Johne's disease and lesions
(DRAFT)

An information paper regarding the value of using the identification of lesion's at slaughter for Johne's disease surveillance

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EXECUTIVE SUMMARY

Johne's disease (JD) is a chronic, contagious and sometimes fatal infection of ruminant animals caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Infected animals shed MAP in their faeces contaminating the environment and propagating infection on a farm. Lesions of the intestine and lymph nodes are a characteristic symptom of the disease and are noted to varying extent in all species where the disease is diagnosed. Clinical JD is comparatively easy to diagnose, while milder infections are progressively more difficult to detect. Evidence suggests that early diagnosis maybe helpful to aid in the control of disease therefore an effective low level surveillance method could be of value to aid in proactive management of the disease.

This paper provides a preliminary review of the occurrence and form of lesions associated with Johne's disease in sheep, cattle and deer and their utility as a surveillance method for the early diagnosis of JD affected herds and flocks.

While the review concludes that it is not possible to reliably detect lesions in stock in the early stages of disease, evidence suggests that animals in advanced stages of JD are highly likely to have lesions visible to the naked eye that would provide evidence of the disease at slaughter. Lesion detection is therefore theoretically useful as a low level surveillance tool for JD, particularly as there are existing monitoring and data capture systems in place for farmed deer that would support cost effective implementation of the tool. However it is noted that lesion detection is likely to be of limited practical if animals with visual signs of disease are not being sent for slaughter.

Further work would be required to assess how many sheep and cattle with lesions are being sent to abattoirs for processing in New Zealand before concluding whether lesion detection at slaughter would be of value as a low level surveillance tool for JD in sheep or cattle in New Zealand.

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Glossary

BJD	Bovine Johne's disease
DNA	Deoxyribonucleic acid
DSP	Deer Slaughter Premise
ELISA	Enzyme-linked immunosorbent assay – diagnostic for Johne's disease
JAG	Johne's Advisory Group
JD	Johne's disease
JDRC	Johne's Disease Research Consortium
JDSLN	JD suspect lesions
JML	Johne's Management Limited
MAP	Mycobacterium avium paratuberculosis – the organism that causes Johne's disease
MLN	Mesenteric lymph nodes
OJD	Ovine Johne's disease
OIE	World Organisation of Animal Health
OML	Ovis Management Limited
Paratuberculosis	Johne's disease
PCR	Polymerase chain reaction – diagnostic for Johne's disease
qPC	Quantitative Polymerase chain reaction – diagnostic for Johne's disease
RMP	Risk Management Plan
Se	Sensitivity
Sp	Specificity

1 BACKGROUND

1.1 Johne's Disease

Johne's disease (JD) is a chronic, contagious and sometimes fatal infection of ruminant animals caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Infected animals shed MAP in their faeces contaminating the environment and propagating infection on a farm. Lesions of the intestine and lymph nodes are a characteristic symptom of the disease and are noted in all species where the disease is diagnosed.

MAP Infection can be widespread in a herd or flock but usually only a small number of animals progress through to the clinical stages of disease which results in wasting and death. JD affects both animal health and welfare and has economic consequences on farm through lost productivity and premature death of stock. There is also concern over the possible, unproven link between MAP and human disease, meaning that JD has the potential to affect trade.

In 2013 it was estimated that 75% of sheep flocks[1], 47% of dairy cattle[2], 43% of beef cattle[1] and 46% of deer herds[1] in New Zealand were infected with MAP, but the number of herds and flocks experiencing significant clinical disease was much lower. In all species there was a small number of farms where the disease was severe and causing major stock losses that needed to be managed. While it is highly unlikely that the disease can be eradicated it is believed that it is possible to control its impact on farm through high levels of farmer awareness and the application of good management practice. The ability to detect and diagnose affected animals and problem herds/flocks can be difficult, particularly in the early stages of an outbreak. Evidence suggests that early diagnosis maybe helpful in controlling the disease[3], therefore low cost surveillance methods that could provide assistance to farmers in the early detection of a problem within herds or flocks should be of value to the sector. The identification of lesions at slaughter is one possible method that could be considered for disease surveillance and detection as it is low tech and should be able to be integrated into existing inspection systems operating in slaughter premises with minimal difficulty.

1.2 The Johne's Disease Research Consortium and Johne's Advisory Group

The Johne's Disease Research Consortium was established in 2008 to undertake a coordinated program of research to develop practical and cost effective tools for the management of JD in New Zealand[4]. In 2013 JDRC established the Johne's

1 Epidemiology of Mycobacterium Avium subspecies Paratuberculosis infection on sheep, beef cattle and deer farms in New Zealand. Cristobal Verdugo, Massey University PHD Thesis, November 2013

2 Risk factors for the Presence of Johne's disease in dairy herds: a survey of New Zealand farmers. JDRC Study Report. Jaimie Hunnam, Cognosco, February 2014

3 For example: (a) JDRC 2013, 5.2 Modelling Final report to JDRC. Cord Heuer, Massey University, January 2014; Strategies for time of culling in control of paratuberculosis in dairy herds. (b) Kudahl AB, Nielsen SS, Ostergaard S, Journal of Dairy Science. 94 (8), 3824-3420, Aug 2011

4 Partners in the Consortium include Beef+Lamb New Zealand, DairyNZ, DEEResearch Limited, AgResearch, Livestock Improvement Corporation, Massey University and the

Advisory Group (JAG), a cross-sector committee comprised of JD experts from differing disciplines. The role of the JAG is to review technical information (including research outputs, and management control measures) and formulate consistent information and best practice guidelines to up skill industry and ensure the timely and effective uptake of tools to minimize the impact of JD in New Zealand.

