MEAT & LIVESTOCK
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On farm

Determination of Individual Animal - Level Sensitivity of Abattoir Surveillance for Ovine Johne's Disease

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ABSTRACT

This project aimed to estimate the sensitivity of abattoir surveillance for ovine Johne's disease (OJD) at an individual animal level. Currently abattoir surveillance for OJD occurs in 21 meatworks around Australia and since late 1999 over 16 million sheep have been examined by this method. Abattoir inspection is an extremely efficient and economical method of regional surveillance for OJD but could also be used for negative assurance purposes if the sensitivity were known. The trial involved three inspectors working in different OJD prevalence areas and compared their diagnoses with the gold standard of histopathology for approximately 1200 sheep from known infected properties. The sensitivity level for the best inspector was 87.3%. A "dummy run" of presumed negative sheep yielded a very low false positive rate. This trial has established that for the lines of sheep examined, an average sensitivity of 70% is applicable. However for the purposes of a flock negative assurance system in a low prevalence flock, a sensitivity level of 50% would be more appropriate. This potential use of this methodology for flock assurance purposes relies on an accurate identification system for sheep.

EXECUTIVE SUMMARY

Ovine Johne's disease (OJD) is a fatal enteric infection that is difficult to diagnose and control. In recent years this disease has become increasingly important as surveillance strategies have found infected flocks in different areas in Australia. The disease is highly topical and receives wide coverage in both the rural and mainstream media. Recently funds have been allocated to research many aspects of this disease such as the efficacy of vaccination and organism survival. However an economic, rapid and highly accurate diagnostic test still eludes researchers around the world.

Visual and manual examination of sheep viscera by inspectors as they pass through an abattoir is an extremely rapid and economic method of diagnosing OJD. Unfortunately until this time there has been no rigorously conducted study to estimate the ability of inspectors to detect truly infected sheep. Nationally abattoir surveillance has been increasingly adopted as a methodology for detecting infected viscera and this surveillance activity is now a requirement for maintaining Protected and Control zone status. Since late 1999, approximately 16 million sheep[1] have been examined for OJD at abattoirs with a total of 2398 infected lines detected as positive. In total, 4% of the lines examined were positive with most of these lines emanating from the heavily infected areas of NSW. It is apparent that the vast majority of sheep viscera examined are in fact negative.

In the absence of a perfectly sensitive diagnostic test for OJD, sheep producers wishing to buy uninfected stock have the option of purchasing from a "lower risk" source such as a Market Assurance Program (MAP) accredited flock. There are significant costs and risks associated with being involved in such programs and uptake has not been high. Abattoir surveillance is currently conducted in all states in Australia and much of the data generated is in fact negative. It would seem sensible to utilise this information for the purpose of providing some assurance of freedom from OJD. It is necessary to have a good estimate of the sensitivity of abattoir surveillance to do this.

This project aimed to establish the sensitivity of abattoir surveillance for OJD under conditions encountered in a meat works. Three OJD inspectors were involved in the trial from different OJD prevalence areas in Australia. Approximately 1200 sheep from OJD infected farms were examined by the inspectors and subsequently with follow – up histopathology. The ability of inspectors to detect gross lesions varied from 53% to 87%. The best performing two inspectors (74% and 87%) had a high level of agreement between their diagnoses despite coming from very different prevalence areas (WA and NSW). The third inspector had not undergone the formal OJD inspector training which may have contributed considerably to the lower level of sensitivity. The results suggest that abattoir inspection can detect about 70% - 75% of histologically positive animals However, for the purposes of setting a sensitivity level for a negative assurance scheme, it is reasonable to use a sensitivity of 50% to account for the lower level of heavily infected animals in low prevalence flocks..

A small control line of presumed negative sheep were inspected at the abattoir without confirmatory histopathology. These two hundred sheep yielded an extremely low false positive rate with only one inspector misclassifying one sheep. The false positive rate was higher for the infected lines. Since confirmatory histopathology is performed on the most diagnostic lesions in such circumstances, it is extremely unlikely that a flock of sheep would be falsely labelled as infected because of gross pathology. However the use of gross pathology — or indeed any test based on abattoir surveillance — is contingent upon a widely adopted, accurate method of sheep identification.

The use of gross pathology also relies upon properly trained and accredited OJD inspectors. The best inspector in this trial, with such a high sensitivity, would be well placed to assess other inspectors under line conditions to ensure minimum inspection standards are met.

When a sheep identification system and inspector accreditation system are in place negative abattoir data for OJD could be used in a flock assurance scheme, or as part of providing an assessment of the risk of the flock having OJD (risk-based trading). Use of abattoir surveillance for such purposes would probably occur at the request of the individual producer initially and could supplement other diagnostic testing regimes such as pooled faecal culture and serological check tests. This would aid producers immensely in trading in "OJD assured negative" sheep or in a risk-based trading system with very little, if any cost to the producer.

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1. MAIN RESEARCH REPORT

1.1. Background to Project and Industry Context

Johne's disease is an important disease of ruminants worldwide which is often difficult to control and usually fatal. Aside from production losses due to Johne's disease in farmed animals, recent speculation of a potential zoonotic link with Crohn's disease and hence food safety issues has further enhanced interest in this infection. OJD in Australia has become increasingly topical in recent years due to changes in government policies related to controlling the spread of the disease at both a state and national level. Nationally, an Ovine Johne's Disease Control and Evaluation Program (NOJDP) commenced in 1998. One of the greatest impediments to control of the disease has been the absence of an accurate and inexpensive diagnostic test. Programs such as the Market Assurance Program (MAP) have been undertaken by some producers to provide assurance of disease freedom using either serology or pooled faecal culture as the methodology. Testing a flock can cost producers in the vicinity of $1500 for pooled faecal culture.

