Ministry for Primary Industries
Manatū Ahu Matua

# A study of the distribution of lesions in cattle caused by *Mycobacterium bovis*

MPI Technical Paper No: 2017/69

Prepared for the Ministry for Primary Industries
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ISBN No: 978-1-77665-746-9 (o)
ISSN No: 2753-3923 (o)

November 2017

New Zealand Government

*Growing and Protecting New Zealand*

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# 1 Summary

This project evaluated, relative to current practices, the impacts of alternative post mortem inspection regimes for tuberculosis in cattle presented for slaughter in New Zealand. This included the possible use of animal, herd and farm factors to target "high risk" animals and tissues.

Firstly, a retrospective analysis of slaughterhouse post mortem data collected from *Mycobacterium bovis* infected animals was conducted. A computer model was then developed to determine the sensitivity of gross post mortem inspection. The model was then used to determine the effect that alternative inspection regimes would have on the ability to detect both an individual infected animal and slaughter lines containing infected animals.

Individual animal records from 1989 to 1992 and from 2003 to 2010, extracted from the national tuberculosis information system, were used in the study. A subset of 2,206 records where *M. bovis* had been isolated was used for the analysis.

The mean number of infected animals per slaughter line was 1.9, with a median of 1, and a maximum of 25. In 64% of lines one infected animal was found, in 25% there were two and in 11% there were three or more. Single infected animals were more common in non-reactor (usually called "cull") lines than tuberculin reactor lines.

The mean number of lesions reported per confirmed case was 1.4, with a median of 1 and a maximum of 10. Mature animals generally had more lesions. No statistically significant associations were found between the number of lesions and herd type[1], vector risk area[2], reactor status[3] or herd status[4].

The most commonly reported tissue found with typical gross TB lesions were the mediastinal lymph nodes, followed by the retropharyngeal nodes and then the tracheobronchial nodes; i.e. 44%, 28% and 24% of animals, respectively. Ninety-three per cent (93%) had lesions in head, thorax or gut tissues nodes; 62% had lesions in thoracic tissues; 33% had lesions of the head nodes, and 7% had gut lesions.

The distribution of lesions in animals with single or multiple lesions were different. In the former, only 2.4% had lesions of the gut while in the latter it was 19%.

[1] Dairy or Beef
[2] Vector risk: TB infected wildlife were known to be present on the farm of origin.
[3] Animal had been designated positive to an official test for tuberculosis; e.g. the tuberculin skin test
[4] Herd was designated TB infected or TB Clear.

Statistically significant associations between animal, herd and sex risk factors were found, but in all cases the strength of the association was either small or of little operational value.

A model which accurately simulated the current observed lesion prevalence was developed. It was found that approximately 50% of tissues could be dropped from the examination with only a 2% loss of sensitivity. A group of "operationally relevant" tissues could be dropped with only a 1% loss of sensitivity.

It is estimated that the overall sensitivity of post mortem examination, as conducted in New Zealand slaughter-houses, is around 75% (i.e. the probability that gross lesions will be detected in an *M. bovis* infected animals). The decrease that would occur as a result of dropping around 50% of current sites is small in comparison with this. It is suggested that a more intensive examination of tissues draining the oropharynx and of thoracic tissues might yield a higher sensitivity.

A follow-up analysis of slaughter-house post-mortem data collected over three years from May 2013 was also conducted. Taking a broad view of the results from the early and late periods, the gross pathology observed is generally remarkably similar. Lesions of the abdomen, especially in the ileojejunal lymph nodes, were more prevalent in the more recent group. This arose from an unusual case: the spread of infection to other herds via young stock which had been fed *M. bovis* contaminated milk shortly after birth.

# 2 Introduction

The examination of cattle carcases for evidence of infection with the pathogenic *Mycobacteria*[5] is a time consuming and thus costly activity in slaughterhouses. It is complicated by the frequent occurrence of granulomatous lesions caused by other agents, such as the non-pathogenic *Mycobacteria*, other bacteria, fungi, parasites and foreign bodies.

The prevalence of tuberculosis in cattle in New Zealand has been reduced by a very active national control programme and nowadays the probability that a 'suspect lesion' is the result of *M. bovis* infection is very low. As a result, much of the effort and costs are being expended on non-tuberculous lesions and this poses the question of whether or not current procedures should be modified.

The objective of this project was to evaluate, relative to current practices, the impacts of alternative post mortem inspection regimes for tuberculosis in cattle presented for slaughter in New Zealand. This was to include not only the scope of the examination of carcases, but also the possible use of animal, herd and farm factors to allow a more targeted approach. To achieve this objective, it was seen that it would be necessary:

- To describe in cattle the prevalence of tuberculosis in slaughter lines and the anatomical distribution of typical tubercular lesions.
- To investigate associations between both the prevalence and lesion distribution and animal, herd and farm risk factors.
- To investigate the probability of missing tuberculosis cases with alternative inspection procedures, including if any decrease can be ameliorated by consideration of these risk factors.
- To investigate likely effects with alternative procedures on exposure of consumers to *M. bovis* and surveillance for tuberculosis in cattle.

[5] Generally *M. bovis* but occasionally *M. tuberculosis*

# 3 Materials & Methods

## 3.1 OVERVIEW

There were three sequential phases in this investigation. First, conducting a retrospective analysis of slaughterhouse post mortem data. Second, developing a model of slaughterhouse post mortem examination. Third, using this model to evaluate alternative post mortem examination procedures.

## 3.2 RETROSPECTIVE ANALYSIS OF POST MORTEM DATA

### 3.2.1 Preparation of data for analysis

From the mid 1980's, the results of the examination of individual cattle and deer for tuberculosis have been stored in the 'National TB Database'. Over this period three different databases have been used; the Scientific Information Retrieval tuberculosis and brucellosis recording system (SIR), the National Livestock Database (NLDB) and the Disease Management Information System (DMIS).

Data from the SIR and NLDB systems were retrieved from archives. A download of data from DMIS was provided by the Animal Health Board[6].

Although the data stored in these systems were similar, there are some important differences and ambiguities, especially with respect to recording lesions of the small intestine and caecum.

When gut lesions were seen, one could record the affected tissues as "mesenteric lymph nodes", "ileocaecal lymph nodes", "ileojejunal lymph nodes", "jejunal lymph nodes" and "abdominal grapes". Jejunal can clearly be included in the ileojejunal field, but there are difficulties with mesenteric nodes as these could be either ileojejunal or ileocaecal nodes. This does not present a difficulty with the NLDB data, as the mesenteric option was not offered. In the SIR data 26% (215/827) of gut lesions were recorded as both mesenteric and ileojejunal and 0.4% (1/282) as both mesenteric and ileocaecal. In the DMIS data 14% (813/5809) of gut lesions were recorded as both mesenteric and ileojejunal and none (0/64) as both mesenteric and ileocaecal. It appears that the descriptors ileojejunal and mesenteric were seen as the same and different to ileocaecal; therefore, in the analysis mesenteric was included under ileojejunal.

Only one case of abdominal grapes[7] was recorded; as this was not an important issue the case was deleted.

[6] Now OSPRI

[7] Haematogenous spread of *M. bovis* from the initial point of entry can result in lesions on the pleura and/or mesentery. Spread at these sites is aided by respiratory or intestinal peristaltic movements resulting in diffuse, caseous, plaque-like or clustered, nodular lesions. These are commonly referred to as "TB-grapes", or after calcification as "TB-pearls".

Other changes that were made were:

- Bronchial nodes (as in SIR & NLDB) was grouped under tracheobronchial nodes (as in DMIS)
- Cases with only skin lesions were deleted.

In all other cases where a field to record the presence of a lesion was not offered within the system that field was set to missing.

The records from the three systems were combined in MS Access®. Most statistical analyses were conducted with Minitab®.

Where the field 'Gross Post Mortem' was missing or equal to 'No Visible Lesions' the record was deleted. A subset of the remaining records indicating that *M. bovis* had been cultured was used for the analysis.

### 3.2.2 Outline of the analyses undertaken

*Risk factors*

The association between seven possible risk factors (i.e. possible predictors of *M. bovis* infection) and the number and the anatomical distribution of lesions was investigated. The factors were as follows:

- Sex (male or female)
- Age (immature (i.e. less than 2 to 3 years) or mature)
- Reactor (animal was submitted for slaughter as a result of a positive skin and/or blood test for tuberculosis) or was submitted as part of normal farm management (i.e. culls or prime stock)
- Herd type (dairy or beef)
- TB status of herd-of-origin (clear or infected)
- Vector risk (VR) status of farm-of-origin (TB vectors present/suspected or not)
- Period (1989 to 1992, 2003 to 2005 or 2005 to 2010, as per the source of data shown in Table 1)

A well-documented problem with such analyses is multicollinearity[8], and thus the association between these factors was investigated. Multiple cross-tabulations of the factors were examined. Chance variation was measured via the Pearson Chi-square statistic. The strength of the association was measured via Cramer's V-square statistic[9].

[8] A situation when several putative predictors are highly correlated and thus one cannot decide which one is important in explaining the association among the predictors and response. It can also distort the regression analysis outcome.
[9] Cramer's V is based on adjusting chi-square significance to factor out sample size to gauge the strength of a relationship.

*Analyses based on slaughterhouse lines*

The individual animal records were grouped using the fields 'Herd Number' and 'Slaughter Date'. In addition, the first record values of the fields 'number of animals in line', 'number of animals with lesions', 'vector risk status of farm-of-origin', 'TB status of herd-of-origin', 'herd type', 'animal reactor status' and 'period' were copied into the new records based on the key fields.

A new categorical field was created for the investigation of associations between the number of lesion animals per line and the animal, herd and farm factors; i.e. 1, 2 to 3, 4 to 10, and 11 to 25 lesion animals in the line. Contingency table analyses were used to measure associations with the risk factors.

*Analyses based on animals with confirmed M. bovis lesions*

In the first part of these analyses the number of suspect lesions in each animal was investigated. As in the previous section, a new categorical field was created for the investigation of associations with the animal, herd and farm putative risk factors; i.e. 1, 2 or 3 or more lesions per animal. Contingency table analyses were again used to measure the strength of associations and chance effects.

In the second part the anatomical distribution of suspect lesions was described. The pattern in animals with one, two and three or more lesions was also investigated. In addition, the association between the presence/absence of a lesion at a particular site and the animal, herd and farm risk factor was examined via logistic regression and table contingency analyses. In the former, odds ratios, with 95% confidence intervals, were used to measure both statistical significance and the strength of associations. The initial step was to model presence/absence of the lesion and all the risk factors. If there was insufficient data to run the model, or the modelling results suggested there were no significant ($P > 0.05$) associations, no further work was done. If the results suggested there were one or more significant associations, additional investigations were undertaken using single or multiple contingency table analysis.

### 3.3 DEVELOPMENT OF A MODEL OF THE POST MORTEM EXAMINATION PROCEDURE

A model of the post mortem procedure, in which the key items affecting outcomes can be varied, was developed. The purpose of the model was to investigate alternative examination procedures. The outcomes of interest were the probability that suspect lesions would be observed, given that the infected animal had gross lesions, and the probability that one or more infected animals would be detected in a line submitted for slaughter, again given infected animals with gross lesions were present. As will be discussed, this is against a background of infected animals with no visible lesions also being present.

