# final report

Project code: A.MFS.0152

Prepared by: Dr Roland Cobbold

UniQuest Pty Ltd

Date submitted: June 2009

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

## Bovine Lymph Node Microbiological Survey

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

# Abstract

Incision of specified lymph nodes is routinely performed for all cattle slaughtered in Australia. With the eradication of bovine tuberculosis, this practice requires reassessment. Lymph nodes may be a source of contamination of the carcase, but no microbiological data for cattle nodes exists for Australia. Lymph nodes from cattle at post-mortem inspection were therefore tested. A substantial proportion of nodes contained high levels of bacteria, with indications of presence of food-borne pathogens. There was substantial variation in levels of contamination, associated with a number of factors. The value of routine lymph node incision needs to be considered in terms of risks associated with microbiological cross-contamination, processing efficiencies, and animal disease surveillance requirements. Further studies on the role of bovine lymph nodes as risks to beef safety will enhance the development of evidence-based, effective, and efficient post-mortem inspection procedures.

# Executive summary

Lymph node examination has played a valuable role in the diagnosis and eventual eradication of bovine tuberculosis (bTB) in cattle at slaughter, and apart from animal disease surveillance has other functions in meat quality and safety assurance during post-mortem inspection. However, lymph nodes represent microbiological filters and may therefore be a source of contamination of the carcase during inspection. Processes for meat hygiene inspection and post-mortem examination should be re-evaluated periodically for them to remain responsive to changing foodborne pathogen risks, animal disease surveillance and export certification requirements, as well as for improving the efficiency and sustainability of beef processing. As Australia continues to advance its bTB surveillance from a detection and eradication to a proof of freedom mode, revision of the value of bovine lymph node incision at current rates is justified. In order to effectively determine the benefits and risks of lymph node incision, potential hazards associated with them need to be better evaluated. Although studies on cattle in other countries and for other livestock production systems in Australia indicate that lymph nodes can be a source of carcase contamination, data on bovine lymph node microbiological risks are absent.
The overall aim of this project was to assess the degree of bacteriological contamination of bovine lymph nodes at slaughter with respect to public health and beef marketability. Objectives were to:

1. Collect lymph nodes from a targeted subpopulation of slaughter cattle that offer a higher risk for carriage of pathogens of public health significance.
2. Assess the microbiological safety of these nodes with respect to:
    a. Detection of pathogens of major food safety and veterinary public health importance.
    b. Quantitative assessment of the degree of contamination.
3. Use this data to derive conclusions on the value of discontinuing unnecessary incision of lymph nodes.

Seven abattoirs provided samples representing a total of 5340 lymph nodes, collected over 1068 node pools, from 534 cattle. Sampling was conducted strategically across cattle lots, cattle types and dates so as to optimise representation across processing and production environments. Cull cattle, calves, and feedlot cattle were targeted, however a wide variety of cattle types were included among samples sent. Major head and chest lymph nodes were excised, without being incised, from carcases at post-mortem inspection. Node pools were sent for microbiological processing following surface sterilisation to remove bacteria associated with their collection. *E. coli* and aerobic plate counts (APC) were performed. Enriched node tissues were subjected to standardised and highly sensitive detection methods aimed at the key beef foodborne pathogens, *Salmonella* and Shiga-toxigenic *E. coli* (STEC), with targeted isolation of *E. coli* O157:H7. Descriptive statistics relating to the prevalence and levels of indicator organisms and prevalences of foodborne pathogen contamination were generated. Appropriate statistical tests were applied to compare various factors' associations with lymph node contamination levels.
Data indicated that substantial proportions of lymph nodes were contaminated with bacteria, with all cattle sampled demonstrating infected nodes. These bacteria can exist at considerable levels, with the average APC being approximately 3700 cells/g of node tissue. Included among these bacteria were enteric indicator organisms, which were found at high (averaging approximately 570 cells/g) counts among positive samples. 64% of samples had *E. coli* counts >100 cells/g, and the maximum recorded count was in the tens of millions. STEC and *E. coli* O157:H7 were identified among node pools, although *Salmonella* was not. The prevalence and counts of bacteria in lymph nodes varied depending on date sampled, with peaks evident in summer. Prevalences and counts also varied

significantly between abattoirs, with cattle associated factors (age, production type, etc.) most likely to influence levels of contamination of nodes at carcase dressing. The proportion of

samples positive for *E. coli* and APC collected from cattle head nodes was significantly higher than that for thoracic nodes. *E. coli* counts, APCs, and STEC prevalence were not significantly different for head or thoracic nodes although most of the tested parameters were correlated by anatomical collection site such that presence of bacteria in one type of node was predictive of presence in the other.