Abattoir surveillance for disease has been used to detect conditions such as tuberculosis and contagious bovine pleuropneumonia. This technique of disease surveillance has the benefits of being economical and efficient: large numbers of animals from diverse areas can be assessed over a short period of time. Abattoir surveillance however does have the disadvantage of selecting from a biased population — those animals that are in sufficient health and condition to be presented to a meat works. Therefore abattoir surveillance usually detects subclinical disease.

Abattoir surveillance of sheep intestines ("runners") in Australia for OJD has occurred at 21 meat works in all states except the NT. The National Standard Definitions and Rules for Sheep require that for maintenance of Control or Protected zone status more than 20% of flocks must be subjected to abattoir surveillance. Since October 1 1999, 55 000 sheep lines have been examined by this method comprising approximately 16 million sheep[1]. A total of 2398 infected lines (originating mainly from NSW) have been detected by abattoir surveillance in this time. There are a very large number of sheep viscera that are being examined for OJD with negative results. Being able to utilise this negative data is hampered by not knowing the sensitivity of abattoir surveillance.

Previous work examining the sensitivity of abattoir surveillance includes MLA Project OJD-007 conducted by NSW Agriculture[2] which examined line - level sensitivity of inspection and part of a larger project performed at VIAS – Attwood[3]. The NSW work looked at 35 lines of sheep from infected farms. Inspectors detected gross lesions suggestive of OJD in 97% of the lines. The VIAS – Attwood work compared gross examination of viscera by untrained staff (not under abattoir conditions) with histopathology. The sensitivity from this work was 30.6% (95% CI 22.1 – 40.3). As some of the staff assessing viscera in this trial had never seen a set of sheep viscera, this result should be interpreted with caution.

1.2. Project Objectives

The objective of this project was to determine the sensitivity of ovine Johne's disease abattoir inspection at an individual animal level. The sensitivity will depend on the inspector's ability and so three inspectors with varying experience in OJD inspection were involved in the trial.

1.3. Detailed Methodology

Potentially eligible sheep farms were sought from districts in NSW with known high prevalence of OJD in their flocks. Farmers from infected properties were approached directly by their local RLPB vet or sent a letter from the project leader and local RLPB vet. A bounty of $1000 was provided as an incentive for farmers to become involved in the project. Farmers had to subject their sheep to serology, agar gel

immunodiffusion test (AGID) paid for by the project, and agree to consign their sheep to Southern Meats in Goulburn over a specified 2-day period if they were deemed suitable for the trial.

Initial eligibility criteria for farms to enter the trial included: a presumed high prevalence of OJD from previous abattoir surveillance data or on–farm flock profiling, a minimum line size greater than 150 and sheep aged 2 years or older. For statistical reasons (to maintain narrow confidence intervals around the point estimate of sensitivity) it was important to gain an overall true prevalence of OJD for sheep in the trial of over 10%. It would have been possible to set a lower prevalence but, as histopathology was the main cost of the trial and was performed on all viscera, the cost of such a trial would have been prohibitive. Sheep to be consigned to the works were blood tested by private practitioners and subjected to an AGID for *M. paratuberculosis.* The minimum seroprevalence for the trial was 3% in the line of sheep which, allowing for a sensitivity of around 30%[4] for this test, would indicate a prevalence of about 10%. Time constraints and the known higher level of infection in one line resulted in 2 lines being accepted slightly below this cutoff. It was not possible logistically to correlate individual blood results with individual inspector diagnoses or histopathology.

There were 3 inspectors: X, Y and Z who varied in their experience with working in abattoirs inspecting sheep viscera for OJD. Inspector X had previously worked for 14 years as a meat inspector, had been working full - time as an OJD inspector for NSW Agriculture for 2.5 years, and had undergone the formal NSW training program for inspection. Inspector Y was located in Western Australia, had undergone formal training for OJD inspection and worked for 3 years as an inspector but only 20 – 30 full days per year. Inspector Z was based in Victoria, had been a meat inspector for 15 years, had not undergone formal training and had been working for 6 months as an inspector on a fulltime basis.

Ideally, to test the hypothesis that different exposure to OJD infected sheep affects the ability of inspectors to detect the disease (ie the sensitivity of the test) replicates from each different prevalence area would be enlisted. Logistically this was not possible due to space constraints on the line at the works. The inspectors were aware that they were involved in the trial but did not know ahead of time what proportion of the sheep were positive nor which individual sheep were. The inspectors were instructed to simply “call the lesions as they saw them”, as they would in their own work place.

All sheep intestines (runners) were tagged sequentially with numbers from 0001 to 1200. The task of the inspector was to read the tag number and give a diagnosis for each set of viscera as it moved along the line. Their results were recorded on small cassette recorders using an amplifying microphone attached to their lapel. The inspectors were separated from each other by between one and four workers on the line, and so were unable to see or hear the other inspectors making their assessment. Their observations were therefore independent. The inspectors worked for less than an hour each morning and afternoon over 2 days. The line speed in the works was 10 runners per minute and in nearly all cases the inspectors were able to examine every set of guts as they presented on the line. At the end of the consigned “trial” sheep, the inspectors (without foreknowledge) examined 200 sheep that were not part of the trial but were from a farm where OJD was not known to occur – the “dummy run”. This farm was located in the Protected Zone for OJD, but was not a MAP flock and had no extra testing for OJD conducted on this property.