In 2013 a media report noted that an Australian Meat Processor in Tasmania was using the observation of JD-like lesions in slaughtered sheep as a surveillance tool to alert farmers to the possibility that JD was affecting their flocks[5]. This is not an unfamiliar concept in New Zealand as the deer industry uses lesion status as a surveillance tool for farmed deer (see section 3.1). The JAG recommended that JDRC undertake a review of the use of this technique as a potential low level surveillance tool for cattle and sheep farmers in New Zealand, noting that the observation of lesions at slaughter could be recorded on Meat Processor kill sheets and used as a means to alert farmers of a potential issue in their flocks/herds. Kill sheets are completed for all stock slaughtered by processing works and are used as a record for both the company and the farmer.

This information paper provides a preliminary review of lesions associated with Johne's disease as a diagnostic tool in sheep, cattle and deer and the likely usefulness of the observation of lesions as a surveillance tool for Johne's disease in sheep and dairy cattle.

1.3 Scope of the review

This is a preliminary review to assess the premise that lesion detection may be useful for both industry and farmers as a surveillance technique in New Zealand. It is based on information available in published literature, JDRC documentation and personal communication with a selection of NZ based experts, but does not include evaluation by all stakeholders (e.g. Livestock Industry, Meat Companies, Farmers or Veterinarians).

2 Diagnosis of Paratuberculosis

The diagnosis of JD is not always straightforward. While there is a range of tests available, they all suffer from limitations, with the speed, accuracy, sensitivity and costs for each test varying widely. The ability to detect disease also varies as the disease progresses, with sensitivity generally increasing as the disease progresses. There are no reliable diagnostics for the early detection of MAP infection. Developing an economical method for disease surveillance is therefore difficult, and a low priority in New Zealand as the impact of the disease is low and severe effects localized to a limited number of farmers. Given the potential impact of the Johne's disease through on farm losses and the potential for trade issues, it is important that the prevalence of disease remains low. As the disease is primarily controlled by good management practice, maintaining an awareness of good practice is a key

University of Otago, with observers from the Ministry of Business Innovation and Employment, Dairy Companies Association of New Zealand and the Meat Industry Association. Landcorp Farming Limited, Johne's Management Limited (JML) and NZ Merino Limited are all associated with the Consortium through Research Partnerships.

5 Stopping the spread of sheep diseases is a winner, Rosemary Grant, ABC Rural. 13 September 2013. http://www.abc.net.au/news/2013-09-12/sheep-disease-tqm-biosecurity/4953218.

factor for achieving this goal. Research**Error! Bookmark not defined.** and anecdotal evidence also suggests that early detection and management of affected stock to reduce the contamination risk for other stock and the environment is another important factor for successful control and management of JD. A cost effective low level surveillance method may therefore be of considerable value for providing early alerts for farmers of a potential problem within flocks or herds to aid in proactive management of the disease.

2.1 Diagnostic Methods for Johne's Disease

The World Organisation of Animal Health (OIE) notes that clinical diagnosis of paratuberculosis is made on "demonstration of *Mycobacterium avium subspecies paratuberculosis* (MAP) in the faeces by microscopy, culture, or by the use of DNA probes and the polymerase chain reaction (PCR)". However diagnosis can also be made post mortem by finding lesions in the intestine that are characteristic of JD (pathognomonic), that are either visible to the naked eye or identified by histology (acid fast organisms in impression smears or the isolation of MAP in culture). The test chosen for diagnosis "depends on the circumstances and degree of sensitivity required at the individual animal and herd level"[6].

A list of commercially available tests for the detection of Johne's disease is given in Table 1 along with an assessment of their utility for disease surveillance.

Table 1: Diagnostic Tests

Test	Samples	Pro's/Cons	Utility as Surveillance tool
ELISA	Blood	Ante mortem; rapid test, sensitive for clinical animals, low cost. False positives & negatives an issue	Possible to use as screening tool but not favoured due to sample collection costs. Would need validation
	Milk	Ante mortem, rapid test, can identify affected herds, low cost. False positives & negatives an issue	Useful screening tool for dairy herds if validated; would need confirmation analysis on individual animals
Culture	Faecal, tissue, environmental	Ante mortem, very slow test method, moderate costs; largely superseded by PCR. Bactec Culture medium for sheep strains is obsolete making culture less reliable for sheep strains	Poor tool due to time/cost and impractical sample collection
PCR	Faecal, tissue, environmental	Ante mortem, rapid test, moderate cost, high	High utility in on-farm surveillance for

6 Manual of Diagnostic Tests and Vaccines for Terrestrial Animal 2013, World Organisation for Animal Health (OIE); Chapter 2.1.11, Paratuberculosis http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.11_PARATB.pdf

Test	Samples	Pro's/Cons	Utility as Surveillance tool
		sensitivity, high specificity, ability to pool samples, quantification of shedding	identifying highly infectious animals and prioritising for culling. No practical utility as a national surveillance tool.
Gross pathology	Carcass	Post Mortem, Low cost as routine part of carcass inspection, useful as a broad (national scale) form of monitoring, very low sensitivity, moderate specificity confirmation of diagnosis requires additional testing	Low cost practical tool for surveillance; operators must be trained to recognise lesions

An analysis of global control programmes for Johne's disease (shown in Attachment 1) lists the testing methods in use globally for surveillance and monitoring of Johne's disease. Most nations with surveillance systems are monitoring dairy cattle and rely on a serological test (ELISA's) for preliminary screening of animals for Johne's disease. ELISA's are rapid, inexpensive and the test is relatively sensitive for the detection of clinical animals, but the sensitivity does fall away in cases of sub-clinical disease. The test can be run on both blood and milk samples. Bulk milk testing for detection of disease in dairy herds is an attractive, non-invasive surveillance method for dairy cattle. PCR and Faecal culture are then often used as supplementary tests to confirm detection of disease. Environmental samples have also been used to monitor disease levels in Australia and the US.

There are currently no nations routinely using lesion detection for surveillance purposes, with the exception of New Zealand, where the deer industry has implemented a surveillance program for slaughtered deer (see section 3.1). Visual inspection of lesions at slaughter is considered an attractive surveillance tool as it can be added into existing carcass inspection procedures at slaughter houses with minimal training of staff, it is low tech and does not require expensive laboratory procedures and requires relatively little in the way support systems to be implemented at works over and above routine recording of inspection findings.