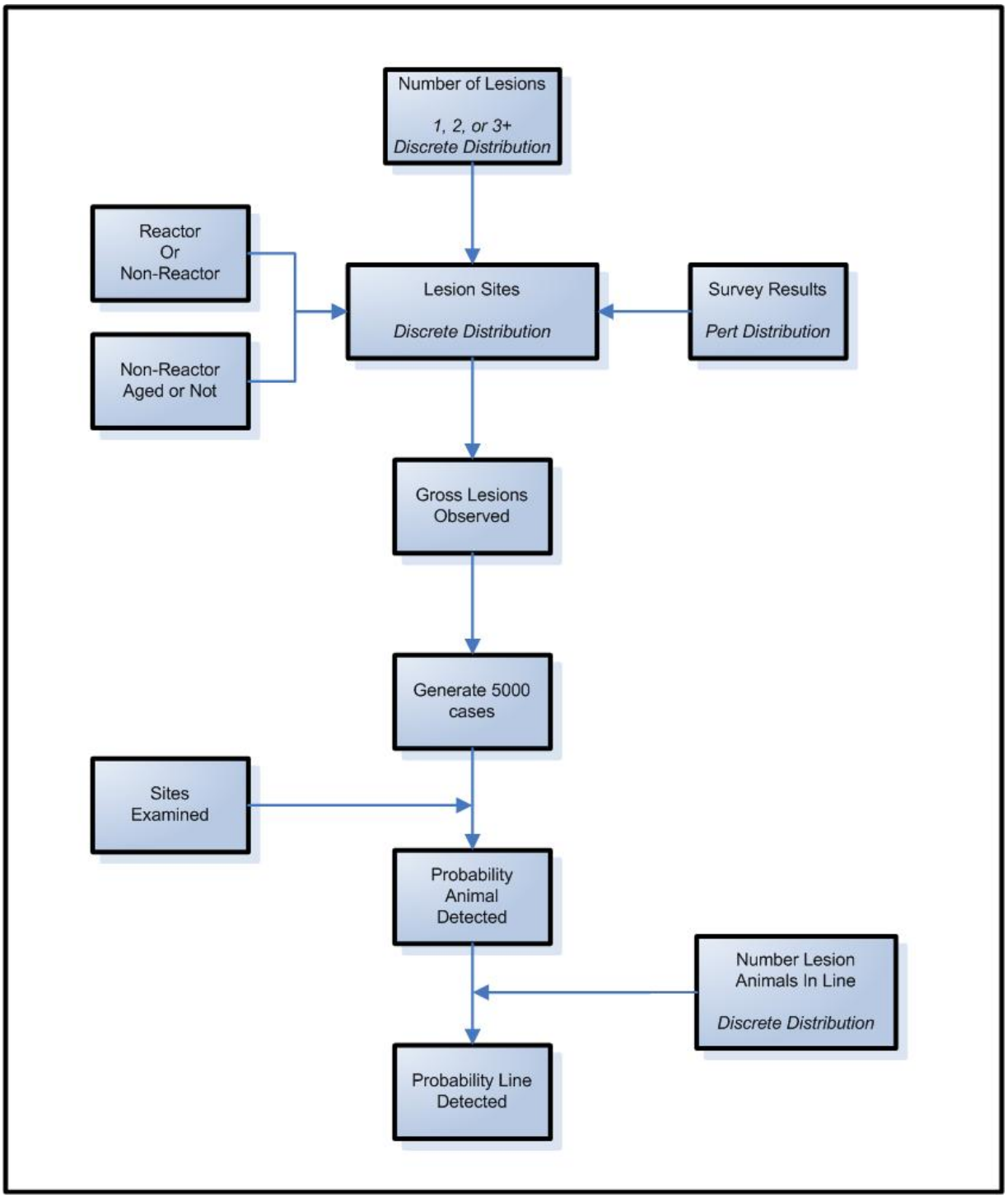
The inputs and outputs of the model are shown in Figure 1.

For each animal, a probability distribution of gross lesions is selected with reference to that found in the latest dataset when there are one, two or three or lesions present. The probability of a particular site is the outcome of a beta-pert distribution, with the most likely value being the prevalence and with the minimum and maximum being the upper and lower 95% confidence limits of the prevalence. As reactors are subject to higher intensity inspection, the scope of visible lesions is reduced in animals with only one lesion present, in line with the current policy. The outcome at this stage in the modelling is a list of sites where suspect lesions in an animal have been observed.

The process is repeated 5,000 times; i.e. a list is generated of 5,000 animals each with one, two or three lesion sites. These data are read into a spreadsheet in which it is possible to set one or more lesion sites to 'not examined'. The calculated outcome is the probability that an animal with a lesion (lesion animal) will be observed.

Finally, using the formulae $1-(1-p)^n$ , where p = the probability that a lesion animal will be observed and n = the number of lesion animals in a slaughter line, the probability that a line containing one or more lesion animals will be detected is calculated. The number of lesion animals per line is entered as a discrete probability distribution as found in the DMIS data. The model was validated by comparing the observed lesion distribution with the model outputs.

**Figure 1. Flow diagram showing the logic the the model of postmortem examination for tuberculosis lesions.**



# 4 Results

## 4.1 RETROSPECTIVE ANALYSIS OF POST MORTEM DATA

### 4.1.1 An overview of the data

Reports on the post mortem examination for tuberculosis from 14,097 cattle were available. Thirty-one percent (31%) were derived from the DMIS, 11% from the NLDB and 58% from the SIR database. The periods covered are shown in the following table; records from a continuous period from July 2003 to June 2010 were analysed.

**Table 1. The source of post mortem records for this analysis**

| Source | From | To | Period |
|---|---|---|---|
| Disease Management Information System (DMIS) | 04/07/2005 | 01/06/2010 | 4.9 years |
| National Livestock Database (NLDB) | 01/07/2003 | 30/06/2005 | 2.0 years |
| Scientific Information Retrieval Database (SIR) | 20/12/1989 | 30/09/1992 | 2.8 years |

A file of records from those animals from which *M. bovis* had been isolated was formed for this analysis. This contained 2,206 records; of these 783 (35%) were from DMIS, 315 (15%) were from NLDB and 1,108 (50%) from SIR. The percentages of records from each source were 18%, 20% and 14% for DMIS, NLDB and SIR respectively.

### 4.1.2 *Mycobacteria* other than *M. bovis* isolated

No other *Mycobacteria* were reported as being isolated from lesions submitted from these animals.

### 4.1.3 The association between putative risk factors

The results of the analysis are shown in Table 2.

In only four cases were there non-significant ($P > 0.05$) differences between the factors; i.e. between age and herd status, age and vector status, sex and vector status, and between herd status and period. In all the other cases the probability that the differences observed were a "chance association" was very small ($P < 0.01$). However, the strength of the association, as shown by Cramer's V-square statistic, is in all cases weak or very weak. The highest are in the range 0.1 to 0.2; these are between sex and age, sex and reactor status, sex and herd type, age and period and, lastly, herd type and period.

**Table 2. The association between risk factors used as predictors of the anatomical distribution of lesions.**

Cells to the right of the shaded cells show the probability that the observed differences could have been the result of chance (calculated from the magnitude of the Chi-sq

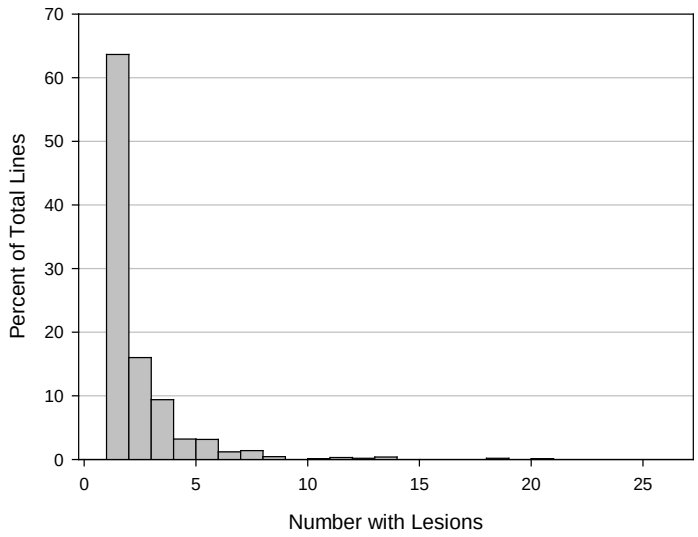
statistic). Cells to the left of the shaded cells show the strength of the association (Cramer's V-square statistic, 0 = no association 1=perfect association).
H-Type = Herd Type, H-Status = TB status of the herd-of-origin, VR-status = TB vector risk status of farm-of-origin, Period = 1989-1992, 2003-2005 or 2005-2010.

| | Sex | Age | Reactor | H-Type | H-Status | VR-Status | Period |
|---|---|---|---|---|---|---|---|
| **Sex** | | <0.001 | <0.001 | <0.001 | 0.001 | 0.199 | <0.001 |
| **Age** | 0.152 | | 0.007 | <0.001 | 0.290 | 0.300 | <0.001 |
| **Reactor** | 0.140 | 0.004 | | <0.001 | <0.001 | 0.007 | 0.006 |
| **H-Type** | 0.140 | 0.083 | 0.083 | | <0.001 | <0.001 | <0.001 |
| **H-Status** | 0.006 | 0.001 | 0.007 | 0.015 | | <0.001 | 0.362 |
| **VR Status** | 0.001 | 0.001 | 0.003 | 0.029 | 0.074 | | 0.007 |
| **Period** | 0.048 | 0.109 | 0.005 | 0.220 | 0.001 | 0.005 | |

### 4.1.4 Numbers of cattle with "suspect" lesions per line in which one or more had been confirmed as having *M. bovis* infection

The fields "number in line" and "number in line with lesions" were not present in NLDB. For the balance of the data (i.e. SIR and DMIS data), aggregation of the file of *M. bovis* confirmed animals on the basis of herd number and slaughter date yielded 1,820 slaughter lines. In approximately two thirds (64%) the count was one, and in 25% it was two; i.e. in 90% of lines only one or two animals with suspect lesions, later to be confirmed as due to *M. bovis*, were observed (Figure 2). The mean was 1.9, median one and range one to 25.

**Figure 2. Histogram showing frequency (%) of lines containing one or more animals with suspect TB lesions**




Although the difference between DMIS and SIR line data was significantly different (P = 0.004), it was small. Sixty percent (60%, 428/717) of lines in the former had a single one

lesion animal whereas the figure was 67% (582/870) in the latter. In both 11% of lines were reported as having three or more lesion animals.
The association between the status of the animals in terms of tuberculin reactors and non-reactor ("culls") and counts per line was investigated. As shown in Table 3 cull lines exhibited more singleton infected animals (73% versus 55%). The differences shown in Table 3 are statistically significant ($X^2 = 62.191$, df = 4, $P < 0.001$).

**Table 3. Counts of animals with TB suspect lesion per line, presented for slaughter by tuberculin reactor and non-reactor status**

| Line Status | 1 | 2 to 3 | 4 to 5 | 6 to 10 | 11 to 25 |
|---|---|---|---|---|---|
| Reactor | 442 (55%) | 250 (31%) | 60 (7%) | 38 (5%) | 17 (2%) |
| Non-Reactor | 568 (73%) | 153 (20%) | 41 (5%) | 13 (2%) | 5 (1%) |

Likewise the differences associated with the herd-of-origin TB status, infected or clear, were statistically significant ($X^2 = 129.355$, df = 4, $P < 0.001$). More lines from clear herds had singleton TB cases (78% versus 51%).

**Table 4. Counts of animals with TB suspect lesion per line, presented for slaughter by the TB status, infected or clear, of the herd-of-origin**

| Line Status | 1 | 2 to 3 | 4 to 5 | 6 to 10 | 11 to 25 |
|---|---|---|---|---|---|
| Clear | 514 (78%) | 114 (17%) | 18 (3%) | 6 (1%) | 7 (1%) |
| Infected | 432 (51%) | 277 (33%) | 76 (9%) | 45 (5%) | 15 (2%) |

The difference between beef and dairy herds was not significant ($X^2 = 3.471$, df = 2, $P = 0.176$).

Although the counts per line in the older SIR between vector free and vector risk areas was marked (85% one per line versus 60% respectively), in the recent dataset it was very similar (57% versus 61%, $X^2 = 2.643$, df = 2, $P = 0.267$)

A "low risk" line for TB would thus be one that originated from a clear status herd and the animals were not tuberculin reactors. The association between these combined factors and numbers of TB cases in a line, for only the DMIS data, is shown in Table 5. As can be seen in "high risk lines" there was a marked reduction in lines with only one lesion animal (52% versus 86%) and a marked increase in lines with more than five lesion animals.

**Table 5. Counts of animals with TB suspect lesion per line, by low and high risk line status (see above text) - recent DMIS data only.**

| Line Status | 1 | 2 to 3 | 4 to 5 | 6 to 10 | 11 to 25 |
|---|---|---|---|---|---|
| Low Risk | 143 (86%) | 24 (34%) | 0 | 0 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| High Risk | 284 (52%) | 188 (34%) | 48 (9%) | 23 (4%) | 6 (1%) |

### *4.1.5* The number of lesions in animals that had been confirmed as being infected with *M. bovis*

A total of 3,160 lesions were reported in the 2,206 animals, a mean of 1.4 lesions per animal. The range was from one to ten, with a median and mode of one (Figure 3, Table 5).