Following inspection on the line, all runners fell down into the “runners room” where they were individually bagged (with tag attached) and then processed by Department of Primary Industry animal health staff. All staff were provided with laminated sheets outlining the specimens required and given a brief training session on site. The greatest logistical problem in specimen collection was processing the samples into formalin before autolysis occurred. Approximately ten staff worked in the “runners room”. The samples collected were: the ileocaecal lymph nodes, the ileal (caudal jejunal) lymph node, 5cm of ileum adjacent to the IC valve, 2 further pieces of ileum about one metre from the IC valve, a piece of caecum and any other tissue with suggestive lesions. These specimens were placed in 10% buffered formalin in 250ml jars for histopathological examination (the “gold standard”). The standard collection protocol according to the Abattoir Surveillance Policy is as follows: "Where gross lesions are detected, histology samples are taken from up to 3 sets of viscera. Required samples are: up to 10 cm of terminal ileum, ileocaecal lymph node, caudal jejunal lymph node, section of caecum, any other tissue with suggestive gross lesions." Pathologists in NSW examine sections according to how many samples are submitted as follows: if only one sheep is sampled all tissues are examined, if two sheep are sampled terminal ileum and one lymph node are examined from each sheep; if 3 sheep are sampled, terminal ileum alone is examined from

each sheep (Patrick Staples pers comm).

The samples were dispatched to Gribbles Veterinary Pathology Group in Melbourne and processed generally as 6 tissue pieces on 3 slides with both haematoxylin and eosin (H&E) stains and Ziehl - Neelsen (ZN). The samples were sent to a number of pathologists in both Australia and New Zealand along with a recording proforma and a guide to reading the slides. The pathologists were requested to record the case number, tissue type examined and the presence of giant cells, epithelioid macrophages and ZN organisms (the latter three being ranked on a scale of "–" to "+++"depending on how many were seen). Comments on slide quality or alternative diagnoses were also sought. As per the Australian and New Zealand Standard for Diagnostic Tests (ANZSDT's) if there was no suggestion of OJD pathology on the haematoxylin and eosin (H&E) slide and the tissue appeared well preserved, then there was no need to examine the ZN slide. However if autolysis was present or there were any signs of pathology consistent with OJD then the instruction was to examine the ZN processed slides for 10 minutes per tissue to establish the whether acid fast organisms were present. A positive diagnosis was made where one or more ZN – staining organisms were viewed. A negative diagnosis was made when there was no pathology consistent with OJD on H&E and an inconclusive diagnosis was made where there was pathology consistent with OJD on H&E but no organisms could be viewed on ZN slides.

Pathologists at VIAS Attwood re-read approximately 10% of cases read by Gribbles. This was used as a quality control exercise and, it was hoped, to resolve some of the inconclusive pathology results. These cases were not selected randomly and were either "suggestive" results or biased towards cases where all three inspectors agreed on a diagnosis that was not supported by the histopathological diagnosis.

Data was entered into an "Excel" spreadsheet alongside the transcribed diagnoses made by each inspector.

The sensitivity for each inspector was calculated with 95% binomial exact confidence intervals. Inconclusive results and sheep for which all 3 inspectors did not make a diagnosis were excluded from analysis.

2. Results

2.1. Diagnostic Investigations

Four separate lines were consigned for the trial, with the first and largest line considered to be two separate parts since it was examined over two separate sessions, and very different prevalence rates in the two halves were expected based on the farmer's clinical assessment of the sheep.

The sheep in Line 1 were mixed age and sex Merinos, in Line 2 they were 3 year old cross - bred sheep of mixed sex, in Line 3 they were 2-year-old Merino wethers (including 3 vaccinates) and Line 4 were mixed age and breed females.

In total, there were 1196 sheep viscera that passed along the chain at the abattoir during the 2 days of the trial. Thirty-nine viscera were excluded because either identification tags were lost during processing or a diagnosis was not made by one or more inspector. Twenty-six sets of viscera were excluded because the final histopathology result was inconclusive.

The results of the trial are shown in Table 1 and Table 2. All inconclusive diagnoses were excluded.

2.1.1. Prevalence

Histopathological examination showed that the line prevalence varied from 6.6% to 25.0%: Line 1a – 8.3%, Line 1b – 25.0%, Line 2 – 15.3%, Line 3 – 6.6%, and Line 4 – 8.9%.

2.1.2. Serology Testing

The serology blood results (AGID for OJD) were as follows: Line 1 – 9.0%, Line 2 – 6.0% Line 3 – 2.0% and Line 4 – 2.8%. Unfortunately it is not possible to provide seroprevalence details for lines 1a and 1b as they were not consigned in the exact same order as they were bled.