2.2 Johne's Disease Lesions

Lesions of the intestine and lymph nodes are a characteristic symptom of paratuberculosis and have been noted in all species where the disease is diagnosed, including sheep[7], cattle[8], deer[13], goats[9] and other ruminant species[10].

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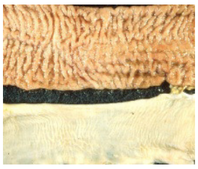
7 Sequential Development of Histologic Lesions and Their Relationship with Bacterial Isolation, Fecal Shedding, and Immune Responses during Progressive Stages of Experimental Infection of Lambs with Mycobacterium avium subsp. Paratuberculosis. N. P. Kurade, B. N. Tripathi, K. Rajukumar and N. S. Parihar. Vet Pathol 41: 378, 2004

8 Isolation of Mycobacterium avium subsp. paratuberculosis from muscle tissue of naturally infected cattle. Alonso-Hearn M, Molina E, Geijo M, Vazquez P, Sevilla I, Garrido JM, Juste RA. Foodborne Pathog Dis. May;6(4):513-8. 2009.

The development of lesions is progressive through the course of the disease, with the number and severity increasing as the disease progresses. However there is not always a close correlation between the severity of clinical signs of disease and the presence and extent of intestinal lesions**Error! Bookmark not defined.**.

Lesions form as a result of the animal's inflammatory response to the invading MAP bacteria[11]. MAP ingested by an animal travels to the small intestine and enters into the walls of the intestine through "M" cells located in small masses of lymphatic tissue (Peyer's patches), where it lodges in cells that fight infection (macrophages). Depending on the resistance of the individual animal, the infection can either be eliminated at this stage or the animal can remain infected as a healthy or "sub-clinical" carrier. Subclinical carriers excrete variable numbers of MAP in the faeces. In the early stages of infection MAP lesions usually only occur in the walls of the small intestine and the draining mesenteric lymph nodes. A thickening and cording of lymphatics is normally seen and the mesenteric lymph nodes will usually be enlarged and filled with fluid (oedematous).



Thickened corrugated mucosa in sheep

MAP appears to be able to turn off the normal responses of macrophages and the immune system through complex signalling between cells and the release of proteins (cytokine's), creating a hospitable environment for itself in the gut. However at some point, in some animals, the bacteria trigger an inflammatory response and lesions (or granuloma's) start to form as macrophages gather and coalesce to fight the infection. At this point the disease progresses from sub-clinical to clinical phase. The number of bacteria multiplies, which leads to the extension of lesions, interference with gut metabolism and clinical signs of disease. In the later stages of disease lesions may be found in the ileum, jejunum, terminal small intestine, caecum and colon, and in the mesenteric lymph nodes. MAP is present in these lesions and will be spread by the blood throughout the rest of the body. MAP can also be found within muscle tissue at this stage of the disease. Clinical animals usually shed large numbers of MAP in their faeces.

Uncontrolled inflammation of the gut is the primary reason Johne's disease is fatal. The gastrointestinal tract becomes severely damaged, usually thickened, corrugated and reddened and is no longer capable of absorbing nutrients. Lymph nodes become swollen and pale. It is at this point that the animal begins to show signs of diarrhoea or weight loss.

2.2.1 Diagnosis of JD from Lesions[12]:

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9 Classification of lesions observed in natural cases of paratuberculosis in goats. Corpa JM, Garrido J, García Marín JF, Pérez V. J Comp Pathol. May;122(4):255-65, 2000.

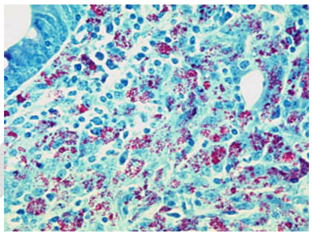
10 For example: (a) Identification of Mycobacterium avium subsp avium in an alpaca with lesions resembling paratuberculosis. Lucas JN, Cousins DV, Mills AJ, Van Wijk JG. Aust Vet J. Sep;81(9):567-9, 2003. (b) Paratuberculosis in small ruminants, deer, and South American camelids. Stehman SM. Vet Clin North Am Food Anim Pract. Jul;12(2):441-55,1996.

11 http://www.johnes.org/general/pathology.html#inflammation

12 Paratuberculosis (Johne's Disease). DV Cousins et al. Australia and New Zealand Standard Diagnostic Procedures, April 2002

The OIE notes that "Paratuberculosis cannot be diagnosed on superficial examination of the intestines for signs of thickening. The intestines should be opened from the duodenum to the rectum to expose the mucosa. There is not always a close correlation between the severity of clinical signs and the extent of intestinal lesions".Error! Bookmark not defined. Diagnosis of the (severity of) disease is best confirmed by the collection of multiple intestinal wall and mesenteric lymph node samples followed by histological examination.

In all species, a wide spectrum of cellular responses has been described on histological examination. In advanced cases of disease with obvious gross lesions, the histological changes are obvious and very characteristic with vast numbers of acid-fast bacilli (shown as red in the diagram opposite) readily identifiable ("multibacillary"), but in animals with subclinical or limited infections, the changes are less distinct and acid-fast bacilli may be infrequent and difficult to detect ("paucibacillary"). In sheep, some advanced cases have granulomatous lesions with very few acid-fast bacilli.



Acid fast bacteria

In New Zealand a positive diagnosis of lesions consistent with *MAP* infection is indicated if a minimum of 3 acid fast bacteria with morphologically consistent with *M paratuberculosis* are sighted on histological examination.