**Figure 3 Number of lesions per animal - Percent in each class**

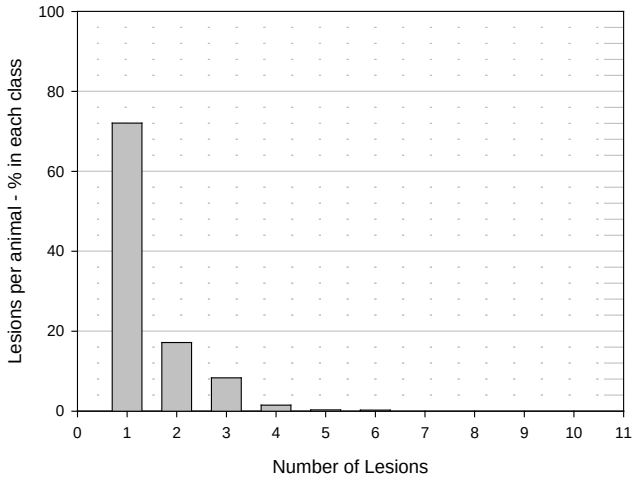



**Table 6 Lesions per animal, counts of animals with percent of total (2206) shown**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| 1590 (72%) | 379 (17%) | 184 (8%) | 33 (1.5%) | 7 (0.3%) | 6 (0.3%) | 2 (0.1%) | 1 (0.1%) | 3 (0.2%) | 1 (0.1%) |

The count of lesions per infected animal in the three different databases was not significantly different (P = 0.100). The effects of the factors; herd type (dairy or beef), vector risk area (farm within zone or not), tuberculin reactor (reactor or not) and herd TB status (infected or clear) were also not significant (P > 0.05).

In the univariate analysis both age (P = 0.011) and sex (P = 0.051) showed significant associations with lesion counts. However, when entered into a multivariate ordinal logistic analysis only age reached the 0.05 limit. The odds ratio (OR) for "mature" versus "immature" (i.e. < 2 years) was 0.78 (P=0.030, 95% CLs 0.62 and 0.98). The interpretation of this result is that there is a 22% decrease in the odds that an adult will have 1 lesion versus 2, and, likewise, 2 versus 3 or more; i.e. multiple lesions are more common in adults than immature animals. The model fit is significant but poor (P ~ 0.15).

### *4.1.6* The anatomical distribution of lesions in animals that had been confirmed as being infected with *M. bovis*

The proportion (%) of cases reported as having a typical gross TB lesion in each site[10] is presented in Figure 4 and Table 7.

Some aggregated affected tissues statistics are as follows:

- 92.3% had lesions either of the head, gut or thorax, the so-called primary sites for tuberculosis.
- 90.2% had lesions of either the head or thorax.
- 61.6% had lesions of the thorax (i.e. mediastinal, tracheobronchial nodes or lung).
- 33.1% had lesions in the head (i.e. retropharyngeal, mandibular or parotid lymph nodes).
- 7.1% had gut lesions (i.e. ileojejunal or ileocaecal nodes)

**Figure 4. Prevalence (%) of lesion sites in cases.**
**(Total cases = 2,206 Ln = Lymph Node)**

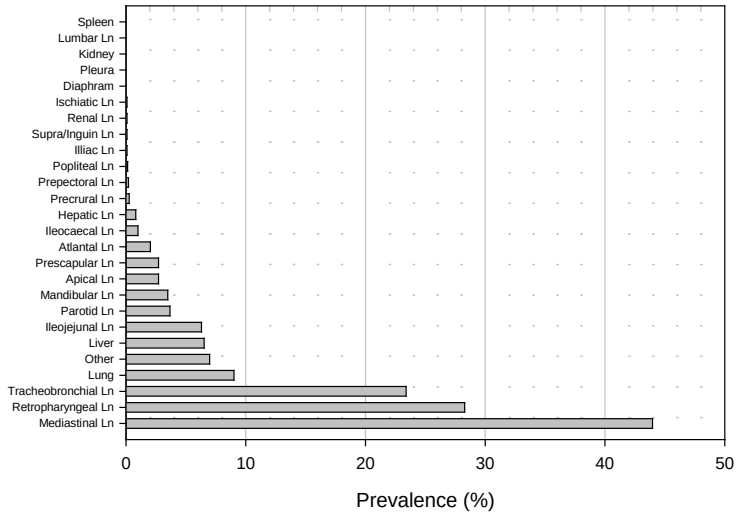



**Table 7. Prevalence (%) of lesion sites in cases.**
**(Total = 2,206 cases)**

| Tissue | Prevalence | 95% Confidence Limits | |
|---|---|---|---|
| Diaphragm | 0.00 | 0.00 | 0.38 |
| Pleura | 0.00 | 0.00 | 0.14 |
| Kidney | 0.00 | 0.00 | 0.14 |
| Lumbar Lymph Nodes | 0.00 | 0.00 | 0.21 |
| Spleen | 0.00 | 0.00 | 0.27 |
| Iliac Lymph Nodes | 0.09 | 0.00 | 0.33 |

[10] i.e. the prevalence of lesion sites in cases

| Tissue | Prevalence | 95% Confidence Limits | |
|---|---|---|---|
| Supramammary/Inguinal Lymph Nodes | 0.09 | 0.00 | 0.33 |
| Renal Lymph Nodes | 0.09 | 0.00 | 0.50 |
| Ischiatic Lymph Nodes | 0.09 | 0.00 | 0.50 |
| Popliteal Lymph Nodes | 0.13 | 0.81 | 2.08 |
| Prepectoral Lymph Nodes | 0.21 | 0.04 | 0.61 |
| Precrural Lymph Nodes | 0.27 | 0.01 | 0.59 |
| Hepatic Lymph Nodes | 0.82 | 0.48 | 1.29 |
| Ileocaecal Lymph Nodes | 1.00 | 0.63 | 1.51 |
| Atlantal Lymph Nodes | 2.04 | 1.49 | 2.72 |
| Apical Lymph Nodes | 2.72 | 2.08 | 3.49 |
| Prescapular Lymph Nodes | 2.72 | 2.08 | 3.49 |
| Mandibular Lymph Nodes | 3.49 | 2.76 | 4.34 |
| Parotid Lymph Nodes | 3.67 | 2.92 | 4.54 |
| Ileojejunal Lymph Nodes | 6.30 | 5.30 | 7.40 |
| Liver | 6.53 | 5.53 | 7.64 |
| Other | 6.98 | 5.95 | 8.13 |
| Lung | 9.02 | 7.86 | 10.29 |
| Tracheobronchial Lymph Nodes | 23.39 | 21.64 | 25.21 |
| Retropharyngeal Lymph Nodes | 28.29 | 26.41 | 30.22 |
| Mediastinal Lymph Nodes | 43.97 | 41.89 | 46.07 |

"Other" was recorded in 7% of the cases. Field staff were asked what sites this might cover. They replied that this is used when lesions are seen in the lumbar, ischiatic and precrural lymph nodes, all of which are not offered in DMIS. In addition, there appeared to be some minor confusion about the label "tracheobronchial", as some said they coded bronchial node lesions as "other" (Ira Stapp, personal communication).

### 4.1.7 The distribution of lesions in animals with single or multiple lesions

In animals with single or multiple lesions the proportion of affected tissues did not increase in a simple "additive" manner. As shown in Table 8 and Figures 5 to 7, in all cases lesions of the mediastinal, retropharyngeal and tracheobronchial lymph nodes still predominated, but in those with multiple lesions, lung, liver and the ileojejunal nodes were relatively more commonly affected. Likewise, lesions in "other sites" were relatively more common.

**Table 8. Prevalence (%) of lesion sites in cases, sub-divided into those with one, two or three or more lesions.**

| Tissue | 1 lesion | 2 lesions | 3+ lesions |
|---|---|---|---|
| Apical Ln | 0.94 | 4.75 | 11.39 |
| Atlantal Ln | 0.69 | 3.69 | 8.44 |
| Hepatic Ln | 0.31 | 1.06 | 3.8 |

| Tissue | 1 lesion | 2 lesions | 3+ lesions |
|---|---|---|---|
| Ileocaecal Ln | 0.5 | 0.79 | 4.64 |
| Ileojejunal Ln | 1.89 | 12.93 | 25.32 |
| Iliac Ln | 0.06 | 0 | 0.42 |
| Ischiatic Ln | 0.12 | 0 | 0 |
| Liver | 1.51 | 10.82 | 33.33 |
| Lung | 1.7 | 18.73 | 42.62 |
| Mandibular Ln | 1.89 | 4.75 | 12.24 |
| Mediastinal Ln | 35.79 | 61.21 | 71.31 |
| Other tissues | 2.39 | 13.19 | 27.85 |
| Parotid Ln | 2.77 | 4.49 | 8.44 |
| Popliteal Ln | 1.53 | 0.89 | 0.67 |
| Precrural Ln | 0.06 | 0.79 | 0.84 |
| Prepectoral Ln | 0 | 0.44 | 1.34 |
| Prescapular Ln | 2.52 | 2.11 | 5.06 |
| Renal Ln | 0 | 0 | 0.87 |
| Retropharyngeal Ln | 28.81 | 22.16 | 34.6 |
| Supramammary/Inguinal Ln | 0 | 0.53 | 0 |
| Tracheobronchial Ln | 16.35 | 35.88 | 50.63 |