2.1.2.i. Overall Sensitivity Estimates

The proportion (with 95% binomial confidence intervals in brackets) of the histologically positive animals that were detected as having lesion was:

Inspector X: **87.3%** (82.5 – 91.2)

Inspector Y **74.1%** (66.5 – 80.7)

Inspector Z: **52.5%** (44.1 – 60.2)

Table 1. Comparison between inspectors' diagnoses

Number of animals diagnosed as H+ = histopathology positive, H– = histopathology negative, G+ = gross pathology positive, or G– = gross pathology negative by the three inspectors

Pathology	Inspector			Line number					
Histo	**X**	**Y**	**Z**	**1a**	**1b**	**2**	**3**	**4**	**Total**
H–	G–	G–	G–	275	217	150	106	180	928
H–	G–	G–	G+	0	1	0	0	0	1
H–	G–	G+	G–	8	8	2	8	1	27
H–	G–	G+	G+	0	0	0	0	0	0
H–	G+	G–	G–	3	7	3	0	2	15
H–	G+	G–	G+	0	0	0	0	0	0

H–	G+	G+	G–	0	1	0	0	1	2
H–	G+	G+	G+	0	0	0	0	0	0
H+	G–	G–	G–	3	4	3	1	5	16
H+	G–	G–	G+	0	0	0	0	0	0
H+	G–	G+	G–	0	1	2	0	0	3
H+	G–	G+	G+	0	1	0	0	0	1
H+	G+	G–	G–	9	2	5	1	3	20
H+	G+	G–	G+	1	2	1	0	1	5
H+	G+	G+	G–	7	18	4	3	4	36
H+	G+	G+	G+	6	50	13	3	5	77
Total				312	312	183	122	202	1131

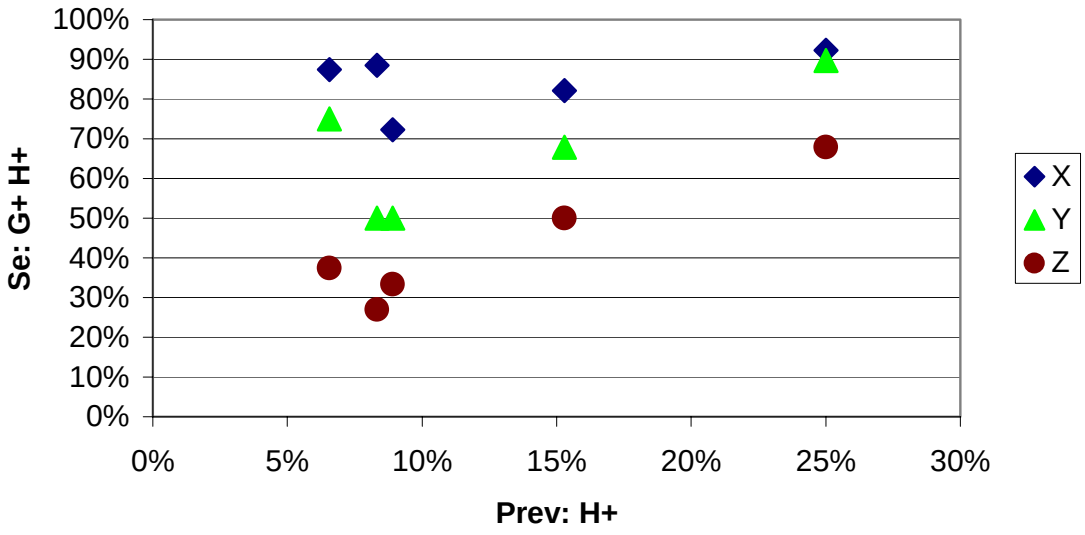
Table 2. Summary of results by inspector and line (abbreviations are as for Table 1).

Inspector	Line	**Total**	**H+**	**G+**	**% H+**	**%G+**	**% G+ not H+**	**% H+ are G+**
X	1a	**312**	26	26	8.3%	8.3%	11.5%	88.5%
X	1b	**312**	78	80	25.0%	25.6%	10.0%	92.3%
X	2	**183**	28	26	15.3%	14.2%	11.5%	82.1%
X	3	**122**	8	7	6.6%	5.7%	0.0%	87.5%
X	4	**202**	18	16	8.9%	7.9%	18.8%	72.2%
Y	1a	**312**	26	21	8.3%	6.7%	38.1%	50.0%
Y	1b	**312**	78	79	25.0%	25.3%	11.4%	89.7%
Y	2	**183**	28	21	15.3%	11.5%	9.5%	67.9%
Y	3	**122**	8	14	6.6%	11.5%	57.1%	75.0%
Y	4	**202**	18	11	8.9%	5.4%	18.2%	50.0%
Z	1a	**312**	26	7	8.3%	2.2%	0.0%	26.9%
Z	1b	**312**	78	54	25.0%	17.3%	1.9%	67.9%
Z	2	**183**	28	14	15.3%	7.7%	0.0%	50.0%
Z	3	**122**	8	3	6.6%	2.5%	0.0%	37.5%
Z	4	**202**	18	6	8.9%	3.0%	0.0%	33.3%
X	Total	**1131**	158	155	14.0%	13.7%	11.0%	87.3%
Y	Total	**1131**	158	146	14.0%	12.9%	19.9%	74.1%
Z	Total	**1131**	158	84	14.0%	7.4%	1.2%	52.5%

From Table 1, it can be seen that of the 158 histopathology positive (H+) sheep, 77 (49%) were detected by all three inspectors while 16 (10%) were not detected by any of the three inspectors. Of the remainder, 23 (15%) were detected by just one inspector while 42 (27%) were detected by two of the three inspectors. Pairwise, the inspectors agreed on the diagnosis for about 94% of sheep, ranging from 90–97% across the five lines of sheep.

Figure 1 graphs sensitivity verses prevalence. The correlation between sensitivity and prevalence was 0.43, 0.72 and 0.95 for inspectors X, Y and Z respectively.

Figure 1: Sensitivity versus line prevalence for the 3 inspectors (X, Y, Z)



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2.1.3. Relationship between lesion severity and inspector diagnosis

The number of positive inspector diagnoses was examined for sheep where the pathologist registered a maximum count against the number of acid fast organisms in one or more tissues examined (these “severe” cases also registered a maximal count for “epithelioid macrophages”). There were 55 "severe" cases that fitted this criterion. The remaining 103 cases were classified as "mild".