2.2.2 Specific Lesion Pathology in varying species[7]

In affected sheep, goats and alpaca, the lymphatic vessels are usually knotted and corded. Approximately 10% of clinically affected goats show caseation (where dead tissue is changed into a dry mass resembling cheese) and calcification (hardening) of the lymph nodes. Unlike sheep, goats and deer, there is no caseation or calcification seen in cattle. In sheep and goats, some lesions may be visible as white foci 1–4mm in diameter. In alpaca, enlargement of all mesenteric lymph nodes is striking.

Lesions in Farmed Deer

A notable feature of Johne's disease in farmed deer is large granulomas in mesenteric lymph nodes that are very difficult to distinguish from lesions caused by other mycobacteria (including *M bovis*, the cause of Tuberculosis*).*

3 Lesions for Disease Surveillance

3.1 Deer

The New Zealand Deer Industry has a JD surveillance program for farmed deer that is funded by Processors and managed by Johne's Management Limited (JML). The programme is based on the detection of lesions indicative of but not proven to be JD in slaughtered stock by meat inspectors at processing during routine carcass inspection.

JML maintains a confidential national database of JD-suspect lesions. Over 99% of farmed deer processed since the start of 2007 are in the database and are linked to their farm of origin (See Section 3.4 Data capture systems for further details). JML monitor and analyse the data for disease trends and use the information to identify farms with a new, high, or rising incidence of JD-suspect lesions. JML works with farmers and their veterinarians to establish Risk Management Plans (RMP's) that control JD and integrate with their broader herd health plan for optimising productivity. The database is unique in the world and has proven a valuable surveillance tool for the deer industry. JML is funded via a voluntary contribution on all slaughtered deer. It is well supported by New Zealand based research teams that provide the science which underpins the scheme[13]. It also provides these research teams with data from the database on a contractual basis. The drive to establish the JML database came in response to the emergence, rapid expansion, and severe impact JD in the deer industry. In contrast to this typical outbreak type scenario, the sheep and cattle industries experience a chronic and lower level of disease.

3.1.1 Research supporting the utility of lesion detection for surveillance in farmed New Zealand Deer

For the purpose of JML surveillance abnormal mesenteric lymph nodes (MLN) are defined as those with a circumference measurement of ≥ 55 mm and/or grossly visible pathological changes such as caseation, necrosis and/or mineralisation, while those with a circumference of <55 mm and without gross lesions are defined as normal.

In a study published in 2013 the sensitivity (Se), specificity (Sp) and level of agreement in the detection of abnormal deer MLN by official assessors (meat inspectors) for the purpose of slaughter premise surveillance was determined[14]. Four meat inspectors in two commercial deer slaughter premises (DSP) were assessed and it was concluded that inspectors diagnosed abnormal deer MLN with a high specificity, but low sensitivity. The findings support the premise that visual assessment of MLN characteristics was suitable for national surveillance for paratuberculosis in deer, while highlighting the need for training of inspectors in abnormal lymph node detection.

A 2014 JDRC/JML study to investigate the relationship between lesion rate, farmer concern about JD and perception[15] of the impact of JD concluded that "*there is a clear relationship between Johne's disease suspect lesion (JDSLN) rate and the on-farm severity of Johne's disease. Farmers with a higher JDSLN rate in their deer reported greater severity of the disease, in terms of JD related death rate and higher levels of concern about it*". The report authors note that JDSLN provides information about the status of animals at a herd-level, and further aids in the understanding of the history of a herd with JD; as the development of lesions occurs over a period of time.

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[13] For example: Sensitivity, specificity and level of agreement of meat inspector detection of abnormal lymph nodes of farmed deer (Cervus elaphus) in New Zealand. Hunnam JC, Wilson PR, Heuer C, Stringer L, Mackintosh CG. N Z Vet J. May;61(3):141-6, 2013.

[14] Sensitivity, specificity and level of agreement of meat inspector detection of abnormal lymph nodes of farmed deer (Cervus elaphus) in New Zealand. Hunnam JC, Wilson PR, Heuer C, Stringer L, Mackintosh CG. N Z Vet J. 2013 May;61(3):141-6.

[15] The JML Johne's Disease Slaughterhouse Surveillance Database: The relationship between lesion rate, farmer concern about JD and perception of its impact; prepared for Johne's Management Limited and the Johne's Disease Research Consortium. D Martin-Collado, P Fennessy and T Byrne, Abacus Bio Ltd. September 2014.

3.2 Cattle

To our knowledge there is no national control programme that has implemented routine lesion detection at slaughter as a means of surveillance for JD in cattle. However there have been a number of research trials that have included observation of lesions which indicate that lesion detection at slaughter may have utility as a surveillance tool.

A 2013 research study concluded that the detection of lesions at a slaughterhouse is a reliable diagnostic for cattle in the clinical stages of infection[16] and can detect up to 98% of those animals that are clinically infected. The technique was not as reliable for detecting sub-clinical animals as ~28% of cattle without evidence of lesions, classified as non-suspect by investigators, were found to be infected with MAP on further examination.

Other studies also report a relationship between lesions and the presence of clinical disease in cattle[17], noting that there is not always a direct correlation between the presence of disease (as determined by immunology) and clinical findings[18]. A 2009 study concluded that there is an association between lesion severity, clinical signs of JD, heavy bacterial loads in tissues, faecal shedding and the presence of MAP in diaphragm muscle in cattle[19]. A study investigating the effect of age of exposure to MAP on the development of disease, found that calves exposed at <6 months of age usually had more severe lesions than calves exposed when they were older. Similarly calves infected with a high dose of MAP had more severe lesions than those calves infected with a low dose[20].

Sampling of cattle at slaughter for histological analysis (rather than just visual inspection) was trialled as part of the Scottish Paraban Johne's control programme from 2011-2014. MAP infection was successfully detected in samples of the terminal ileum and draining lymph nodes, even from farms with a low reported incidence of ELISA positive animals. However, the sampling practice was discontinued as it was impractical to routinely collect histological samples in the commercial environment of a working processing plant.