In conclusion, the majority of bovine lymph nodes pose some degree of bacteriological risk to the carcase during inspection procedures that particularly involve incision of nodes. This level of risk may be considerably high, based on typical total bacterial counts seen, as well as the proportion of nodes positive for enteric bacterial indicators and the counts for these. Increased levels of contamination with spoilage bacteria may reduce shelf life and other beef quality parameters. Detection of STEC and *E. coli* O157:H7 confirm that pathogens are present in lymph nodes, and that there is potential for their transfer to beef whenever lymph nodes are handled. Lymph nodes in cattle should be considered high risk points of carcase contamination with bacteria. This needs to be factored in to inspection and dressing procedures occurring in plants currently. But perhaps more importantly, any future revisions of food safety and quality assurance programs and meat inspection processes and regulations should carefully assess how many nodes need to be incised and how reductions in node incision without compromising other components of inspection can be accommodated. Further studies examining factors influencing bacterial contamination of bovine lymph nodes and how these impact food quality and safety are warranted.

# Contents

# Background

## 1.1 Role of Lymph Nodes in Meat Inspection

Examination of lymph nodes during post-mortem inspection of livestock has always been an important component of determining suitability of meat for human consumption. Lymph nodes represent biological filters which trap microbes emanating from local catchment areas, thus limiting their systemic distribution and enhancing the immunological response to infection. The presence of enlarged, inflamed, or abscessated lymph nodes can indicate the presence of infection. Lymph node inspection is also carried out to detect metastatic tumours. Nodes are classically examined organoleptically: i.e. visually, via palpation, and via incision to inspect internal node consistency. Suspect nodes are also excised and referred for more detailed histopathological or microbiological examination where indicated.

Lymph node examination plays a specific role in the diagnosis of bovine tuberculosis (bTB) in cattle at slaughter. Select nodes in the head and thorax are examined in detail for evidence of granulomatous changes associated with bTB. Such examination was a critical component of the Australian Brucellosis and bTB Eradication Campaign (BTEC), which culminated in Australia's designation as bTB free in 1997[1]. Since then, bTB programs (represented by the bTB Freedom Assurance Programs I and II, and on to the current Australian bTB Surveillance Project) progressively moved from a bTB granuloma detection and response mode to a surveillance for bTB freedom mode. Each of these programs was underwritten by the National Granuloma Submission Program (NGSP) which provided the logistic basis for lymph node testing.

## 1.2 Potential Hazards Associated with Lymph Nodes

Because of their microbial filtration function, lymph nodes can be a significant focal point for commensal bacteria and pathogens in the carcase of infected animals. A number of bacterial pathogens of public health significance, including *Mycobacterium avium* subsp. *paratuberculosis*[2], *Rhodococcus equi*[3], and *Salmonella*[4] have been isolated from bovine lymph nodes. Counts of indicator organisms offer an estimate of the degree of contamination or infection, with aerobic plate counts (APCs) indicating the overall bacteriological burden, and *E. coli* counts indicating levels of contamination with enteric pathogens, which constitute the majority of organisms of food-borne significance. *Salmonella* and Shiga-toxigenic *E. coli* (STEC), particularly the O157:H7 serotype, represent the principal pathogens of concern to the beef industry. Studies in the USA have determined that bovine lymph nodes demonstrated APC and *E. coli* counts representative of significant levels of contamination[5, 6]. No such studies exist for cattle in Australia. However, similar studies in swine indicate that bacterial contamination of nodes (both normal and diseased) is substantial and that incisional inspection of nodes is unlikely to be significantly contributing to food safety assurance[7, 8].

# 2 Project Rationale and Objectives

## 2.1 Project Rationale

Processes for meat hygiene inspection and post-mortem examination should be re-evaluated periodically in order to remain responsive to changing foodborne pathogen risks, animal disease surveillance requirements, and export certification requirements. Additionally, commercial and logistical pressures demand continual operational review and refinement of the inspection process without compromising quality and safety assurance. In light of Australia's status of freedom from bovine tuberculosis, a review of lymph node incision practices is warranted. Beyond their value in bTB surveillance, excessive incision of bovine lymph nodes may be detrimental to beef safety and quality as a result of promoting cross-contamination by node-borne pathogens or spoilage organisms to the rest of the carcase during inspection. Data on typical levels of bacterial contamination of bovine lymph nodes does not exist for Australia.