Only 4 of the severe lesions were given a negative diagnosis by all three inspectors. Inspector X diagnosed all the remaining 51 severe lesions as positive, with 40 of these given as positive by all three inspectors There were 6 severe lesions diagnosed as positive by X and Y, and 4 by X and Z.:

Table 3: Inspector diagnoses for “severe” and “mild” histopositive viscera.

Inspector	Number of “severe lesions” detected	Number “mild lesions” detected
X	51 (92.7%)	88 (85.4%)
Y	46 (83.6%)	72 (69.9%)
Z	44 (80.0%)	40 (38.8%)
Overall	55	103

Histological diagnosis rate by tissue type:

Of the three tissue types submitted for pathology (lymph node, small intestine and large intestine, the percentage of positives (of the total number of positive cases) was as follows:

Small intestine alone diagnostic — 23.6%

Lymph node alone diagnostic — 3.7%

Large intestine alone diagnostic— 0.0%

Small intestine and lymph node combined diagnostic— 19.9%

Small intestine and large intestine combined diagnostic — 7.4%

Large intestine and lymph node combined diagnostic — 0.7%

All 3 tissue types combined diagnostic — 44.9%

Small intestine totals are 95.8%, and 69.1% for lymph nodes, and 45.6% for large intestine.

VIAS Attwood rereads of approximately 10% of slides:

Table 4. Comparison between 1st and 2nd pathology diagnoses of slides:

2nd reading (VIAS)		1st reading (Gribbles)	
	Negative	Positive	Suggestive
Negative	10	6	25
Positive	10	13	15
Suggestive	4	2	14

2.1.4.'Inconclusive' Pathology Diagnoses

Causes of inconclusive pathology have not been quantified in any way as pathologists did not consistently give an alternative diagnosis when classifying a case as inconclusive. The causes for pathology suggestive of OJD without the presence of acid fast bacilli are (as recorded by the pathologists): coccidiosis and the presence of foreign material (where epithelioid macrophages contain granular or crystalline material – apparently commonly seen). Interestingly, in NSW where an inconclusive slide has an obvious alternative cause for pathology (other than OJD), it is classified as negative (Laurie Denholm pers comm.)

Of the 26 samples that were histologically inconclusive, 18 were classified negative by all 3 inspectors and 2 were classified as positive by all three inspectors. The remaining 6 samples were split evenly — 3 samples were thought to be positive by two inspectors and 3 samples were thought to be negative by two inspectors.

If all 26 inconclusive samples are classified as H+, the average sensitivity of inspection would drop to 78.8% for X, 66.3% for Y and 46.7% for Z.

2.1.4.i. Negative Control Line

The trial included 200 animals from a flock with no known history of OJD (Table 5). Only one animal (and by only one inspector) was classified as positive from this negative control line. However, the situation was different for the infected lines, in which two of the inspectors diagnosed more than 10% of sheep as "G+ H–" (ie false positive).

Table 5. Negative control line results:

Inspector	Line	Number G-	Number G+	**Total**	% G+
X	Control	200	0	**200**	0.0%
Y	Control	199	1	**200**	0.5%
Z	Control	200	0	**200**	0.0%

3. DISCUSSION

3.1. Sensitivity

The sensitivity of abattoir inspection of viscera for OJD depends on many factors. At the individual animal level it depends on whether the infected sheep responds in a manner that can be detected, and the ability of an inspector to detect that response. It is usually assumed that as disease progresses in an animal, the lesions detectable by histopathology will become more pronounced and uniform with an increased chance of a positive diagnosis. The results from the analysis of lesion severity and inspector detection rates would suggest that this is the case.

The sensitivity of detecting gross pathology indicative of OJD compared to histopathology (the “gold standard”) varied between lines and inspectors, although for all lines the ability of the inspectors to detect JD was ranked in the same order. The best inspector (X) detected 87.3% of the histopathology positive sheep, which compares well with the 89.9% of “H+” animals that were diagnosed as positive (“G+”) by one or more of the three inspectors. Even the inspector that performed worst (Z) detected just over half of the histopathology positive sheep. It seems reasonable to assume that a proportion of sheep (possibly 10%) that are “H+” do not develop lesions that can be detected grossly, regardless of the inspector's ability, thus providing an upper limit to the sensitivity for the method.

Given that it is intended that gross pathology would be used as a screening test, and that histopathology would be used as the definitive test, the "sensitivity" that is required to be estimated is the probability that a histopathologically positive animal is actually detected. This comment applies to any screening test relative to the definitive test being used. If one wanted to estimate the sensitivity of gross pathology at detecting infected animals, a minimum estimate would be the relative sensitivity (as calculated by this trial) multiplied by the sensitivity of histopathology. Because of the lack of a 100% sensitive test, such an estimate would not be able to take into account any "G+ H–" animals that were actually infected.

There was a high level of agreement between inspectors X and Y despite fairly different levels of exposure to OJD. It is unclear why inspector Z achieved a substantially lower overall sensitivity level compared to X and Y, despite a long work history in abattoirs and moderate exposure to OJD. Possibly a lack of training and thus ability to detect lesions may be responsible for this. Alternatively, inspector Z was being extremely conservative and avoiding false positives since interestingly, inspector Z only had one false positive result in all the sheep screened.