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16 Comparison of prevalence estimation of Mycobacterium avium subsp. paratuberculosis infection by sampling slaughtered cattle with macroscopic lesions vs. systematic sampling. Elze J, Liebler-Tenorio E, Ziller M, Köhler H. Epidemiol Infect. 2013 Jul;141(7):1536-44.

17 Association of severity of enteric granulomatous inflammation with disseminated Mycobacterium avium subspecies paratuberculosis infection and antemortem test results for paratuberculosis in dairy cows. Dennis MM, Antognoli MC, Garry FB, Hirst HL, Lombard JE, Gould DH, Salman MD. Vet Microbiol. 2008 Sep 18;131(1-2):154-63.

18 Relationships between clinical signs, pathological changes and tissue distribution of Mycobacterium avium subspecies paratuberculosis in 21 cows from herds affected by Johne's disease Brady C, O'Grady D, O'Meara F, Egan J, Bassett H. Vet Rec. 2008 Feb 2;162(5):147-52.

19 Isolation of Mycobacterium avium subsp. paratuberculosis from muscle tissue of naturally infected cattle. Alonso-Hearn M, Molina E, Geijo M, Vazquez P, Sevilla I, Garrido JM, Juste RA. Foodborne Pathog Dis. 2009 May;6(4):513-8.

20 Evaluation of age-dependent susceptibility in calves infected with two doses of Mycobacterium avium subspecies paratuberculosis using pathology and tissue culture. Mortier RA, Barkema HW, Bystrom JM, Illanes O, Orsel K, Wolf R, Atkins G, De Buck J. Vet Res. 2013 Oct 7;44:94.

The consistent message in all of these reports is that cattle in the advanced stages of disease are likely to show evidence of lesions at slaughter and that lesion detection can be used to reliably detect clinical cases of JD.

It is interesting to note that surveillance of JD in cattle would need to recognise the varying rates of JD between beef and dairy cattle. It is well known that there is a much lower rate of Johne's disease in beef compared to dairy cattle. A surveillance tool should ideally differentiate between beef and cattle so that national trends could be accurately assessed.

3.3 Sheep

There are a number of reports in the literature that demonstrate that the identification of lesions at slaughter provides reliable evidence of clinical disease in sheep. Most studies have been undertaken in Australia where national and regional control programmes are targeted at eradication of the disease. As noted previously this paper was prompted by a 2013 media release[5] reporting that an Australian Meat Processor was successfully using the observation of JD-like lesions in slaughtered sheep as a surveillance tool to alert farmers to the possibility of JD in flocks. It is not known how widespread the use of lesion detection is as a means of JD surveillance in Australia.

A 2005 Australian study analysed the performance of 3 meat inspectors at slaughter to identify JD in sheep[21]. The sensitivity of abattoir surveillance ranged from 57 to 87% and was found to be 97-100% specific for identifying sheep with JD. The ability of inspectors to identify disease was higher in lines where the disease prevalence was high and improved with formal training. The authors concluded that abattoir surveillance was economic and provided a rapid assessment of the ovine Johne's disease (OJD) status of flocks and was therefore a useful ancillary for other diagnostic testing carried out as part of the Australian national monitoring programme.

Similar to other species data in the literature suggest that lesion severity increases as disease progresses[22,23]. However there is also evidence to the contrary; where researchers investigating the histological characteristics of different MAP strains[24], found that lesions induced by C-type MAP strains in lambs showed a regressive character and tended to decrease as the infection progresses, while lesions from S-type MAP strains lesions showed no difference in intensity over time. The study suggested that the strain of MAP had a strong influence over the immune and pathological responses developed by an animal.

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[21] Determining the sensitivity of abattoir surveillance for ovine Johne's disease. Bradley TL, Cannon RM. Aust Vet J. 2005 Oct;83(10):633-6.

[22] Description and classification of different types of lesion associated with natural paratuberculosis infection in sheep. Pérez V, García Marín JF, Badiola JJ. J Comp Pathol. 1996 Feb;114(2):107-122.

[23] Longitudinal study of clinicopathological features of Johne's disease in sheep naturally exposed to Mycobacterium avium subspecies paratuberculosis. Dennis MM, Reddacliff LA, Whittington RJ. Vet Pathol. 2011 May;48(3):565-75.

[24] Experimental infection of lambs with C and S-type strains of Mycobacterium avium subspecies paratuberculosis: immunological and pathological findings. Fernández M, Benavides J, Sevilla IA, Fuertes M, Castaño P, Delgado L, Marín JF, Garrido JM, Ferreras MC, Pérez V. Vet Res. 2014 Jan 16;45(1):5. doi: 10.1186/1297-9716-45-5.

It is also noted that skin quality has been used to identify OJD infected merino breed sheep at slaughter in Australia. Low condition score, carcase weight and poor skin quality were noted as signs visible at slaughter that may be attributable to OJD[25].

While the literature provides evidence that lesions are detectable in sheep at slaughter, data from New Zealand studies suggest that very few animals with observable signs of disease are likely to make it to processing works in New Zealand. A 2008-2009 study to recover DNA samples from JD infected sheep by sampling poor quality ewes at slaughter failed to identify sufficient numbers of animals for the study[26]. The conclusion from this research was that either the prevalence of JD was very low in the areas where these processing works were located (lower South Island) or that JD affected animals did not make it to the processing works. In a 2013-15 JDRC study researchers sampled, carried out post mortem examinations and recorded the number of ewe deaths on 20 properties with suspected high rates of JD in order to determine the cause and impact of ewe death[27]. Incidental data from this study suggests that sheep that progress to clinical stages of disease fade rapidly (within 2-3 months) and once clinical are usually in too poor condition to be sent for processing, and therefore do not make it to the works[28]. Researchers noted that the disease was obvious once the animal was in the clinical stages, but those in the earlier stages would be difficult to detect visually by inspection of the gut, even though there are likely to a number of these animals in affected flocks. Specific work at a number of processing plants would be required to confirm the number of affected animals that could be expected to be sent off-farm for slaughter.