## 2.2 Project Aim and Objectives

The overall aim of this project was to assess the degree of bacteriological contamination of bovine lymph nodes at slaughter with respect to public health and beef marketability. Objectives are to:

1. Collect lymph nodes from a targeted subpopulation of slaughter cattle that offer a higher risk of carriage of pathogens of public health significance.
2. Assess the microbiological safety of these nodes with respect to:
    a. Detection of pathogens of major food safety and veterinary public health importance.
    b. Quantitative assessment of the degree of contamination.
3. Use this data in conjunction with other data to derive conclusions on the value of discontinuing unnecessary incision of lymph nodes.

# 3 Methods

## 3.1 Sample Collection

Seven abattoirs were enrolled to provide samples. Abattoirs comprised both export registered and domestic plants, and were located in Queensland (5), NSW (1), and Victoria (1). A total of 534 cattle were sampled across the abattoirs, with a maximum of 100 cattle sampled from any one plant. The sampling plan requested samples from two cattle per lot, for five lots per day, over 10 sampling days. Sampling was performed generally over five weeks (i.e. two sampling days per week) at each abattoir, although many plants supplied samples opportunistically. The total sampling period ran from September 2008 to June 2009. Cull cattle, calves, and feedlot cattle were targeted for sampling among lots, however a wide variety of cattle types were included among samples sent.

Lymph nodes were excised, without being incised, from carcases at post-mortem inspection by AQIS inspectors and/or on plant veterinarians or company quality assurance staff. Parotid, sub-maxillary and retropharyngeal nodes were collected from each animal and added together into sterile sample bags to represent a Head node pool. Bronchial and mediastinal nodes from respective cattle were similarly collected into a Thoracic node pool. Therefore, for each animal, two pools comprising five nodes were collected, totalling 1068 pools representing 5340 nodes. Sample bags were shipped to the laboratory at 4°C within 24 hours of collection.

### 3.2 Laboratory Methods

Received nodes, maintained in pools, were surface sterilised using an open flame and the parenchyma shelled out aseptically into stomacher bags. Buffered peptone water (BPW) was added to weighed node pools to create approximately 1:10 (w/v) dilutions, and stomached. Appropriate decimal dilutions of homogenates were inoculated onto *E. coli* and Total Aerobic Petrifilm™ plates in duplicate for *E. coli* and aerobic plate counts (APC), respectively. Coliform counts were also recorded from Petrifilm™ plates, but will not form the basis of reported results. Node homogenate was pre-enriched in BPW overnight and inoculated into each of tetrathionate broth, Rappaport-Vassiliadis (RV) broth, and mTSB broth. Enrichments from tetrathionate and RV broths were each inoculated onto xylose lysine desoxycholate (XLD) and brilliant green agar plates. Following overnight culture, suspect *Salmonella* colonies from each plate were subjected to standard biochemical testing (lysine, urease, triple sugar iron agar) for *Salmonella* confirmation. Original samples (stored at 4°C) positive for *Salmonella* were used to determine *Salmonella* counts using decimal dilution plate counts as per the detection methods. Immunomagnetic separation using O157 Dynabeads™ (Dynal) was applied to mTSB broths to concentrate target *E. coli* O157:H7 cells, which were isolated by plating onto sorbitol MacConkey (SMAC) agar plates. Suspect *E. coli* O157:H7 colonies were subjected to latex agglutination testing and PCR[9] to confirm their status as Shiga-toxigenic *E. coli* O157:H7. Enriched broths were also screened by PCR for genes[9] to determine the potential presence of other strains of STEC.

### 3.3 Data Analysis

Data were analysed using Stata statistical software. Chi square and McNemars tests were used for assessing the contribution of key variables to prevalence outcomes, and for comparing prevalence data. As count data demonstrated a non-normal distribution, non-parametric statistical tests were used for comparative analyses: Wilcoxon signed rank tests were used for pairwise comparison of counts, Kruskal-Wallis tests were used for assessing the significance of variable effects on counts. Correlations of counts were performed using Spearman's rank test. Confidence intervals are provided for means cited, with those for proportions being exact binomial intervals. Unless otherwise indicated, statistical significance is inferred by *P* values of less than 0.05.

# 4 Results and Discussion

### 4.1 Levels of Indicator Organisms in Bovine Lymph Nodes

#### 4.1.1 Total Bacterial Counts

Details for bacterial counts are provided in Table 1. Overall, 97 (95 – 98) % of bovine lymph node pools sampled from 100% of cattle demonstrated the presence of culturable bacteria. Among positive samples, the mean bacterial APC was 3.57 (3.48 – 3.66) $\log_{10}$ CFU (colony forming units)/g. A high proportion (60%) of samples had mean APC counts > 1000 CFU/g, and the maximum count recorded was 9.10 $\log_{10}$ CFU/g. Among the aerobic bacteria isolated, the most commonly identified included (in order of frequency of isolation): *E. coli*, coagulase-negative *Staphylococci*, *Proteus* spp., *Streptococcus* spp., *Enterobacter* spp., Pseudomonads, *Bacillus* spp., environmental gram negative bacilli, and *Staphylococcus aureus*.