There was a degree of consistency between the lines for sensitivity, with the ranking of inspectors being the same for each line. The highest detection rate for all inspectors was in the line (1b) with the highest prevalence. Line 4 had the worst overall detection rate, although it did not have the lowest prevalence (Table 2 and Figure 1). Nonetheless, there was a positive correlation between the prevalence and sensitivity for each inspector. The degree of correlation increased as the ability of the inspector decreased, suggesting that the "obviousness" of the lesions might be related to prevalence — the ability of a more skilled inspector to detect lesions varied less between low and high prevalence lines than for a less skilled inspector. While the 3 inspectors retained their ranking to detect histologically positive viscera, for both “severely” and “mildly” affected viscera (Table 3) inspector X was much more able to detect “mild” positives than inspector Y, and both in turn were much better than inspector Z.

Although many factors have been suggested as contributing to the sensitivity of this technique, such as age or breed of sheep, it is more likely that at an animal level, it is the extent of lesions (ranging from extremely slight to very obviously grossly affected viscera) that is the prime determinant of the method's sensitivity. The extent to which these are detected will depend on the ability of an inspector. The severity of lesions is probably related to the time since infection of the animal but could depend on the initial dose of organism which may relate to flock prevalence. The issue of whether, across the whole range of gross lesions from mild to severe, a heavily infected flock would have a higher proportion of severely affected sheep viscera than a less infected flock was not examined in this trial. However, because MAP protocols are based on a prevalence of 2%, an analysis of the data was done to estimate the sensitivity at that prevalence (see later).

All inspectors involved in the trial knew that they were part of a trial. There was no possibility of

incorporating blinding as such in the design of the trial. The inspectors were instructed to work as they would in their own workplace and their observations were completely independent. The conditions of the line were identical to those most would experience in their own locations (with the exception of inspector Y who was used to a slower line speed). However, it is likely that the participants tried a little harder to detect lesions during the trial, although it was stressed to them during briefing that this was not a competition and that there would be a penalty for a high number of false positives. It is possible that both inspectors Y and Z were developing their skills during the trial as they were exposed to the high prevalence lines although there is no way of measuring this effect.

It is apparent from this trial that the ability of individual inspectors to detect OJD in viscera varies, and intuitively it would seem that training and exposure to disease play a role in that ability. There is also standard human variation in the ability to perform any task, a factor that cannot be discounted. The best inspector (X) achieved a very high sensitivity level for abattoir surveillance. Such an inspector would be more than able to act in the role of quality control on the line for training and accreditation purposes. It is reassuring that even an inspector from Western Australia with very little exposure to OJD can achieve such a high sensitivity — for three of the five groups of sheep the inspector's ability was not that much lower than the best inspector.

On the basis of these results it is reasonable to suggest that abattoir inspection can detect about 70% of histologically positive animals. Obviously the use of negative data requires confidence in identifying sheep with respect to their property of origin. However, the sensitivity is likely to be lower in low prevalence flocks because it depends on the relative proportions of easy to hard-to-find lesions in the line, something that will be lower in lower prevalence flocks.

3.1.1. Estimating Sensitivity at 2% Prevalence

Simply fitting a linear regression to the data (Figure 1) for each inspector suggests that at 2% prevalence the sensitivity would still be very high for inspector X (80%). The sensitivity would be 49% for inspector Y and 21% for inspector Z.

The data was analysed in a second way, by classifying each H+ sheep according to the number of inspectors that detected it: hard (all 3 missed), difficult (1 found it), medium (2 found it), and easy (all 3). The proportion of "easy-to-detect" animals increased with increasing prevalence and the proportions of each of the other three categories decreased. Straight lines were fitted to estimate the proportion of each category as a function of prevalence.

For all inspectors, it was assumed that none of the "hard-to-find" animals would be found, and that all the "easy" ones would be detected. The sensitivity figure used for "difficult" and "medium" animals was the proportion of that class that the particular inspector found. This was used to estimate the sensitivity at 2% prevalence for each inspector: X with 75%, Y with 51%, and Z with 23%

This suggests that a sensitivity of 50% would be appropriate to use in the context of assurance from negative findings based on 2% prevalence.

3.1.2. False Positives

Abattoir surveillance for OJD is a screening test for which suspected positive diagnoses are followed up with definitive histopathology. In general, specificity less than 100% for gross pathology simply incurs a cost of unnecessary laboratory follow up. This trial included a “dummy” run of 200 sheep from a property with no known history of OJD. The “dummy” line occurred at the end of the main trial lines and the inspectors were unaware of its existence. Only one inspector (Y) misdiagnosed one sheep as positive in this line (although there was no histopathology performed on this line). Both inspectors X and Y had a false positive rate of greater than 1% with X and Y having comparable levels (about 10%) in three of the five lines, and Y having a much higher level of false positives in the remaining two infected trial lines. It is possible that the inspectors, being knowing participants in a trial, may have over–diagnosed lesions to some extent.

The results of inspector Z must be discounted in this discussion of false positives. The fact that the inspector only classified one histologically negative sheep as positive and detected only half of the histologically positive sheep suggests a too conservative approach in selecting lesions (for a screening test). While some of the false positive sheep reflect an incorrect diagnosis, particularly for inspector Y, the results from X across the 5 lines suggest that in an infected flock the number of sheep that are "G+ H–" is more closely related to the number of histologically positive sheep (correlation 0.97) than to the number of sheep in the line (correlation 0.77). The results for inspector X suggest that about 10% of the "G+" sheep were "H–" fairly consistently across the lines, and presumably actually infected.