3.4 Drivers for Lesion Surveillance in Cattle and Sheep in New Zealand

As noted above there is evidence that providing early warning of disease can reduce the impact of JD in herds and flocks. Disease surveillance is therefore a tool to minimise the impact of JD on farm in New Zealand. Surveillance statistics can be used by industry to monitor disease levels and by farmers and industry to identify potential disease "hot-spots" and assist in the efficient deployment of resource to bring the disease under control at the point of concern.

The following are noted as two primary drivers for reducing the impact of Johne's disease on farm in New Zealand:

- **The cost of JD to the economy both through stock losses and lost production**. The cost of JD to New Zealand sheep flocks has been estimated to be approximately $27 million annually[27]. Data for cattle is not currently available.
- **To protect the food chain and trade**. There is a potential but unproven link between MAP and human disease in susceptible individuals. MAP has been found in raw milk and the meat of infected animals[29]. While there are mitigations available there is the potential for MAP to pass into the food chain.

[25] Financial impact at slaughter of Ovine Johne's disease (OJD) in A Merino flock in Australia. Bell R, Jackson B, Links IJ. 12th ICP Parma, June 22-26, 2014
[26] Milestone 4.1.1 – Ovine JD DNA Archive, 2008-9. Allan Crawford, AgResearch
[27] Final Report (Milestone 5.3.2), JDRC Intervention Phase – Ovine Johne's disease, July 2015. Cord Heuer, Massey University and Peter Anderson, Vet Centre Marlborough.
[28] Personal Communication, Peter Anderson, Vet Centre Marlborough, July 2015
[29] Mycobacterium avium subsp. Paratuberculosis in Dairy products, Meat and drinking water. C.O.Gill, L.Saucier and W.J.Meadus. Journal of Food Protection, 2011, 74(3); 480-499.

> Should the link be proven or public perception about JD change, Johne's disease may become notifiable and has the ability to affect trade. Ensuring that systems are in place to monitor and control disease demonstrates that New Zealand is taking a proactive stance in protecting the food supply and consumers.

This preliminary assessment of lesion surveillance does not include comment from or an assessment by industry stakeholders of the wish or need for surveillance to be instigated for cattle or sheep in New Zealand. Such an assessment would be required as a necessary step should any further work be undertaken to evaluate the proposition around lesion surveillance.

3.5 Lesion Surveillance at Processing Works in New Zealand

3.5.1 AsureQuality Carcass Inspection

AsureQuality is the approved Inspection agency for Ante and Post Mortem Meat Inspection in New Zealand. The agency employs approximately 700 Official Assessors who are warranted under the Animal Products Act to carry out meat inspection across 73 meat processing plants throughout New Zealand. AsureQuality assessors are responsible for recording the presence of JD-suspect lesions at processing works in deer for JML (as discussed in section 3.1 and 3.5.2).

Assessors are currently not trained or required to look for JD-suspect lesions in the viscera of either cattle or sheep, but AsureQuality note that it might be possible to do so in the future if required[30]. For lamb and cattle the gastro-intestinal tract (GI tract) is dumped into a viscera pan and a minimal level of inspection undertaken. In lambs the spleen is examined but the gut is not manipulated, while in cattle inspectors look at the mesenteric lymph nodes. In both species it would be necessary to pick up and fan out the runner to view any lesions in the GI tract and the ability of inspectors to do this would depend on the speed the chain was operating at. Inspection systems are more rigorous in sheep where inspectors are looking for anomalies associated with sheep measles (see section 3.5.2).

The majority of Meat Processors have touch screen data collection systems at the point of inspection, which allow the inspector to tap a single button to identify any defects noted in the viscera contents.

3.5.2 JML Data Capture systems

As discussed in section 3.1 JML undertakes lesion surveillance for all deer slaughtered in New Zealand. Ovis Management Limited (OML) is responsible for managing the data capture systems for Johne's Management Limited, alongside their primary role which is the monitoring and collection of data from sheep at slaughter for sheep measles.

OML receives data regarding JDSLN status and sheep measles via monthly data downloads direct from 17 Meat Processing companies, who operate 34 plants across New Zealand. As noted above, within the plants AsureQuality Official Assessors are

[30] Personal Communication; Ira Stapp, AsureQuality, September 2015

trained to monitor and record the detection of conditions on inspection of the carcase and viscera. The OML database collates the data from Meat Processors and is searchable by farmer name or property identification. The system relies on a simple, fit for purpose piece of software designed specifically for this task. Other systems were considered for managing the data but were found to be expensive in comparison and not fit for purpose. OML have systems approved by the Office of the Privacy Commissioner in place to ensure appropriate protection of information sensitive to Meat Processors and individual farmers. These systems protect the confidentiality of the data. Processes are audited annually to ensure the system maintains the required standard of confidentiality for the industry.

OML believe that it would be a relatively straightforward process to incorporate the monitoring of sheep and/or cattle for JD into their existing data capture systems.

3.5.3 Estimated Costs

Based on the current activities of OML the following is noted regarding the costs:

- **Data collection:**
  The annual running costs for OML is $350,000. OML estimate it would cost a minimum of $56,000 to add monitoring for sheep or cattle lesion information to current systems:
    - Data capture and maintenance and making the information available to industry would cost ~$16,000 per annum
    - Monitoring and feedback to Meat Processing plants would cost ~$30,000 per annum
- **System Development:**
  The cost to develop a system for sheep or cattle is estimated to cost $100,000:
    - Set up at OML and initial training of Meat Inspectors ~$40,000
    - Set up for data capture at Meat company usually undertaken by processor at no cost to OML
    - Project Manager (estimated ~$60,000)
- **Training:**
  Meat Inspectors would require a minimal amount of training to include lesion inspection in their role (1-2 hours training and resources). Training could be done by OML/JML or AsureQuality as part of their existing annual programme for sheep measles and JD in deer. Costs would therefore be minimal and limited to paying for the trainer's time and travel expenses (estimated at ~$10,000).
- **Data Analysis:**
  The implementation of a data analysis system is optional and would be dependent on the level of monitoring and feedback required by industry. For example JML provides a high level of data analysis for the deer industry and a comprehensive industry, farmer and veterinary engagement programme at an annual cost of $350,000 per annum.