### 4.1.2 *E. coli* Counts

*E. coli* was detected in 57 (54 – 60) % of samples, with 75 (71 – 80) % of cattle sampled having *E. coli* present in lymph nodes. Of samples positive, the mean *E. coli* count was 2.76 (2.65 – 2.87) $\log_{10}$ CFU/g. 64% of samples had *E. coli* counts > 100 CFU/g, and the maximum recorded count was 7.59 $\log_{10}$ CFU/g. There was a high degree of correlation between *E. coli* counts and APCs (Spearman's rho 0.7003; $P < 0.001$). Figure 1 demonstrates the distribution of counts for *E. coli* and total aerobic bacteria. Low numbers of identifiable counts at and below the $10^1$ CFU/g level of contamination are likely to reflect the limit of detection of the cultural methods used.

## 4.2 Presence of Pathogens in Bovine Lymph Nodes

### 4.2.1 Shiga-toxigenic *E. coli* (STEC).

Based on detection of Shiga toxin genes by PCR, 3.8 (2.8 – 5.2) % of samples, representing 7.3 (0 – 16) % of cattle, had evidence of STEC present. Of samples positive for STEC by PCR, 3/39 (representing 0.3% of samples and 0.6% of cattle) possessed the full complement of virulence genes that are indicative of human pathogenicity. Two *E. coli* O157:H7 strains were isolated from lymph node pools representing different cattle from different abattoirs. This represents a 0.2 (0.02 – 0.7) % sample prevalence, and a 0.4% cattle prevalence for *E. coli* O157:H7 in lymph nodes. Both *E. coli* O157:H7 isolates possessed the typical virulence markers for this pathogen. The prevalence of STEC tended to increase as *E. coli* count increased, with a maximum STEC prevalence of 8.1% being evident among samples with *E. coli* counts in the $10^3$ – $10^4$ $\log_{10}$ CFU/g range (Figure 2). However, there was no correlation between STEC prevalence and *E. coli* count, as STEC were not isolated from the higher end count samples. PCR evidence of STEC occurred in nodes where no *E. coli* were isolated, indicating the presence of dead or unculturable STEC cells in some lymph nodes.

### 4.2.2 Salmonella

Salmonella was not isolated from any lymph node samples. Based on sample size calculations[10], it is estimated (with 95% confidence) that should *Salmonella* have been present but undetected, the *Salmonella* prevalence would be less than 0.56%.

## 4.3 Variables Influencing Lymph Node Contamination

### 4.3.1 Abattoir Sampled

The proportion of samples positive for aerobic bacteria, total plate counts*, E. coli* counts and the proportion of samples positive for *E. coli* all varied significantly depending on the abattoir providing samples (Table 2). This is most likely related to cattle factors such as age, production type, geographic origin, time in transport/lairage which vary with the different sources of cattle to different plants. It may reflect variable hygiene standards between plants, but only if significant levels of external contamination of nodes was occurring and this variability was not negated by the surface sterilisation of nodes during sample processing.

### 4.3.2 Date of Sampling

The proportion of samples positive for aerobic bacteria, total plate counts*, E. coli* counts and the proportion of samples positive for *E. coli* all varied significantly based on different sampling dates. *E. coli* count, APC and the proportion of samples positive for *E. coli* followed a temporal trend, with peaks evident in summer (Figure 3). This is likely to represent different bacteriological

burdens of cattle presented for slaughter due to variable exposure factors on farm or in lairage relating to climatic effects.
STEC prevalence did not differ significantly between plants sampled, nor between dates of sampling. This is likely to be a result of the relatively small number of STEC positive samples.

### 4.3.3 Lymph Node Site

The proportion of lymph node pools positive for *E. coli* and APC collected from cattle heads was significantly higher than that for thoracic nodes. *E. coli* and aerobic bacterial positivity rates were also correlated (Chi square P < 0.001, odds ratios of 2.3 and 3.7, respectively) such that presence of these bacteria in one set of nodes was predictive of presence in the other nodes. *E. coli* counts, APCs, and STEC prevalence were not significantly different for head or thoracic nodes. APCs between sites were, however, significantly correlated (Spearman's rho 0.34; *P* < 0.001).