**Figure 5. Prevalence (%) of sites in cases. with only one lesion.**

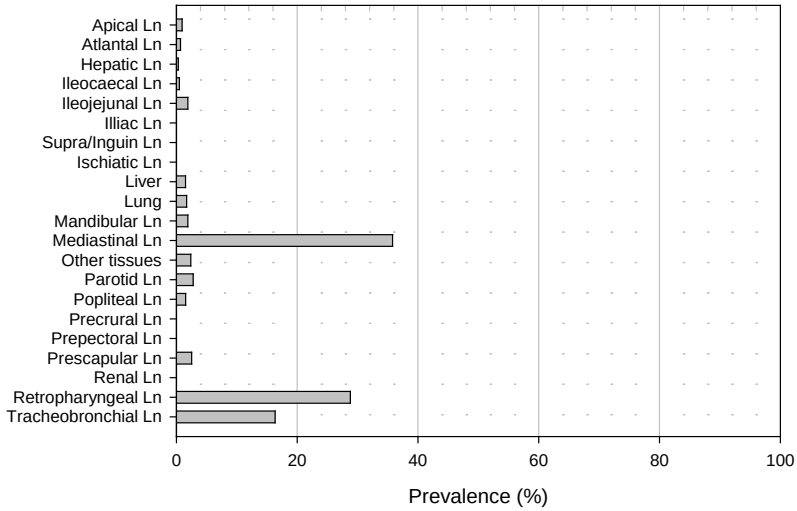


**Figure 6. Prevalence (%) of sites in cases with two lesions**

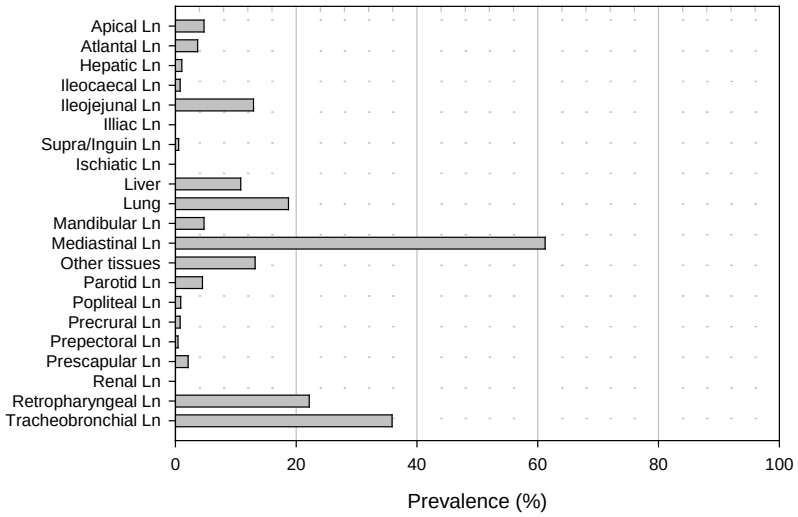
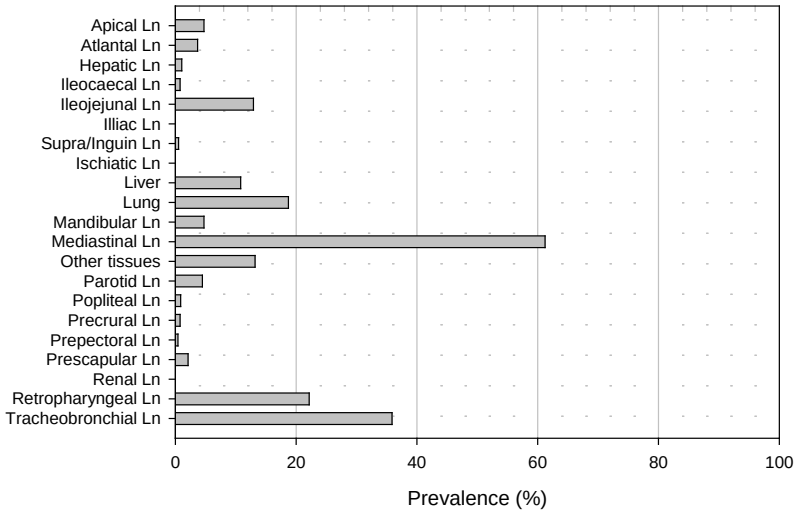
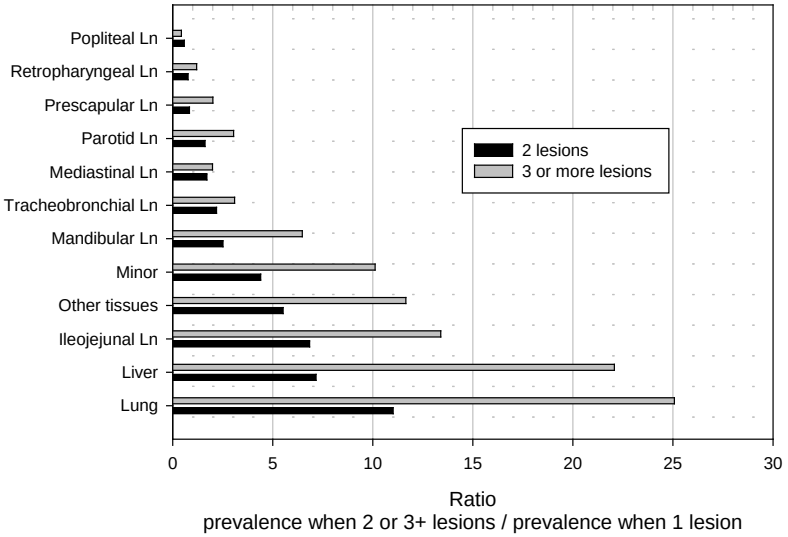



**Figure 7. Prevalence (%) of sites in cases with three or more lesions**




To illustrate this situation more clearly, the proportional increase in prevalence at each site in animals with two and three-or-more, relative to that in animals with only one lesion was calculated. Where the site prevalence was less than 1% in animals with single lesions, data was aggregated under the label "minor". The results (Figure 8) show the marked relative increase in lesions of the lung, liver and ileojejunal lymph nodes.

**Figure 8. The proportional increase in lesions at each site in animals with multiple lesions**




### 4.1.8 Associations with animal, herd and farm factors

The results, expressed in terms of odds ratios, for the degree of association between a lesion site and 'data collection periods' is shown in Table 9.

**Table 9. The association between lesion sites and the period when the data was recorded.**
**S = SIR, N = NLDB**
**Odds ratios shown; * P =< 0.05, ** P =< 0.01, P ***=< 0.001**

| Tissue | Period S | Period N |
|---|---|---|
| Apical Ln | 2.00* | 1.57 |
| Ileocaecal Ln | 2.15 | 20.21** |
| Ileojejunal Ln | 0.74 | 0.31** |
| Other lesions | 1.05 | 1.93** |
| Tracheobronchial Ln | 1.96*** | 1.48* |

With reference to the current period (i.e. DMIS),

- Apical node lesions were more common during the earliest period (SIR).
- Ileojejunal lesions were less common and ileocaecal lesions more common in the period covered by NLDB.
- 'Other lesions' were more commonly recorded in the NLDB data.
- Tracheobronchial node lesions were more commonly reported during the earlier periods (i.e. SIR and NLDB).

With one exception, the odds ratios are close to 1; i.e. the proportional differences in the odds ratios are approximately two times for tracheobronchial, apical and other lesions, and

a third for ileojejunal lesions. However, for ileocaecal node lesions, the odds ratio is 20.21 (95% CI 2.44 to 167.43).

In the DMIS data ileocaecal lesions were recorded in 0.26% (2/783) of cases, in the NLDB data the figure is 2.54% (8/315), while in the SIR data it is 1.08% (12/1108). Proportionally, the difference between DMIS and NLDB is large (i.e. ~ 10 times), but a relatively small number of cases are involved.

The results, expressed in terms of odds ratios, for the degree of association between a lesion site and 'animal, herd and farm factors' are shown in Table 10. Again, the odds ratios are close to one (i.e. minimum = 0.41 and maximum = 2.69, 1st and 3rd quartiles = 0.76 and 1.26). With five sites there was insufficient data to perform the analysis. With the balance there were significant ($P < 0.05$) associations with one or more of the factors, as follows:

- 
  - Mediastinal node lesions were more common in dairy cattle; conversely lesions of the retropharyngeal and mandibular nodes are more common in beef cattle.
  - Cattle from vector free areas less commonly had retropharyngeal node, ileojejunal node and liver lesions
  - In reactors parotid, mandibular and ileojejunal node lesions were more common, with mediastinal and tracheobronchial node lesions less prevalent.
  - If the herd-of-origin was infected, atlantal and retropharyngeal node lesions were more prevalent.
  - Mature animals more commonly had tracheobronchial node, lung and 'other lesions', but retropharyngeal nodes were less prevalent.

No significant associations were found with the sex of the case.

- 

There was also evidence of more complex interactions, for example differences between dairy and beef herds depending on whether or not the herd was located in a vector free or risk area.

| | Dairy Herd | Vector Risk Area | Reactor | Infected Herd | Male | Mature |
|---|---|---|---|---|---|---|
| Atlantal Ln | 0.59 | 0.89 | 1.54 | 2.69* | 1.12 | 0.76 |
| Ileojejunal Ln | 1.27 | 0.53** | 2.22*** | 1.24 | 0.81 | 1.61 |
| Liver | 0.94 | 0.49*** | 0.87 | 1.25 | 1.05 | 1.40 |
| Lung | 1.16 | 1.21 | 0.72 | 0.77 | 1.24 | 1.71* |
| Mandibular Ln | 0.42* | 1.37 | 2.14** | 0.52 | 0.41 | 0.44 |
| Mediastinal Ln | 1.46** | 0.97 | 0.66*** | 0.81* | 0.79 | 0.86 |
| Other lesions | 1.02 | 1.34 | 1.13 | 0.93 | 1.06 | 2.19** |
| Parotid Ln | 0.90 | 1.28 | 1.84* | 0.90 | 0.69 | 1.13 |
| Retropharyngeal Ln | 0.59*** | 0.74* | 0.92 | 1.21*** | 0.97 | 0.65*** |
| Tracheobronchial Ln | 1.21 | 1.24 | 0.78* | 0.72** | 1.06 | 1.54** |

**Table 10. The association between lesion sites and the putative animal, herd and farm risk factors.**
**Odds ratios shown; * P =< 0.05, ** P =< 0.01 P ***=< 0.001**

These results suggest that a "low risk animal" (i.e. from a TB clear herd and not a reactor) would be more likely to have thoracic lesions. These data were cross-tabulated; 71% (328/463) of the low risk animals had lesions in these sites versus 59% (1030/1743) of the other animals. This is statistically significant (P = 0.03) but the strength of the association is very weak (Cramer's V-square = 0.01).

## 4.2 DEVELOPMENT OF A MODEL OF THE POST MORTEM EXAMINATION PROCEDURE

The model outputs compared well with the field data (Figures 9 and 10). The correlation coefficient (r) was 0.998 (P < 0.001). For those tissues where the prevalence was less than 10%, r = 0.981. The poorest fits were with the ileojejunal nodes, 'other' tissues, and the mediastinal nodes (4% versus 6%, 6% versus 7%, and 43% versus 44% respectively)
There is a cluster of points where the field data were zero or near zero, but the model outputs are somewhat greater. This is due to 95% confidence intervals being included for the model inputs.

**Figure 9. Scatter plot showing the fit between lesion prevalence in cases from the field and the model output**

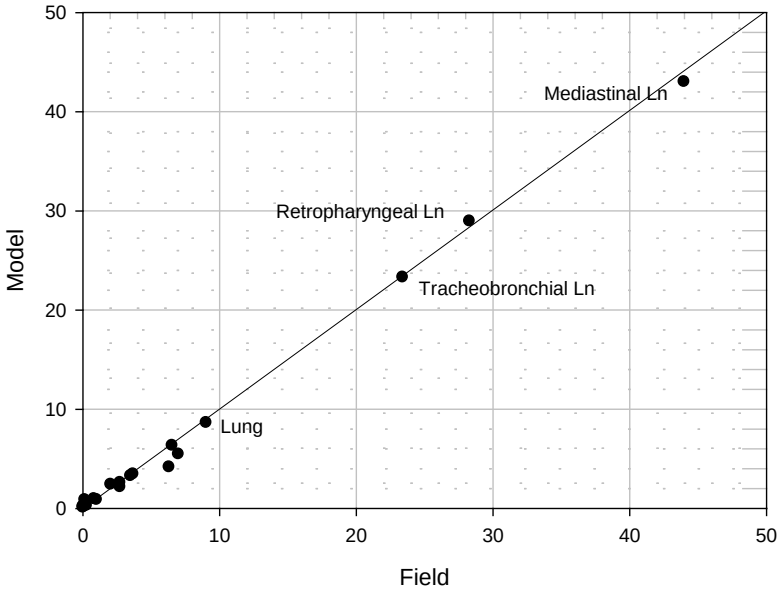
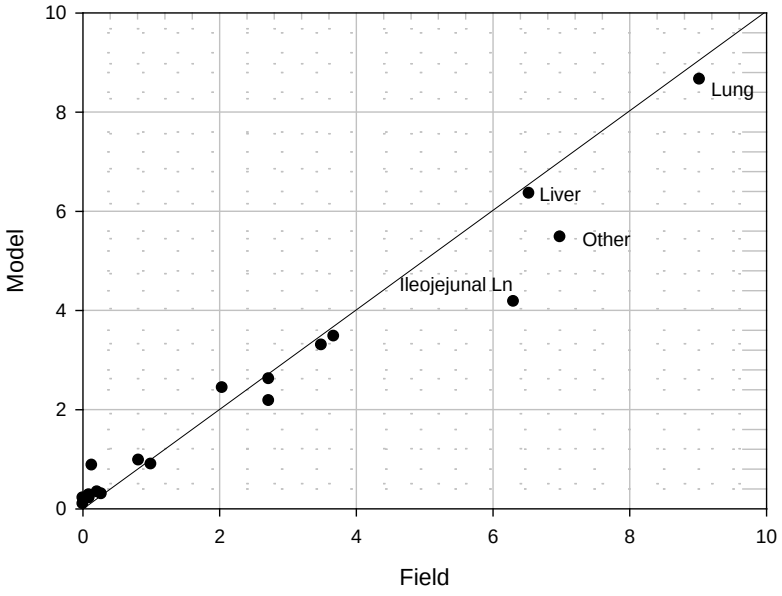



**Figure 10. Scatter plot showing the fit between lesion prevalence from the field and the model output, where the model prevalence is less than 10%**




## 4.3 MODELLING OUTCOMES OF ALTERNATIVE PROCEDURES

### 4.3.1 Detection of infected animals following reduction of tissues examined

A situation where one tissue at a time is "dropped out" of examination was simulated. As can be seem in Figures 11 and 12, with the exception of the most common lesion sites (i.e. tracheobronchial, retropharyngeal and mediastinal nodes) the probability of detection remains high (> 98%).