In discussing false positives within the context of abattoir surveillance it is important to differentiate between infected and non-infected lines. Analysis of data available from OJD surveillance at abattoirs indicates that across Australia the average number of "G+ H–" sheep found per 10 000 sheep slaughtered is about 3.5, ranging from less than 1 in lines from states with no or low JD to about 10 for lines from residual zones. The abattoir data suggests that up to 10-20% of sheep sampled in infected lines can be histologically negative.

Line Level Sensitivity:

The line-level sensitivity Se_{line} — the probability that abattoir monitoring will detect at least one infected animal in the line — depends on a number of factors and is given by:

$$Se_{line} = 1 - (1 - Se_{inspector} \times Flock\_prev)^{Number\_inspected}$$

where

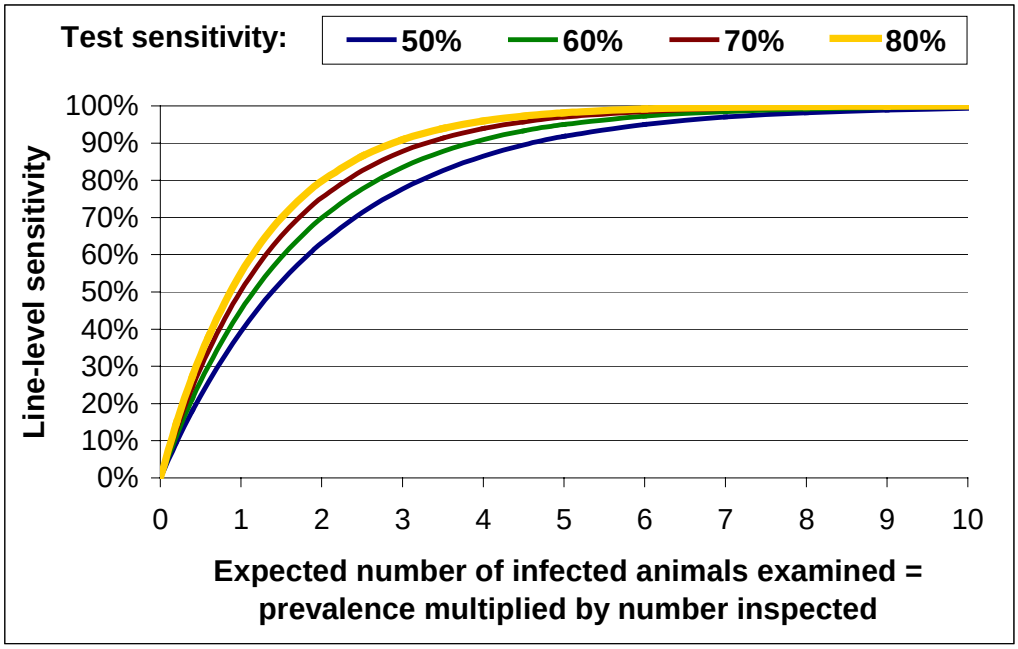
- Number_inspected is the number of animals actually inspected;
- Flock_prev is the proportion of infected animals (H+); and
- $Se_{inspector}$ is the probability that an infected animal will be detected by abattoir surveillance.

An approximation to this formula is:

$$Se_{line} = 1 - \exp(- Se_{inspected} \times Flock\_prev \times Number\_inspected).$$

Figure 2 uses this approximation to illustrate how line-level sensitivity depends on the various parameters.

Figure 2: Line-level sensitivity



3.1.3.i. Submitting a Maximum of 3 Samples

Normally false positives detected by a screening test do not affect the line-level sensitivity — they simply add to the overall cost. However in the protocol for the abattoir surveillance only a maximum of three (the most suggestive) specimens per line are submitted. If the proportion of “G+” animals that are “H–“ is high, as occurred for inspector Y in two lines, it is possible that all three samples are by chance “G+ H–“. This reduces the sensitivity of the method (and the formula given above for calculating line-level sensitivity needs to be adjusted). At low prevalence, the maximum number of submissions has little effect because it is unlikely to be reached. There is also little difference if most of the “G+” animals are also “H+”. However, as Figure 3 illustrates, increasing the maximum number of samples submitted (from 3 to 4) would improve the line sensitivity for an inspector like Y who occasionally had a high level of false positives. Whether the maximum number should be increased would depend on how typical such a pattern of inspection was.

Figure 3. Relationship between line-level sensitivity and the maximum number of samples submitted