# 5 Success in Achieving Objectives

## 5.1 Objective 1

*Collect lymph nodes from a targeted subpopulation of slaughter cattle that offer a higher risk for carriage of pathogens of public health significance.*

This objective was completed, although challenges were faced. Unanticipated compliance, logistical and commercial pressures created some delays in enrolment of sampling abattoirs, and in reliable supply of samples by participating plants. Enrolled plants were over-represented by lower throughput operations where sample collection had less impact on staff resourcing and chain disruption. Where possible, collection of further data on lymph node contamination from more diverse cattle populations and from a wider range of slaughter operations would be beneficial. Although the targeted high risk cattle were sampled, the final range of cattle for which samples were submitted and the sampling plan design suggests that results are reasonably representative of the general cattle population.

## 5.2 Objective 2

*Assess the microbiological safety of these nodes with respect to:*

*a. Detection of pathogens of major food safety and veterinary public health importance.*

*b. Quantitative assessment of the degree of contamination.*

This objective was successfully completed. All samples were subjected to pathogen screening using the most sensitive isolation methods available. *E. coli* counts and APCs were readily estimated from a large proportion of samples, providing valuable quantitative data for use as baseline information for on-going studies or within formal risk assessments. *Salmonella* counts were not derived as no samples were identified as being *Salmonella* positive. Results obtained did not differ substantially from those expected, suggesting no major anomalies in approaches or technical methodology. Data obtained was appropriate to deriving applied conclusions and recommendations.

## 5.3 Objective 3

*Use this data in conjunction with other data to derive conclusions on the value of discontinuing unnecessary incision of lymph nodes.*

This objective was successfully completed. Data obtained represent a valuable resource for the beef industry, and provide the basis for conclusions and recommendations of practical significance to the processing sector.

# 6 Impact on the Meat and Livestock Industry

## 6.1 Impact on the Meat and Livestock Industry – now

Results indicate that bovine lymph nodes have substantial levels of bacterial contamination. APCs determined within the current study are similar to those from the USA[5], although *E. coli* positivity rates and counts were higher in the current study. All of the major parameters measured (APC, *E. coli* positive rates, *E. coli* counts) for lymph nodes in the current study were substantially higher than those for Australian beef carcases and frozen beef[11]. High levels of bacterial prevalence and counts for lymph nodes should not be surprising, considering their biological function. The higher bacterial loads, and specifically the substantially high levels of enteric bacteria found in lymph nodes, suggest that they represent a significant potential source of bacterial contamination of carcasses. Improvements in the microbiological quality of beef carcases and boxed beef over the last 15 years[11] suggest that operational hygiene and quality assurance systems are working to reduce levels of beef contamination. However, reducing initial loads of bacterial contamination, such as from lymph nodes, remains a key area of control. Pre-slaughter interventions are more likely to be important in reducing lymph node bacterial levels, and therefore risks of carcase cross contamination.
The presence of STEC, and *E. coli* O157:H7 specifically, within bovine lymph nodes indicates their potential for transfer to the carcase during inspection procedures, particularly with routine incision of nodes. STEC and *E. coli* O157:H7 prevalences in Australian carcase and meat surveys reflect this potential[11]. STEC have been identified in bovine lymph nodes previously[12], with their isolation being associated with intestinal carriage in cattle. The current identification of STEC in head and thoracic nodes, as opposed to tonsils and mesenteric lymph nodes[12], is significant as it indicates STEC migration through the systemic circulation beyond the immediate enteric drainage system. *Salmonella* were not identified among bovine lymph nodes in the current study. However, based on typical Australian carcase and boxed beef prevalences[11] and detection rates for *Salmonella* in similar studies performed overseas[6], in earlier Australian studies[4], and in other production forms in Australia[7], it is likely that bovine lymph nodes routinely harbour *Salmonella*.

## 6.2 Impact on the Meat and Livestock Industry – in five years time

Current meat inspection practices require the incision of the parotid, submaxillary, retropharyngeal, bronchial and mediastinal lymph nodes[13]. For as long as this remains industry practice, the carcase contamination potential of lymph nodes needs to be considered. This contamination has two major implications: reduced meat quality through greater levels of spoilage organisms being deposited[6], and increased risk of carcase contamination with foodborne pathogens. Operational procedures, hygiene practices and quality assurance monitoring should be revised such that lymph nodes are treated as are other high risk contamination sources such as the hide and gastrointestinal tract. For instance, it may be advisable that knives and other equipment coming into contact with incised nodes be decontaminated (e.g. by dipping in 82°C water) prior to use on other areas of the carcase, that lymph nodes be removed from the carcase prior to incision, or that some other form of logistic processing be implemented that separates handling of lymph nodes from handling of other

carcase components. However, practical, logistical and resourcing restrictions are likely to limit such operational modifications.