**Figure 11. Simulation of the probability of detection of an infected animal after dropping out examination of one tissue at a time. Scale 0% to 100%**

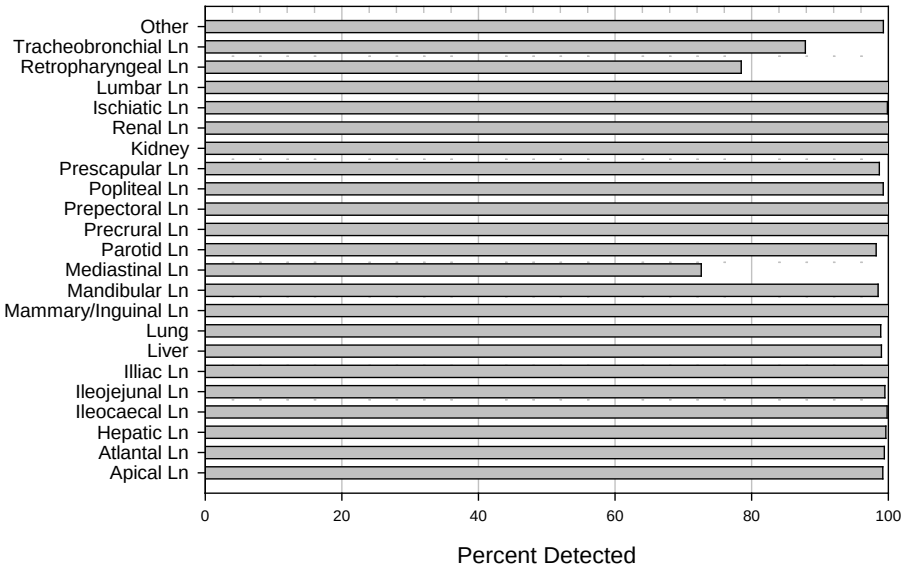
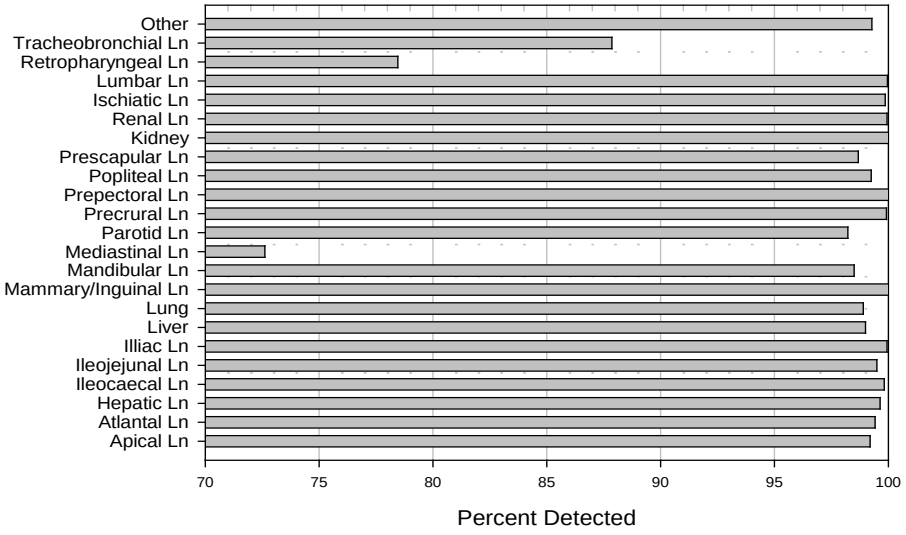



**Figure 12. Simulation of the probability of detection of an infected animal after dropping out examination of one tissue at a time. Scale 70% to 100%**




Another simulation was undertaken where tissues are sequentially dropped out in the ascending order of the prevalence. The results are shown in Figures 13 and 14. The effects of dropping out low prevalence lesions is small. The detection rate up to "prescapular node" is > 96%.

**Figure 13. Simulation of the probability of detection of an infected animal after sequentially dropping out examination of a tissue. Scale 0% to 100%**

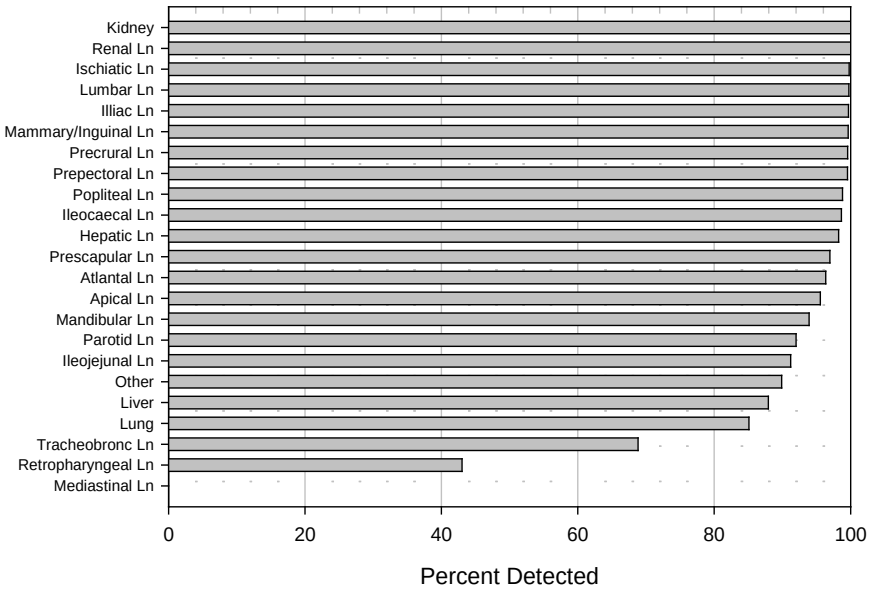
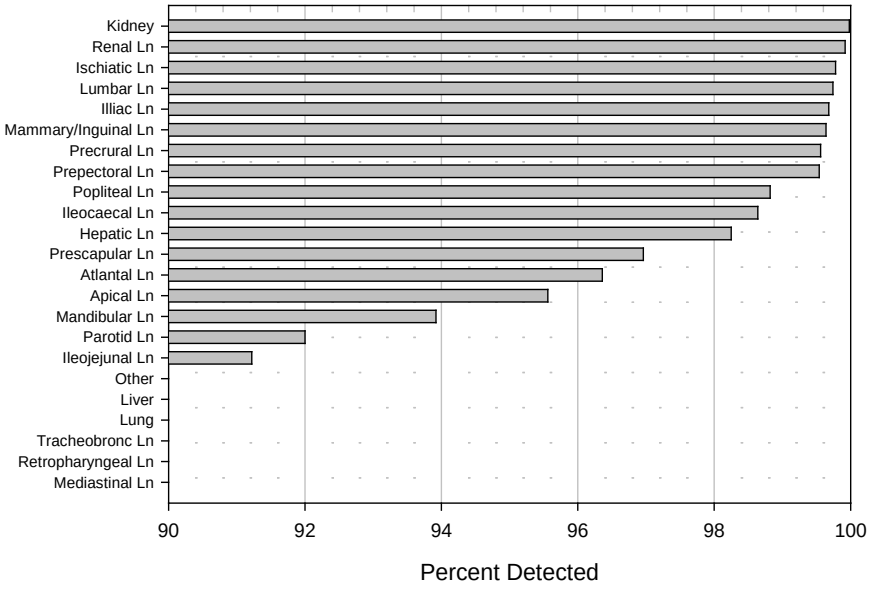



**Figure 14. Simulation of the probability of detection of an infected animal after sequentially dropping out examination of a tissue. Scale 90% to 100%**




### 4.3.2 Detection of lines containing infected animals following reduction of tissues examined

As there may be more than one infected animal with lesions in a line of cattle, the probability of detection will be enhanced. This effect is shown in Figures 15 and 16. In figure 17 the difference between the probability of detecting an infected animal and an 'infected line' is shown.

**Figure 15. Simulation of the probability of detection of an infected line after sequentially dropping out examination of a tissue.**

**Black fill = animal detection probability (as in figure 12)**
**Grey fill = line detection probability**
**Scale 0% to 100%**

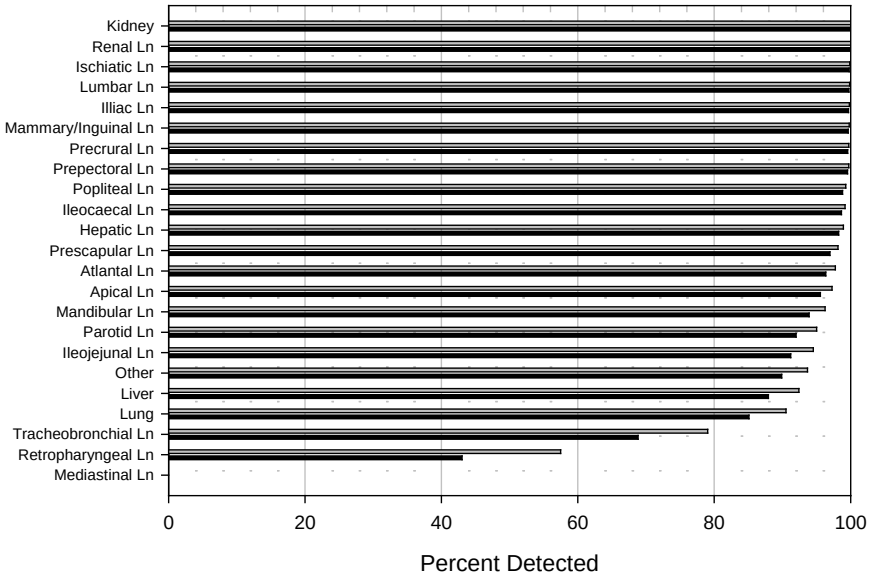



**Figure 16. Simulation of the probability of detection of an infected line after sequentially dropping out examination of a tissue.**
**Black fill = animal detection probability (as in figure 12)**
**Grey fill = line detection probability**
**Scale 90% to 100%**

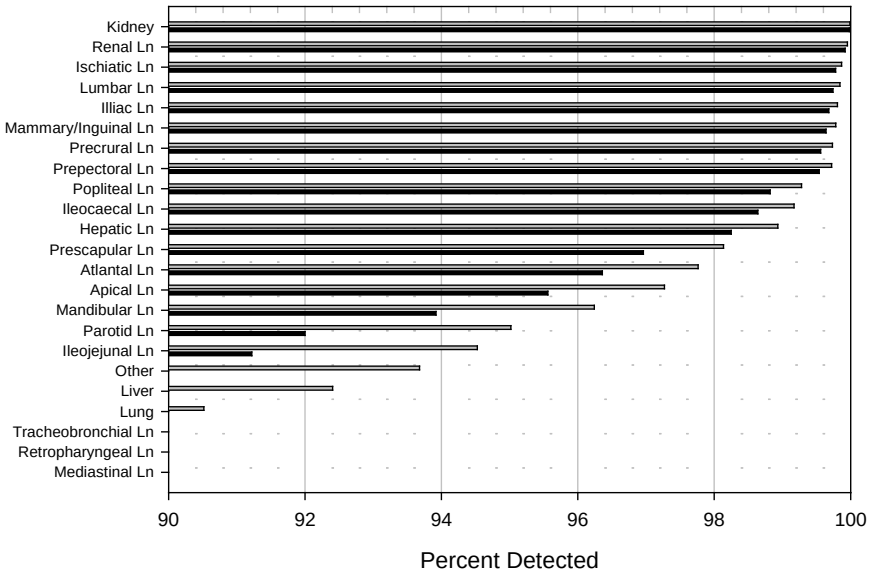
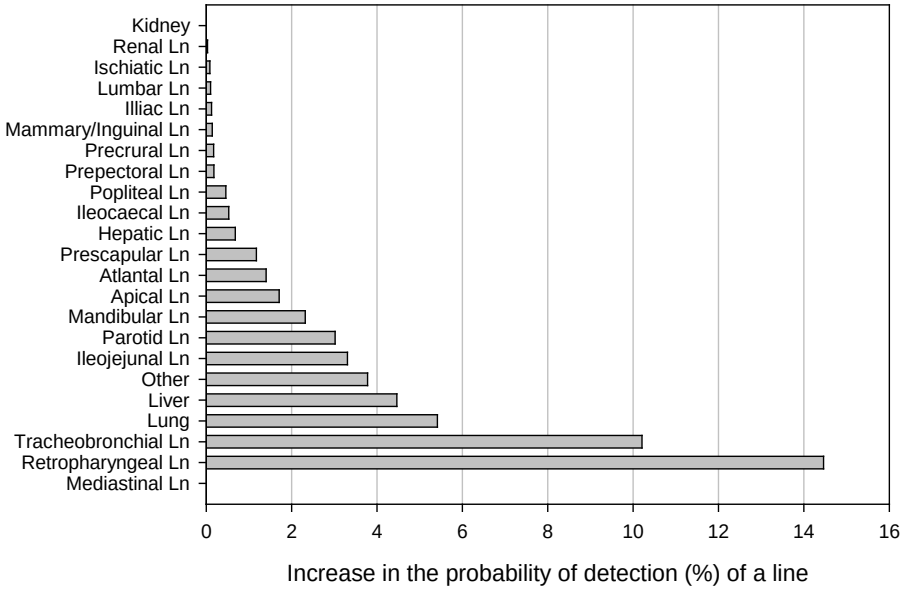


**Figure 17. Simulation of the probability of detection of an infected line after sequentially dropping out examination of a tissue. The bars show the difference between the probability of detecting an infected animal and an 'infected line'.**




### 4.3.3 Detection of infected animals and lines following elimination of 'operationally relevant' groups of tissues.

In discussion with operational staff, tissues that contribute significantly to the time taken to inspect carcases, but usually yield little information, were identified. The model was used to estimate the loss of sensitivity if these tissues were removed from examination in all cattle and in non-reactors only (Table 11). In all cases it was less than 2%.