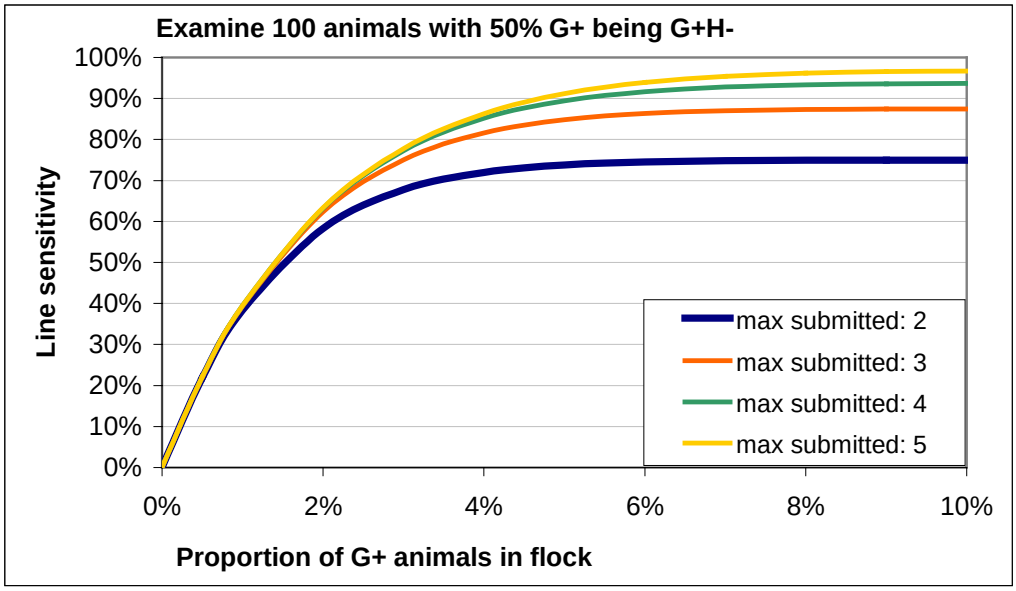


Figure 3 is based on the proportion of "G+" animals found in the flock when 100 animals are submitted and examined by an inspector for whom half the G+ animals are falsely classified as positive.

3.2. Practical Applications for Abattoir Surveillance for OJD

Along with other diagnostic tests and testing regimes for OJD such as pooled faecal culture, serology (AGID or ELISA) and individual faecal culture, negative abattoir surveillance data can be built into an overall confidence-of-freedom program.

Testing two animals with a 50% sensitivity test has approximately the same chance of detecting disease as testing one animal with a 100% sensitive test. If a fraction of a point (equal to the sensitivity of the test) is assigned for each animal inspected/tested, the total number of points gained approximates the equivalent number of 100% sensitive tests[5].

Such a points–based system could be used to combine the confidence from several different types of tests. For example, to achieve a target expressed as a number of "100%-sensitive" tests that should be done in a year, the results from lines of sheep subjected to abattoir surveillance could be "topped up" with a part-round of pooled faecal culture tests or sufficient serological check tests. Alternatively, the confidence obtained by a specified regime of MAP testing could be increased by including the points resulting from the amount of abattoir monitoring. The measure of assurance of freedom from disease would be obtained by totalling the number of points allocated for each "round" of testing, provided that the number of sheep tested and the test sensitivity were known.

3.2.1. Comparison with MAP Testing Protocols

The MAP protocols are based on detecting disease when the within-flock prevalence is 2%. The sensitivities of the tests used have been lowered to reflect the lower likelihood of a detectable response in a lower prevalence flock.

The existing protocols are based on pools of 50 animals tested using a pooled faecal culture test with 56% sensitivity at the animal level. Seven clear pools have 98% confidence of detecting infection if the flock prevalence is 2%.

The nature of the PFC test means that the sensitivity cited for PFC is not relative to histopathology. Consequently the sensitivity of histopathology needs to be taken into account when comparing other tests whose sensitivity is usually given relative to histopathology.

The equivalent number (875) of AGID tests to the 7 PFC pools can be calculated from a relative sensitivity of 30% for AGID and 75% sensitivity for histopathology, making an overall sensitivity of 22.5%. A lower sensitivity for histopathology was thought to be appropriate for a lower prevalence flock.

If the relative sensitivity of abattoir inspection relative to histopathology is taken to be 50% for a low prevalence flock, the overall sensitivity is 37.5% = 75% $\times$ 50%. Hence, 525 animals need to be examined to give the same level of confidence as 7 PFC pools.

There is a convenient way to calculate the number of samples required by different tests to achieve the same level of confidence — the number required is almost inversely proportional to the sensitivity of the test[5]. In comparing two tests, the number N_2 of the second test that achieves the same level of confidence as N_1 of the first test is given by

$$Se_1 \times N_1 = Se_2 \times N_2 \qquad \text{or} \qquad N_2 = N_1 \times Se_1 / Se_2$$

For example 2 pools (100 animals) tested with a PFC is equivalent to

248.9 = 100 $\times$ 56% / 22.5% AGID tests or

149.3 = 100 $\times$ 56% / 37.5% animals inspected at abattoir.

This would be rounded to convenient numbers (100 PFC = 250 AGID = 150 abattoir).

As another example, 200 animals inspected at an abattoir are equivalent to 333 AGID tests or 133 animals tested by PFC. Each corresponds to a 78% confidence of detecting 2% prevalence. A typical negative line in NSW has 314 killed with 213 inspected (M Evers, pers. comm.)

4. CONCLUSION

4.1. Impact on Meat and Livestock industry – now and in five years time

The use of OJD abattoir surveillance for the purposes of negative flock assurance depends on use of a sheep identification system and properly trained and accredited inspectors. Logistically there may be some administrative details to attend to before the system could be used. However once these above-mentioned issues were addressed, producers could benefit from the assurance information gained from abattoir surveillance. How negative data fits in with other programs such as MAP would depend on national agreement. The time - line for complete adoption of abattoir surveillance for negative assurance depends on how quickly the other issues are resolved. With the added incentive of being able to use abattoir data, producers may be encouraged to embrace identification systems or in some cases individual meat works may insist upon the use of identification.

4.2 Recommendations

It is apparent that abattoir surveillance is potentially a highly sensitive methodology. In low prevalence lines it is also highly specific (although specificity is less important as positive cases are resolved with histopathology). For the negative data that is already generated from abattoir surveillance to be utilised, a good sheep identification system needs to be in place and inspectors need to be properly trained and accredited. There would also be a requirement to ensure lines of sheep consigned were of an appropriate age, given the likelihood that the sensitivity would be lower when applied to sheep less than 2 years of age.

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