Better use of the current findings will come from consideration of lymph node contamination potential in revising regulatory requirements. The best option for reducing cross-contamination from lymph nodes is to restrict their incision. The primary incentive for routine incision of lymph nodes in cattle is for bTB surveillance. Australia has been formally declared bTB free for 12 years[1]. Some continuing degree of lymph node inspection is necessary to ensure proof of freedom. However, based on both microbiological contamination risks and the continual incentive within industry to refine the inspection process for logistical and economic reasons, lymph node inspection practices are a good target for revision. This may take the form of incising only a proportion of nodes inspected, switching to visual or palpation based methods of inspection, or a combination of both.

Findings in the current study relating to factors that appear to influence lymph node contamination levels are relevant. Data suggest that avoidance of head node incision may have a greater impact on protecting meat hygiene, as compared to thoracic nodes, and may not significantly reduce bTB diagnostic potential. Temporal trends for lymph node bacteriological levels are likely to reflect climatic effects on incoming node bacterial loads. Between-abattoir variation similarly likely reflects plant to plant variation in the cattle being sourced for slaughter, with cattle factors such as age, geographic origin, production type likely to contribute to such variation, although controllable factors such as time in transport, stress reduction, and lairage operations may influence lymph node bacterial loads. Further data on cattle that contributed the lymph node samples based on NLIS codes and processing logs is currently being assessed. Further studies examining contamination risks associated with lymph nodes are warranted in order to develop evidence-based and needs-based approaches to regulatory and operational practices.

# 7 Conclusions and Recommendations

## 7.1 Conclusions

1. The majority of lymph nodes in cattle at slaughter have some degree of bacteriological contamination.
2. Levels of contamination can be substantially high, and lymph nodes may be sources of spoilage organisms and other microbes that will compromise the quality of beef.
3. Enteric bacterial loads can also be substantial, and pathogens such as Shiga-toxigenic *E. coli* are evident in bovine lymph nodes. Contamination of the carcase with foodborne hazards following handling of nodes is therefore probable.
4. Rates and levels of contamination are highly variable, with factors such as date, anatomical node site, and abattoir potentially influencing potential node-borne hazard risks.

## 7.2 Recommendations

1. Lymph nodes in cattle should be considered high risk points of carcase contamination with bacteria, including foodborne pathogens.
2. On-going incision of nodes as part of inspection and animal health surveillance procedures should either be:
    a. more effectively justified, and appropriate contamination risk mitigation strategies introduced into processing practices involving nodes
    b. more carefully considered as part of any future revisions to programs and regulations involving food safety and quality assurance, meat inspection processes, and animal disease surveillance. Specifically, consideration of

reductions in the number of nodes to be incised or alternatives to incision would be appropriate.

3. Further studies examining factors influencing bacterial contamination of bovine lymph nodes and how these impact food quality and safety are warranted.

# 8 Bibliography

1. Radunz, B., 2006. Surveillance and risk management during the latter stages of eradication: experiences from Australia. Vet. Microbiol. 112 (2-4): 283-290.

2. McKenna, S. L.; Keefe, G. P.; Barkema, H. W.; McClure, J.; Vanleeuwen, J. A.; Hanna, P. and Sockett, D. C., 2004. Cow-level prevalence of paratuberculosis in culled dairy cows in Atlantic Canada and Maine. J. Dairy Sci. 87 (11): 3770-3777.

3. Flynn, O.; Quigley, F.; Costello, E.; O'Grady, D.; Gogarty, A.; Mc Guirk, J. and Takai, S., 2001. Virulence-associated protein characterisation of *Rhodococcus equi* isolated from bovine lymph nodes. Vet. Microbiol. 78 (3): 221-228.

4. Samuel, J. L.; O'Boyle, D. A.; Mathers, W. J. and Frost, A. J., 1980. Isolation of *Salmonella* from mesenteric lymph nodes of healthy cattle at slaughter. Res. Vet. Sci. 28 (2): 238-241.

5. Sofos, J. N.; Kochevar, S. L.; Bellinger, G. R.; Buege, D. R.; Hancock, D. D.; Ingham, S. C.; Morgan, J. B.; Reagan, J. O. and Smith, G. C., 1999. Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. J. Food Prot. 62 (2): 140-145.

6. Arthur, T. M.; Brichta-Harhay, D. M.; Bosilevac, J. M.; Guerini, M. N.; Kalchayanand, N.; Wells, J. E.; Shackelford, S. D.; Wheeler, T. L. and Koohmaraie, M., 2008. Prevalence and characterization of *Salmonella* in bovine lymph nodes potentially destined for use in ground beef. J. Food Prot. 71 (8): 1685-1688.