**Table 11 Simulation of the probability of detection of an infected animal and infected line after dropping out examination of the following 'operationally relevant' tissues.**

| Tissues | Dropped in both reactors and non-reactors | Dropped in non-reactors only |
|---|---|---|
| Atlantal Lymph Node | 99.42% / 99.65% | 99.78% / 99.87% |
| Hepatic Lymph Nodes | 99.64% / 99.78% | 99.82% / 99.89% |
| Atlantal & Hepatic Lymph Nodes | 99.04% / 99.41% | 99.60% / 99.76% |
| Iliac, Lumbar, Renal & Supramammary/Inguinal Lymph Nodes | 99.80% / 99.88% | 99.90% / 99.94% |
| All these nodes dropped | 98.86% / 99.30% | 99.50% / 99.70% |

# 5 Discussion

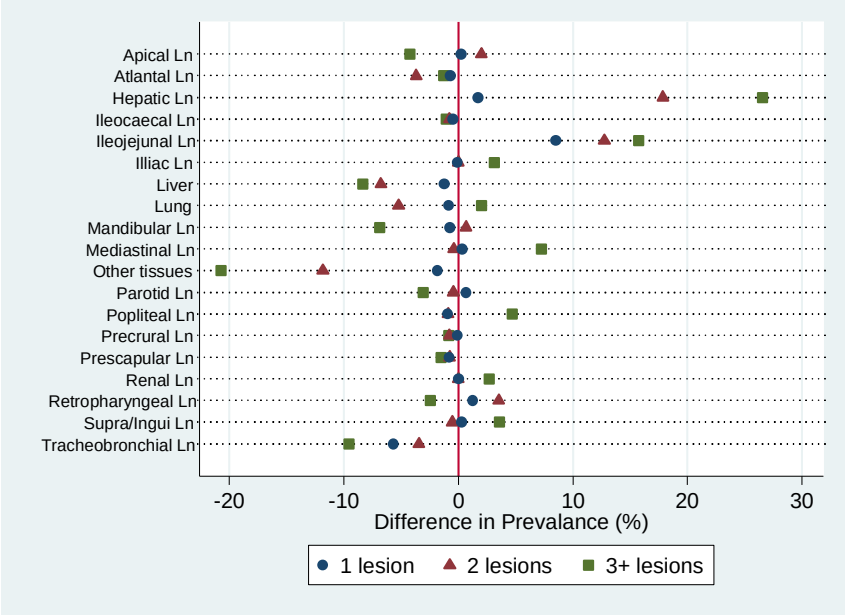
The most important conclusion arising from this is analysis is the reaffirmation that the primary route of entry for *M. bovis* in cattle is via the oropharynx and the lower respiratory tract. Around 90% of animals were found to have lesions in nodes draining these sites or in tissues in these sites. These tissues should therefore be the major focus in an effective post mortem examination. Interestingly, 57% had thoracic but no head lesions reported, 28% had head but no thoracic lesions, and only 5% with lesions in both areas. This suggests that both are important independent initial sites of infection.

The role of the gut as a primary site of infection appears less certain. In animals with only one lesion, ileojejunal or ileocaecal node lesions were reported in only 2.5% of cases, increasing to 20% and 30% in animals with two and three or more lesions, respectively. This suggests that gut lesions are generally part of the progression of disease; perhaps the result of swallowing large numbers of organisms either draining or coughed up into the pharynx.

The anatomical distribution of lesions is broadly in line with that reported from other studies in New Zealand [1], the United States of America [2], Australia [3], England [4] and Ireland . Of note is that Corner *et al*. [3] reported that with animals with only one lesion, in only 2.9% were the mesenteric nodes affected. Similarly, Liebana *et al*. [4] found that only 3% (4/133) 'TB-confirmed' cases had only lesions in the abdomen.

Notwithstanding the above results, and the quoted literature, more recent data from New Zealand, indicates that in some circumstances the ileojejunum is a more significant route of entry (see Appendix 1). Hepatic and, especially, ileojejunal lymph node lesions were more common. This can be seen in Figure 18 which shows the difference in the lesion prevalence found in the two datasets. Field staff were queried about this; their response was that this arose from a serious spread of infection via infected calves which had been fed milk from a cow with *M. bovis* mastitis (Kevin Crews, *personal communication*).

**Figure 18. The difference between the prevalence (%) of lesion sites in cases from 2013 to 2016 and 2003 to 2010.**




This demonstrates that the distribution of lesions seen in animals will be significantly influenced by the mode of transmission; e.g. via an aerosol or via contaminated milk. Furthermore, when there are low numbers of cases, local variations can produce unusual differences in the prevalence of lesion sites.

An important feature of tuberculosis in cattle is the sporadic nature of the disease in both reactor and non-reactor slaughter lines. In only 11% were there three or more lesion animals. From a disease surveillance perspective (i.e. detecting new infected herds) this is a limiting factor. One has few opportunities to identify infection among animals that may also have other non-tuberculous granulomas, for example lesions caused by *M. avium*.

The investigation of putative risk factors was not hampered by multicollinearity. Although there were significant associations, the correlations were not strong and therefore would not have had a profound effect on the multivariate analysis.

A strong association between an animal, herd or farm risk factor could have been used to design a targeted post mortem examination. This might have increased sensitivity and offered cost-benefit advantages. Although statistically significant associations were found, in none was the strength of the association of practicable use. However, the results do offer some insight into the epidemiology of tuberculosis. For example, dairy cattle (herding together) are associated with thoracic lesions while mature animals (duration of infection) are associated with more extensive lesions. The reason for some is not so obvious. For example, if the farm-of-origin is in a vector risk areas, gut lesions are more common. A possible explanation for this is licking dead TB possums or the eating grass which has been contaminated by infected possums.

The results of reducing the tissues examined needs to be looked at in terms of the wider aspect of detecting all *M. bovis* infected animals. During the 2009/2010 year, 260 cattle with grossly visible tuberculous lesions were reported from slaughterhouses in New Zealand (Paul Livingstone, *personal communication*). A key question is 'how many infected NVL[11] animals and how many lines with infected animals were missed?' To resolve this, an estimate of the conditional probability 'given that animal is infected what proportion will be VL[12]' is required; in other words, the sensitivity of gross post mortem examination. Because of the costs involved, these data are not readily available, but some are as shown in Table 12.

**Table 12. Data from infected herds where animals with both visible lesions (VL+) and no visible lesions were cultured for M. bovis**
**AHB = Animal Health Board, FSA = Food Standards Authority**

| Source | Species | Visible Lesions | No Visible Lesions | Sensitivity of VL+ |
|---|---|---|---|---|
| NZ. AHB | Cattle | 47 | 7 | 0.87 |
| NZ AHB | Deer | 22 | 2 | 0.92 |
| UK FSA [5] | Cattle | 20 | 19 | 0.51 |
| USA [2] | Cattle | 12 | 3 | 0.80 |
| USA [6] | Cattle | 37 | 6 | 0.86 |

Clearly, the data published by the UK Food Standards Authority is out-of-line with the other reports. An expert in the area of tuberculosis in animals considers the sensitivity to be around 0.75 (Paul Livingstone, Animal Health Board). This is lower than the above NZ and USA data. Thus, a conservative estimate would be from 0.70 to 0.80, with a most likely value of 0.75.

There has been much discussion about the sensitivity of slaughterhouse examination for tuberculosis infection in cattle, both in terms of exposure of consumers and reduced effectiveness of surveillance for national control programmes. A paper commonly quoted is that by Corner et al. [3] who estimated that "abattoir inspection failed to detect an estimated 47% of cattle with lesions". However, this was a comparison between a "detailed necropsy procedure" and "predominantly visual examination and palpation", which is unlike that currently used in New Zealand.

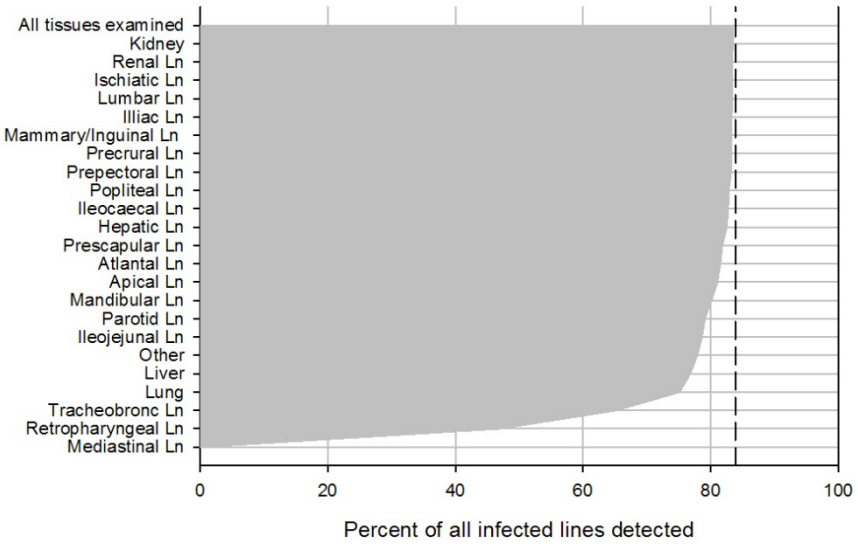
The negative binomial function[13] can be used to estimate the total number of *M. bovis* infected animals missed; this is 87, with 5 and 95 percentiles of 66 and 110. That is a median of 25%, but potentially up to 30%, of infected animals missed. The probability of missing an infected line at slaughter would be approximately 16%[14]. Thus, the reduction in sensitivity consequent to dropping a large number of tissues from the current procedure is small in relation to the missed cases and lines currently occurring (Figure 19). An alternative

[11] No Visible Lesions
[12] Visible Lesion
[13] Using the software @Risk®
[14] Assuming the distribution of infected animals per line is the same as that of animals with visible lesion.

approach would be to re-direct resources away from examining so many 'non-productive' sites to a more intensive examination of the prime early sites; i.e. the tonsils and lymph nodes draining the oropharynx, lower respiratory tract and the gut.

The exposure to consumers has recently been considered by technical groups within the European Union [5, 7]. Despite their very low estimate of the sensitivity of gross post mortem inspection (Table 12), they concluded that 'the risks to the public health through the consumption of meat from TB reactor animals are very low'. The European Food Safety Authority did not recommend any changes to existing meat hygiene controls. In the New Zealand context, the apparently higher sensitivity of inspection would even further minimise any public health risk. A redirection of resources to a more detailed examination of high risk sites should increase the sensitivity of gross post mortem inspection and thus further reduce the exposure of consumers to *M. bovis.*

**Figure 19. Simulation of the probability of detection of all infected lines after sequentially dropping out examination of a tissue.**
**Filled area = lines detected**
**Dotted vertical line = probability (84%) when all tissues are examined**



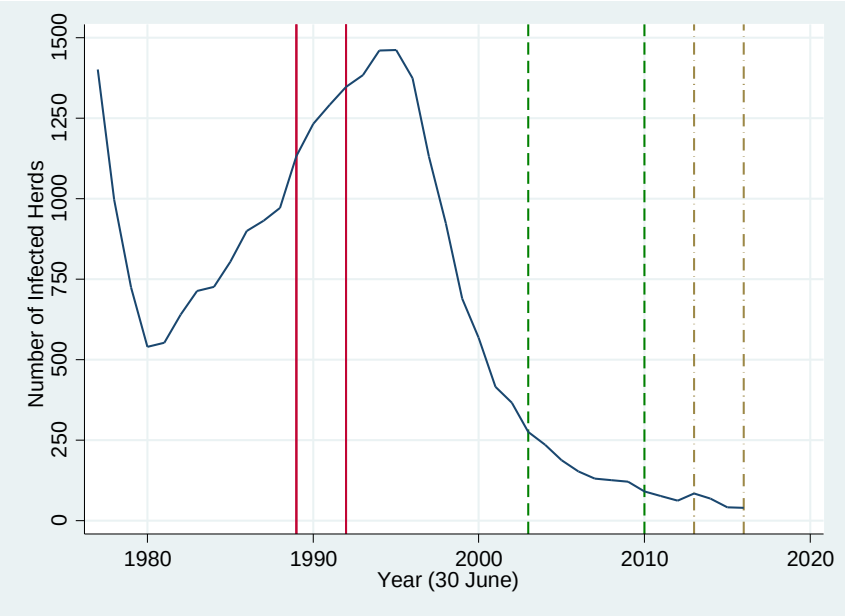

# 6 Appendix 1: Follow-up analysis 2013 to 2016

## 6.1 INTRODUCTION

Over the last five decades, the control of tuberculosis control in cattle (and for a shorter period in deer) has undergone some remarkable changes. Initially there was confidence that a "traditional" test and slaughter programme, as practiced in many other countries, would be effective. However, in the 1960's and 1970's there were considerable reverses, as shown in Figure 20. There was confusion why this was occurring and pessimism that tuberculosis could ever be eradicated from livestock. Intensive field and laboratory studies pointed to infected possums (*Trichosurus vulpecula*) being the primary problem, a so-called "wildlife

tuberculosis vector". Control of possums, together with a targeted disease control programme in cattle and deer herds, was successful. As at 30 June 2016 there were only 39 infected herds remaining, and there are now plans to eradicate *M. bovis* from New Zealand [8].