4 Conclusions: Monitoring of Stock in New Zealand

The information reviewed suggests that there is sufficient data to support the premise that visual inspection of the intestines and lymph nodes of animals at slaughter can be used as a means to monitor for (primarily) clinical Johne's disease in both sheep and cattle, as it does in deer. Animals in advanced stages of disease are highly likely to have macroscopic lesions that provide visible evidence of the disease at slaughter.

We conclude, based on the information reviewed, that while lesion detection at slaughter may have utility as a low level surveillance tool, further information is required regarding the frequency that lesions are seen in cattle or sheep at slaughter in New Zealand. JDRC studies suggest that very few sheep with visual signs of disease are sent for slaughter at a processing plant in New Zealand, and no-one has assessed the frequency with which lesions are observed in cattle.

The following is also noted:

- It is not possible to reliably detect lesions and therefore evidence of Johne's disease in stock in the early stages of MAP infection. The value of lesion detection is in the identification of clinical animals, which signals there may be a problem within that herd or flock. A programme or system is then required to act on that information to follow up on those herds or flocks.
- While lesions are highly indicative of Johne's disease, immunological or histological methods are required to confirm the presence of MAP and therefore a diagnosis of Johne's disease. A lesion surveillance programme is therefore best tailored to report "suspect" cases of JD to avoid the additional time and expense required to confirm the presence of disease.
- Studies at abattoirs and JML experience suggests that meat inspectors can find stock with JD like lesions, but that inspectors need to be trained to recognise the disease.

4.1 Issues for Consideration

When considering the practicalities of implementing lesion identification as a surveillance tool for sheep and cattle in New Zealand the following issues need to be considered:

- Does the prevalence/impact of JD in NZ support the need for surveillance?

  Industry needs to make an assessment of whether disease surveillance would be of benefit to New Zealand.

  The prevalence and impact of clinical Johne's disease in New Zealand is low and the bacteria endemic. There is a possibility that changing farm practices, poor farm management, or inattention to Johne's risk factors could lead to higher rates of disease.

  There is benefit, from an international trade perspective, for New Zealand to have proactive systems in place to manage any risk (either real or perceived) from JD. If an effective methodology could be introduced to provide early warning of issues, this would help maintain or reduce the low level of disease *in New Zealand.*

- What would be the objective of undertaking surveillance?

  Should surveillance be considered then Industry would need to define the objective of any surveillance programme, which may have a range of uses, for example:
    - *To provide a means to notify farmers of potential issues in their stock which may need to be followed up with specific testing or professional advice*
    - *To allow industry to collect data and monitor the incidence of JD in New Zealand*

  Defining the objective is important to determine what level of monitoring and support would be required for surveillance.

- What alternative forms of surveillance could be used?

  Are there more effective methods of surveillance that could be implemented that would achieve the objective proposed?

- What monitoring would be required to support the proposed objective and what systems would be needed to do so? Could the system be developed that was practical, cost-effective and fit-for-purpose?

  As noted above the objective chosen would determine the level of monitoring and support required for the surveillance. Discussions with OML suggest that a practical, low-cost, fit-for-purpose monitoring of stock for JD via lesions could be modelled on existing systems for sheep measles and JD in deer that have established inspection and data capture systems.

- What research/preparation work would be needed before implementing lesion surveillance?

  Assessment of the need to implement disease surveillance. Does industry want a means to monitor disease levels in New Zealand? Does the prevalence and economic impact justify implementation?

  Understanding what the rate of JD-like lesions in sheep and cattle are and whether they are indicative of on-farm disease problems.
    - *As noted above, there are doubts that clinically affected sheep, which are those most likely to have gross lesions, would be sent to the works for processing as it is thought that most are culled on farm. A small study, across a number of abattoirs, recording how many animals were detected with lesions at slaughter would be useful to confirm these observations.*
    - *Furthermore if lesion detection for cattle were to be considered, a pilot study would also be recommended as necessary to confirm the utility of lesion detection in cattle as a surveillance tool, to provide New Zealand based data on which a surveillance tool could be designed.*

- What research would be needed to provide ongoing support for lesion surveillance?

  Ongoing support work for a surveillance tool would depend on the level of monitoring and validation required to achieve the proposed objective for monitoring stock.