7. Pointon, A. M.; Hamilton, D.; Kolega, V. and Hathaway, S., 2000. Risk assessment of organoleptic postmortem inspection procedures for pigs. Vet. Rec. 146 (5): 124-131.

8. Hamilton, D. R.; Gallas, P.; Lyall, L.; Lester, S.; McOrist, S.; Hathaway, S. C. and Pointon, A. M., 2002. Risk-based evaluation of postmortem inspection procedures for pigs in Australia. Vet. Rec. 151 (4): 110-116.

9. Paton, A. W. and Paton, J. C., 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for $stx_1$, $stx_2$, *eaeA*, enterohemorrhagic *E. coli hlyA*, $rfb_{O111}$, and $rfb_{O157}$. J. Clin. Microbiol. 36 (2): 598-602.

10. Cannon, R. M. and Roe, R. T., 1982. Livestock Disease Surveys: A Field Manual for Veterinarians. Australian Bureau of Animal Health.

11. Phillips, D.; Jordan, D.; Morris, S.; Jenson, I. and Sumner, J., 2006. A national survey of the microbiological quality of beef carcasses and frozen boneless beef in Australia. J. Food Prot. 69 (5): 1113-1117.

12. Bonardi, S.; Foni, E.; Chiapponi, C.; Salsi, A. and Brindani, F., 2007. Detection of verocytotoxin-producing *Escherichia coli* serogroups O157 and O26 in the cecal content and lymphatic tissue of cattle at slaughter in Italy. J. Food Prot. 70 (6): 1493-1497.

13. Agriculture and Resource Management Council of Australia and New Zealand, 2002. Canberra. Australian Standard for the Hygienic Production and Transport of Meat and Meat Products for Human Consumption. AS 4696-2002.

# 9 Appendix

Table 1. Summary data for bacteriological counts from bovine lymph nodes.

| Counts | Min | Median | Mean | Standard deviation | 90th % | 95th % | 99th % | Max | % samples positive |
|---|---|---|---|---|---|---|---|---|---|
| *E. coli* | 0.35 | 2.56 | 2.76 | 1.35 | 4.74 | 5.25 | 6.20 | 7.59 | 57% |
| Coliform | 0.57 | 2.61 | 2.87 | 1.35 | 4.83 | 5.50 | 6.25 | 7.11 | 68% |
| APC | 0.69 | 3.41 | 3.57 | 1.50 | 5.42 | 6.14 | 7.77 | 9.10 | 97% |

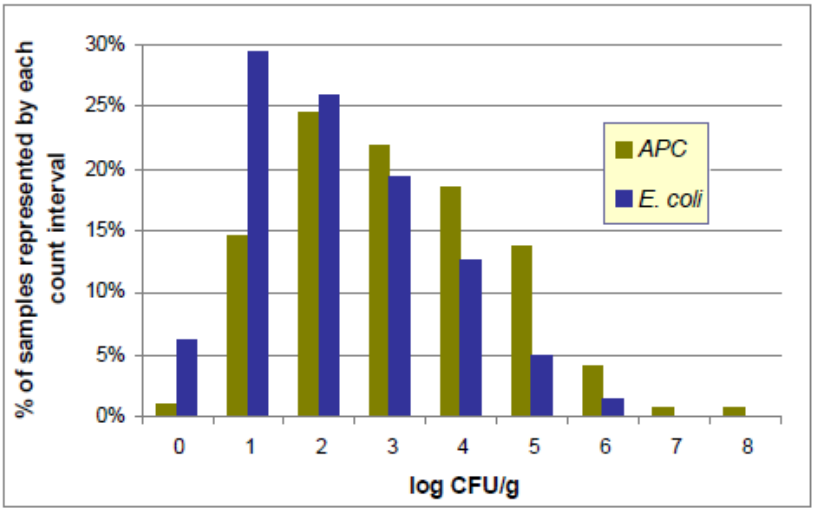


Figure 1. Distribution of *E. coli* Counts and Aerobic Plate Counts (APC) in Bovine Lymph Nodes.