**Figure 20. Number of tuberculosis infected herds at 30 June from 1977 to 2016. The vertical lines show the periods over which data on infected cattle was analysed; i.e. Between the red solid lines (1989 to 1992) Between the green dashed lines (2005 to 2010) Between brown the dash-dot lines (2013 to 2016)**




The data for the original study of the distribution of lesions in *M. bovis* infected animals was from cattle slaughtered over two periods as listed in Table 1. These are also shown in Figure 20. A pertinent question is whether the pathology at these different times has been influenced by the changes in the epidemiology. In particular, how relevant are the findings in the low incidence environment that exists today? To provide insight into this, an additional analysis was conducted of animals slaughtered between 2013 and 2016 (see Figure 20) and the results were compared with those from the earlier study.

## 6.2 METHOD AND MATERIALS

Data was extracted from the OSPRI tuberculosis control database. In this system, the Area Disease Managers (ADM) flag the animals that have been reported as having typical or suspicious lesions and that he/she considers is due to *M. bovis* infection. This subset of records was used in the analysis.

## 6.3 RESULTS

### 6.3.1 Overview

The whole data file contained the post mortem records of 2,132 animals, with 94% being slaughtered over three years from May 2013.

Approximately a quarter (476/2132, 22%) were flagged by the ADM as having one or more *M bovis* lesions. These animals were used in the analysis.

Approximately a quarter (501/2136, 24%) were official tuberculin reactors.

Seventy one percent (355/501, 71%) of the reactors were reported as being tuberculous, but only 7% (121/1631) of the culls. (Table 13)

**Table 13 Cross-tabulation of TB status and reactor / cull (non-reactor) status**

| Reactor | TB Infected | Not TB Infected | Total |
|---|---|---|---|
| Yes | 355 (71%) | 146 (29%) | 501 (100%) |
| No | 121 (7%) | 1510 (93%) | 1631 (100%) |
| Total | 476 | 1656 | 2132 |

Twenty eight (28) animals were reported as having "skin lesions". Two of these had other tuberculous lesions and were included in the analysis. The skin lesions were not included in the analysis.

### 6.3.2 Gross pathology of TB animals

Eleven animals were recorded as having "TB grapes". All these animals were reported as being tuberculosis cases, and additional typical lesions were found in lymph nodes and/or organs.

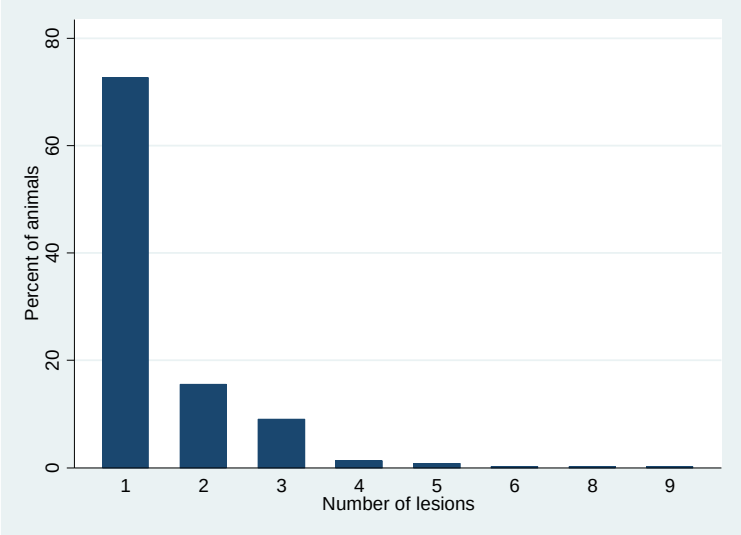
The number of typical tuberculous lesions reported in each case is listed in Table 14 and illustrated in Figure 21. As in the previous study, most (89%) of the animals were found to have only one or two lesions.

**Table 14. Lesions per animal, counts of animals with percent of total (476) shown**

| Number of lesions | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Animals % of | 346 (73%) | 74 (16%) | 43 (9%) | 6 (1%) | 4 (1%) | 1 (0.2%) | 0 (0%) | 1 (0.2%) | 1 (0.2%) | 0 (0%) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| total | | | | | | | | | | |

**Figure 21. Lesions per animal, percent of total animals (476) shown**



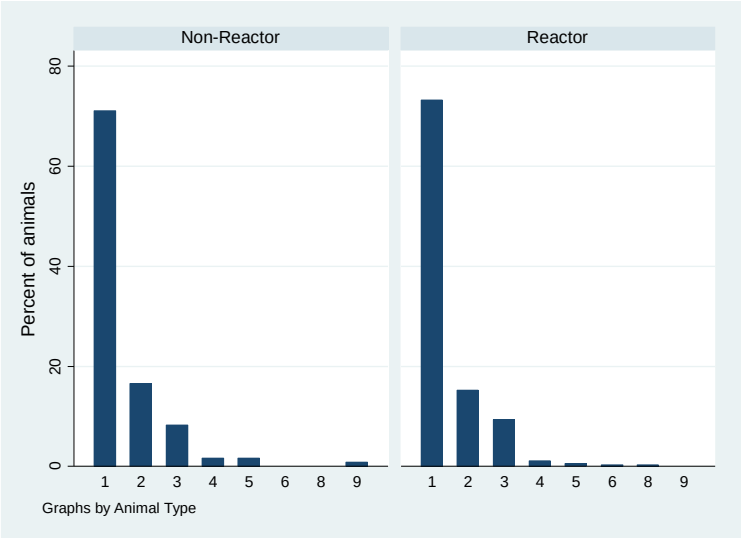

A comparison of the prior (Period 1) and the more current data (Period 2), with respect to number of lesions per animal, is presented in Table 15.

**Table 15. Number of TB lesions found in each animal**

| Lesions per animal | Period 1 | Period 2 |
|---|---|---|
| 1 | 1590 (73%) | 346 (73%) |
| 2 | 379 (16%) | 74 (16%) |
| 3 or more | 237 (11%) | 56 (12%) |

Considering the more recent period, the distributions of the number of lesions (i.e. one, two or three or more) in reactors and non-reactors (culls) are very similar (Figure 22). A Chi-squared analysis yielded a non-significant result ($\chi^2 = 0.2141$, $P = 0.898$).

**Figure 22. Lesions per animal in official TB reactors and non-reactors. Percent of total animals (121 non-reactors, 355 reactors) shown.**




The anatomical sites where typical TB lesions were found is presented in Figure 23 and Table 16. This is expressed as an animal prevalence; i.e. the percent of all animals that were found with a lesion in each of the 19 named locations and as "other". The results from the prior period have also been included to allow direct comparison.

**Figure 23. The percent of all TB animals with a lesion at each of the named sites. Prior data (1989 to 2010) bars are blue. Recent data (2013 to 2016) bars are red.**

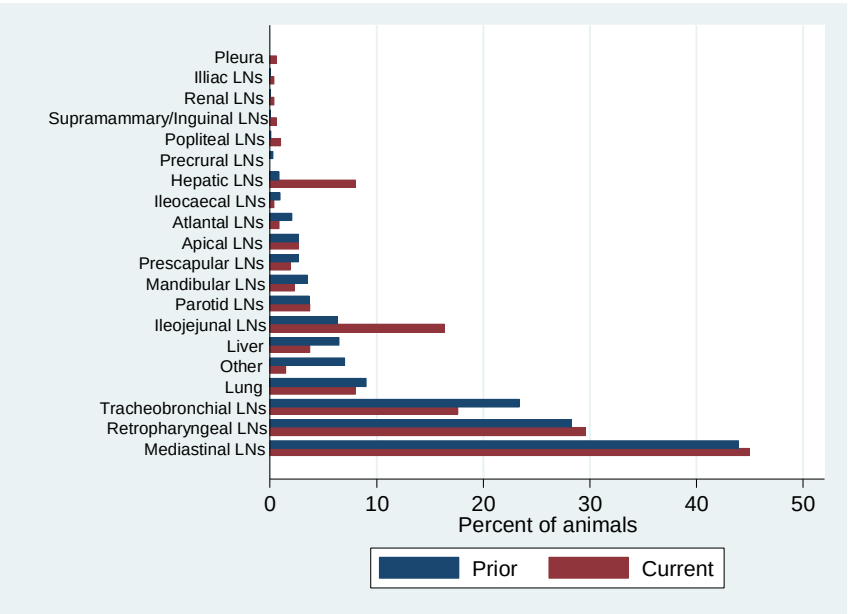


**Table 16. The percent of all TB animals with a lesion at each of the names sites, in the prior dataset (1989 to 2010) and recent dataset (2013 to 2016). NR = Not Reported**

| Tissue | 1989 to 2010 | 2013 to 2016 |
|---|---|---|
| Grapes | NR | 2.31% |
| Pleura | 0.00% | 0.63% |
| Kidney | 0.00% | NR |
| Lumbar Lymph Nodes | 0.00% | NR |
| Spleen | 0.00% | NR |
| Diaphragm | 0.00% | NR |
| Ischiatic Lymph Nodes | 0.09% | NR |
| Iliac Lymph Nodes | 0.09% | 0.42% |
| Supramammary/Inguinal Lymph Nodes | 0.09% | 0.63% |
| Renal Lymph Nodes | 0.09% | 0.42% |
| Popliteal Lymph Nodes | 0.13% | 1.05% |
| Prepectoral Lymph Nodes | 0.21% | NR |
| Precrural Lymph Nodes | 0.27% | 0.00% |
| Hepatic Lymph Nodes | 0.82% | 7.98% |
| Ileocaecal Lymph Nodes | 1.00% | 0.42% |
| Atlantal Lymph Nodes | 2.04% | 0.84% |
| Apical Lymph Nodes | 2.72% | 2.73% |
| Prescapular Lymph Nodes | 2.72% | 1.89% |
| Mandibular Lymph Nodes | 3.49% | 2.31% |
| Parotid Lymph Nodes | 3.67% | 3.78% |
| Ileojejunal Lymph Nodes | 6.30% | 16.39% |
| Liver | 6.53% | 3.78% |
| Other | 6.98% | 1.47% |
| Lung | 9.02% | 7.98% |
| Tracheobronchial Lymph Nodes | 23.39% | 17.65% |
| Retropharyngeal Lymph Nodes | 28.29% | 29.62% |
| Mediastinal Lymph Nodes | 43.97% | 44.96% |

With the exception of three sites[15] (the hepatic lymph nodes, the ileojejunal lymph nodes and the tracheobronchial lymph nodes), the prevalence figures for each site are similar. In addition, the "other lesion" category in the older data is five times greater than in the more recent data.

In a further analysis of the old and new data, the lesion sites were first aggregated into five groups; as follows:

1. Tissues of the head (Table 17)

[15] See red font in Table 16.

2. Tissues of the neck and chest (Table 18)
3. Tissues of the abdomen (Table 19)
4. Tissues of the carcase body (Table 20)
5. Other tissues (Table 21)

A statistical analysis was then conducted with the aim of determining if the animal prevalence was the same in the old and new data. The contingency tables and Chi-squared results are presented below.