Attachment 1: Global Control Programmes – Comparison table
September 2014

Country	Species	Approach	Farmer participation	Aim	Goals	Testing[31]	Herd Classification	Components of Programmes[32,33]
Australia[34]	Cattle (BJD) Sheep (OJD)	Nationally coordinated	Voluntary but legal requirements imposed	NBJDSP - To minimise contamination of farms/farm products and to protect non-infected herds while minimising disruption to trade and the social, economic & trade impact of BJD at herd, regional & national level	Food quality Assurance, Protecting areas/herds, Reducing impact infection	BJD: Serum Elisa screening of >2yr olds; FC of individuals or histology follow up of positives. Environmental samples for maintenance		National JD Control programmes (strategic plans) for BJD and OJD - Cooperative programme of livestock industries, government and veterinary professionals - Managed by Animal Health Australia. - Provides government support & compensation for farmers - Separate management of OJD & BJD via Market assurance based programmes SheepMAP & ▪ Audits and certifies herds/flocks for purchase decisions based on multiple herd/flock sco ▪ Prevalence based geographic zoning ▪ Legally required movement controls ▪ Technical notes for best practice ▪ Additional BJD control activities within endemically infected states - Vaccination used for control (OJD -Gudair®) - Some states monitor OJD at abattoirs
Canada[34]	Dairy Cattle	Multiple provincial programmes	Voluntary	CVJDCP - To reduce prevalence, reduce impact on animal health and economics, to reduce or eliminate MAP in milk, beef cattle & the environment & provide certification of low risk BJD herds	Reducing # infected herds, Accreditation low risk herds, Reducing impact infection	CVJDCP – proposed Milk/pooled serum Elisa ID screening of >2yr olds. PFC for screening in some provinces		Two major programmes – Canadian JD initiative (2006) and Canadian Voluntary JD prevention (CVJDCP, 2006) Producer driven Programmes; largely independent of each other Have attempted national coordination with little success All are management based systems with four major components - Education - Annual risk assessments - Optional testing with some compensation - Permanent removal for all high positive cows with some compensation
Denmark[34]	Dairy Cattle	Single national Programme (Operation Paratuberculosis)	Voluntary	To provide tools to dairy farmers that wish to control BJD to reduce the overall prevalence of BJD	Reducing # infected herds, Tools for producers, Accreditation low risk herds, Eradication	Quarterly milk ELISA screen of all milking cows.		Programme coordinated by Knowledge centre for Paratuberculosis - Standard educational and on-farm material - Quarterly individual animal testing and herd classification - Training of herd advisors - Risk management plans - Voluntary removal of test positive animals - High test frequency Milk Elisa
France	Dairy Cattle	Two integrated programmes	Voluntary			Not known		Two integrated programmes; one that controls JD in infected herds and a monitoring programm - Herd testing - Monitoring and certification of test negative herds
Germany	Dairy Cattle	No Programme	Notifiable disease			Not known		No control programme Guidance available for control of JD is issued by the federal government

[31] ELISA – enzyme-linked immunosorbent assay; PCR – Polymerase Chain Reaction; FC – Faecal Culture; PFC – pooled Faecal Culture
[32] JD – Johne's disease; BJD – Bovine Johne's disease; OJD – Ovine Johne's disease
1 [33] Paratuberculosis: Organism, Disease, Control. Edited by Marcel A. Behr, Desmond M. Collins

[34] A review of bovine Johne's disease control activities in 6 endemically infected countries. Geraghty T. et al. Preventative Veterinary Medicine, 116, 1-11, 2014.

Country	Species	Approach	Farmer participation	Aim	Goals	Testing[31]	Herd Classification	Components of Programmes[32,33]
Ireland	Dairy Cattle	Single national programme	Voluntary			Not known		National voluntary control programme for JD run by Animal health Ireland - Veterinary on-farm Risk Assessment and Management planning (RAMP)[35] - Animal hygiene, focus on calving, culling of test positive animals - Herd sampling
Italy	Dairy Cattle	Unknown	Voluntary			Not known		Focus on farmer education On-line Risk Management and management planning tool available
The Netherlands[34]	Dairy Cattle	Intensive Paratuberculosis Programme (IPP, 1998) and Milk quality assurance programme (MQAP, 2006)	Required for delivery of milk to processors	IPP - To enable low risk trade of cattle between herds and to facilitate eradication of PTB from known infected herds MQAP – to reduce the concentration of MPA in milk delivered to processing units	Food quality Assurance, Protecting areas/herds, Reducing # infected herds, Accreditation low risk herds, Reducing impact infection, Eradication	IPP: serum ELISA >3yr, FC or PCR >2yr as screens MQAP: as IPP or milk ELISA of all milk animals annually, FC or PCR to follow positive ELISA		Main goal of programme to reduce shedding which will impact herd transmission and the food c - Dairy Producer participation requirement of terms of delivery to dairy processors (2010). Mo take milk from test positive herds - Assignment of test statuses to herds - Generous subsidies by government - Testing and management of test positive animals - Low test frequency and options to investigate positive test results
United Kingdom[34]	Dairy Cattle	Multiple independent initiatives	Voluntary	Certification: to provide a graded system of accreditation which enables herds to maintain or move to clear herd tests Controls: Implement a control programme to reduce the detrimental effects on herd productivity	Tools for producers, Accreditation low risk herds, Reducing impact infection	Milk serum ELISA quarterly for >2 years old. FC/PCR on individuals for screening and following positive ELISA		Multiple groups look at herd certification and control guidelines run by breed societies and labor Cattle Health certification Standards operating to common standard (Cattle Health Certification schemes) Multiple engagement programmes that aim to increase awareness and uptake of controls run b organisations , vet practices, animal health and milk processing companies e.g Healthy Herd P encompassing all diseases on farm: - Full herd health assessments - Milk yield as trigger for JD testing - Engagement with farmers and vets Some milk retailers require regular testing of supplier herds Development of web based economic health evaluator Scotland - Paraban project (2010-13) to determine cost effective approaches and optimise cont beef and dairy farms, components include testing, slaughterhouse monitoring (trial only), mana
United States[34]	Cattle	State programmes adhering to minimum standards	Voluntary	To provide national standards for the control of BJD and to reduce prevalence, impact and risk of introducing BJD to non-infected herds	Reducing # infected herds, Reducing impact infection	Milk ELISA and serum ELISA, PFC/PCR females >36 months and males>24 months for screening. FC or TC on individuals for screening and following positive ELISA. FC/PCR Environmental samples for maintenance		Voluntary Bovine Johne's disease control program. Incorporates "Uniform Program standards" minimum standards (2002). Program has three components: Education, Management and her - State by state management may differ slightly. Statewide Johne's coordinators available - Level of producer engagement increases from education, through to management and finally without herd classification - Johne's certified veterinarians or animal health officials develop RAMP (risk assessment and each farm which must be reviewed every 3 years to remain in program - Focus on calving and animal hygiene, removal of test positive animals - Some Government support available

[35] http://www.animalhealthireland.ie/page.php?id=30
[36] http://www.johnesdisease.org/Educational%20Material/Educational%20Materials/Brochure%20PDFs/Voluntary%20Bovine%20Control.pdf