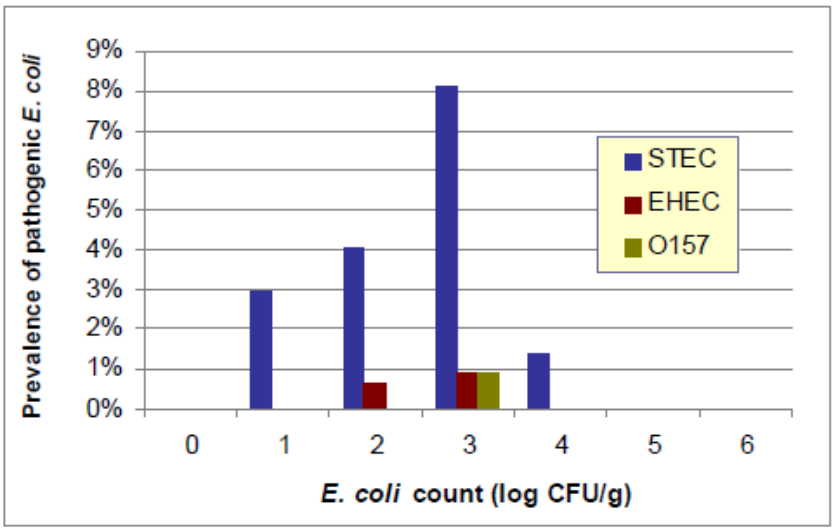


Figure 2. Distribution of pathogenic *E. coli* prevalence relative to *E. coli* count.

**Table 2. Microbiological parameters for bovine lymph nodes collected from seven abattoirs.**

| Abattoir | *E. coli* count | % samples positive for *E. coli* | Aerobic plate count | % samples positive for aerobic bacteria | STEC prevalence | EHEC * prevalence |
|---|---|---|---|---|---|---|
| A | 3.02 | 84.9% | 4.06 | 100.0% | 4.5% | 0.50% |
| B | 1.77 | 13.3% | 2.36 | 83.3% | 3.3% | 0.00% |
| C | 2.39 | 64.6% | 3.54 | 99.0% | 6.1% | 0.51% |
| D | 1.51 | 40.0% | 2.63 | 98.3% | 3.3% | 0.00% |
| E | 2.25 | 40.6% | 3.10 | 97.1% | 1.4% | 0.00% |
| F | 2.36 | 30.9% | 3.07 | 94.1% | 3.2% | 0.53% |
| G | 3.46 | 77.9% | 4.62 | 96.5% | 3.5% | 0.00% |
| TOTAL | 2.76 | 56.8% | 3.57 | 96.7% | 3.8% | 0.30% |

*EHEC were STEC strains also possessing ancillary virulence markers

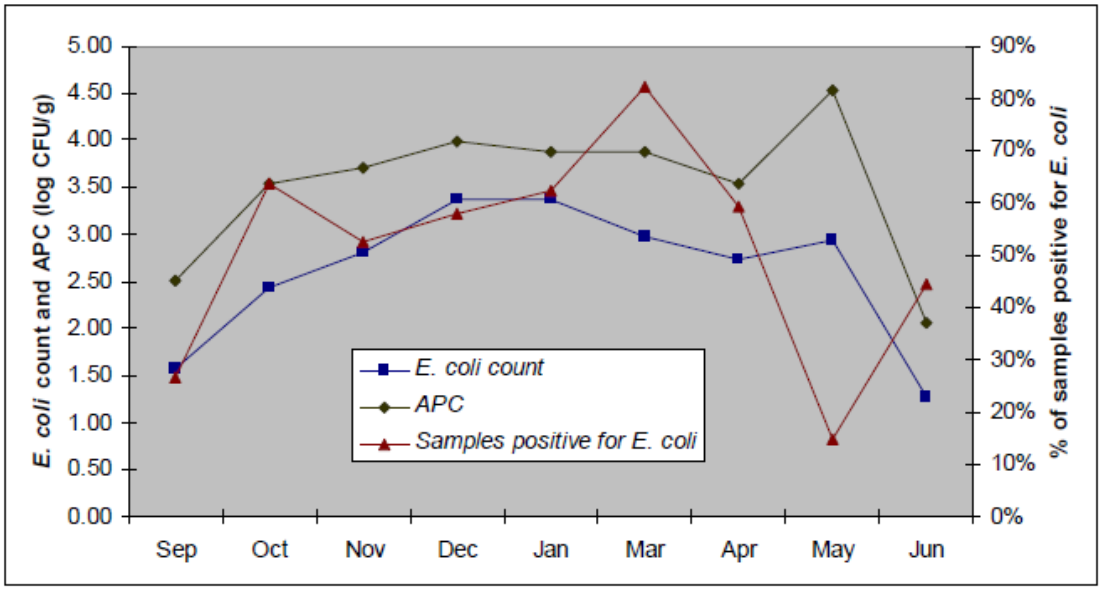


**Figure 3. Temporal trends for *E. coli* counts and prevalence, and APC, among bovine lymph nodes collected over a nine month period.**