**Table 17. Crosstabulation of presence/absence of head lesions over the periods 1989 to 2010 (Period 1) and 2013 to 2016 (Period 2)**

| Head Lesions | Period 1 | Period 2 | Total |
|---|---|---|---|
| No | 1476 (67%) | 311 (65%) | 1787 |
| Yes | 730 (33%) | 165 (35%) | 895 |
| Total | 2206 (100%) | 476 (100%) | 2682 |

$\chi^2 = 0.435$, P = 0.509

**Table 18. Crosstabulation of presence/absence of chest lesions over the periods 1989 to 2010 (Period 1) and 2013 to 2016 (Period 2)**

| Chest Lesions | Period 1 | Period 2 | Total |
|---|---|---|---|
| No | 848 (38%) | 198 (42%) | 1046 |
| Yes | 1358 (62%) | 278 (58%) | 1636 |
| Total | 2206 (100%) | 476 (100%) | 2682 |

$\chi^2 = 1.639$, P = 0.200

**Table 19. Crosstabulation of presence/absence of abdominal lesions over the periods 1989 to 2010 (Period 1) and 2013 to 2016 (Period 2)**

| Abdominal Lesions | Period 1 | Period 2 | Total |
|---|---|---|---|
| No | 1915 (87%) | 365 (77%) | 2280 |
| Yes | 291 (13%) | 111 (23%) | 402 |
| Total | 2206 (100%) | 476 (100%) | 2682 |

$\chi^2 = 31.518$. P = 0.000

**Table 20. Crosstabulation of presence/absence of carcase lesions over the periods 1989 to 2010 (Period 1) and 2013 to 2016 (Period 2)**

| Carcase Lesions | Period 1 | Period 2 | Total |
|---|---|---|---|
| No | 2099 (95%) | 459 (96%) | 2558 |
| Yes | 107 (5%) | 17 (4%) | 124 |
| Total | 2206 (100%) | 476 (100%) | 2682 |

$X^2 = 1.452$, P = 0.228

**Table 21. Crosstabulation of presence/absence of "other" lesions over the periods 1989 to 2010 (Period 1) and 2013 to 2016 (Period 2)**

| Other Lesions | Period 1 | Period 2 | Total |
|---|---|---|---|
| No | 2052 (93%) | 469 (99%) | 2521 |
| Yes | 154 (7%) | 7 (1%) | 161 |
| Total | 2206 (100%) | 476 (100%) | 2682 |

$X^2$ = 21.069, P = 0.000

As shown, there were statistically significant (P < 0.001) differences with respect to the proportion of animals reported with abdominal lesions and with "other" lesions.

In the earlier study the prevalence of lesions in specific tissues in animals with one lesion, two lesions and three or more was investigated. The rationale for this was that with a greater number of lesions there would be a lower risk of missing an infected animal. The same information has been extracted from the later data and is listed in Table 22. In addition, dot graphs of both the recent and older data are presented in Figures 24 and 25 respectively.

**Table 22. The prevalence (%) of animals with lesions in specific tissues where one, two or three or more lesions were reported.**

| Tissue | 1 lesion | 2 lesions | 3+ lesions |
|---|---|---|---|
| Apical Ln | 1.16 | 6.76 | 7.14 |
| Atlantal Ln | 0.00 | 0.00 | 7.14 |
| Hepatic Ln | 2.02 | 18.92 | 30.36 |
| Ileocaecal Ln | 0.00 | 0.00 | 3.57 |
| Ileojejunal Ln | 10.40 | 25.68 | 41.07 |
| Iliac Ln | 0.00 | 0.00 | 3.57 |
| Ischiatic Ln | NR | NR | NR |
| Liver | 0.29 | 4.05 | 25.00 |
| Lung | 0.87 | 13.51 | 44.64 |
| Mandibular Ln | 1.16 | 5.41 | 5.36 |
| Mediastinal Ln | 36.13 | 60.81 | 78.57 |
| Other tissues | 0.58 | 1.35 | 7.14 |
| Parotid Ln | 3.47 | 4.05 | 5.36 |
| Popliteal Ln | 0.58 | 0.00 | 5.36 |
| Precrural Ln | 0.00 | 0.00 | 0.00 |
| Prepectoral Ln | NR | NR | NR |
| Prescapular Ln | 1.73 | 1.35 | 3.57 |
| Renal Ln | 0.00 | 0.00 | 3.57 |
| Retropharyngeal Ln | 30.06 | 25.68 | 32.14 |
| Supramammary/Inguinal Ln | 0.29 | 0.00 | 3.57 |
| Tracheobronchial Ln | 10.69 | 32.43 | 41.07 |

**Figure 24. The prevalence (%) of animals with lesions in specific tissues where one (blue circle), two (maroon triangle) or three or more (green square) lesions were reported. Data from 2013 to 2016.**

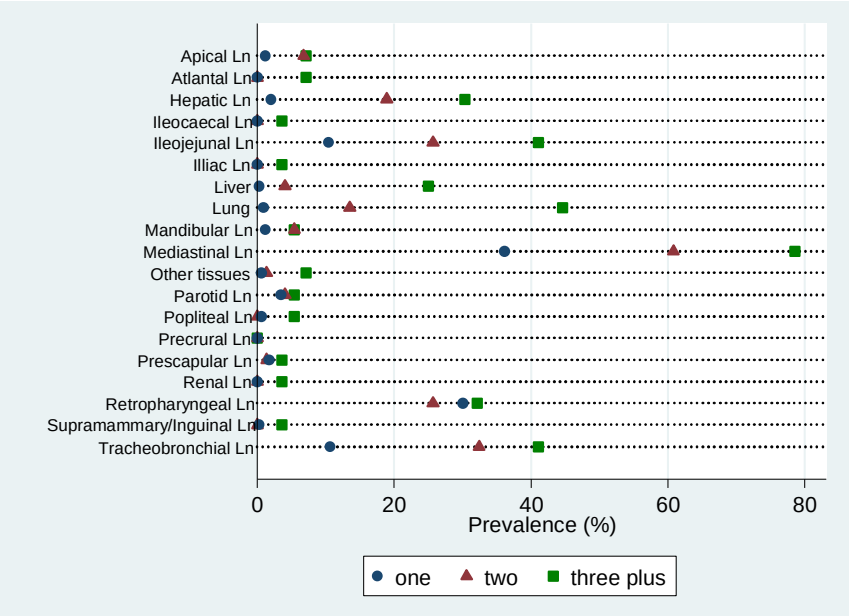
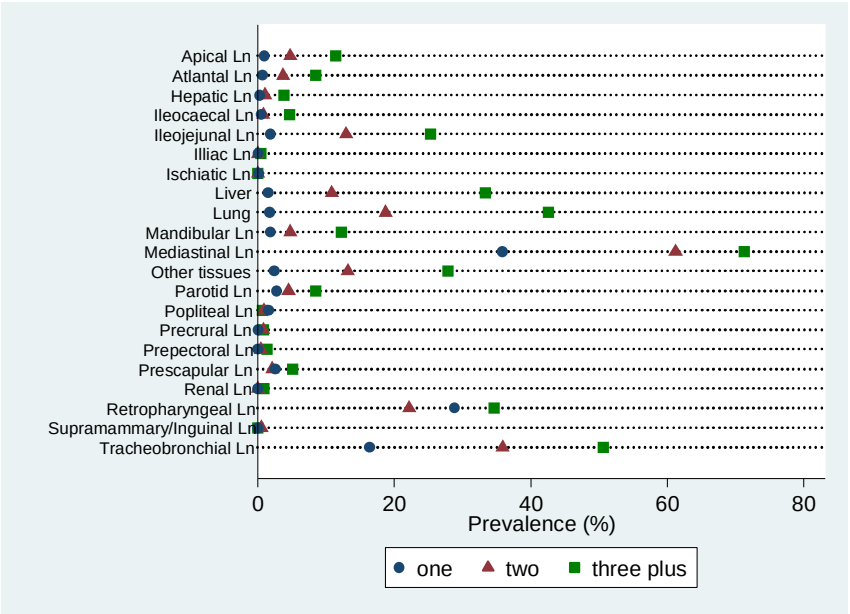



**Figure 25. The prevalence (%) of animals with lesions in specific tissues where one (blue circle), two (maroon triangle) or three or more (green square) lesions were reported. Data from 1989 to 2010.**




## 6.4 CONCLUSIONS

Taking a broad view of the results from the early and late periods, the gross pathology observed is generally remarkably similar.

The only marked differences are:

- The higher prevalence of animals with abdominal lesions, especially the ileojejunal nodes, in the recent group; i.e. 6.3% versus 16.4%.
- The higher prevalence of animals reported with "other lesions" in the older group; i.e. 7.0% versus 1.4%.

It is a well-documented phenomenon that towards the end of a successful disease control programme, minor routes of infection become more prominent. Over the last 3 years some herd breakdowns arose from the dissemination of calves from a herd where they had been fed milk from a cow with an *M. bovis* mastitis. This is not common but in the low incidence environment it made a considerable impact (Kevin Crews, *personal communication).*

The difference in the prevalence of "other" lesions is most likely due to limitations in the "pick-list" presented to data entry personnel. For example, "grapes" could not be recorded in the DBMS and SIR systems. It appears that there was also confusion about the name of some tissues among frontline-staff.

# 7 Appendix 2: Recording lesion data

Affected tissues that can be recorded in the DMIS, NLDB and SIR TB information systems. The instruction given in the NZFSA manual is also listed (O = Incise only in heavily muscled young bulls & aged cattle, P = Palpation, S = TB Suspects, V = Visual Inspection)

| Tissue | DMIS | NLDB | SIR | NZFSA List |
|---|---|---|---|---|
| Apical Lymph Nodes | Yes | Yes | Yes | Incise |
| Atlantal Lymph Nodes | Yes | Yes | Yes | Incise |
| Bronchial (Tracheobronchial) Lymph Nodes | Yes | Yes | Yes | Incise |
| Diaphragm | Yes | | | VP |
| Abdominal Grapes | Yes | | | V |
| Hepatic Lymph Nodes | Yes | Yes | Yes | Incise |
| Ileocaecal Lymph Nodes (see Mesenteric LN below) | Yes | Yes | Yes | |
| Ileojejunal Lymph Nodes (see Mesenteric LN below) | Yes | Yes | Yes | |
| Iliac Lymph Nodes | Yes | Yes | Yes | Incise |
| Inguinal Lymph Nodes (♂) | Yes | Yes | Yes | Incise |
| Ischiatic Lymph Nodes | | Yes | Yes | Incise(S) |
| Jejunal Lymph Nodes (see Mesenteric LN below) | Yes | | | |
| Kidneys | Yes | | | VP |
| Liver | Yes | Yes | Yes | VP |
| Lumbar Lymph Nodes | | Yes | Yes | Incise |
| Lungs | Yes | Yes | Yes | VP |
| Mandibular (Submaxillary) Lymph Nodes | Yes | Yes | Yes | Incise |
| Mediastinal Lymph Nodes | Yes | Yes | Yes | Incise |
| Mesenteric Lymph Nodes | Yes | | Yes | Incise (S) |
| Parotid Lymph Nodes | Yes | Yes | Yes | Incise |
| Pleura | Yes | Yes | | V |
| Popliteal Lymph Nodes | | Yes | Yes | Incise(S) |
| Precrural (Prefemoral) Lymph Nodes | Yes | Yes | Yes | Incise(OS) |
| Prepectoral (Axillary) Lymph Nodes | | Yes | Yes | Incise(S) |
| Prescapular (Superficial Cervical) Lymph Nodes | Yes | Yes | Yes | Incise (O S) |

| Tissue | DMIS | NLDB | SIR | NZFSA List |
|---|---|---|---|---|
| Renal Lymph Nodes | Yes | Yes | Yes | Incise |
| Retropharyngeal Lymph Nodes | Yes | Yes | Yes | Incise |
| Skin | Yes | Yes | Yes | |
| Spleen | | Yes | Yes | VP |
| Supramammary Lymph Nodes (♀) | Yes | Yes | Yes | Incise |
| Tonsils | Yes | | | V |
| Other Tissues | Yes | Yes | Yes | |

# 8 Acknowledgements

The Animal Health Board provided the data for this study. In addition, Dr Paul Livingstone provided valuable expert opinion on a number of issues.

Drs Neil Cox and Harold Henderson, AgResearch Ruakura, provided statistical advice, especially with regard to regression analyses.

Ira Stapp, AsureQuality, provided valuable feedback on field recording of TB lesions.

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