirs, post-farmgate contamination could be a problem for any culture method and hence visual/tactile examination is likely to be the best method.

Conversely, for abattoirs that draw sheep only from regions of low to nil OJD prevalence, visual/tactile examination is likely to be less efficient and contamination will not be a problem. In these abattoirs, surveillance by pooled faecal culture may well be optimal. Further research in this area is clearly needed, but it must be acknowledged that such research will not be easy to design or cheap. What is needed is an estimate of the effect upon the estimated sensitivity of detection of OJD infected lines of variable factors such as (1) within-line disease prevalence, (2) inspector skill, (3) number of sheep in the line, (4) proportion of sheep in the line that are inspected and (5) any variation in pathology between different regions or disease situations (for example, do lesions develop less frequently in infected sheep from persistent low prevalence flocks?). It is unlikely that any single estimate of sensitivity will be appropriate for all situations that are likely in the course of the national OJD program. Estimates under several different prevailing situations are needed.

2.8 Success in achieving objectives

This project was successful in achieving three of the four objectives.

1. The project demonstrated the practicality of implementing post-mortem surveillance for OJD in lines of slaughter sheep at NSW meatworks that slaughter significant numbers of caste-for-age sheep. However, the reliability of conclusions in relation to this objective is reduced significantly due to the data set comprising results from only 35 of the intended 100 lines of OJD affected sheep.

2. The project compared two alternative techniques for OJD surveillance in abattoirs for practicality, relative sensitivity and cost. These techniques were (1) screening by visual inspection of intestinal tracts followed by histological examination of suspect lesions and (2) BACTEC culture of pooled ileal mucosal scrapings from 10 randomly selected sheep in each line of slaughter sheep. In this trial, the pooled mucosal scraping test was shown to be less sensitive than visual examination, completely impractical as a routine surveillance method and expensive.

3. The project obtained crude estimates of the sensitivity of these two alternative detection techniques in a sheep population with a high prevalence of flocks with longstanding infection, utilising culled sheep from known infected properties. These estimates were 97% for visual examination and 58% for pooled intestinal culture. However, the reliability of these estimates is significantly reduced due to the data set used to make these estimates comprising results from only 35 of the anticipated 100 lines of OJD affected sheep.

4. Due to logistical problems in abattoirs, matched samples of caudal jejunal lymph node, rectal faeces and terminal ileum were not obtained as had been planned. To collect such specimens would have required an extra person on the abattoir line to collect the samples.
Moreover, collection of these specimens would have required arrangements that either alerted the inspectors to the identity of the trial lines, thereby removing the “blind” inspection of trial lines, or collection of specimens from all lines killed on the shift, with subsequent disposal. In the event it was not deemed to be feasible to collect samples to comply with the fourth supplementary objective of the project without potentially compromising the other three primary objectives. Collection of these samples can be done any time in the future now that routine abattoir surveillance is in place.

2.9 Impact on Meat and Livestock Industry

In 1999 the future success of the sheep industries' OJD program was critically dependent on the development of a cost-effective surveillance technique. This has been achieved. However, given the expected wide application of abattoir surveillance for OJD in Australia in coming years, the further refinement of abattoir surveillance techniques and obtaining more reliable estimates of their sensitivity in different disease prevalence situations is warranted.

2.10 Conclusions and recommendations

1. In regions with a moderate to high prevalence of OJD infected flocks, abattoir surveillance for OJD using combined visual and tactile examination of viscera from adult sheep is an effective and cost-effective method for the detection of infected flocks in which cases of OJD are occurring.

2. Pooled culture of intestinal mucosal scrapings is not a practical, cost-effective or sensitive method for the detection of OJD infected flocks and should be abandoned.

3. In regions where OJD infected flocks are known to be present, post-farmgate contamination of the intestinal contents of sheep may cause false positive errors in the detection of OJD infected flocks where the method of surveillance relies on mycobacterial culture rather than demonstration of lesions of OJD. This potential problem however should not be an issue in regions where OJD is rare or unknown.

4. The limited data for "low prevalence flocks" (<2%) obtained in this trial suggests that visual/tactile examination of viscera may be a more sensitive technique for detection of low prevalence infected lines than was previously believed and probably more sensitive than the current estimate of 30%. Further investigation of the utility of this method of abattoir surveillance for OJD in low prevalence flocks and low prevalence regions is warranted.

5. Although not directly a part of this project, it was concluded from observations of inspectors at work in abattoirs that the detection of OJD lesions and tracking of sheep within abattoirs is a skilled procedure best undertaken by experienced meat inspectors. Inspectors who have not had formal training and considerable prior experience as meat inspectors are unlikely to detect OJD lesions, particularly subtle lesions, as reliably as inspectors who have been trained in this way. This is likely to be more critical when inspectors are operating for long periods in regions where OJD is rare or unknown. It is recommended that national standards for abattoir OJD inspectors should be established by the NOJDP to include tertiary qualifications and experience with AQIS as a meat inspector, completion of a nationally accredited OJD abattoir inspection course and passing an OJD inspection competency test.

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